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Multiplexed site-specific electrode functionalization for multitarget biosensors

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ABSTRACT

Multitarget biosensors hold great promise to improve point-of-care diagnostics as they enable simultaneous detection of different biomolecular markers. Multiplexed detection of different markers, like genes, proteins, or a combination of both, propels advancement in numerous fields such as genomics, medical diagnosis and therapy monitoring. The functionalization of these biosensors, however, necessitates patterned immobilization of different bioreceptors, which remains challenging and time-consuming. We demonstrate a simple method for the patterned multiplexing of bioreceptors on a multi-electrode chip. By using the lithographically defined electrodes for surface functionalization, additional patterning steps become obsolete. Using the electrodes for self-aligned immobilization provides a spatial resolution that is limited by the electrode patterning process and that cannot be easily obtained by alternative dispensing or coating techniques. Via electrochemical reduction of diazonium salts combined with click chemistry, we achieved site-specific immobilization of two different ssDNA probes side by side on a single chip. This method was experimentally verified by cyclic voltammetry (CV), Fourier transform infrared spectroscopy (ATR-FTIR) and X-ray photoelectron spectroscopy (XPS), and specific target recognition was visualized by fluorescence microscopy. The combination of the electroaddressability of electrografting with the chemoselectivity of click chemistry, offers a versatile platform for highly efficient site-specific functionalization of multitarget biosensors.

1. Introduction

In the last decade, covalent immobilization of diazonium compounds has received considerable attention as an alternative to self-assembled monolayers (SAMs) for surface functionalization [1–5]. This approach, popularly known as electrografting, uses diazonium salts that are electrochemically reduced in order to generate reactive radicals. These radicals enable covalent binding to the electrode surface. Electrografting offers several advantages over alkanethiol SAMs on gold [6]. The generation of an aryl radical results in a strong covalent bond between the diazonium compound and the electrode surface. Furthermore, functionalization of the surface using electrografting only takes a few seconds, which is in sharp contrast to various silane or thiol chemistries that typically take several hours [7]. The approach also offers easy control over the surface coverage and the ability to functionalize electrodes in an electroaddressable way. As a result, electrografting has been shown to be a straightforward, stable and effective method to functionalize a variety of different materials, such as metals, diamonds and silicon [2,8,9].

The covalent immobilization of diazonium compounds can function as a first preparation step to couple biomolecules on a solid surface. Biomolecule immobilization based on electrografting has previously been shown via two approaches. In a first approach, diazonium-modified biomolecules are synthesized and directly grafted onto the surface [10–13]. However, the difficulties associated with introducing the reactive groups present a clear drawback of this technique. A second approach indirectly couples biomolecules on the surface by means of a crosslinker after diazonium grafting, often using carbodiimide chemistry [14–16]. As the carbodiimide approach struggles with certain problems, such as low yield and difficult modification, 1,3 cycloaddition click chemistry has become a more attractive alternative to couple biomolecules onto a diazonium-functionalized layer. Click chemistry is a selective, reproducible and fast coupling method that overcomes the difficulties associated with carbodiimide chemistry [17–20].

In numerous applications, such as molecular electronics and biosensors, the patterned immobilization of biomolecules is essential. Patterned surface functionalization based on electrografting has previously been shown using local silicon doping as a promoter [21], by local electrografting using scanning probe microscopy [22], using lithographic techniques [23] and by microcontact printing [24]. Patterned functionalization with two different modifiers has been

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demonstrated by Downard et al. via both scanning probe lithography [25] and soft lithography using a PDMS mold [26]. A drawback of these methods is that they require additional patterning steps, which are costly and time consuming. In electrochemical biosensor devices – that rely on the use of well-defined electrodes for readout [27–30] – the need for these additional patterning steps can be overcome by using the electrodes not only for readout, but also for the surface functionalization.

In this work, we demonstrate a versatile and straightforward method to immobilize different biomolecule probes on neighboring electrodes (Schematic 1). By taking advantage of the electroaddressability of the electrografting approach, we have grafted two different aryl diazonium salts – 4-ethynylaniline and 4-azidoaniline – adjacently on an electrode array chip. As a next step, alkyne- and azide-modified ssDNA was site-specifically coupled onto the functionalized electrodes, based on the chemoselectivity of click chemistry. As proof of concept, an array of five 700 μm wide electrodes was used for clear visualization. The proposed method is easily scalable to larger arrays with smaller electrode sizes [23]. This constitutes a simple and versatile platform for site-specific multiplexing of biomolecules on an electrode array chip.

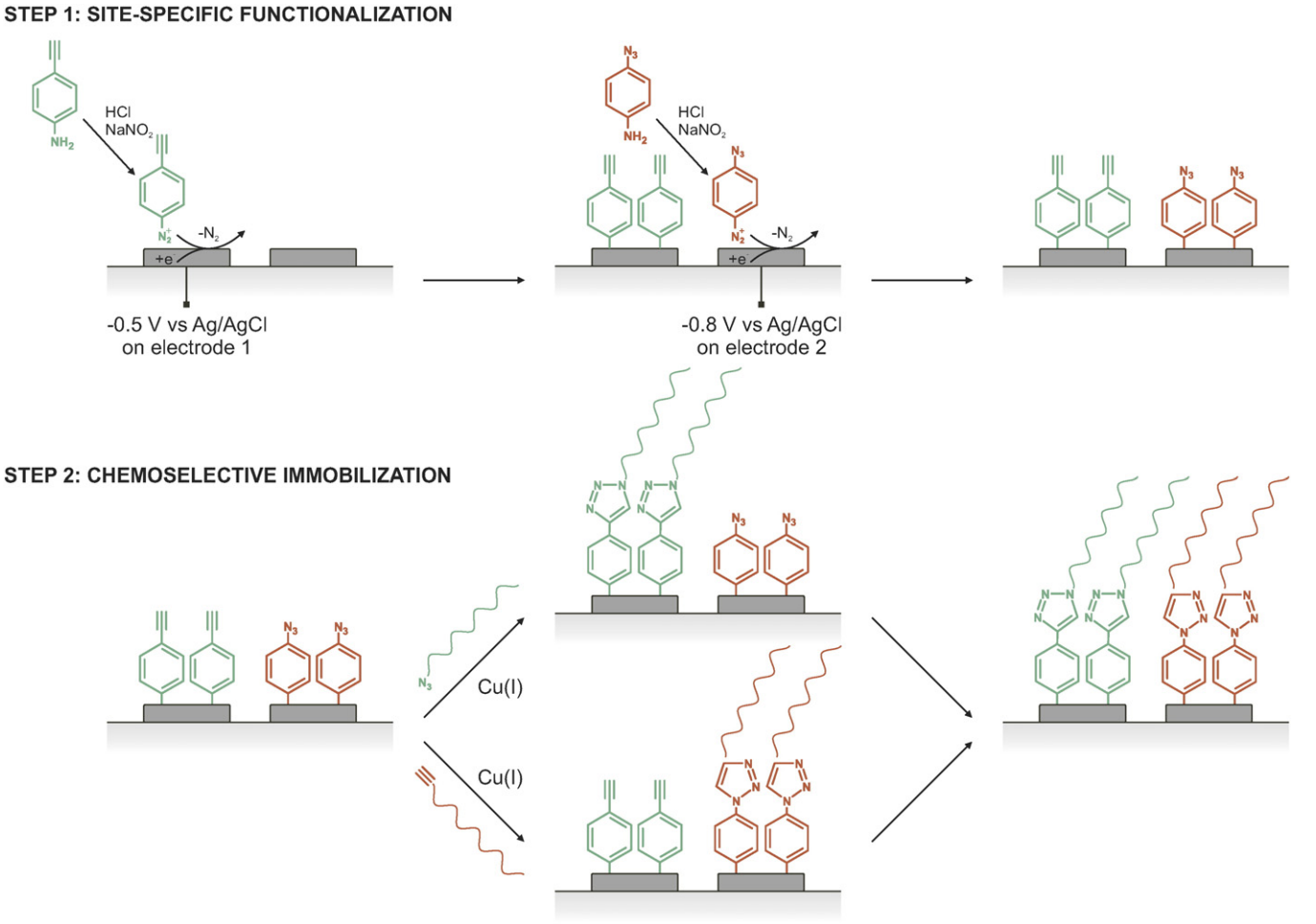
2. Materials and methods

2.1. Chemicals

4-ethynylaniline ($HC{\equiv}CC_6H_4NH_2$), 4-azidoaniline hydrochloride ($N_3C_6H_4NH_2{\cdot}HCl$), sodium nitrite ($NaNO_2$), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA), tetrakis(acetonitrile)copper(I) hexafluorophosphate (TCH), (+)-sodium L-ascorbate (SA), dimethyl sulfoxide (DMSO), acetonitrile (ACN), iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), potassium chloride (KCl), hydrochloric acid (HCl, 37%), isopropyl alcohol (IPA) and acetone were purchased from Sigma Aldrich (Belgium). All reagents were used without further purification. Alkyne- and azide-modified oligonucleotides (25mer) were purchased from IDT (Belgium) (sequences in Supporting Information).

2.2. Electrode array fabrication

The electrode array consisted of five 700 μm wide gold line electrodes with a separation of 700 μm, that terminate in large clamping pads (Schematic 2). The electrodes were fabricated using photolithography on standard glass microscope slides (VWR, Belgium) of 76 × 26 × 1 mm. The glass slides were cleaned by 20 min ultrasonication in acetone, followed by 5 min immersion in hot IPA (60 °C) with thorough drying under a stream of nitrogen after each step. In order to remove any remaining contamination, a 15 min oxygen plasma treatment was performed. Immediately after cleaning, the samples were soft baked at 190 °C for 5 min to remove water vapor. Lift-off resist (LOR1A, MicroChem, France) was spincoated at 2000 rpm (WS-650-8NPP spincoater, Laurell, USA) for 45 s and soft baked at 190 °C for 5 min. A next layer of photoresist (IX845, JSR, Belgium) was spincoated at 4000 rpm for 60 s and soft baked at 120 °C for 1 min. After 9 s vacuum-type exposure in a Karl Suss (Germany) MA6 mask aligner, the patterned samples were immersed in developer (OPD5262, Fujifilm,



Schematic 1. Schematic illustrating multiplexed site-specific electrode functionalization. (Step 1) Site-specific electrografting of 4-ethynylaniline and 4-azidoaniline on neighboring gold electrodes. (Step 2) Chemoselective immobilization of azide- and alkyne-modified ssDNA (depicted as wavy lines).