Microfabricated Devices to Study Single Vesicle Release

CNF Project # 848-00

Principal Investigator(s): Manfred Lindau

Abstract:

Many cell types, including neurons, chromaffin cells of the adrenal gland, and mast cells, release transmitter molecules stored in vesicles by a process called exocytosis. In this process, an intracellular vesicle loaded with molecules to be secreted, fuses with the cell membrane, forming an initial opening called a fusion pore, and emits its contents into the extracellular space. The process of fusion pore formation and expansion is not well understood.

We are developing microchip devices that detect exocytosis of oxidizable molecules from single vesicles electrochemically, to better understand the process of exocytosis and the role of drugs in the modulation of loading and release of single vesicles.

Summary:

Release of the catecholamines dopamine, adrenaline, and noradrenaline from single vesicles can be detected amperometrically as a brief spike of oxidation current using carbon fiber electrodes as well as microfabricated platinum electrochemical detector (ECD) arrays on microscope cover-glasses to obtain also spatial information about the release sites. The ECD arrays detect quantal noradrenaline release events of single vesicles from chromaffin cells. Release positions were determined based on the relative fraction of molecules that is detected by each of the four electrodes of the array and verified by simultaneous fluorescence microscopy.

The time course of the signal was analyzed to determine the release kinetics from single vesicles and apparent diffusion constants of the molecules. We found that adrenaline molecules leave their vesicles more quickly than previously thought, but diffuse much slower near the cell surface (D = 0.8-1.3x10^{-6} cm^2/s) then they do in bulk medium (D = 6x10^{-6} cm^2/s) [3]. This method opens the door to experiments where we can observe the actions of fluorescently labeled molecules involved in exocytosis, while concurrently recording and then co-localizing electrochemical information from the same events.

The new generation of detectors uses SiO_2 as the insulating layer instead of photoresist, which makes the detectors much more robust. They can be cleaned multiple times using solvents or HCl and reused. It also eliminates fluorescence background of the photoresist. The new ECD arrays measure release using three instead of four electrodes, using the fourth electrode at a retracted position to measure noise for later subtraction. The features of this new design are finer than in the old ECD arrays (minimum feature ~0.9 µm), which makes the use of projection lithography necessary (5x-stepper).

Various drugs affect release from single vesicles (also called quantal release). As an example, the drug L-Dopa, used to treat Parkinson’s disease, increases quantal size, while drugs such as reserpine and amphetamines decrease quantal size. Using patch amperometry, a method developed in our laboratory [4], we found that changes in quantal size are associated with changes in vesicle size [5]. Since many drugs affect quantal size, we are developing amperometric electrode active pixel arrays with integrated electronics that can perform high throughput measurements of quantal size and the effect of drugs on the spatio-temporal properties of single vesicle exocytosis.

The design involves a regulated cascode amplifier (RCA) pixel circuit which allows the determination of electrical charge released due to the oxidation with two electrons transferred per oxidised molecule. In our circuit, since the electrode is held at a reference potential, common to all pixels, we use a scheme called shared-buffered direct injection that has been employed in infrared imaging systems [6, 7]. Our circuit provides pico-amp resolution and can detect currents up to 1nA-600pA. Pt electrodes were deposited on the CMOS die using FEl 611 FIB system at the CNF.

References:

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- Fabrication of electrochemical detector arrays for simultaneous electrochemical and total internal reflection fluorescence (TIRF) to investigate molecular events leading to neurotransmitter release.

- High-speed electrochemical imaging of fusion pore opening.

- Discovery that time course of catecholamine release is due to low apparent diffusion coefficient near the cell surface.

- Development of electrochemical detector active pixel arrays with integrated electronics providing pA sensitivity.

- Simulation results of the pixel output and the output at the end of the signal processing chain are shown in Figures 1a and 1b.

- Figure 1a shows the simulated response of the CMOS chip at a recording site, to a typical exocytotic event.

- Figure 1b shows the input current at the recording site and the output current reconstructed using the IV characteristic of the pixel. This is how we intend to use the active pixel array to record events at multiple sites.

Figure 1a

We fabricated a test chip in a submicron CMOS technology through the Metal-Oxide Semiconductor Implementation Service (MOSIS), containing individual devices and amplifiers characterizing the properties of our shared amplifier circuit experimentally.

Figure 1(a), top left: Output voltage of the chip at a single recording site to a typical exocytotic event.

Figure 1(b), top right: Exocytotic event reconstructed from the output voltage of the chip and the actual input current.

Figure 2, bottom: Section of the chip fabricated through MOSIS using AMIS 0.5μm technology showing the 5 x 5 array. Shows the 5 x 5 test array of the CMOS die fabricated at MOSIS.