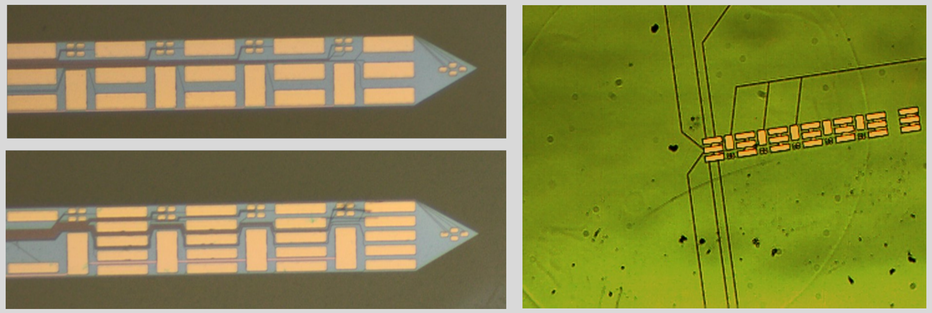
Simultaneous Interferent Blocking and Target Detection for Nano-scaled Electrochemical Neural Probes

Eleanor Rackoff [1], Yoo-Jin Hwang [1], Xinyu Liu [2], Warren Fon [2], Michael Roukes [2], Jessica Arlett[1]

Harvey Mudd College[1], California Institute of Technology[2]

Introduction

Neurological disorders are correlated to irregularities in neurotransmitter and neuromodulator release. However, existing technology doesn't allow neuroscientists to easily explore chemical irregularities, meaning experimental neuroscientists typically focus on electrophysiology studies. Thus, we're developing implantable neural probes to assist in concurrent electrophysiological and neurochemical measurements. This project aims to improve the accessibility and effectiveness of these devices for neurological research.

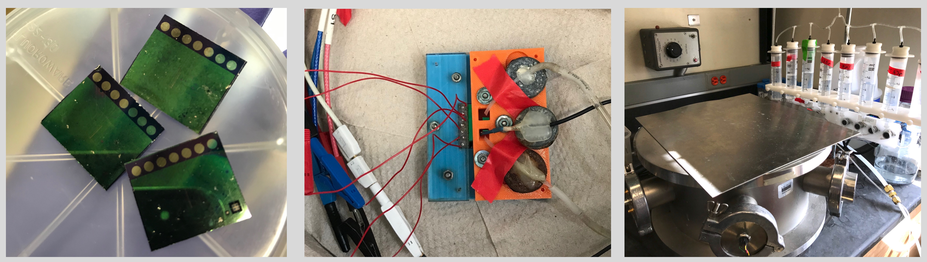


Probe shanks are 125µm wide and 6mm long. Sensors are 25 x 100µm on top left probe and 15 x 100 µm on bottom left probe. Before working with probes, we worked with chips that had identical sensor geometries.

Objectives

Our goal was to demonstrate simultaneous interferent blocking and target chemical detection on a 25µm x 100µm electrochemical sensor without cross-talk on neighboring sensors.

Methods



(Left) Small chips we use to develop our protocols; (Middle) Flow cell set-up to perform chemical measurements; (Right) Faraday cage that holds our flow cell during experimentation, the eight channels for flowing in different chemicals

Protocols for target detection and interferent blocking were developed on test chips that share the same rectangular pad size as those on the probes (25µm x 100µm). Enzymatic functionalization to sensitize the array to target chemicals was carried out using a micro-plotter, while interferent blocking was done through the electrodeposition of a polymer layer. Testing was then performed with an automated, eight channel pump system that allowed us to check various concentrations of targets. Before applying enzymes, we test the detection of peroxide, since that is what is generated by the target/enzyme reaction.

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Target Specification

In order to measure the target chemicals, the pads must be functionalized with an enzymatic layer. The challenge with functionalization is that the enzyme layer must only cover a single pad; this is to enable an array of sensors functionalized for multiple distinct target chemicals without cross-talk from neighboring sensors. Our current approach for functionalization is through the use of a Sonoplot micro-plotter, which allows us to precisely pipette the enzyme onto each pad. Testing so far has been focused on glucose functionalization, but our goal is to explore other chemical targets such as nicotine, acetylcholine, lactate, glutamate.

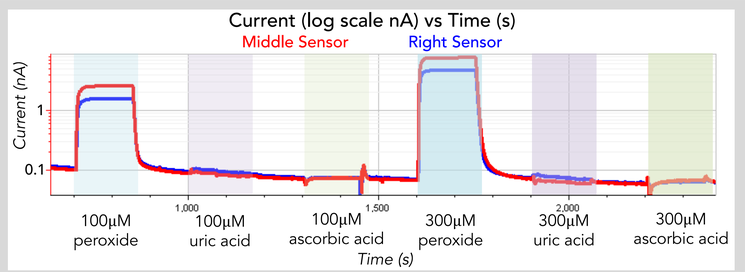
(Left to right) Functionalizing a sensor using the Sonoplot; Sonoplot pipette above a sensor; different camera angle of pipette over chip; microscope view of functionalized chip

Interferent Blocking

Summary of Blocking Results

Protocol	Time	Peroxide to dopamine signal ratio		Peroxide to ascorbic acid signal ratio	
		100µM	300µM	100µM	300µM
40mM Phenol in 0.05X PBS, 0.9V	2 min	1.7	1.6	2.0	2.0
	3.5 min	1.1	1.5	8.0	14.4
	4.5 min*	6.5	102.5	(blocked)	(blocked)
	4.5 min	2.3	4.7	3.2	21.0
	5 min**	3.7	7.4	5.5	19.7
40mM Chlorophenol in 0.05X PBS, 0.9V	5 min	4.0	7.9	(blocked)	(blocked)
40mM p-Cresol in 0.05X PBS, 0.9V	17 min	1.5	0.3	(blocked)	(blocked)
100mM PPD in 0.01X PBS, 0.7V	20 min	7.2	10.0	(blocked)	(blocked)
	10 min	25.0	27.2	(blocked)	226.7

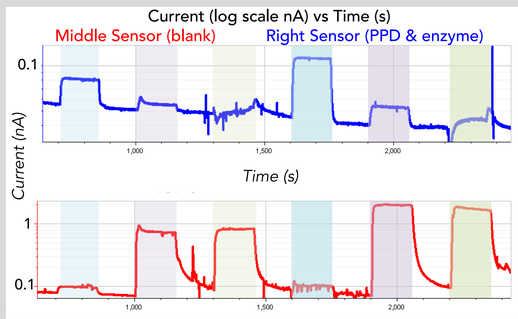
Cells with "(blocked)" mean that concentration was below the detection level of the device
**4.5 min was completed by applying an extra minute to the previous 3.5 minute application*
*** 5 min application was completed in two rounds of 2.5 min*



Flow measurement results after applying PPD to two adjacent sensors. Between each concentration, we flow in phosphate buffered saline (PBS)

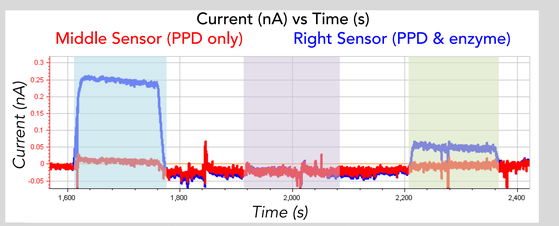
When the probes are implanted *in vivo*, the pads will be exposed to various redox-reactive chemicals in the brain, leading to confounding signals. To eliminate this, we electrodeposit a blocking layer onto the electrochemical pads to stop the interferents from reaching the surface. We then assess the performance of these layers by comparing the ratios of target to interferent signals. We found that m-phenylenediamine (PPD) gave us the best signal ratio and was easiest to work with.

Simultaneous Blocking & Target Detection



Concentrations (left to right): 100µM glucose, uric acid, ascorbic acid, 300µM peroxide, uric acid, ascorbic acid

Blocking interferents (uric acid, ascorbic acid) while measuring the target chemical (glucose) was achieved by applying a PPD blocking layer and crosslink on adjacent pads before functionalizing one pad. The blocking layer, as well as the parylene well of the sensors, allowed us to minimize cross talk. Future work will go into optimizing the functionalization method in order to fit the enzyme completely inside of the well.



Concentrations (left to right): 300µM glucose, uric acid, ascorbic acid; Blocking on both pads before applying the enzyme layer helped to reduce crosstalk in glucose detection