

Electrochemical Biosensor Arrays

ELECTRICALLY INITIATED SURFACE FUNCTIONALIZATION

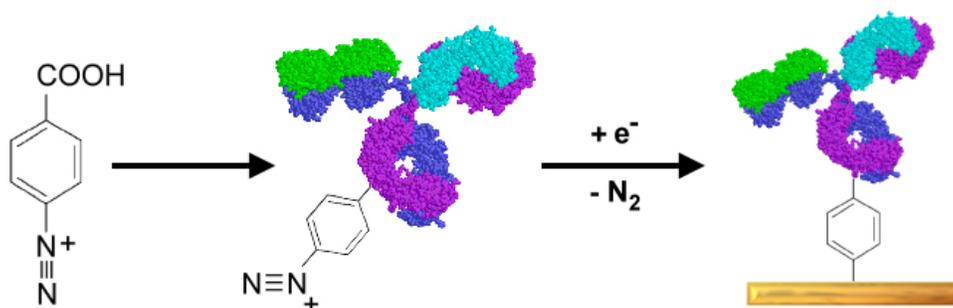
Overview

The reliable and definitive detection of multiple biowarfare agents on a single robust platform would be a significant asset for the defense of our nation and the safeguarding of warfighters. Multiple signature based biosensors can meet this need as they not only allow for multianalyte detection, but also substantially increase confidence in the sensor output as whole cell, genomic, and proteomic data can be interrogated for each target analyte. We have developed a method that allows for controlled and selective immobilization of biorecognition elements onto electrodes allowing simultaneous multianalyte detection of DNA and proteins.

Approach

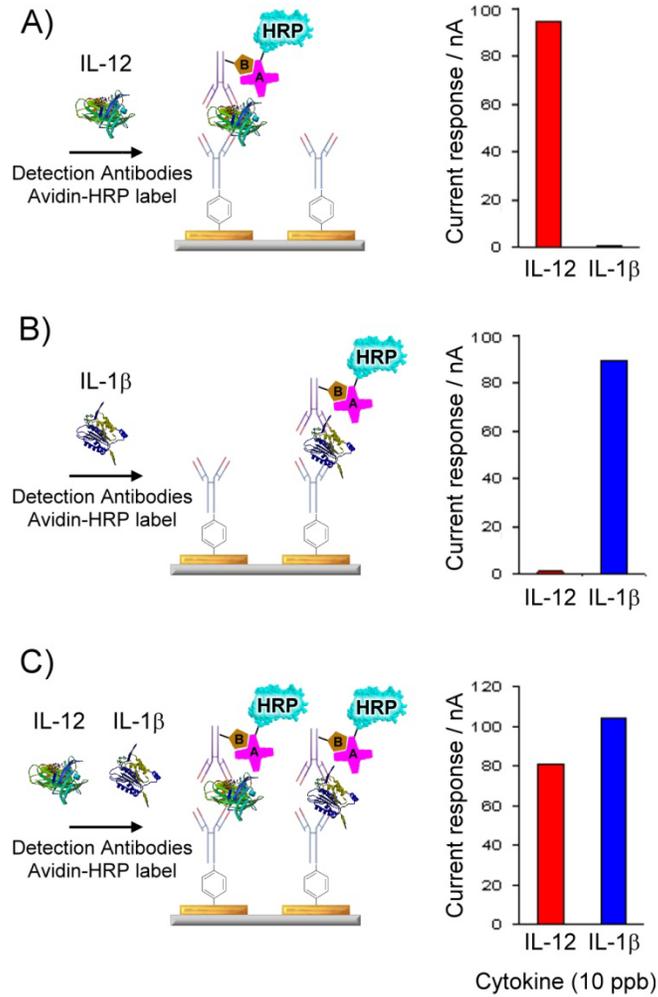
Selective immobilization of biomolecules onto electrodes was obtained using aryl diazonium salts. This patternable surface chemistry forms a highly stable covalent bond with semiconductive or conductive substrates. We have shown the selective functionalization of glassy carbon electrodes with diazonium-horseradish peroxidase conjugates [1] providing facile direct electron transfer and electrochemical detection of peroxide. We have also recently reported the control over surface functionalization density, electron transfer kinetics, and catalytic NADH detection using diazonium modified gold electrodes [2].

Modification of antibodies with diazonium chemistry allows for the selective electrodeposition of antibodies on closely-spaced microelectrodes for multi-target protein detection in an array format, as shown below.



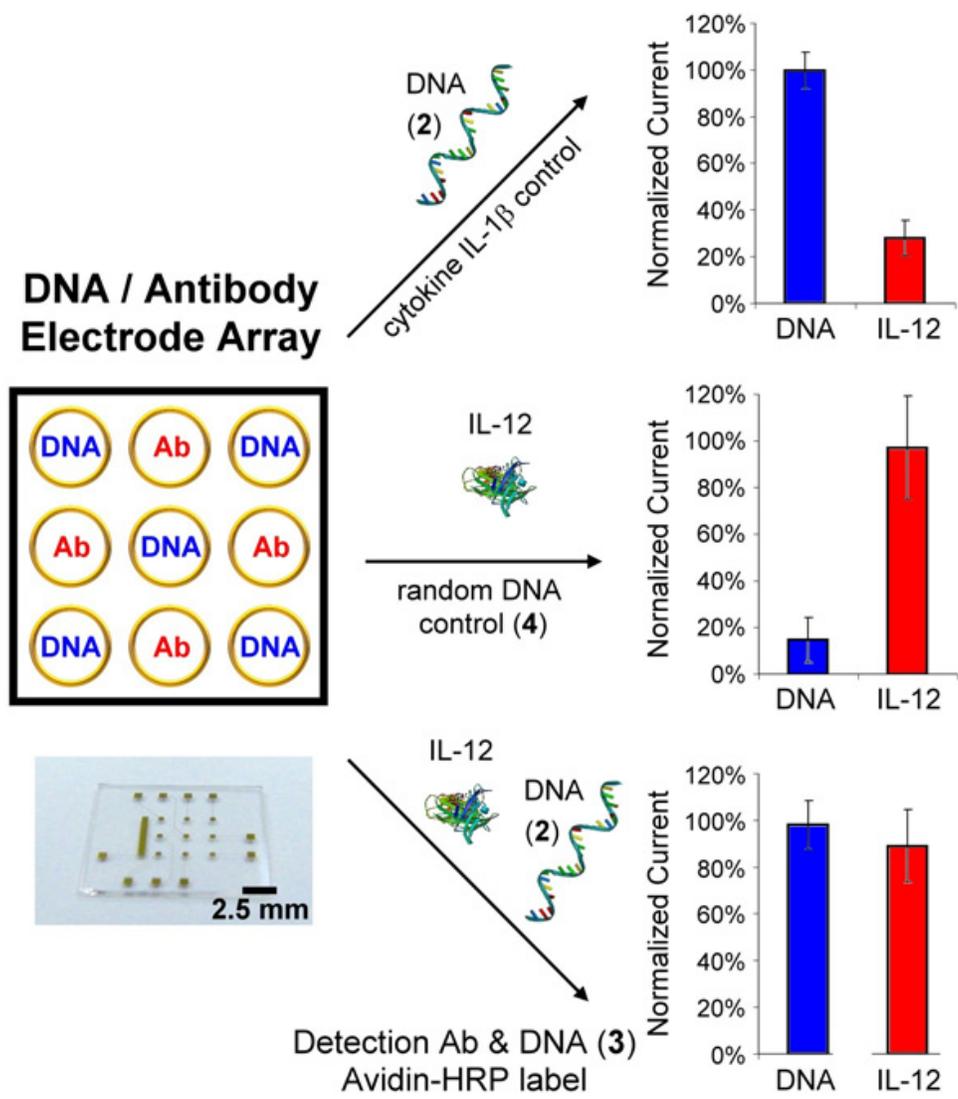
Antibody Mod Scheme

Using this method we have demonstrated simultaneous detection of three different cytokine proteins on a three element array selectively modified with three different diazonium functionalized antibodies [3]. Control experiments, shown below, used a two element array selectively patterned with two different antibodies. These results show the ability to selectively detect either target with minimum cross-talk between electrodes.



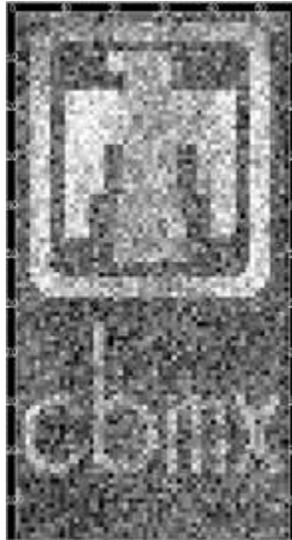
Multianalyte Immunoassay Controls

We have also demonstrated the selective immobilization of DNA probes and antibodies on a 9 element electrode array using diazonium chemistry. The figure below shows the simultaneous electrochemical detection of DNA and protein on a single platform. (Gold array with 5 electrodes patterned with DNA and 4 with anti-TNF- α antibodies)



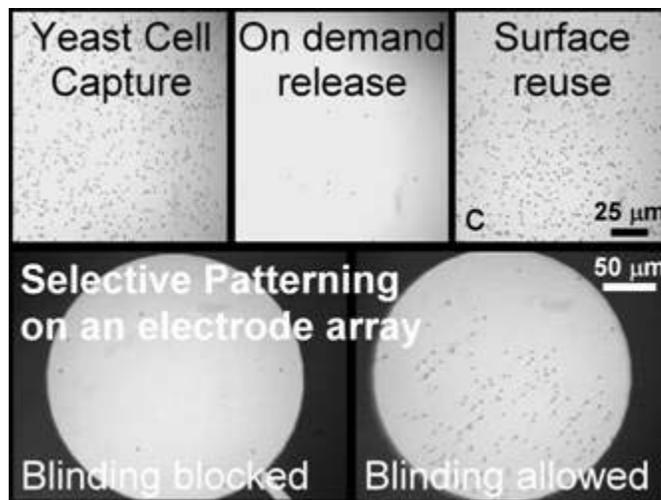
Multianalyte DNA & Ab array controls

Presented below is the result of a recent collaboration between our group and CombiMatrix Corp. illustrating the use of aryl diazonium salts for selective modification of high density arrays [5]. CombiMatrix CustomArray™ chips possess 12,544 individually addressable electrode sites, each 44 μm in diameter. We produced a FLAG peptide probe surface on 2,151 individual electrodes in the pattern of the Sandia Thunderbird and the CombiMatrix logo (CBMX). The resulting “false” grey-scale image correlates to the current measured at each electrode due to the electrochemical detection of anti-FLAG antibodies. This result clearly demonstrates the feasibility of selective patterning of biomolecules onto a commercially available high density electrode array with the potential of detecting hundreds or thousands of analytes.



Combi Chip with FLAG – small

We also introduced a new method which allows for electrically addressable immobilization of whole cells [6]. A phenyl boronic acid diazonium salt was used to activate individual gold electrodes for facile and reversible (on demand release) immobilization of eukaryotic cells (yeast and macrophage). This platform shows great promise for use in single cell or arrayed cell studies including cell signalling, host-pathogen interactions, and other cellular function studies.



Yeast capture, release, array

References:

1. "Diazonium-Functionalized Horseradish Peroxidase Immobilized via Addressable Electrodeposition: Direct Electron Transfer and Electrochemical Detection", R. Polsky, J. C. Harper, S. M. Dirk, D. C. Arango, D. R. Wheeler, S. M. Brozik, *Langmuir*, 2007, 23, 364-366.
2. "Electro-Addressable Selective Functionalization of Electrode Arrays: Catalytic NADH Detection Using Aryl Diazonium Modified Gold Electrodes", J. C. Harper, R. Polsky, S. M. Dirk, D. R. Wheeler, S. M. Brozik, *Electroanalysis*, 2007, 19, 1268-1274.
3. "Electrically Addressable Diazonium Functionalized Antibodies for Multianalyte Electrochemical Sensor Applications", R. Polsky, J. C. Harper, D. R. Wheeler, S. M. Dirk, S. M. Brozik, *Biosens Bioelectron*, 2008, 23, 757-764.
4. "Selective Immobilization of DNA and Antibody Probes on Electrode Arrays: Simultaneous Electrochemical Detection of DNA and Protein on a Single Platform", J. C. Harper, R. Polsky, D. R. Wheeler, S. M. Dirk, S. M. Brozik, *Langmuir*, 2007, 23, 8285-8287.
5. "Multi-Functional Electrode Arrays: Towards a Universal Detection Platform", R. Polsky, J. C. Harper, D. R. Wheeler, S. M. Brozik, *Electroanalysis*, 2008, 20, 671-679.
6. "Electrically Addressable Cell Immobilization Using Phenyl Boronic Acid Diazonium Salts", R. Polsky, J. C. Harper, D. R. Wheeler, D. C. Arango, S. M. Brozik, *Angew. Chem., Int. Ed.*, 2008, 47, 2631-2634.

For additional information or questions, please email us at BioNano@sandia.gov



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