Electrochemistry

DOI: 10.1002/anie.201000046

Building Addressable Libraries as Platforms for Biological Assays by an Electrochemical Method**

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addressable libraries · cross-coupling · diblock copolymers · electrochemistry · microelectrode arrays

Since the discovery of the Kolbe coupling in the 19th century, this electrochemical method has served as a powerful and environmentally benign way of synthesizing organic compounds in both laboratory synthesis and industrial production.[1,2] Various new strategies and techniques have been developed in organic electrochemistry. They are used not only for conventional synthesis, but also for generating molecular diversity.[3]

The recent emergence of microelectrode-array technology has opened up a new aspect of organic electrochemistry. Microarrays have great potential for use in a variety of biological assays.[4] For example, this technology enables the assembly of large libraries of potential ligands within a diminutive area and hence the development of systematic strategies for the evaluation of complex mixtures of proteins.[5] One of the advantages of microelectrode-array technology is that each electrode in an array is individually addressable and can therefore be used to monitor a unique member of a molecular library that is associated with its surface. Furthermore, microelectrode-array technology provides a direct label-free method to measure small-molecule–protein interactions. To functionalize microelectrode arrays, molecular libraries need to be immobilized on the surface of the electrode through anchoring molecules.[6] For this purpose, it is necessary to develop the synthetic tools necessary for the site-selective construction and placement of molecules.[7–9] One approach to this problem is to take advantage of the electrodes themselves to trigger chemical reactions.

Moeller and co-workers have made a number of breakthroughs in the construction of addressable libraries as platforms for biological assays on microelectrode arrays (Figure 1). In 2004, they reported a system for the construction of molecular libraries through a combination of electrochemistry and Pd chemistry.[10] In the first step, the array was coated with a porous hydroxylated polymer membrane and then treated with the ester **1** of 10-undecenoic acid (Scheme 1). The substrate was concentrated on the chip in the region close to the electrodes by a reaction catalyzed by the base generated by the reduction of vitamin B_{12} in solution by the electrodes on the chip. Selected electrodes were poised at a potential difference of −2.4 V versus the Pt counter electrode for periods of 0.5 s separated by off periods of 0.1 s for 300 cycles. Following the coupling reaction, any free hydroxy groups remaining on the surface of the chip were capped by exposing the chip to acetic anhydride under the same reaction conditions.

In the next step, Wacker oxidation was performed at selected electrodes by reversing the electrode polarity. The triarylamine-mediated electrochemical oxidation, which was

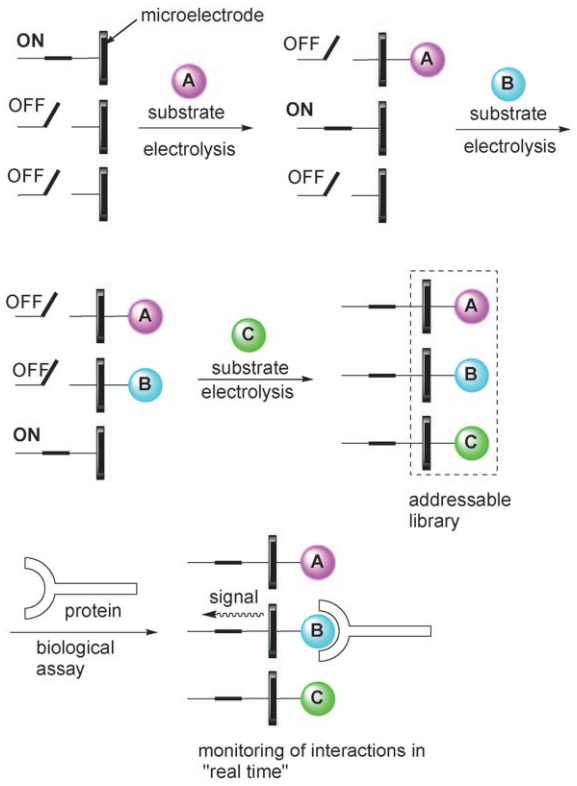

Figure 1. Preparation of an addressable molecular library on a microelectrode array and electrochemical monitoring of interactions with a protein.

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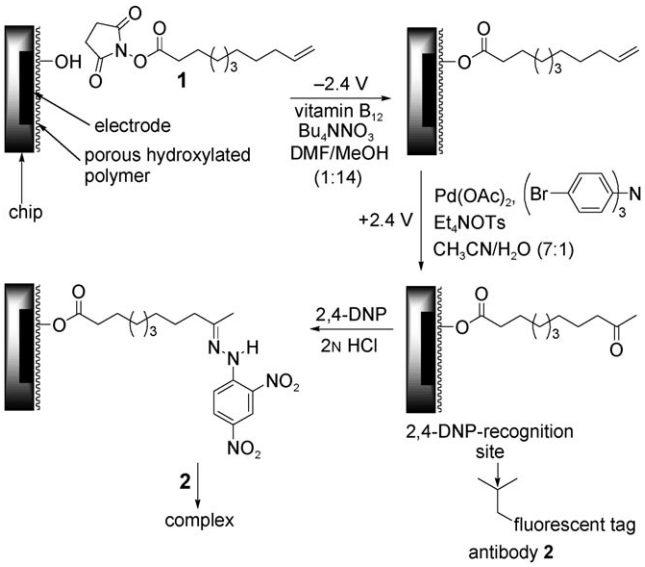
[**] J.Y. is grateful for a Grant-in-Aid for Scientific Research and financial support from the NEDO Project.



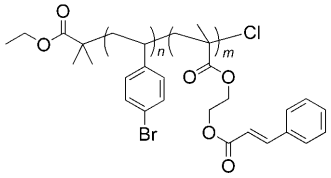
Scheme 1. Use of a combination of electrochemistry and Pd catalysis for the development of addressable molecular libraries on microelectrode arrays. DMF = *N*,*N*-dimethylformamide, 2,4-DNP = 2,4-dinitrophenylhydrazine, Ts = *p*-toluenesulfonyl.

carried out by pulsing the selected electrodes as anodes for 0.5 s at +2.4 V and 0.5 s at 0 V for either 300 or 600 cycles, converted Pd^0 into Pd^{II}. Pd^{II} oxidizes the carbon–carbon double bond to give the ketone and regenerate Pd^0. To confine the Pd^{II} to the preselected sites on the chip, ethyl vinyl ether was added to the solution to reduce any Pd^{II} that diffused into the solution phase. The ketone was then converted into the corresponding 2,4-DNP derivative. The chip was incubated with a solution of bovine serum albumin (BSA) containing a commercially available rabbit antibody conjugated to a fluorescent probe (**2**). After washing of the surface of the chip to remove excess antibody, the chip was imaged with an epifluorescence microscope. The image indicated the formation of ketones at the selected electrodes on the chip. This successful combination of electrochemistry and organometallic chemistry opened new possibilities for the construction of chip-based molecular libraries.

The synthesis of coumarin derivatives on a microelectrode array by a similar method was also developed. In this case, the binding assay with the anticoumarin antibody was performed by monitoring the current associated with a ferrocene–ferrocinium ion redox cycle.[11] Other reactions, such as the Heck reaction,[12] the Suzuki reaction,[13] the removal of a *tert*-butoxycarbonyl group,[14] the generation of reactive *N*-acyliminium ion intermediates,[15] a hetero-Michael reaction,[16] Lewis acid catalyzed reactions,[17] and copper(I)-catalyzed click reactions,[18] were also found to be effective for building libraries by this approach.

The microelectrode arrays discussed above were coated with a porous reactive layer, mainly agarose[17,18] and sucrose,[11] for the surface attachment of substrates. However, there are significant problems associated with these conventional coating methods. Agarose delaminates from the surface of the array with time, dissolves in a variety of solvents, and reacts with a number of the reagents used for site-selective synthesis.[19] The use of sucrose solves these problems because it provides a stable surface for the generation of functionalized arrays. However, the polyhydroxylated surface provided by both agarose and sucrose coatings limits the use of microelectrode arrays for monitoring the behavior of small molecules that are synthesized by using protected amine and alcohol functional groups.

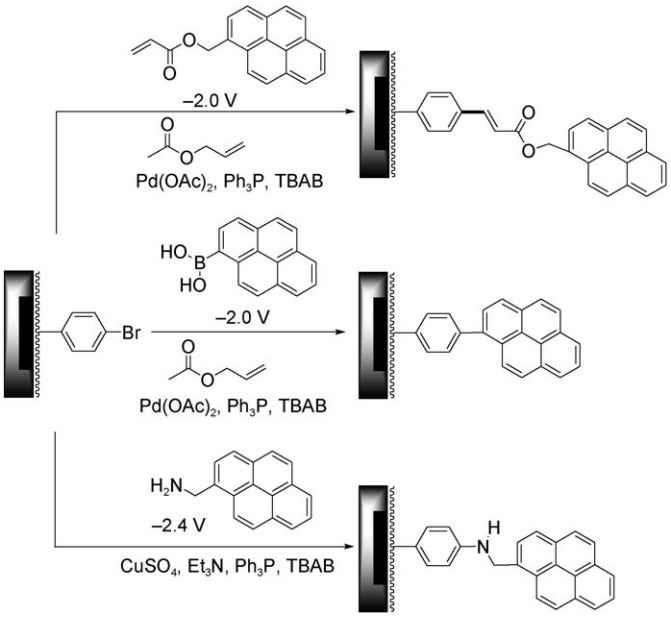
In 2009, Moeller and co-workers reported a solution to this problem in the use of the diblock copolymer shown in Scheme 2.[20] The surface of an array was coated with the



Scheme 2. Diblock copolymer used to form a porous reactive layer for the development of an addressable molecular library on a microelectrode array.

polymer (prepared by atom-transfer radical polymerization) by a spin-coating technique with a solvent system composed of a solvent that solubilizes both blocks of the copolymer and a solvent that solubilizes only the polystyrene block of the copolymer. The coated microelectrode array was then subjected to irradiation with a 100 W Hg lamp to effect cross-linking through the cinnamyl moiety to give a porous polymer with pore sizes on the order of (19 ± 3) nm.

Several reactions were performed on this new surface by using the *p*-bromophenyl group to develop addressable molecular libraries. For example, a Suzuki coupling with a Pd^0 catalyst, which was generated at selected electrodes by using them as cathodes to reduce Pd^{II}, was used to introduce the pyrene structure (Scheme 3). The polymer was stable



Scheme 3. Combination of electrochemical and chemical reactions on the porous surface made from the diblock copolymer shown in Scheme 2. TBAB = tetra-*n*-butylammonium bromide.

during 15 consecutive experiments, each of which involved 300 cycles, without any sign of delamination from the surface. The pyrene structure could also be introduced through Heck and copper(I)-catalyzed reactions (Scheme 3). Following the reaction, the array was removed from the solution and washed to remove any unbound substrates; it could then be imaged with a fluorescence microscope (Figure 2).

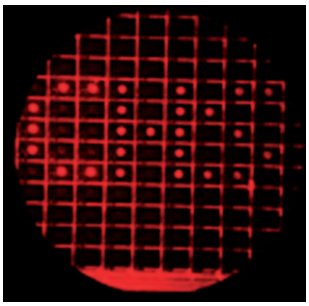


Figure 2. Site-selective pattern on a microelectrode array.

The compatibility of the surface with the desired electrochemical signaling was also examined by first measuring the current associated with an iron species in the solution above the array and then adding a protein to the solution and monitoring drops in the current at the microelectrodes. Strong impedance was observed upon the nonspecific binding of BSA to the unfunctionalized polymer-coated electrode surface. This result proved that the diblock copolymer was compatible with the signaling experiment. Similar results were obtained when an antibody was used in place of BSA.

The present electrochemical approach made it possible to build addressable libraries on arrays of microelectrodes. It is hoped that such libraries will be used for the "real-time" probing and monitoring of binding events between potential ligands and various biological receptors.

Received: January 5, 2010
Published online: April 20, 2010

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