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Highly-sensitive and label-free indium phosphide biosensor for early phytopathogen diagnosis

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ABSTRACT

The development of highly-sensitive and label-free operating semiconductor-based, biomaterial detecting sensors has important applications in areas such as environmental science, biomedical research and medical diagnostics. In the present study, we developed an Indium Phosphide (InP) semiconductor-based resistive biosensor using the change of its electronic properties upon biomaterial adsorption as sensing element. To detect biomaterial at low concentrations, the procedure of functionalization and covalent biomolecule immobilization was also optimized to guarantee high molecule density and high reproducibility which are prerequisite for reliable results. The characterization, such as biomolecular conjugation efficiency, detection concentration limits, receptor:ligand specificity and concentration detection range was analyzed by using three different biological systems: i) synthetic dsDNA and two phytopathogenic diseases, ii) the severe CB-form of *Citrus Tristeza Virus* (CTV) and iii) *Xylella fastidiosa*, both causing great economic loss worldwide. The experimental results show a sensitivity of 1 pM for specific ssDNA detection and about 2 nM for the specific detection of surface proteins of CTV and *X. fastidiosa* phytopathogens. A brief comparison with other semiconductor based biosensors and other methodological approaches is discussed and confirms the high sensitivity and reproducibility of our InP based biosensor which could be suitable for reliable early infection diagnosis in environmental and life sciences.

1. Introduction

The development of highly-sensitive and label-free operating biosensors with conjugated biomolecules has important applications in areas such as environmental science, biomedical research and medical diagnostics (Lazcka et al., 2007; Su et al., 2011; Xue et al., 2011). Due to difficulties associated with the sensitivity, reproducibility, chemical stability, and non-specific physisorption of biomolecules to surfaces such biosensors are not available from commercial sources. In order to circumvent these problems new approaches have to be developed to create new biosensor platforms. Most biosensing devices rely on metal-oxide-semiconductor structures based on silicon (Gonçalves et al., 2008; Jane et al., 2009; Lazcka et al., 2007; Nicu and Leïchlé, 2008); few studies have used III–V semiconductors for biomolecular detection. In fact, several studies indicate that these materials show a high sensitivity to detect gases (Chattopadhyay et al., 2009; Kimura et al., 2006; Wierzbowska et al., 2008), due to the ample conductance change upon gas adsorption. In particular, for gas detection, Indium Phosphide (InP) is more convenient as potentiometric and amperometric sensor than silicon (Sato et al., 2010; Talazac, 2001). So far, for biomolecular detection applications in liquid environment, n-type InP porous nanostructures were successfully used to detect a glucose oxidase enzyme (Sato et al., 2010). In this study we show the development of a new type of resistive biosensor using InP as biosensing platform for liquid environment.

In the general context of semiconductor biosensors, the surface properties and, consequently, the functionalization quality play an increasingly important role for the overall device sensing behavior (Sturzenegger et al., 1999). A standard method of semiconductor surface functionalization specify the use of alkoxysilanes by esterification of surface silanole groups to create amino grafting sites for further biomaterial immobilization (Lenci et al., 2010; Logatcheva and Horton, 2008). However, non-homogeneous and micro-structured poly(siloxane) surface coatings are frequently obtained (Vandenberg et al., 1991; Wang et al., 1992; Wang and Jones, 1993). Surface

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functionalization using ethanolamine hydrochloride is described in the literature (Ebner et al., 2007) as an alternative method to create an acceptable number of amino grafting sites. In contrast to published work (Sturzenegger et al., 1999; Park and Ivanisevic, 2007; Zerulla and Chassé, 2009) regarding InP functionalization, in this study ethanolamine and poly(ethylene glycol) (PEG) were used to ensure a homogeneous, dense and reproducible immobilization of DNA, peptides and proteins (Janissen et al., 2009).

Physically and chemically inert linear PEG chains have been commonly used as flexible linkers to attach single biomolecules to different surfaces (Albrecht et al., 2003; Hinterdorfer et al., 2002; Kamruzzahan et al., 2006; Walsh et al., 2001). These linkers allow a rapid and free re-orientation of the attached receptor biomolecule and also shield the surface from non-specific physisorption (Blank et al., 2003; Yong-Mei et al., 2007). The carboxylic group of the custom-synthesized heterobifunctional NHS-PEG-COOH (N-hydroxysuccinimide-PEG-carboxylic acid) applied here can be used to immobilize different types of biomolecules and reacts non-specifically less often compared to the more commonly used amino and aldehyde end groups (Janissen et al., 2009; Krautbauer et al., 2003; Lindroos et al., 2001).

The sensing element of this biosensing device is the change of the electronic properties of the crystalline semiconductor (Seker et al., 2000) due to the immobilized biomaterial on the surface and can be compared with the operation of a field-effect transistor (FET) in which the channel of charge carriers is controlled capacitively by an electric field (Fig. 1).

In an n-type device as used in this study, a negative voltage of the gate causes an expansion of the depletion region width and pinches off the conduction channel, reducing the flow of charge carriers. Consequently, the change in gate voltage also changes the resistance of the conduction channel while the source-drain current is proportional to the applied voltage within a voltage range, in which case the device is operating under linear ohmic regime (Galup-Montoro and Schneider, 2007; Sze and Kwok, 2007). The biosensing

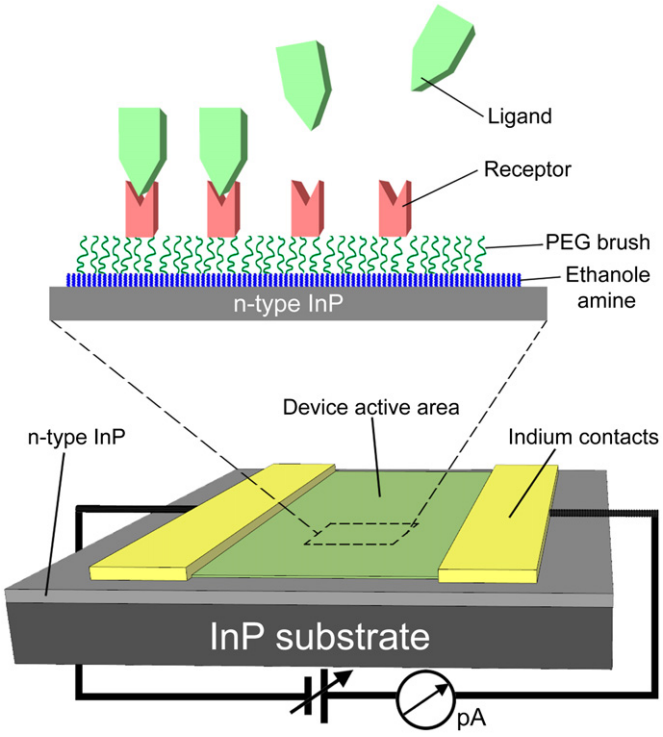

Fig. 1. Schematic presentation of the InP-biosensor setup and functionalization method via amination, PEGylation and covalent coupling of biomolecular receptor to detect specific ligand interaction via electrical resistance change measurement.

conduction channel is represented as an InP epitaxial film with indium contacts defining the active biosensing device area at which the receptor biomolecules are covalently coupled. The change in charge distribution resulting from specific receptor:ligand interactions corresponds to the gate voltage modulation on the device active area and thereby increase or decrease the overall resistance. Thus, it is possible to detect specific molecular interaction events and the amount of the ligand material by measuring the associated changes in resistance (Seker et al., 2000).

In this study we present the development and characterization of the first label-free InP biosensor for liquid environment using three different biomolecular receptor:ligand systems: i) dsDNA oligonucleotides as a highly-reproducible and comparable standard, ii) *Citrus Tristeza Virus* (CTV) capsid CB-22 protein and iii) *Xylella fastidiosa* XadA1 adhesion protein, both demonstrating a direct application to detect phytopathogens which have been associated with a large number of diseases, causing large economic losses (Chatterjee et al., 2008; Peroni et al., 2009) worldwide.

2. Material and methods

2.1. Materials

Super pure-grade materials were used, when commercially available. NHS-PEG-COOH (MW3400) was custom-synthesized by LaysanBio (USA). Semi-insulating, nominally (0 0 1) oriented InP wafers were purchased from InPAct (France). Used DNA oligonucleotides (receptor-ssDNA:5′-NH2-CCACTCGTGACGCATTCACCTCAGCAG-CACTCCTCCTCGG-3′; complementary Atto647n-fluorophor labeled ssDNA:5′-CCGAGGAGGAGTGCTGCTGAGGTGAATGCGTCACGAGTGGA-tto647n-3′; non-complementary Atto647n-fluorophor labeled ssDNA: 5′-GGCTCCTCCTCACGACGACTCCACTTACGCAGTGCTCACCAtto647n-3′) were synthesized by PURIMEX (Grebenstein, Germany). Deionized water was obtained from *Barnstead* (USA) water system (NANOpure). Goat anti-mouse and anti-rabbit antibodies conjugated with a rhodamine fluorophore were purchased from Rheabiotec (Brazil).

2.2. Indium phosphide biosensor preparation

A ~300 nm thick n-type layer of InP was grown on semi-insulating InP substrates by *Chemical Beam Epitaxy* (CBE); the layer thickness was measured by profilometry and atomic force microscopy (Cotta et al., 1995a, 1995b; Bortoleto et al., 2002). The as-grown n-type layer presents a residual electron carrier concentration of $\sim 10^{16}$ cm^{-3} (measured by Hall effect) which is commonly used for InP gas sensors (Talazac, 2001). The sample was cut into 9×8 mm^2 dimensions. The ohmic contacts using indium at the edges of the sample were annealed at 450 °C for 15 min in a nitrogen atmosphere defining the active area of the sensor device. Thin copper wires (400 μm in diameter) were welded onto the ohmic contacts and connected to the picoammeter for electrical measurements. Since the InP layers in the active region are not intentionally doped, carrier concentration can vary in the range 9.10^{15}–2.10^{16} cm^{-3} from run to run. This variation does not strongly affect the resistance of the sample which also includes the series resistance of electrical contacts. All sensor electrical measurements are normalized to rule out these effects.

2.3. Production of CB-22, Xf.XadA1 and specific antibodies

The production of the *CTV* CB-22 capsid protein, the *Xylella fastidiosa* adhesion protein Xf.XadA1 and their specific antibodies is described in the supplemental information.