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Hybrid organic/inorganic interfaces as reversible label-free platform for direct monitoring of biochemical interactions

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ABSTRACT

The combination of organic and inorganic materials to create hybrid nanostructures is an effective approach to develop label-free platforms for biosensing as well as to overcome eventual leakage current-related problems in capacitive sensors operating in liquid. In this work, we combine an ultra-thin high-k dielectric layer (Al_2O_3) with a nanostructured organic functional tail to create a platform capable of monitoring biospecific interactions directly in liquid at very low analyte concentrations. As a proof of concept, a reversible label-free glutathione-S-transferase (GST) biosensor is demonstrated. The sensor can quantify the GST enzyme concentration through its biospecific interaction with tripeptide reduced glutathione (GSH) bioreceptor directly immobilized on the dielectric surface. The enzymatic reaction is monitored by electrical impedance measurements, evaluating variations on the overall capacitance values according to the GST concentration. The biosensor surface can be easily regenerated, allowing the detection of GST with the very same device. The biosensor shows a linear response in the range of 200 pmol L^{-1} to 2 μmol L^{-1}, the largest reported in the literature along with the lowest detectable GST concentration (200 pmol L^{-1}) for GST label-free sensors. Such a nanostructured hybrid organic-inorganic system represents a powerful tool for the monitoring of biochemical reactions, such as protein-protein interactions, for biosensing and biotechnological applications.

1. Introduction

The combination of materials of different nature and dimensionalities to form functional elements is an effective approach to address sensing- and biosensing-related challenges (Zheng et al., 2005; Lin et al., 2009; Stern et al., 2007; Casalini et al., 2015). The immobilization of organic layers on nanostructured materials such as silicon nanowires (Ahn et al., 2010; Gao et al., 2010), semiconducting layers (Ashkenasy et al., 2002; Bof Bufon et al., 2011), carbon nanotubes (Lin et al., 2004; Nguyen et al., 2002), high-k oxides films (Bof Bufon et al., 2010; Vervacke et al., 2014, 2012) and, more recently, graphene (Liu et al., 2012; Shan et al., 2009) has been recognized as key to produce devices with novel functionalities and improved performance. In sensing and biosensing, additional advantage is achieved by making use of solid-liquid interfaces for the detection of specific analytes. Insulator/electrolyte (Bousse and Bergveld, 1983) interfaces, for instance, have been widely exploited in transistor-like structures for the detection/monitoring of organic solvents (Grimm et al., 2013), heavy metal ions (Cobben et al., 1992) and biomolecules (Cui, 2001), to mention a few. In order to use such interfaces to target specific molecules, receptor elements are immobilized on top of the insulating layer (Berggren et al., 2001; Cui, 2001).

In capacitive sensors, the observation of changes in the solid/liquid interface requires the capacitance of the insulating layer to be as high as possible (Berggren et al., 2001). This strategy is adopted to enhance the capability of measuring the changes on the electrical double layer capacitance as a result of recognition events occurring at the solid/liquid interface. Consequently, to monitor the concentration of specific analytes in this type of devices, which is proportional to the charge density at the interface, the sensor has to operate at low frequencies (< 100 Hz) (Taylor and Macdonald, 1987), which is known to be unstable (Maupas et al., 1997). The incorporation of insulating organic molecules on top of conducting substrates has also been investigated and modeled (Finklea et al., 1993; Góes et al., 2012). Nevertheless, the permeability of the organic tail to the plethora of ions present in

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solution may bring serious instabilities to device operation (Góes et al., 2012).

The understanding of biochemical interactions is also of fundamental importance for both biosensor's development and the identification of biological mechanisms responsible for a set of physiological activities. Reduced glutathione (GSH), for instance, is an ubiquitous tripeptide associated with vital functions in the body, including the regulation of cell's redox system (Martos-Maldonado et al., 2015). The protection of cells against damage occurs via a detoxification process driven by the conjugation of GSH with xenobiotics, which is mediated by the glutathione S-transferase (GST) enzyme (Martos-Maldonado et al., 2015, 2012). In this sense, the GSH/GST pair is key for detecting different exogenous substances, such as herbicides and insecticides (Kapoli et al., 2008), fungicides (Singh et al., 2009) and anticancer drugs (Materon et al., 2014). Furthermore, the GSH concentration, along with the GST expression and activity, have been associated to the neurodegenerative diseases like Parkinson's and Alzheimer's (Liu et al., 2015; Mazzetti et al., 2015). In addition, variable expressions of GST have been correlated to various types of cancer (Chuang et al., 2005), as well as to the capability of cells to respond to anticancer drugs (Townsend and Tew, 2003; Tsuchida and Sato, 1992). In all cited cases, the evaluation methodology consists on comparing healthy and pathological cells, where the relative changes of GSH and GST quantities are of interest.

Complex or labeled methods of analysis, such as radioimmunoassays (Howie et al., 1989), gene chips (Chuang et al., 2005) and ELISA (Daukantienė et al., 2014) are usually employed to follow the probe/target molecule interaction. These methods, however, usually cannot provide real time measurements, are more expensive and bulkier then label-free systems (Tsouti et al., 2011). The development of a label-free GST biosensor, i.e., an analytical device to directly monitor the presence of the target molecule (GST) without requiring additional reagents, usually luminescent and electrochemical compounds, for signal generation, is a relevant case as a proof of concept for the monitoring of biochemical interactions. In the literature, just a very limited number of methods, including surface plasmon resonance (Jung et al., 2006), silicon nanowire field-effect transistors (Lin et al., 2009) and water-gated organic transistors (de Oliveira et al., 2016) have reported the label-free detection of GST. To the best of our knowledge, if all reported GST label-free sensors (Jung et al., 2006; Lin et al., 2009; Martos-Maldonado et al., 2012; Qin et al., 2015) are combined, GST can be monitored in a range of concentration of three orders of magnitude (from 2×10^{-9} mol L^{-1} to 4.2×10^{-6} mol L^{-1}). In addition, most of these devices cannot be reused since they employ innovative but complexe functionalization methods that may lead to difficulties in obtaining reproducible manufacturing/regeneration processes as well as routes for GSH/GST monitoring that are not reversible (Jung et al., 2006; Martos-Maldonado et al., 2012; Qin et al., 2015).

In this work, we demonstrate a label-free, reversible and highly sensitive hybrid organic-inorganic biosensor to monitor GSH/GST at a wide range of GST concentration (from 200 pmol L^{-1} to 2 μmol L^{-1}). The sensor's architecture and operation rely on the combination of an ultra-thin high-k dielectric layer (3.3 nm Al_2O_3) with the functional bioactive tail to monitor variations of the net charge at the hybrid solid/liquid interface caused by the GSH/GST biospecific interaction. Here, the organic-inorganic combination allowed us to overcome two critical drawbacks in capacitive biosensors: (a) the instabilities arising from low frequency operation (Maupas et al., 1997; Taylor and Macdonald, 1987) and (b) the high ionic permeability of the biorecognition layer, which may lead to electrical instabilities and irreproducible measurements (Berggren et al., 2001). The conformational Al_2O_3 coating of metallic electrodes is capable of suppressing eventual leakage currents and enables the device to continuously operate in aqueous buffered medium.

The immobilization of biomolecules in biosensors and related biotechnological applications commonly utilizes functionalization agents bearing thiol and silane groups (Lin et al., 2009; Casero et al., 2002). Here, we employed an alternative functionalization route capable of delivering high quality self-assembled phosphonic acid monolayers (SAM) chemically bonded to the Al_2O_3 nanocoating. Such a layer is part of the organic functional tail of our device that allows the GSH immobilization. Once the functionalized device is exposed to the GST in aqueous phosphate buffer solution (PBS), the GSH/GST reversible interaction can be precisely monitored. The very same biosensor was successfully employed to quantify the GST concentration from 200 pmol L^{-1} to 2 μmol L^{-1}; the broadest linear operation range reported so far to this enzyme. For comparison, even combining all label-free devices reported in literature, our device outperforms their operation range in one order of magnitude. In addition, to the best of our knowledge, we also achieved the lowest detectable concentration of GST (200 pmol L^{-1}) among such devices (Jung et al., 2006; Lin et al., 2009). From the manufacturing point of view, the fabrication process is compatible with standard microfabrication techniques and the scaling-up production is simple and straightforward. Finally, the devices exhibited a stable operation, with the possibility of being constantly regenerated for further use.

2. Materials and methods

2.1. Fabrication of the biosensor's inorganic structure

The inorganic part of the device comprises nickel interdigitated electrodes, prepared by standard photolithography and thin-film deposition processes (see Section 1 and Fig. S1 in the Supplementary material for details), coated with a conformational Al_2O_3 insulating layer. Therefore, the device's electrodes are protected from PBS used for the device functionalization and characterization. The gap between electrodes (~21 μm) is filled with PBS. In such a configuration, the device electrical impedance depends on the insulator thickness, the aqueous medium (PBS) ionic strength, and the device interfacial characteristics. The equivalent circuit shown in Fig. 1b represents the inorganic part of the device when exposed to the liquid medium. The modification created by the immobilization of the bioactive layer at the oxide surface is expected to induce changes in the electrical double layer capacitance (C_{dl}) at the solid/liquid interface. Therefore, to assess such changes, the capacitance of the insulating layer (C_{ox}) has to be maximized. For simplicity, two variables were initially considered: the insulating layer dielectric constant (ε) and thickness (d). Here, we opted to use an ultrathin Al_2O_3 film deposited by atomic layer deposition (ALD) at 150 °C as the device insulator. This choice is made based on the following reasons: firstly, ALD guarantees an excellent conformational coating within the monolayer precision level (Groner et al., 2004); secondly, amorphous Al_2O_3 layers with thicknesses smaller than 10 nm can exhibit both high ε (~9) and low leakage currents (Bof Bufon et al., 2010). Different from what has been recently reported (Correa et al., 2015), no signature of degradation of the Al_2O_3 nanocoating in the presence of aqueous solution was observed. Here, stability is achieved after conditioning the Al_2O_3 layer using an ac voltage with amplitude 5 times higher than the signal used to characterize the devices (200 mV). The procedure consists of immersing the Al_2O_3 coated interdigitated electrodes in PBS while applying 1 V to the devices pads. This process takes approximately 30 min and provides the stability necessary to operate the devices in aqueous medium (see Section 2 in the Supplementary material).