Design Framework for the Electrical Characterization of Alcohol Sensors

A Thesis Presented in Partial Fulfillment of the Bachelor’s Degree in Bioengineering (Bioelectronics)

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Abstract

The goal of this project was to develop a biosensor capable of detecting toxic alcohol concentrations in the blood. I created an array of organic electrochemical transistors that can sense varying concentrations of ethanol in solution, and plan on extending this functionality to sense other toxic alcohols such as methanol, isopropanol, and ethylene glycol. I quantified the sensitivity and functionality of organic electrochemical transistors by applying bias voltages to the drain of the transistor, and measuring the respective output currents. After running several characterization tests on small, medium, and large transistor sizes, transconductance was optimized by finding device geometries that would maximize the ratio between the change in current and gate voltage. Maximizing the ratio between current and gate voltage was essential in using these transistors as toxic alcohol sensors. Ethanol concentrations in solution were quantified by measuring PEDOT:PSS polymer conductivity on electrochemical transistors functionalized with alcohol dehydrogenase and its cofactor NAD\(^+\). Characterization of these organic electrochemical transistors indicates that small voltage fluctuations (mV scale) in gate voltage modulate polymer conductivity. Using these transistors as sensors for ethanol, I concluded that changes in gate voltage due to the metabolic breakdown of ethanol in the presence alcohol dehydrogenase and NAD\(^+\) leads to a noticeable changes in polymer conductivity. Although the sensitivity of the devices was compromised by enzyme functionalization, current responses were quantifiable within the microamp range.

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1 Problem Statement

I am building an array of organic electrochemical transistors (OECTs) that is capable of performing rapid detection of the commonly consumed toxic alcohols methanol, ethylene glycol, ethanol, and isopropanol. Specifically, my goal is to electrically characterize these organic transistors for optimal sensitivity and functionality. Toxic alcohols can commonly be found in household products such as hand sanitizer, rubbing alcohol, mouthwash, automotive products, and alcoholic beverages. Because these products are so commonplace, many people are exposed to these toxic chemicals through inhalation or ingestion. In 2016 alone, there were over 40,000 cases of toxic alcohol exposures filed by the American Association of Poison Control Centers [8]. Symptoms of toxic alcohol exposures, include abdominal swelling, renal and cardiovascular failure, seizures, and in severe cases, death. Timely detection of these species is required for determining the correct choice of treatment for a patient suffering from alcohol poisoning. The aim of my project is to develop a point-of-care alcohol sensor, that is capable of quantifying amounts of toxic alcohols in blood serum, as well as differentiating between methanol, ethylene glycol, ethanol, and isopropanol within the samples. For this application, organic electrochemical transistors are optimal because of their low cost, compatibility with various substrates and polymers, and stability within aqueous environments.

Organic electrochemical transistors (OECTs) consist of a channel, composed of an organic conjugated polymer, and three electrodes labeled the source, drain, and gate electrodes. The source and drain are connected via the conductive channel, while its conductivity is modulated by the gate electrode. The voltage at the gate electrode controls the amount of ions in an electrolyte gel that is injected into the conductive polymer, causing a change in current between the source and drain electrodes. Entrapped within the gel are specific enzymes (alcohol oxidase, glycerol dehydrogenase, alcohol dehydrogenase, and NADP-dependent isopropanol dehydrogenase), that break down alcohols and consequently, release electrons to the gate electrode. Each OECT is functionalized with a different enzyme, allowing the differentiation between the toxic alcohols sensed.

Design of each OECT starts with choosing appropriate geometries for the gate, drain, and source electrodes, as well as determining an optimal channel thickness of the conductive polymer, poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) anions. Doing this plays a critical role in reducing the noise of the device output current and increasing the sensitivity of the device. After device fabrication and enzyme entrapment, characterization of the devices gives key insight into components of the OECT that need to be modified, such as amounts of enzymes used and voltage applied to the gate and drain electrodes. Finding the right balance between enzyme reaction and the voltages applied is essential in determining if current modulation can be attributed to noise. I will use a parameter analyzer with custom LabVIEW software to measure drain current and transconductance curves for each OECT, and apply appropriate voltages at the gate and drain accordingly. Characterization of these OECTs includes tuning
the bias voltages at the gate and drain electrodes for appropriate transconductance values. For the best sensitivity, the highest amount of change in current divided by change in gate voltage, gives the optimal range for operating the transistor. I will also be using the Metrohm AutoLab potentiostat to observe current responses (amperometry measurements), voltage responses (cyclic voltammetry), and impedance (impedance spectroscopy). These steady state input and output characteristics of the OECTs will help in determining the best configuration for current modulation, and will ensure that our current responses recorded by the transistor can be attributed to the enzymatic reactions that are happening within the enzyme entrapped electrolyte gel.

2 Background

Methanol, isopropanol, ethylene glycol, and diethylene glycol are toxic alcohols found in household products such as antifreeze, hand sanitizer and degreasing agents. Toxic alcohol poisoning occurs when these alcohols are metabolised by the human body via ingestion or inhalation. Alcohol dehydrogenase, an enzyme secreted by the liver, breaks down these compounds, and toxic byproducts are released into the blood [7]. Because a high mortality rate is associated with the exposure of toxic alcohols, it is important for healthcare providers to quickly diagnose and treat patients that show symptoms of metabolic acidosis, acute renal failure, and unexplained neurologic disease [10].

Serum osmolality, the measure of different solutes in blood plasma, is one of the key indicators for diagnosing alcohol poisoning. After exposure to toxic alcohols, low molecular weight substances in the serum raise serum osmolality, leading to elevated anion and osmolar gaps [11]. Although it is easy for healthcare providers to assess changes in serum osmolality through blood tests, it is difficult to determine what substances lead to such changes.

Gas chromatography is the standard for measuring alcohols in blood serum [2], but the process of analyzing compounds by evaporation can take days to complete. Because the equipment needed for gas chromatography is costly, specialized laboratories are often used to test blood samples for toxic analytes. Treating symptoms of alcohol poisoning requires quick recognition of toxic alcohols. Using the initial concentrations of toxic alcohols in blood samples, healthcare providers are able to plan sufficient dialysis therapy [9]. With that in mind, organic electrochemical transistors (OECTs) coupled with the functionalization of encapsulated enzymes, look promising in their ability to rapidly detect metabolites in the blood.
Figure 1: Organic electrochemical transistors are composed of three electrodes labeled the gate, source, and drain. A conductive channel made of organic polymers connects the source and drain electrodes, where a bias voltage (DC voltage) is applied at the drain and the source is connected to ground. The gate electrode modulates the current through an electrolyte.

2.1 Organic Electrochemical Transistors

Transistors are three terminal electronic devices that are able to operate as switches, amplifiers, and sensors. Within electronic circuits such as the smartphone, transistors are made using the semiconductor materials silicon and germanium in bipolar junction transistors (BJTs) and more commonly, metal oxide field effect transistors (MOSFETS). When interfacing with enzymes and biological compounds, certain types of transistors, namely organic electrochemical transistors, use the conductive properties of organic polymers to transduce environmental changes into electrical signals.

The use of organic conductive polymers provides a platform for ionic and electrical properties to be translated between organic materials and electronics. That being said, organic electrochemical transistors operate in an analogous manner to semiconductor transistors. OECTs are often composed of a drain and source electrode connected through a conductive polymer in contact with an electrolyte. A gate electrode, also connected through the electrolyte, is used to modulate the flow of ions between the electrolyte and conductive polymer.
2.2 Device Physics of OECTs

Organic electrochemical transistors (OECTs) are three terminal devices that are able to transduce small changes in gate voltage into large output currents. This change in source/drain current with respect to gate voltage is often represented by a transfer curve, where current amplitude increases as gate voltage increases. At a given gate voltage, the steeper this transfer curve is, the larger the output current is with respect to voltage. This change in current over change in voltage is known as transconductance. In order to get the largest current response from a change in voltage, transconductance must be maximized. With respect to OECTs, there are several ways in which transconductance can be altered. Source, drain, and gate electrode geometries have been shown to greatly alter the output characteristics of OECTS. Rivnay et al [14] concluded that the transconductance of OECTs is dependent on the width/length ratio (W/L) and thickness of the conductive polymer channel.

Using these parameters, the Bernards Model [4] gives an accurate representation of how OECTs transconductance can be modeled with respect to channel thickness and polymer conductivity.

\[ g_m = \frac{W}{L} \ast d \ast C \ast \mu \ast (V_{th} - V_G) \] (1)

Within this model, W, L, and d refer to the width, length, and thickness of the channel, C refers to the capacitance per unit volume of the channel, and \(\mu\) refers to the charge carrier mobility.

Although OECTs have high transconductance values, this advantage comes at a cost of slow operation. According to the Bernards Model, the resistance of the electrolyte and the capacitance of the transistor channel influence the time it takes for ions to change the transconductance of the OECT. For biosensor applications, response time happens within the tens of microseconds [4], magnitudes faster than speeds that are actually needed.

2.3 Conductive Polymers

Organic electronic materials can exhibit mixed (electronic and ionic) conductivity [13], a key property for devices used in electrochemical and bioelectronic devices. Within the context of polymers, this is important because the conductivity of organic materials can be directly correlated to ion transport from certain electrolytes. In a study conducted by Aoki et al [3], optical transmissions of a film were modulated by ion injection from an electrolyte. As the electron carrier density of the organic film began to decrease, optical absorption of the material was measured and quantified in terms of ion transport from the electrolyte to the polymer film. The organic film Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate)( PEDOT:PSS) is a
Figure 2: Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate), PEDOT:PSS, has a PEDOT backbone, connected with a PSS chain. The PSS chain has sulfonate groups with a high affinity for holes (positive charge). The movement of holes gives PEDOT:PSS its conductive properties. Image taken from Sigma Aldrich [1].

PEDOT:PSS is composed of a PEDOT backbone connected with a PSS chain. Because there are uncompensated sulfonate anions on the PSS chain, this polymer is considered p-type, where the sulfonate ions play the role of hole acceptors. When modeling the ion transport and conductivity of these polymers, Stavrinidou et al [17] were able to show the movement of ions from electrolyte to the polymer by applying a voltage to the material.

2.4 Biosensing with Entrapped Enzymes

Without the correct functionalization and encapsulation of enzymes, organic electrochemical transistors would not be able to operate in unstable environments. Known for their metabolic diversity, enzymes provide a biological interface between sensors and their external environments. Biosensors that are functionalized with enzymes show promise in their ability to use enzymes as a catalyst for quantifying concentrations of
metabolites in media. Although enzymes are easily used within controlled environments, their use with biosensors is more complicated due to environmental changes that occur on the sensor interface. When an enzyme breaks down compounds, there is a rapid deactivation of biomolecules causing unwanted changes in pH and chemical structure of the enzyme [5].

One way of protecting enzymes within unstable environments is enzyme entrapment within calcium alginate gels. Enzyme entrapment is a process in which covalent or non-covalent bonds are created between an enzyme and certain gels or fibers. Calcium alginate gels prevent enzyme leakage, and provide increased mechanical stability of enzymes [16].

3 Work Done

3.1 Device Design

The first step in creating an array of electrochemical transistors involved developing the correct geometries for the photomasks used during fabrication. When I started designing the first iteration of these photomasks, there were several parameters I needed to keep in mind before creating a template for the gold layer. An important limitation was the distance between each organic electrochemical transistor. Each transistor needed to be roughly 1 mm apart in order to reduce the amount of noise that could be amplified by the gold electrodes in the circuit. In addition, gold wiring connections could not be more than 20 micrometers in distance from each other because it would cause errors in the micro-fabrication process. Keeping these things in mind, I had plenty of room to create an array of 12 separate transistors on an area that was 1,875 mm$^2$, an appropriate size for the glass substrates used in fabrication. When determining the orientation of each of the transistors with respect to one other, I had to keep in mind the drop casting methods used for enzyme functionalization. That being said, a considerable amount of room (1mm) was left around each transistor to make the process of dropcasting hydrogels easier.

Quantifying the sensitivity of each transistor involves choosing appropriate ratios of gate size with respect to channel area. Current modulation of organic electrochemical transistors increases as this ratio is increased [14]. With this in mind, I wanted to test three different layouts with different aspect ratios. The first and smallest configuration I mapped out had a gate area of 0.06 mm$^2$, and a channel area of 0.002 mm$^2$. In the second transistor configuration, the gate area was 0.5 mm$^2$ with a channel area of 0.01 mm$^2$. Finally, the last and largest configuration had a gate area of 6 mm$^2$, and a channel area of 0.5 mm$^2$. These three layout sizes were considered because they are small enough for fast current responses, and large enough for any mistakes in the fabrication process. Other gate to channel ratios were considered, but I chose to stick with the ratio of 10:1 between the gate and channel areas because it would maximize transconductance [14].
3.2 Electrical Characterization of PEDOT:PSS

The conductive polymer PEDOT:PSS plays a critical role in how an organic electrochemical transistor performs. Although many publications measure the conductivity of PEDOT:PSS [6], especially with conductivity boosting agents such as ethylene glycol [15], I wanted to quantify the conductivity of PEDOT:PSS using impedance spectroscopy. This process involves running a current through the polymer, and analyzing its resistive properties. In addition, impedance spectroscopy can be used to determine conduction of a polymer as a function of material composition. I spin coated a thin layer (10 micrometers) of PEDOT:PSS, ethylene glycol (EG), GOPS, and Dodecyl Benzene Sulfonic Acid (DBS) onto an impedance characterization device, where two gold plates are separated by a 20 micrometer channel gap on a glass substrate. EG and DBS are used to increase the conductivity of the organic polymer PEDOT:PSS, while GOPS is used to provide mechanically stable connection between the polymer and the gold and glass.

After preparing my samples with PEDOT:PSS, I was able to vary current through the polymer and record impedance data using the Metrohm AutoLab Potentiostat. I made an electrical connection with two electrodes connected across the gold terminals and fluctuated a current from -100 mA to 100 mA. Electrochemical impedance was calculated by the potentiostat with this small current signal, and simultaneously fitted with a series of equivalent electrical circuits within the AutoLab software. This process compares the electrochemical impedance of the polymer with an equivalent circuit consisting of a voltage source, resistors,
and inductors. Although conductivity of the PEDOT:PSS polymer could be quantified by characterizing the electrochemical transistor, this step ensures that the PEDOT:PSS used for the electrochemical transistors is conductive within the voltage ranges that we plan to apply to it.

### 3.3 Setting Data Acquisition Software and Instrumentation

Before I could start working with the newly fabricated electrochemical transistors, I wanted to make sure that I could measure the transconductance of a working MOSFET as well as its transient and steady state output characteristics with my LabView and DAQ setup. This data would give me a good overview of the functionality of my data acquisition system. Although current vs voltage curves could be easily be made by sweeping a voltage across a transistor and measuring the current across a load resistor, I wanted to test the sweeping of my custom LabView modules. Instead of using straight sweep from 0 to 1 V, I need to apply a negative voltage to the gate electrodes between every step up in gate voltage\(^1\).

I linked the data acquisition system with the transistor to record the output current characteristics in terms of changing gate voltages. Attached to this setup were three electrodes where I could ground the transistor, set bias voltages at the gate and drain electrodes, as well as record a current through the source and drain terminal on the transistors.

Using this setup, the MOSFET was operating as expected after applying a gate voltage higher than its threshold voltage. As voltage increased beyond the threshold voltage, the current response of the transistor began to decrease. This current modulation and amplification is evident of a working MOSFET transistor. With that in mind, I ensured that the I/V curves measured were on par with nominal values for biasing within the triode and saturation regions\(^2\).

### 3.4 Electrical Characterization of OECTs

Within the Rolandi Group, fellow undergraduate Nebyu Yonas and graduate student John Selberg are in charge of device fabrication. After the transistors were fabricated, I characterized each OECT configuration using LabView in tandem with the NI PXIe-1062Q Data Acquisition System (DAQ).

For the first round of devices, I wanted to see how the current response changed with respect to the different sized OECTs that were fabricated. To do this, I applied a constant drain source voltage bias of 0.5

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\(^1\) This application of a negative gate voltage will be used with the OECT. This is needed in order to flush-out ions that have not diffused out of the conductive polymer when increasing the gate voltage.

\(^2\) These measurements were done to ensure that my Data Acquisition System was working. I mainly wanted to test my custom voltage sweeping configured within LabView.
Figure 4: This is the full characterization setup which includes the NI Data Acquisition System and Electrode Probing Station.

Figure 5: This is the probe setup used to connect each of the terminals of the transistor to the data acquisition system and the digital multimeter. The sample is vacuum sealed onto the center disk, and three probes with micromanipulators are used to accurately make contact with the sample. As seen in the photo, a microscope helps in guiding the probes for good contact.
Figure 6: As increasing various gate voltages ranging from 0.1 to 0.5 V are applied to the gate electrode, the magnitude of the drain source current goes down as expected. This is a result of cations being pushed into the conductive polymer, decreasing its affinity for free-moving holes. The voltage applied the gate is roughly proportional to current measured across the channel.

V, and varied the gate voltage from 0 to 0.5 V. Although this process seemed simple at first glance, there were many difficulties in measuring a system that relies on the movement of ions to change current. When changing the bulk ionic conductivity of PEDOT:PSS, it is important to flush ions out of the organic polymer before reapplying a gate voltage. At first, I was only applying positive gate voltages to the gate electrode causing my new measurements to be inaccurate. The drain source current measurements were not dropping with an increase in gate voltage as expected. Seeing this, I realized that I needed to apply a negative voltage of at least -0.5 V for 1 second before I could reapply a different gate voltage. After applying a gate voltage, within a range of 0 to 0.5 V, I applied another negative gate voltage of -0.5 V, before reapplying a gate bias. Although this process was time consuming, it provided an accurate current response from a changing gate voltage. Using Labview, I was able to take this same set of measurements with an automated process that changed the applied various gate voltages after increments of 100 milliseconds. From these experiments, I concluded that the medium sized transistor, with a gate area of 0.5 $mm^2$ and channel area of 0.01 $mm^2$ was the most stable, and performed under ideal current ranges around 0.50 mA to 1 mA. With this configuration, it was easy to see that as gate voltage was increased by 0.1 V, current would decrease by a magnitude of 100 uA. This current response is observed because the positive gate potentials push cations into the conductive polymer, decreasing the movement of holes across the channel. From these values, transconductance was able to be measured by the change in drain current over the change in gate voltage.
Figure 7: Each day, the current response of my OECTs goes down with its respective gate voltage bias. Even though the drain current is decreasing, it stay within the range of 500 microamps.

3.5 Degradation of PEDOT:PSS

As I was characterizing the electrochemical transistors, I started to notice a trend in the current response of the transistors with respect to time. Every day, the current response started to lower by a small factor. Although this is negligible after a couple of days, there was a noticeable amount of current loss after 2 weeks. In order to test and quantify the amount of degradation that was happening, I took daily current measurements of each size OECT configuration over the course of two weeks. With help from other members of the Rolandi Lab, Brian Nguyen and Nebyu Yonas, I was able to see a steady decrease in current response after each day. I believe that this decrease in current can be attributed to the natural oxidation of the sulfonate groups on the PSS chain. As PEDOT:PSS is exposed to forms of energy such as UV light and voltages above 1 V, the sulfonate groups begins to oxidize and lose the ability to transfer and carry holes. Although I wanted to maximize current response, I did not believe that this problem was significant because the output current range was still above 500 milliamps. In addition, the sensitivity, or transconductance, of the transistor was stable over the course of two weeks.

3.6 Second Iteration of OECTs

With the first round of characterization completed for the version one of the OECTs, a new photomask needed to be designed for testing with functionalized enzymes. I concluded that the medium sized transistors with a gate area of 0.5 $mm^2$ and a channel area of 0.01 $mm^2$ was optimal because of its stable output current around the range of 0.5 to 1 mA. In order to maximize the amount of transistors that can be made during fabrication, these electrochemical transistors were fabricated on quartz wafers instead of glass slides. Approximately 4
From this graph, we can assume that transconductance values will stay stable over time. After 2 weeks, the sensitivity of our device stays above 0.0002 mS. This value is enough to give tangible sensitivity readings given small fluctuations in gate voltage.

3.7 Functionalizing Devices With Entrapped Enzymes in a Sodium Alginate Gel

After finalizing the second iteration of electrochemical transistors, these transistors were ready to be functionalized with enzymes entrapped in a sodium alginate gel. In order for these OECTs to function as a biosensor, an enzyme must be used to act as the sensing interface between the transducer (OECT) and toxic alcohols. For sensing ethanol, the enzyme alcohol dehydrogenase and its cofactor \( NAD^+ \) are entrapped in a sodium alginate gel and placed on top of the OECT.

3.8 Characterizing OECTs with Sodium Alginate (Control)

Before characterizing OECTs functionalized with enzymes in the presence of ethanol, it was important to first establish a control set of OECTs with only sodium alginate gels and no enzyme. This control experiment would ensure that decreases in polymer conductivity are only due to the presence of enzymes. For this round of characterization, I exposed the OECT covered with a sodium alginate gel to a DI water and ethanol solution ranging from 0 to 10 mM. The measured current ranges show that without the presence of enzyme,
Figure 9: The second iteration of the organic electrochemical transistors involves fabrication on quartz wafers. Using this configuration, many more transistors available compared to fabricating the devices on glass slides.

Figure 10: A sodium alginate gel was used to entrap alcohol dehydrogenase and $\text{NAD}^+$ to sense ethanol concentrations. This gel can be placed directly on top of the OECT configuration as shown.
Figure 11: A layer of sodium alginate gel was applied to the OECT and ethanol concentrations ranging from 0 to 10 mM were exposed to the device. As ethanol concentration was increased polymer conductivity (Drain Current) increases (Keep in mind the Y axis is inverted). This change in drain current is negligible compared to the changes in conductivity seen in the presence of alcohol dehydrogenase and $NAD^+$. 

polymer conductivity increases slightly.

3.9 Sensing Ethanol

Once a control data set was established, I wanted to characterize the enzyme functionalized OECTs in the presence of ethanol. After exposing the OECT to a 10 mM ethanol solution, the polymer conductivity decreased steadily over the course of 30 minutes. As ethanol is broken down by alcohol dehydrogenase and its cofactor, electrons are released into the gel as a byproduct. I hypothesize that these electrons are collected at the channel decreasing polymer conductivity.
Figure 12: A 10 mM (0.08% BAC) solution was exposed to an enzyme functionalized OECT. Over the course of 30 minutes polymer conductivity steadily decreases. For this experiment, the hydrogel contains 2 mg of alcohol dehydrogenase, and 2 mg of $NAD^+$
4 Conclusion

I have created an array of organic electrochemical transistors that can detect ethanol in solution. Characterization of these devices reveals that current can be modulated with the appropriate voltages applied at the gate and drain electrodes of the transistor. I characterized an OECT functionalized with an enzyme and an OECT missing an enzyme, and established that the sensing mechanism of these OECTs is caused by the presence of an enzyme.

4.1 Future Work

To finish this project, I will characterize two more sets of enzyme functionalized transistors. Using different enzymes (alcohol oxidase, glycerol dehydrogenase, alcohol dehydrogenase, and NADP-dependent isopropanol dehydrogenase) I aim to use the same process to detect methanol, isopropanol, and ethylene glycol. Currently, I am using a NI PXI Data Acquisition System that can operate one transistor at a time. In the future, I would like to redesign the characterization of these devices for use with a microcontroller capable of operating multiple transistors in parallel. The use of a microcontroller would also automate the process of recording polymer conductivity and increase the portability of our devices. Implementing a data recording system for a set of four functionalized enzymes would greatly increase the efficiency of my current data acquisition methods. Although the NI PXI Data Acquisition System has accuracy magnitudes greater than a microcontroller, the portability and multi-use functionality of microcontrollers would make these biosensors usable as a point of care device. Because these electrochemical transistors are enzyme specific, I believe these transistors can be used to detect other metabolites in the blood based on the type of enzyme used to functionalize the device. I would like to explore the use of these types of transistors in wearable technology to detect analytes in the sweat such as glucose, lactate, and electrolytes.
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I would like to thank the following people for their contributions to this project: Marco Rolandi, John Selberg, Brian Nguyen, Nebyu Yonas, and Tom Yuzvinsky.

For this project, my main focus was on the characterization of organic electrochemical transistors for sensing toxic alcohols. With that in mind, Nebyu Yonas focused on device fabrication, while Brian Nguyen specialized in enzyme functionalization.
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