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Triggering the Electrolyte-Gated Organic Field-Effect Transistor output characteristics through gate functionalization using diazonium chemistry: Application to biodetection of 2,4-dichlorophenoxyacetic acid

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ABSTRACT

We investigated an Electrolyte-Gated Organic Field-Effect transistor based on poly(N-alkyldiketopyrrolo-pyrrole dithienylthieno[3,2-b]thiophene) as organic semiconductor whose gate electrode was functionalized by electrografting a functional diazonium salt capable to bind an antibody specific to 2,4-dichlorophenoxyacetic acid (2,4-D), an herbicide well-known to be a soil and water pollutant. Molecular docking computations were performed to design the functional diazonium salt to rationalize the antibody capture on the gate surface. Sensing of 2,4-D was performed through a displacement immunoassay. The limit of detection was estimated at around 2.5 fM.

1. Introduction

2,4-dichlorophenoxyacetic acid (2,4-D) was one of the ingredients in Agent Orange, an herbicide used during the Vietnam War, and is still one of the most widely used weed killer in agriculture. However, since 2015, it has been suspected to be carcinogenic and has been banned in several countries. It is therefore important to find easy, costless and efficient methods to measure 2,4-D levels in soils or water. Apart from classical separative methods such as gas chromatography and high-performance liquid chromatography (Eller and Cassinelli, 1994), sensors constitute an interesting solution. Without any intention of being exhaustive, some reported 2,4-D sensors are listed below.

Two main approaches are typically deployed to detect low molecular weight organic targets such as 2,4-D, i.e. the use of molecular-imprinted polymers (MIP) or specific antibodies. Examples of detection devices based on one of these two receptors have been published since the seminal article of Skladal and Kalab (1995), who described an electrochemical immunochemical assay (in a competitive format) made of a nitrocellulose membrane into which antibodies against 2,4-D were adsorbed. A 2,4-D molecule conjugated to Horseradish peroxidase (HRP) was used as a tracer (labeled target) with H_2O_2 and hydroquinone (HQ) as substrates for HRP. The limit of detection (LoD) for 2,4-D in water was extremely low, 0.1 pg L^{-1} (0.45 fM). Subsequently, several developments and improvements of this principle have been published (Dtantiev and Zherdev, 1996; Dequaire et al., 1999). Another approach consisted in using molecularly imprinted polymers (MIP) where the polymer acts as a preconcentration matrix into which the target is detected by electrochemical methods (Xie et al., 2010; Shi et al., 2011; Kroger et al., 1999). However, the high potentials needed to detect 2,4-D may lead to lack of selectivity due to the possibility of reducing the interferents always present in real samples such as dissolved oxygen. Impedimetric transduction (Navratilova and Skladal, 2004; Prusty and Bhand, 2017) allows to overcome such problem. To reach higher sensitivity without loss of selectivity, most of the recent works are based on optical transduction (Wang et al., 2016; Jia et al., 2017; Wagner et al., 2018; Feng et al., 2017).

If we look at transistor-based devices for 2,4-D detection, no recent works have been published but one was pioneer nearly 25 years from now. The approach proposed by Khomutov et al. (1994), was significantly distinct from the classical electrochemical techniques; they proposed an immunodetection using a pH-sensitive Field-Effect Transistor (pH-FET). Their strategy was based on the use of a competitive

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binding, using a 2,4-D-peroxidase conjugate for binding with antibodies being immobilized on porous cellulose membranes. These membranes were attached to the gate region of the pH-sensitive FET. A mixture of ascorbic acid (0.1 mM), *o*-phenylenediamine (1.0 mM) and H_2O_2 (1.0 mM) was used as substrate solution for the enzyme, the activity of which being detected by a pH-shift at the gate; the LoD reached $1\ \mu g\ L^{-1}$ (4.5 nM).

If we consider previous works reported on Electrolyte-Gated Organic Field-Effect Transistors (EGOFETs), the seminal works were those from Taniguchi and Kawai (2004), Bäcklund et al. (2004), and Panzer and Frisbie (2006). They demonstrated the possibility of fabricating transistors where the classical dielectric is replaced by a polymer electrolyte, and where the semiconductor and the gate, in direct contact with the electrolyte, are impermeable to ions. Polarization of the gate causes migration and accumulation of ions (formation of an electrical double layer, EDL) at the gate/electrolyte and semiconductor/electrolyte interfaces. The latter EDL causes accumulation of carriers at the OSC surface thus forming the channel. Kergoat et al. (2010), demonstrated that polymer electrolytes could be replaced by aqueous solutions as simple as phosphate buffers or even aerated deionized water. Since this date, the interest for EGOFET-based biosensors has kept growing. At first, publications focused on different methods able to biofunctionalize the semiconductor/electrolyte interface (Cotrone et al., 2012; Kergoat et al., 2012; Suspène et al., 2013; Palazzo et al., 2015; Piro et al., 2017). However, such approaches were challenging because they implied covalent or non-covalent functionalization of the semiconductor, which led to significant degradation of its electric properties. That is why the most recent works reported on application of EGOFETs for sensing have been rather based on gate modification (Casalini et al., 2013, 2015; Mulla et al., 2015). As shown by these works, EGOFETs are not only sensitive to changes at the organic semiconductor/electrolyte interface, but also to those occurring at the electrolyte/gate interface.

Indeed, it was shown that the drain current I_D of an EGOFET depends on the overall capacitance (C) of the device (**Eq.** (1)), which is itself given by **Eq.** (2).

$$I_D = \mu \frac{W}{2L} C (V_{GS} - V_{Th})^2 \tag{1}$$

$$C^{-1} = C_{OSC/Elec}^{-1} + C_{Elec/Gate}^{-1} \tag{2}$$

with W the channel width, L its length, C the total interface capacitance per unit area, V_{GS} the operating gate potential, V_{Th} the threshold voltage, μ the mobility of the charge carriers, $C_{OSC/Elec}$ the OSC/electrolyte capacitance and $C_{Elec/Gate}$ the electrolyte/gate capacitance. Therefore, physicochemical processes occurring at the electrolyte/gate interface, such as changes in interfacial capacitance induced by molecular recognition of a target molecule onto an immobilized receptor, can be transduced into a drain current variation. This requires the use of low molecular weight receptors that localize the binding reaction within the EDL over the gate, which excludes the utilization of large receptors such as antibodies (ca. 12 nm in height), for which (smaller) target molecules bind outside the EDL and therefore cannot be sensed (Kergoat et al., 2012; Huang et al., 2015). To overcome this problem, we proposed in a previous work a displacement immunoassay (Nguyen et al., 2017), inspired by previous work of Wijaya et al. (2010). Competition occurs between a target mime (hapten) immobilized onto the organic conducting polymer film and the native target present in the sample. In a first step, the antibody specific to the target binds the target mime immobilized on the polymer; when the target is present in solution, a competitive exchange occurs between the immobilized mime and the diffusing target, which displaces the equilibrium and removes the antibody from the surface (Piro et al., 2017). The large size of the antibody, compared to the small size of the immobilized hapten, allowed a thorough reorganization of the electrolyte/gate interface.

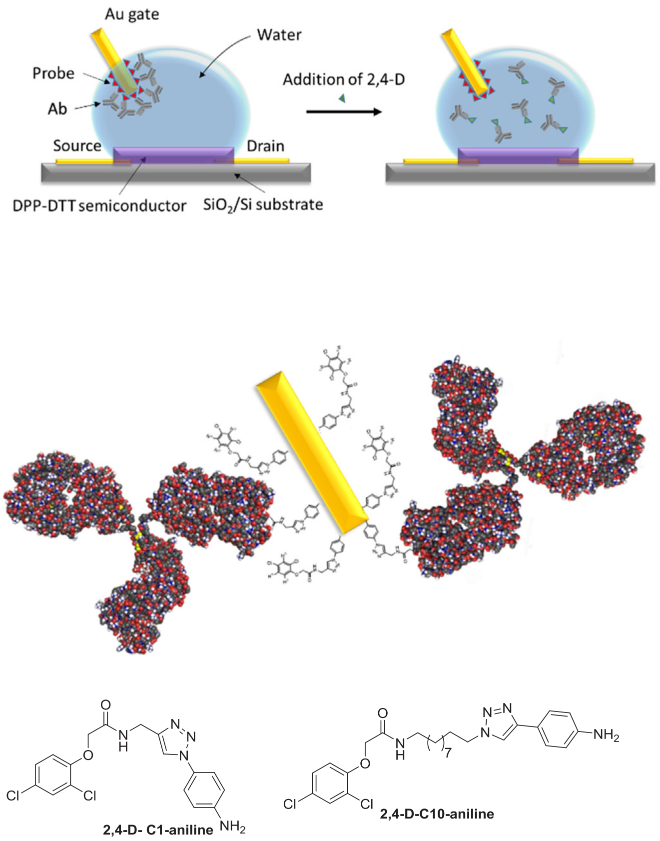

Fig. 1. (Top) Schematic view of the gate-modified EGOFET, before (left) and after (right) addition of the target molecule 2,4-D in the electrolyte. **(Middle)** Antibodies immobilized by affinity interactions on the 2,4-D-modified gate. **(Bottom)** Structure and synthesis of 2,4-D-C_1-aniline and 2,4-D-C_{10}-aniline, the two probe molecules grafted on the gate electrode.

In the present work, for an easy and robust functionalization of the gate, we investigated the electroreduction of a diazonium salt, a methodology which has never been applied for gate modification in EGOFETs, for the covalent immobilization of the molecular probe onto which the specific antibody binds. As a practical example, we used the device for immunodetection of 2,4-D in water samples. A computational investigation was performed, at the atomic scale, to decipher the recognition processes between the immobilized probe and the antibody, through molecular docking computations in order to rationalize the position of the anchoring function with a minimum impact on the probe affinity for 2,4-D. The overall strategy (gate functionalization, antibody immobilization and displacement assay) is illustrated on Fig. 1. Concerning the organic semiconductor, it is the first time since its first description by Li et al. (2010) and Li et al. (2012) that poly(N-alkyldiketopyrrolo-pyrrole dithienylthieno[3,2-b]thiophene) (DPP-DTT) has been used in an EGOFET. This semiconductor is particularly pertinent in this type of device for its high hole mobility (ca. $1\ cm^2\ V^{-1}\ s^{-1}$), its low HOMO (−5.2 eV) and its high molecular weight (M_w = 280 ± 10 kDa) and well-ordered compact lamellar structure which make it stable in water environment.

2. Materials and methods

2.1. Chemicals and reagents

2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were purchased from Sigma-Aldrich. Poly(N-alkyldiketopyrrolopyrrole dithienylthieno[3,2-b]thiophene) (DPP-DTT) was purchased from Ossila (England), with M_w = 280 ± 10 kDa and PDI = 3.8 ± 0.1. Phosphate buffered saline