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Label-free detection of DNA hybridization with light-addressable potentiometric sensors: Comparison of various DNA-immobilization strategies

T. Bronder[a], C.S. Wu[a,b], A. Poghossian[a,c]*, C.F. Werner[a], M. Keusgen[d], M.J. Schöning[a,c]

[a]*Institute of Nano- and Biotechnologies (INB), FH Aachen, 52428 Jülich, Germany*
[b]*Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Ministry of Education, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, China*
[c]*Peter Grünberg Institute (PGI-8), Research Centre Jülich GmbH, 52425 Jülich, Germany*
[d]*Institute of Pharmaceutical Chemistry, University of Marburg, 35032 Marburg, Germany*

Abstract

Light-addressable potentiometric sensors (LAPS) consisting of a p-Si-SiO_2 and p-Si-SiO_2-Au structure, respectively, have been tested for a label-free electrical detection of DNA (deoxyribonucleic acid) hybridization. Three different strategies for immobilizing single-stranded probe DNA (ssDNA) molecules on a LAPS surface have been studied and compared: (a) immobilization of thiol-modified ssDNA on the patterned Au surface via gold-thiol bond, (b) covalent immobilization of amino-modified ssDNA onto the SiO_2 surface functionalized with 3-aminopropyltriethoxysilane and (c) layer-by-layer adsorption of negatively charged ssDNA on a positively charged weak polyelectrolyte layer of poly(allylamine hydrochloride).

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Keywords: LAPS, lable-free detection, DNA hybridization, field-effect sensor

1. Introduction

Most of the current DNA-hybridization detection techniques require labeling of either the probe or target DNA and have been proven to be time-consuming, complicated, and expensive. Semiconductor field-effect sensors are

* Corresponding author. Tel.: +49-2461-61-2605; fax: +49-241-6009-53235.
E-mail address: a.poghossian@fz-juelich.de

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charge-sensitive devices [1-4] and therefore, are able to detect charged molecules (e.g., DNA [5-8], proteins [8-10], polyelectrolytes [11], dendrimers [12], etc.) by their intrinsic molecular charge that opens wide opportunities for the creation of label-free DNA arrays and protein chips with direct electrical readout. Recently, we demonstrated the feasibility of a multi-spot LAPS, which is a kind of field-effect device, for the label-free detection of DNA hybridization for the first time [13]. LAPS provide the possibility of spatially resolved readout of different spots on the sensor surface of a single chip. In general, the hybridization signal strongly depends amongst others on the distance between the DNA charge and the LAPS gate surface and consequently, on both the surface functionalization and DNA immobilization technique. In this work, two types of LAPS consisting of a p-Si-SiO_2 and p-Si-SiO_2-Au structure have been fabricated and tested for the DNA-hybridization detection. Three different strategies have been used to immobilize probe ssDNA molecules (20 bases) on the LAPS surface (see section 2.2).

2. Experimental

2.1. Sensor fabrication

The LAPS chips were manufactured by means of conventional microfabrication processes. The starting material was a 400 µm thick p-doped Si wafer (<100>, 1-10 Ωcm) with a 30 nm SiO_2 layer, grown by thermal dry oxidation. In the next step, the SiO_2 layer on the rear side of the silicon wafer was removed and an aluminum layer with a thickness of 300 nm was deposited to create an Ohmic contact. For the immobilization of thiol-modified ssDNA, a part of the LAPS structure was covered with a patterned gold layer of 10 nm thickness. After fabrication, the wafer was cut into pieces with sizes of 2.0 cm × 2.0 cm. On the rear side of the LAPS chip, a window was opened by removing the aluminum layer to allow for the light illumination from the back side of the chip. For the electrochemical experiments, the LAPS chip was fixed within the detection chamber and connected to the measurement system for photocurrent measurements.

2.2. DNA immobilization

(a) Immobilization of thiol-modified ssDNA on the patterned Au surface via gold-thiol bonds

After mounting the chip into the measurement chamber, 150 µL of 5 µM thiol-modified probe ssDNA dissolved in 100 mM phosphate buffered saline (PBS) with a pH 8.5, was pipetted on the sensor surface. After ssDNA immobilization, the chip was washed to remove unattached DNA molecules.

(b) Covalent immobilization of amino-modified ssDNA onto the SiO_2 surface functionalized with 3-aminopropyltriethoxysilane (APTES)

For the covalent immobilization of 5'-end amino-modified ssDNA probes, the LAPS surface was first treated with 0.1% (v/v) 3-aminopropyltriethoxysilane (APTES) in toluene solution at room temperature for 1 h to form a smooth thin film of self-assembled APTES. After rinsing with toluene and ethanol, the LAPS surface was incubated with 5% glutaraldehyde for 12 h at room temperature. In the next step, the LAPS surface was exposed to 10 µM probe ssDNA solution (0.1 M PBS, pH 8.5) for 12 h.

(c) Layer-by-layer adsorption of negatively charged ssDNA on a positively charged weak polyelectrolyte layer of poly(allylamine hydrochloride) (PAH)

For probe ssDNA adsorption, the SiO_2 surface was pretreated by piranha solution and then, 150 µL PAH solution (50 µM PAH in 10 mM NaCl, pH 5.4) was applied to the chip for 10 min.

A schematic diagram of different strategies for ssDNA immobilization and hybridization on the LAPS surface is shown in Fig. 1.

2.3. DNA hybridization

For DNA hybridization, two protocols were used: For immobilization strategy (a) and (b), 150 µL solution of complementary target ssDNA (1 µM DNA diluted in 0.1 M PBS, 0.9 M NaCl, pH 7.0) was applied to the chip

surface for 15 min. For strategy (c), the same concentration of target DNA was diluted in 1x Tris-EDTA buffer, pH 8.0 and the incubation time was increased up to 45 min.

2.4. Measurement setup

Fig. 2 shows a schematic structure and measurement setup of the DNA-LAPS. The LAPS signal was recorded after each surface functionalization step by means of photocurrent-voltage measurements. The measurements were carried out in the solution of 0.2 mM PBS, pH 7.0 (for immobilization technique (a) and (b)) or 10 mM NaCl, pH 5.4 (for immobilization technique (c)).

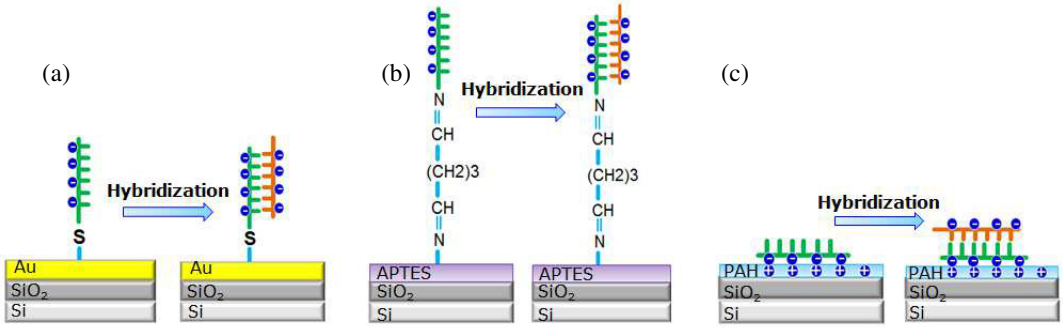

Fig. 1. Different strategies for ssDNA immobilization and hybridization on a LAPS surface. (a) immobilization of thiol-modified ssDNA on the patterned Au surface via gold-thiol bond; (b) covalent immobilization of amino-modified ssDNA onto the SiO_2 surface functionalized with APTES; (c) layer-by-layer adsorption of negatively charged ssDNA on a positively charged PAH. All ssDNA sequences used in this study contain 20 bases.

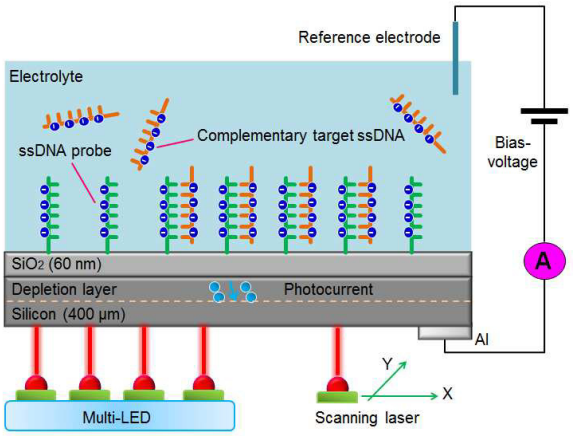

Fig. 2. Schematic of the DNA-LAPS structure as well as multi-spot and scanning-beam setup.

3. Results and Discussion

For all three kinds of DNA immobilization strategies, significant shifts of the photocurrent-voltage curves of the LAPS were observed after the hybridization of probe ssDNA molecules with complementary target ssDNA. As an example, Fig. 3 shows the shift of the photocurrent-voltage curve of the LAPS after hybridization of target ssDNA with thiol-modified probe ssDNA. The highest hybridization signal of about 100 mV was recorded for the LAPS with probe ssDNA immobilized onto the APTES-modified SiO_2 surface.

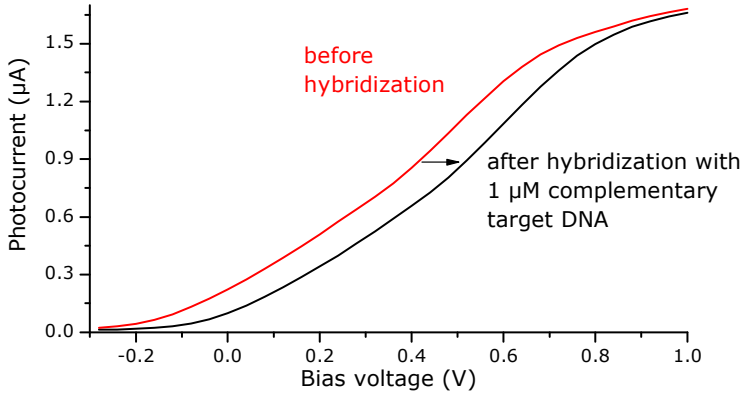

Fig. 3. Shift of the photocurrent-voltage curve of the LAPS after hybridization of target ssDNA with amino-modified probe ssDNA.

4. Conclusion

Three different methods for ssDNA immobilization on a LAPS surface have been tested. The obtained results demonstrate the distinct potential of LAPS devices for a label-free detection of the DNA-hybridization event with direct electrical readout.

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