Electrochemical Detection Array Combining Amperometry and Total Internal Reflection Fluorescence

CNF Project Number: 2260-13

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Primary CNF Tools Used: ABM contact aligner, YES Asher, AJA sputtering system, Aura 1000 resist stripper, Image Reversal Oven, Oxford PECVD, Oxford 81 etcher

Abstract:

Neurotransmitters are released in a quantal event by fusion with membranes. The mechanism of this fusion event remains unknown but is crucial for molecular manipulation and various kinds of disease. We develop and fabricate an electrochemical detection array capable of combining amperometry measurement and total internal reflection fluorescence (TIRF). Amperometry provides the information for the releasing neurotransmitters from vesicles in the cell while TIRF enables direct visualization of vesicles with appropriate fluorescence labels. The combination of the two methods offers a new way for studying the exocytosis process.

Summary of Research:

Exocytosis is the process where neurotransmitters are released into the extracellular space [1]. The amperometry measurement provides precise details about the released transmitters in a single quantal event. While amperometry has the above-mentioned advantages, it measures the releasing contents reaching the electrodes and cannot directly characterize the releasing mechanisms. The total internal reflection fluorescence (TIRF) can detect the fluorescence signals at the substrate surface to visualize the foot print of the cell with its generated evanescence wave. The combination of the two methods offers the availability of monitoring vesicle releasing events and amperometry spikes simultaneously.

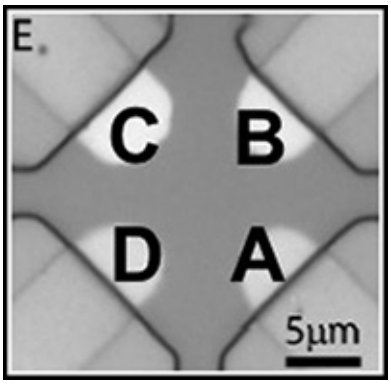

Figure 1: The micrograph showing the geometry of the 4-electrode ECD device.

To fully utilize the TIRF technology, the specific site of release must be known to locate the fluorescence signal. Therefore, we developed the electrochemical detection (ECD) array with four electrodes between which a cell can be placed, as shown in Figure 1 [1]. Individual fusion events can be detected amperometrically with ~ 200 nm precision, utilizing a map of random walk simulations while the cell surface can be imaged with TIRF microscopy [3].

A 4-inch, 175 μm thick glass wafer was used for the fabrication of the ECDs. Patterns were transferred onto the wafer through general lithography with NFOL 2020 negative photoresist. The photoresist was spin-coated on the wafer with 3000 rpm for 30s, resulting in a thickness of 2 μm. After the soft bake, alignment and exposure was performed with ABM contact aligner for 7s at 12.14 mJ/s. Following the post exposure bake, the photoresist was developed using 726 MIF for 70s. Then 10 nm Ti/150 nm Pt were deposited on the device using the AJA sputtering

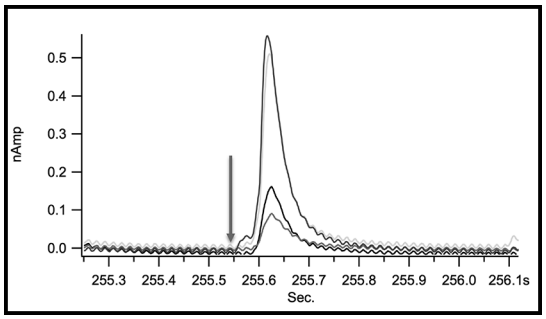

Figure 2: An amperometric spike related to the fluorescent signal in Figure 3. The arrow shows the event start point.

system followed by a lift of process using 1165. A 300 nm thick SiO_2 layer was deposited using Oxford PECVD. By using general lithography and Oxford 81 ether, a window was opened at the tips of the four electrodes to expose them to the environment.

To determine the role of Syntaxin clusters in fusion events in chromaffin cells, fluorescent labelled Syntaxin-CSYS construct was expressed in the cells by overnight infection of semliki forest virus harboring the gene for the expression of Syntaxin-CSYS. Syntaxin-CSYS was cloned into pSFV vector to make virus for the expression in bovine chromaffin cells. A fluorescent image of cell footprint on ECD arrays was collected by the TIRF microscopy. Transmitter release from the single fusion event was detected by the four ECD electrodes as correlated amperometric spikes with different amplitudes, depending on the diffusion distance between the release site and the respective electrode. Fluorescent movie by the TIRF microcopy and release events by the ECD array were recorded simultaneously, A combinatorial fluorescence change with the start of amperometric spike was observed.

A time superresolution analysis of fluorescence loss and amperometric spike start time by ECOM method shows the dispersal of syntaxin clusters coincide with the fusion pore formation.

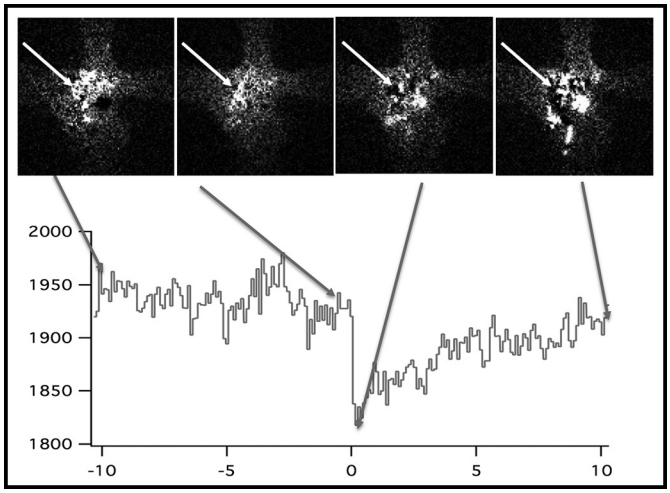

Figure 3: Fluorescent signal correlated with the amperometric spike in Figure 2. The arrows indicate the position of the event and corresponding signal strength.

References:

[1] Kisler, K., et al., J. Biomater. Nanobiotechnol., 2012. 3(2): p.243-253.

[2] Liu, X., et al. Analytical Chem., 2011, 83: p. 2445-2451.

[3] Zhao, Y., et al., PNAS., 2013. 110(35): p.14249-14254.