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Sensing of double-stranded DNA molecules by their intrinsic molecular charge using the light-addressable potentiometric sensor

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ABSTRACT

A multi-spot light-addressable potentiometric sensor (LAPS), which belongs to the family of semiconductor field-effect devices, was applied for label-free detection of double-stranded deoxyribonucleic acid (dsDNA) molecules by their intrinsic molecular charge. To reduce the distance between the DNA charge and sensor surface and thus, to enhance the electrostatic coupling between the dsDNA molecules and the LAPS, the negatively charged dsDNA molecules were electrostatically adsorbed onto the gate surface of the LAPS covered with a positively charged weak polyelectrolyte layer of PAH (poly(allylamine hydrochloride)). The surface potential changes in each spot of the LAPS, induced by the layer-by-layer adsorption of a PAH/dsDNA bilayer, were recorded by means of photocurrent-voltage and constant-photocurrent measurements. In addition, the surface morphology of the gate surface before and after consecutive electrostatic adsorption of PAH and dsDNA layers was studied by atomic force microscopy measurements. Moreover, fluorescence microscopy was used to verify the successful adsorption of dsDNA molecules onto the PAH-modified LAPS surface. A high sensor signal of 25 mV was registered after adsorption of 10 nM dsDNA molecules. The lower detection limit is down to 0.1 nM dsDNA. The obtained results demonstrate that the PAH-modified LAPS device provides a convenient and rapid platform for the direct label-free electrical detection of in-solution hybridized dsDNA molecules.

1. Introduction

DNA (deoxyribonucleic acid) biosensors and microarrays are considered as powerful tools for a wide variety of applications, including, for instance, DNA sequencing, gene expression analysis, clinical diagnostics, pathogen identification, drug and food industry, forensic and parental testing or detection of biowarfare and bioterrorism agents [1–4]. The most technologies for the development of DNA microarrays are currently based on labelling strategies and therefore, they utilize various labels (e.g., fluorescence, redox, enzymatic) for a signal readout and sensitivity enhancement [5]. Although the labeling procedure provides a high sensitivity, however, it requires additional sample preparation steps and has been proven to be complicated, time-consuming and might be not suited for portable point-of-care or mobile diagnostic systems [6]. Therefore, various label-free strategies (e.g., quartz crystal microbalance, surface plasmon resonance, heat transfer, faradaic and non-faradaic impedimetry) have been developed and applied in DNA analytics [7,8]. Especially, electrical detection of DNA molecules by their intrinsic molecular charge using semiconductor field-effect devices (FED) based on an electrolyte-insulator-semiconductor (EIS) system represents a promising label-free platform. It has been attracted much attention owing to the well-established semiconductor technologies available for the fabrication of miniaturized FED-based genosensors and DNA chips. In these devices, the adsorption and binding of charged molecules (e.g., DNA, proteins, polyelectrolytes) or charged nanoparticles on the gate surface of the FED changes the space-charge distribution in the semiconductor, resulting in a change in the output signal of the FED [9–11]. In previous studies, various kinds of FEDs, like capacitive EIS sensors, ion-sensitive field-effect transistors, Si-nanowire transistors and light-addressable potentiometric sensors (LAPS), have been applied for the detection of DNA binding events; this includes adsorption, hybridization, single nucleotide polymorphisms, DNA extension or amplification by polymerase chain reaction (PCR) as well as DNA sequencing [11–19]. In addition, owing to their surface-

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charge-sensitive properties, FEDs are widely implemented for the detection of pH, ions and analyte concentrations in liquids [20–28].

For the detection of specific sequences from an unknown DNA sample, the highly selective base-pairing reaction known as hybridization reaction is mainly used, by which a single-stranded probe DNA (ssDNA) molecule binds specifically to its complementary single-stranded target DNA (cDNA), forming a double-stranded DNA (dsDNA). The vast majority of DNA-FEDs reported in literature detect the so-called on-chip hybridization event: typically, probe ssDNA molecules of known sequences are immobilized onto the FED surface by adsorption or covalent attachment and the subsequent hybridization event is either detected *ex situ* by measuring the sensor signal before and after hybridization or *in situ* by monitoring the sensor signal during the hybridization process. At the same time, very little is known about the application of FEDs for direct label-free electrical detection of dsDNA formed after hybridization reaction occurred in the solution (further referred as in-solution hybridization). In some cases, this could offer several advantages over detection by on-chip hybridization, especially when FEDs are used for the detection of DNA amplification by PCR [29–31]. Since PCR generates dsDNA, no extra sample preparation steps such as the heating of the PCR product to generate cDNA for the on-chip hybridization followed by the rapid cooling to prevent re-hybridization, are required. Moreover, by direct dsDNA detection, the surface modification procedure can significantly be simplified, because no probe ssDNA have to be immobilized onto the sensor surface. Thus, direct dsDNA detection could reduce the detection time and costs and might even increase the reproducibility of DNA analysis.

In this work, a multi-spot (16 spots) LAPS modified with a positively charged weak polyelectrolyte layer of PAH (poly(allylamine hydrochloride)) was applied for the direct label-free electrical detection of in-solution hybridized dsDNA molecules by their intrinsic molecular charge for the first time. It can be expected that in the presence of a positively charged polyelectrolyte layer, the electrostatically adsorbed dsDNA molecules will be preferentially flat-oriented on the LAPS surface. This results in molecular charges positioned near the gate surface within the Debye length (the Debye length defines the distance at which the electrostatic potential drops 1/e), yielding a reduced charge-screening effect and a higher detection signal. During experiments, a consecutive layer-by-layer (LbL) adsorption of PAH and dsDNA molecules was monitored by means of photocurrent–voltage (I_{ph}–V_g) and constant-photocurrent measurements. For comparison, the adsorption of dsDNA directly on a bare LAPS surface (without PAH layer) has been studied, too. In addition, the surface morphology of the adsorbed PAH and PAH/dsDNA layers was investigated by atomic-force microscopy (AFM), while fluorescence measurements were used to verify the successful dsDNA adsorption onto the PAH-modified LAPS surface.

2. Materials and methods

2.1. LAPS chip fabrication

LAPS chips consisting of an Al–Si–SiO_2 structure with sizes of 2 cm × 2 cm were fabricated using a ~400 μm thick p-doped Si wafer (resistivity 1–10 Ωcm). A 60 nm high-quality SiO_2 layer was prepared by thermal dry oxidation of the Si. To create an Ohmic contact to Si, the SiO_2 layer on the rear side of the silicon wafer was etched and then, a 300 nm thick Al layer was deposited on the rear side of the silicon wafer and patterned to open a window for the backside illumination of the Si. After fabrication, each chip was cleaned in ultrasonic bath with acetone, isopropyl alcohol, ethanol, deionized (DI) water and conditioned in 0.66 mM phosphate buffer solution (PBS), pH 7.5, 10 mM NaCl (further referred as measurement solution) for at least 12 h, in order to reduce the drift of the SiO_2-gate LAPS devices.

2.2. Multi-spot LAPS setup

Fig. 1 shows a schematic of the multi-spot LAPS consisting of an Al–Si–SiO_2 structure and measurement setup. Functioning of the multi-spot LAPS has been described in detail in Ref. [12]. Briefly, since the LAPS represents a potential (charge)-sensitive device, the adsorption or binding of charged molecules on the gate surface of the LAPS will modulate the flatband voltage (the voltage at which the energy bands in the semiconductor continue horizontally up to the surface) and the space-charge capacitance in the semiconductor. In order to detect the changes in the space-charge or depletion capacitance induced by the molecular adsorption, the LAPS is illuminated with a modulated light, which generates an alternating photocurrent as the sensor signal. In the multi-spot LAPS setup, multiple regions (16 spots) on the back-side of the Si have been illuminated in parallel by using an array of 4 × 4 infrared light-emitting diodes (LED) with a wavelength of 950 nm, where each LED is modulated at a different frequency ranging from 1 to 1.75 kHz [12]. The diameter of the spot illuminated by a single LED was about 2 mm,

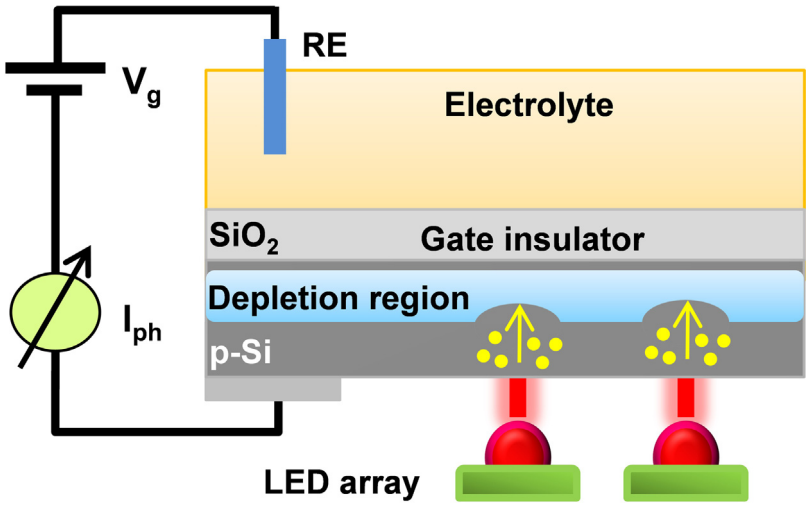

Fig. 1. Schematic of the multi-spot LAPS consisting of an Al–Si–SiO_2 structure and measurement setup. RE: reference electrode; I_{ph}: photocurrent; V_g: gate voltage.