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# Fully integrated graphene electronic biosensor for label-free detection of lead (II) ion based on G-quadruplex structure-switching

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ABSTRACT

This work presents a fully integrated graphene field-effect transistor (GFET) biosensor for the label-free detection of lead ions ($Pb^{2+}$) in aqueous-media, which first implements the G-quadruplex structure-switching biosensing principle in graphene nanoelectronics. We experimentally illustrate the biomolecular interplay that G-rich DNA single-strands with one-end confined on graphene surface can specifically interact with $Pb^{2+}$ ions and switch into G-quadruplex structures. Since the structure-switching of electrically charged DNA strands can disrupt the charge distribution in the vicinity of graphene surface, the carrier equilibrium in graphene sheet might be altered, and manifested by the conductivity variation of GFET. The experimental data and theoretical analysis show that our devices are capable of the label-free and specific quantification of $Pb^{2+}$ with a detection limit down to 163.7 ng/L. These results first verify the signaling principle competency of G-quadruplex structure-switching in graphene electronic biosensors. Combining with the advantages of the compact device structure and convenient electrical signal, a label-free GFET biosensor for $Pb^{2+}$ monitoring is enabled with promising application potential.

## 1. Introduction

Lead ion ($Pb^{2+}$), a widespread heavy metal pollutant in the water environment (Guo et al., 2014), has become a severe threat to human health, due to its long-lasting, cumulative detrimental effects (Needleman, 2004). To strengthen the control of $Pb^{2+}$ pollutions, efficient and reliable detection techniques are of great significance. Nowadays, $Pb^{2+}$ detections in aqueous-media commonly rely on instrumental methods, such as atomic absorption spectroscopy (AAS), atomic emission spectrometry (AES) and inductively coupled plasma mass spectrometry (ICP-MS) (Duarte et al., 2016; Foltynová et al. 2014; Sixto et al., 2016). Although these techniques are capable of the accurate quantification of $Pb^{2+}$, the needs for expensive equipment and professional operations still hinder further applications. As a result, novel sensor techniques, as well as analytical tools, for rapid, simple and convenient $Pb^{2+}$ detections, have been attracting more and more research interests.

Recently, biosensors exploiting specific biorecognition principles for $Pb^{2+}$ detection have been reported. As the functional nucleic acid researches revealed (Beissenhirtz and Willner, 2006; Liu et al., 2009), selected oligonucleotide sequences specifically responsive to $Pb^{2+}$(i.e., DNAzyme and G-quadruplex) can be employed as the pivot receptors (Han et al., 2016; Li et al., 2009). Herein, similar to the DNAzyme-based $Pb^{2+}$ biosensors relying on the specific binding or cleavage of designated site (Wang et al., 2015b), the G-quadruplex-based methods can achieve the signaling of $Pb^{2+}$ recognition via the DNA structure-switching (Kong et al., 2009; Neidle and Read, 2000; Parkinson et al., 2002). Although the utility of G-quadruplex has been demonstrated in the development of $Pb^{2+}$ biosensors, the signal readout still required laboratory analytical instruments, such as electrochemical and optical platforms (Guo et al., 2012; Li et al., 2010; Lin et al., 2011). To our knowledge, integrated G-quadruplex biosensor, which is freed from the instrument restriction and capable of convenient $Pb^{2+}$ detections like the DNAzyme-based electronic $Pb^{2+}$ biosensor (Wang et al., 2016a), is yet to be fulfilled.

Here, we present a novel biosensor that first incorporates the G-quadruplex structure-switching signaling principle in a graphene electronic nanodevice for the label-free $Pb^{2+}$ detection in aqueous-media. We consider that DNA molecules in aqueous-media are naturally charged with negative electricity (DNA isoelectric point is about pH 5) (Stotzky, 2000), and the charge distribution is related to the DNA secondary structure. Thereby, the DNA structure-switching

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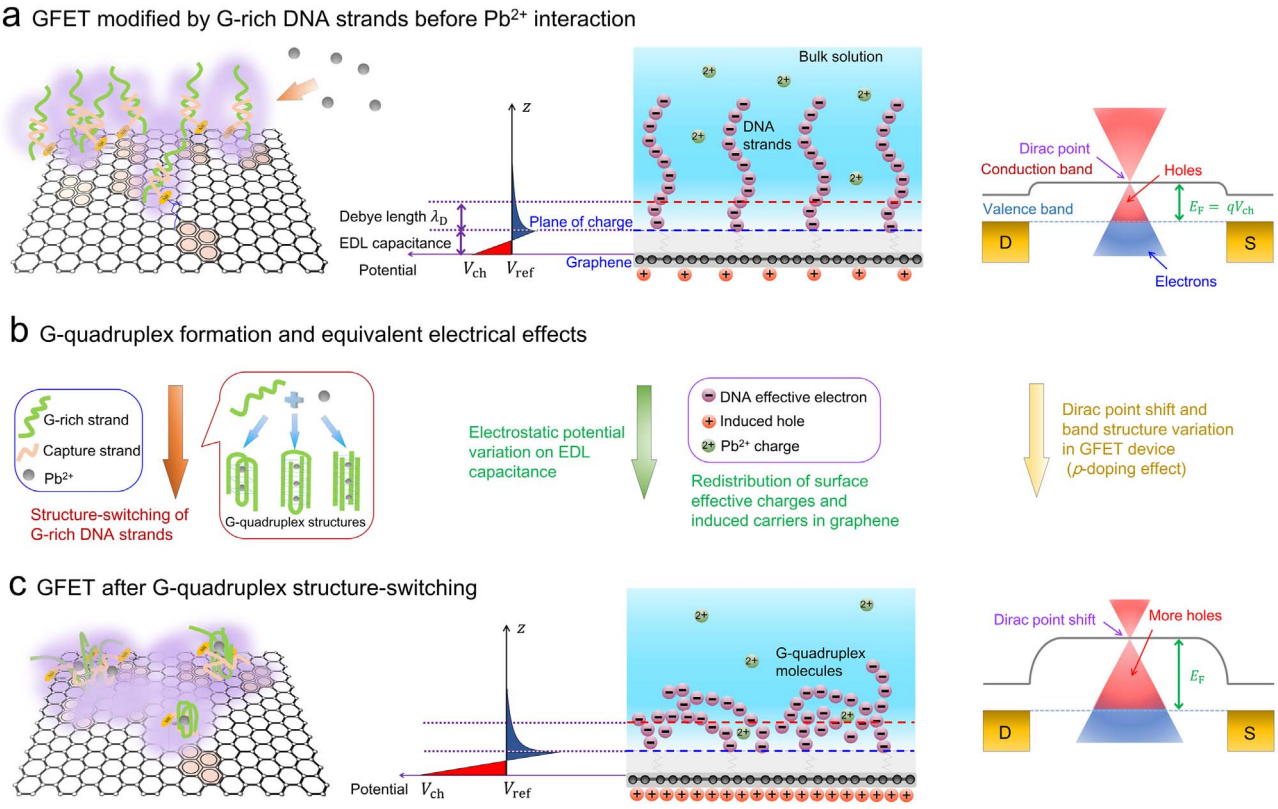


**Fig. 1.** G-quadruplex structure-switching principle and schematic illustrations of electrical response mechanism in GFET. (a) Before G-quadruplex formation: Along *z* axis (upward direction perpendicular to graphene surface), charges distributing on the distal ends of DNA strands cannot effectively apply electrostatic potential onto the charge plane of EDL capacitance due to the Debye screening. The effective hole density and chemical potential ($V_{ch}$) of graphene are relatively low. The GFET exhibits a slight *p*-type doping performance. (b) In the presence of $Pb^{2+}$: G-rich DNA strands switch into the G-quadruplex structures, and thus lead to changes of electric properties. (c) After G-quadruplex structure-switching: More DNA charges move closer to the charge plane of EDL that increase the hole density in graphene, and thus strengthens the *p*-type doping of GFET.

may enable a label-free sensing principle that transduces biomolecular interactions into electrical signals via the charge redistribution on semiconductors (Chen et al., 2008). To realize this principle, we choose graphene, the most sensitive electronic material people ever discovered (Novoselov et al., 2005), to fabricate sensor devices. As a two-dimensional semiconductor with extraordinarily high carrier mobility (Ponomarenko et al., 2009), the carrier equilibrium fluctuation in graphene reflecting interfacial biomolecular interactions can be observed through conductivity variations under the graphene field-effect transistor (GFET) configuration (Cai et al., 2015; Cai et al., 2014; Chen et al., 2013; Kwak et al., 2012; Ohno et al., 2010a, 2010b; Saltzgaber et al., 2013; Wang et al., 2016a; Yan et al., 2014; Yin et al., 2011). In addition, to offer our devices a fully integrated structure for the convenient application, a high-$\kappa$ solid-gate GFET configuration (Wang et al., 2016b; Zhu et al., 2015) is utilized in this work (Fig. 2). Compare to the conventional liquid-gate GFET biosensors, our devices eliminate the structural instability due to the external wire gate electrode (Wang et al., 2015a; Zhu et al., 2016), and thus fundamentally overcome the device reliability hindrance in practical applications.

The sensing principle is designed as shown in Fig. 1. While the graphene sheet is immerged in aqueous-solution (Fig. 1a), an electrical double layer (EDL) is formed on the hydrophobic graphene surface (Bonthuis et al., 2011; Rafiee et al., 2012). Changes of electric properties arising from the interfacial biomolecular interactions can be designed to enable the $Pb^{2+}$ detection (Hepel, 2012; Hepel et al., 2012; Stobiecka et al., 2010; Stobiecka and Hepel, 2011). From the viewpoint of GFET electronics, the one-end immobilized DNA strands carry negative charges by ionization in solution (Stotzky, 2000), and electrostatically induce holes in graphene through the equivalent EDL capacitance. Hence, the graphene sheet may exhibit a positive chemical potential $V_{ch}$ (Novoselov et al., 2005), which renders the GFET a *p*-type doping performance (see electrical modeling and energy band diagrams in Fig. 1a). Meanwhile, the Debye screening effect on DNA charges has to be taken into account. For instance, in physiological saline solutions (ionic strength $I$ ~150 mM), the electrostatic potential of a point charge exponentially decreases with distance and approaches its $1/e$ at the Debye length $\lambda_D \approx 0.7$ nm. Thereby, the charged nucleotides away from the charge plane of EDL farther than the induction range (~2 nm) may not effectively induce holes in graphene (Sorgenfrei et al., 2011). While the DNA strands interact with $Pb^{2+}$ ions and fold into G-quadruplex structures (Fig. 1b) (Kong et al., 2009; Neidle and Read, 2000; Parkinson et al., 2002), more charged nucleotides might be brought into the induction range that increase the hole density in graphene (Wang et al., 2016b). As a result, the GFET may exhibit an increasing *p*-doping effect and corresponding conductivity responses that enable the label-free biosensing of $Pb^{2+}$(Fig. 1c). In addition, we note that the G-quadruplex formation may also incorporate $Pb^{2+}$ ions into the induction range, which is prone to neutralize a part of the negative charges. Here, on the basis of previous investigations (Kong et al., 2009; Neidle and Read, 2000; Parkinson et al., 2002), we conjecture that the hole density increase in graphene led by G-quadruplex structure-switching plays the dominant role.

## 2. Materials and methods

### 2.1. Chemicals and materials

Copper foil (99.8%, 25 μm thick) for the chemical vapor deposition (CVD) graphene synthesis was purchased from Alfa Aesar (Ward Hill, MA). Silicon wafers (*n*-type, 1 μm $SiO_2$-coated) for device substrate were purchased from Crystal-Silicon Electronics (Suzhou, China). Polydimethylsiloxane (PDMS) for microfluidics was purchased from