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High Resolution Multiplexing for DNA Arrays using a Multi-Electrode Chip

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Abstract

We present a versatile and straightforward method to achieve multitarget DNA arrays using the combination of electrochemical functionalization and individually addressable electrodes of a multi-electrode chip. By using the lithographically defined electrodes directly for surface functionalization, additional patterning steps become obsolete. The proposed functionalization method relies on the electrochemical reduction of diazonium salts, so called electrografting, which enables site-specific immobilization of different bioreceptors onto the same chip. As reported previously, click chemistry can be used to chemoselectively couple oligonucleotide probes. This strategy is however limited to the immobilization of two different probes. Alternatively, electroclick chemistry allows coupling of an unlimited amount of different bioreceptors using individually addressable electrodes. The main drawback of this method is the need to synthesize the different probes prior to deposition. Instead of using pre-synthesized probes, on-chip DNA synthesis is highly promising as it enables in-situ parallel synthesis of a custom DNA sequence on each electrode. To this end, we demonstrate here that electrodes functionalized via electrografting can act as a suitable solid support for on-chip DNA synthesis (using a commercial DNA synthesizer).

1. Introduction

Multitarget biosensors hold great promise to improve numerous fields such as genomics, proteomics, drug discovery, medical diagnosis and therapy monitoring. The simultaneous detection of different biomolecular markers, like nucleic acid targets or proteins, helps pave the way towards advanced point-of-care diagnostics. The functionalization of these multitarget biosensors, however, necessitates patterned immobilization of different bioreceptors, which remains time consuming and challenging, especially for small feature sizes.

In this work, we present a fast and straightforward method for the multiplexing of bioreceptors on a multi-electrode array via a two-step procedure (Fig. 1). The first step consists of electrode functionalization via electrochemical reduction of diazonium salts at the surface, known as electrografting (Fig. 1(a)). In a second step, bioreceptors - in this case DNA - can be immobilized onto the functionalized electrode surface (Fig. 1(b)). It was reported earlier in [1] that click chemistry enables site-specific immobilization of different oligonucleotide probes side by side on a single chip (Fig. 1(b).I). As click chemistry relies on the chemoselective coupling of azide and alkyne groups, this method is however limited to the deposition of two different probes. To overcome this limitation, we are developing an alternative approach based on electroclick chemistry (Fig. 1(b).II). The electrodes are used to electrochemically activate the coupling catalyst [2], which allows to site-selectively bind the presented oligonucleotide to a specific electrode. The different oligonucleotide probes have to be synthesized prior to immobilization, which constitutes the main drawback of this method. We are therefore developing a strategy to perform DNA synthesis directly on chip, with a commercial DNA synthesizer, instead of using pre-synthesized probes (Fig. 1(b).III). The focus of this paper is to prove that electrodes functionalized via electrografting can serve as a suitable solid support. As a next step, we are implementing electrochemically induced phosphoramidite deblocking [3], which enables the assembly of a custom DNA sequence on each electrode. It is clear that on-chip DNA synthesis offers promising perspectives for multitarget DNA arrays, as it eliminates the need for pre-synthesized oligonucleotide

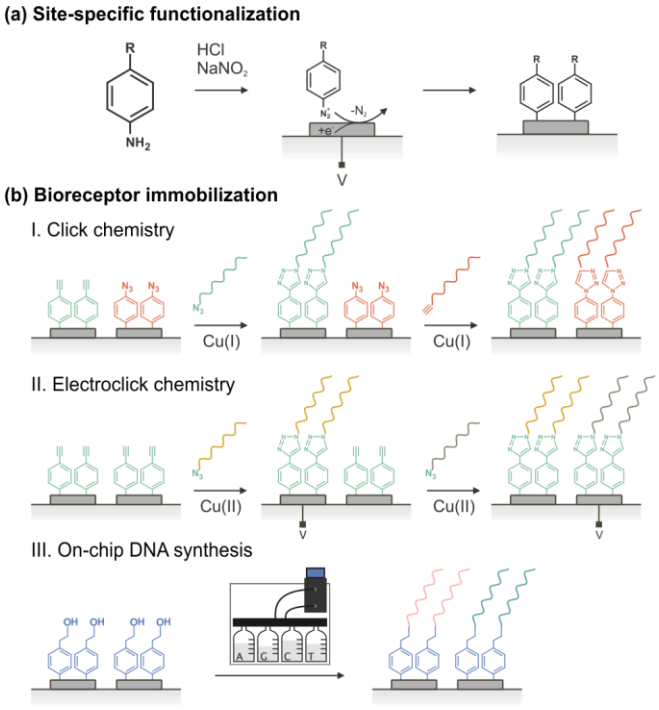

Fig. 1 Schematic illustrating site-specific electrode functionalization via (a) site-specific electrografting of diazonium compound on electrodes; and (b) bioreceptor immobilization (DNA depicted as wavy lines).

probes. Furthermore, using the electrodes not only for readout, but also for surface functionalization, overcomes the need for additional patterning steps. The proposed method for self-aligned immobilization offers a spatial resolution that is solely limited by the lithographic electrode patterning process and that cannot be easily obtained by alternative dispensing or coating techniques.

2. Methods

In a commercial DNA synthesizer, the oligonucleotide is assembled onto a solid support (often controlled pore glass or macroporous polystyrene beads [4]) contained in a synthesis column. In this work, we have replaced the column by a custom flow cell, into which the electrode chip can be inserted via a simple clamping system. The functionalized electrodes of the chip serve as the solid support for DNA synthesis, replacing the commonly used beads. In order for the electrode to function as support, hydroxyl groups must be introduced to accommodate coupling with the first phosphoramidite moiety via a phosphite triester linkage [4]. To this end, electrografting of aminophenethyl alcohol (Sigma Aldrich, Belgium) was performed via cyclic voltammetry (CV) using the method previously reported in [1]. After functionalization of the electrodes with hydroxyl groups, the electrode chip was inserted into the DNA synthesizer. To prove the suitability of the electrografted layer as solid support for DNA synthesis, four different synthesis runs were performed on functionalized gold electrodes. In the first three runs, poly-T probes of different lengths were synthesized onto the functionalized electrodes.

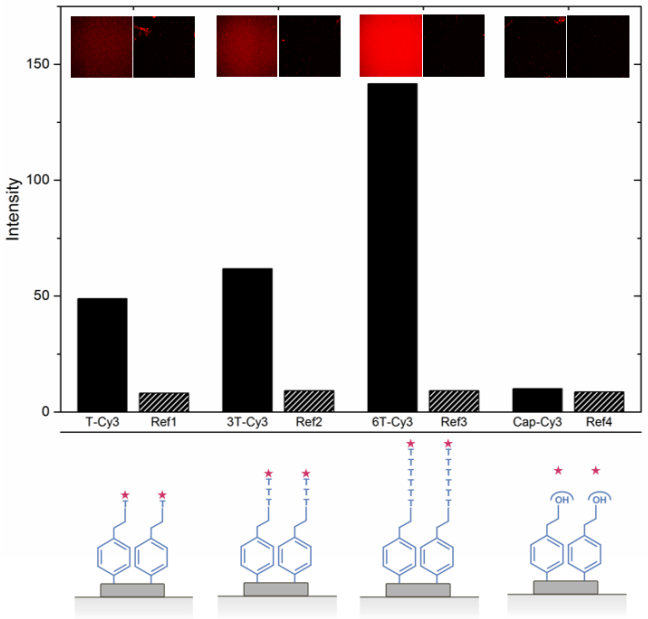

Fig. 2 Fluorescence intensity of functionalized gold electrodes after DNA synthesis in a commercial DNA synthesizer. Three different poly-T lengths with a terminal Cy3 group were synthesized on samples 1, 2 and 3. Sample 4 was first capped, after which a synthesis run with Cy3 phosphoramidite was performed. The negative reference electrode in each synthesis run was a non-functionalized electrode. The fluorescence microscopy images are shown in inset.

Each poly-T probe contained a terminal Cy3 phosphoramidite (Link Technologies, UK), enabling readout via fluorescence microscopy (see schematic illustrations in Fig. 2). In contrast, in the fourth run, a hydroxyl capping step was performed prior to introducing the Cy3 phosphoramidite. In standard phosphoramidite synthesis, this capping step is used to block any uncoupled 5'-OH groups to prevent formation of incomplete oligonucleotides [4]. During all four synthesis runs, a negative reference electrode was included, that was not electrografted with aminophenethyl alcohol, and therefore did not contain hydroxyl groups. After DNA synthesis, the different electrodes were taken out of the DNA synthesizer and checked via fluorescence microscopy.

3. Results and Discussion

The results are shown in Fig. 2. Samples 1, 2 and 3, containing poly-T oligos of different lengths with a terminal Cy3 group, clearly show a higher fluorescence intensity compared to the negative reference electrodes. A higher fluorescence intensity can be observed for the longer oligo lengths. This can be explained by the varying distance of the Cy3 fluorophore from the metal surface. In case of longer probe length, the fluorescence experiences less quenching by the metal, as the fluorophore is further away from the electrode surface [5]. Sample 4 shows a fluorescence intensity comparable to that of the negative reference electrodes, which proves that the capping step in the standard phosphoramidite cycle fully blocks all non-reacted hydroxyl groups, ensuring correct synthesis of each oligonucleotide.

4. Conclusions

We have demonstrated that the electrografting approach offers a versatile platform for the functionalization of electrochemical biosensors. The proposed method leverages individually addressable electrodes for site-specific functionalization to enable multitarget bioreceptor immobilization. To eliminate the need for pre-synthesized oligonucleotide probes, as is the case when using (electro)click chemistry, we have shown that electrografted electrodes can act as a suitable solid support for on-chip DNA synthesis. Future work will focus on implementing electrochemical deblocking, as described in [3], to achieve the synthesis of a custom DNA sequence on each electrode, with a lithographically defined resolution. Preliminary results are promising, and will be reported elsewhere.

Acknowledgements

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