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PNNL-38996

Multiplexed Toxin Activity Detection

February 2026

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U.S. DEPARTMENT of ENERGY

Prepared for the U.S. Department of Energy
under Contract DE-AC05-76RL01830

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Abstract

There is a need for field forward biothreat detection methods for highly lethal marine toxins, such as conotoxins, to protect the warfighter and civilians in contested environments. These toxins arise from harmful algal blooms which impact the warfighter, pets, and civilians. The need for detection platforms that can be fielded on drones or unmanned vehicles is paramount to successful biothreat detection to serve as an early warning system for those impacted in these environments.

We have developed biomimetic devices for characterizing host-toxin interactions. This technology provides an authentic membrane and sensing strategy that is robust, sensitive, scalable, and fieldable. Membranes are harvested from human cells expressing the toxin target of interest, such as an ion channel, then deposited onto an electronic device that can provide the electrical responses of membranes. This cell free approach has been shown to faithfully recapitulate the response of a live cell to omega-conotoxin GVIA (Figure 1). However, the devices only enable one activity assay per experiment. The goal of this effort is to fabricate a device that enables multiplexing these toxin assays such that multiple readings can be obtained in one experimental measurement.

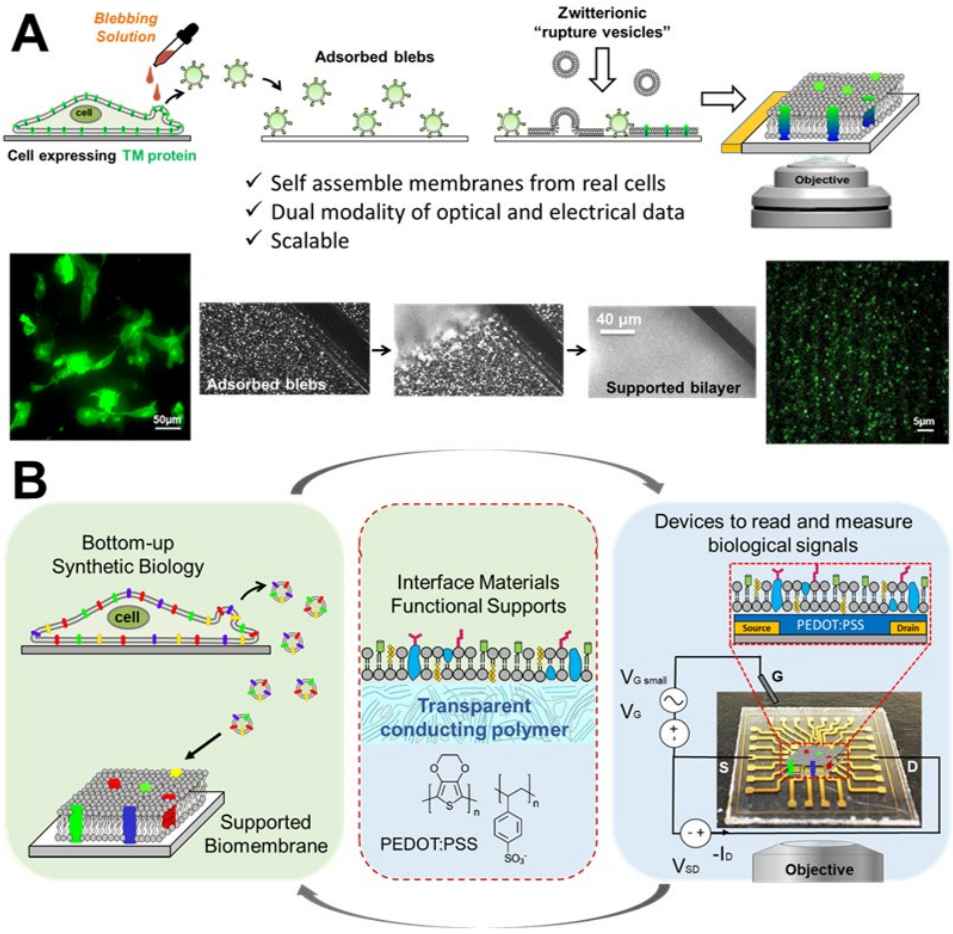

Figure 1. From cells to electrical circuit: integrating a cell membrane with the device. As shown in ***A***, the membrane from a cell containing the receptor of interest (transmembrane protein) can be harvested and deposited on a solid support. ***B***: Using this approach, we can deposit intact membranes onto an electronic device and use electronic impedance spectroscopy (EIS) to measure changes in activity of the transmembrane protein.

Acknowledgments

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Contents

1.0 Introduction

Two of the major goals of the National Biodefense Strategy are: 1) Enable risk awareness to inform decision making across the biodefense enterprise; 2) Rapidly respond to limit the impacts of biological incidents. Assessing toxin activity from an unknown sample or source is critical to mounting an appropriate response to a biothreat agent. Toxins are of interest because they cannot be identified using traditional genome sequencing platforms or polymerase chain reaction (PCR) assays due to their molecular composition. In addition, current methodologies for identifying a toxin do not inform the user if the toxin is active. Therefore, toxin activity assays are in critical need to inform decision making for countermeasure use, personal protective equipment needs, and assessing the safety of a given environment.

2.0 Objective and Approach

2.1 Objective

We have developed biomimetic devices for characterizing host-toxin interactions. This technology provides an authentic membrane and sensing strategy that is robust, sensitive, scalable, and fieldable. Membranes are harvested from human cells expressing the toxin target of interest, such as an ion channel, then deposited onto an electronic device that can provide the electrical responses of membranes. This cell free approach has been shown to faithfully recapitulate the response of a live cell to omega-conotoxin GVIA. We will leverage these biomimetic electronic devices for the development of a threat agnostic sensing platform, capable of detecting toxin activity for any toxin that has interactions with a membrane. We expect this platform could serve in the areas of detection, identification, and development of countermeasures. In this effort, we propose to recapitulate mammalian cell membranes to faithfully capture toxin interactions, thereby creating a toxin activity assay that is based on a cell free platform.

2.2 Approach

We will develop a biomimetic device that faithfully recapitulates the activity between a toxin and a human host membrane target.
Supported lipid bilayers (SLB's) are representative cell membrane models that allow us to characterize and study the interactions of the transmembrane proteins for host/toxin interactions. These SLBs are harvested from cells expressing the receptor of interest, then are deposited onto an electronic device that can provide impedance and transient electrical responses of the membranes. Specifically, we are focusing on ion channels which are important for toxin targets, as shown in figure 1.

The objective of this study is to measure distinct biological responses of host cell membrane interactions with various agents, specifically toxins, and convert these responses into measurable signals for analysis and identification. To achieve this, we will assemble membrane blebs derived from the HEK-293 cell line onto plasma-cleaned chips, and expose the membranes to 1x PBS (negative control), 5 μM and 25 μM α-Hemolysin, and 30 μM verapamil. Verapamil induces membrane changes by binding to and blocking specific calcium channels, which we anticipate will lead to an increase in membrane resistance as ion channel activity is inhibited. In contrast, α-Hemolysin disrupts membrane integrity by forming pores in the lipid bilayer, and we expect to observe a decrease in membrane resistance as ion permeation increases. For the PBS negative control, we anticipate little to no change in membrane properties; however, minor variations may arise due to the ionic capacity of the solution. These expected changes in membrane resistance will provide insight into how these agents interact with the lipid bilayer and modulate its biophysical properties.

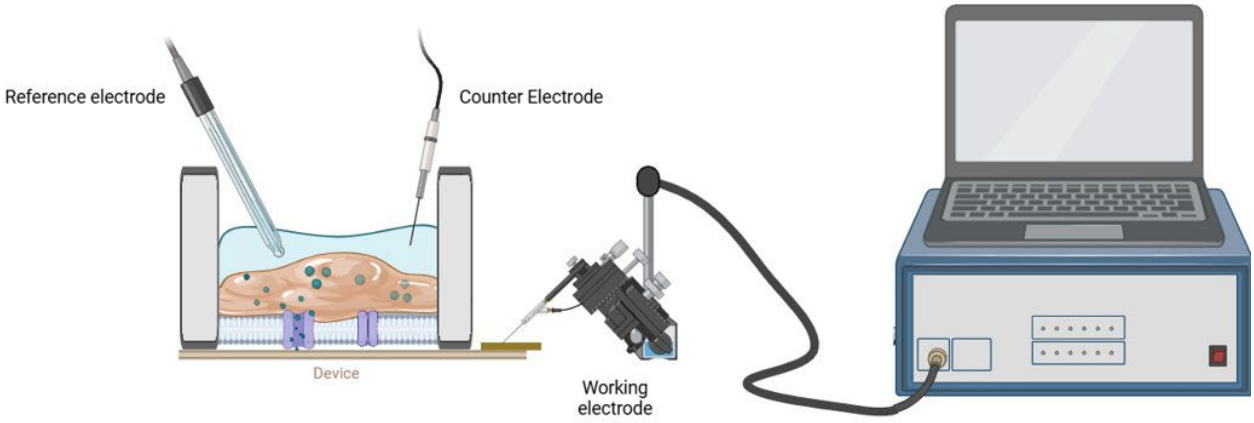

Figure 1. Illustration of the function of a pre-fabricated device integrated with a supported lipid bilayer (SLB) used to study the effects of different agents on ion channel activity. The SLB mimics a biological membrane, and specific agents are introduced to either open or close ion channels embedded within the membrane. These changes in ion channel activity are measured using electrochemical impedance spectroscopy (EIS) which detect alterations in electrical properties such as resistance and capacitance across the membrane. The data provides insights into how the agents modulate ion channel behavior, with distinct patterns corresponding to channel activation or inhibition.

Methods

Chip preparation:
Pre-fabricated chips will be provided and are ready for immediate use. To ensure reliable performance, chips must be handled with care to prevent damage. Special attention should be given to proper orientation during setup, and the contact pads should be inspected for any defects such as scratches, warping, or deformities.

Preparation of Liposomes:
POPC vesicles were prepared by transferring 160 µL of a 200 mg/mL POPC stock solution in chloroform into a pre-cleaned 20 mL scintillation vial. The vial was placed under vacuum for several hours to ensure the complete evaporation of chloroform, leaving behind a dried lipid film. The film was then rehydrated and resuspended in 2 mL of Tris-KCl buffer (10 mM Tris-HCl, 150 mM KCl) to achieve a final lipid concentration of 2 mg/mL. To promote uniformity, the lipid suspension underwent three rapid freeze-thaw cycles. The resulting solution was subsequently extruded through a 100 nm filter membrane to obtain vesicles of uniform size and homogeneity. The prepared solution was stored at 4 °C and remained stable for up to 30 days.

Blebs:
HEK-293 WT cells were cultured on P100 dishes until they reached approximately 90% confluency. The cells were gently washed twice with GPMV buffer "A" (2 mM $CaCl_2$, 10 mM HEPES, and 150 mM NaCl, pH 7.4). Following the washes, 4 mL of freshly prepared GPMV buffer "B" (GPMV buffer "A" supplemented with 25 mM formaldehyde and 2 mM dithiothreitol) was added to each dish, and the cells were incubated at 37°C in a 5% O_2 environment for 2 hours. After incubation, the supernatant containing the membrane blebs was carefully aspirated and transferred into a conical tube. The tube was then placed on ice for 30 minutes to allow cell debris to settle and separate from the blebs. The top 80% of the solution was carefully collected

and centrifuged at 10,000 RPM for 10 minutes to further clarify the sample. Finally, the top 70% of the resulting supernatant was collected and stored at 4°C, where it remained stable for up to 10 days.

Preparing the chip for impendence measurements:
Blebs and POPC vesicles were mixed at a 1:10 ratio and sonicated on ice for 20 minutes on the day of treatment. For chip preparation, chips were cleaned by washing with ethanol and water, followed by drying with house nitrogen gas. The plasma cleaning chamber was preconditioned before chip treatment by lowering the pressure to 105 mTorr, flooding the chamber with pure oxygen gas three times, and running the plasma cleaner on the "low" setting for 3–5 minutes. To plasma clean the chips, the chamber preparation was repeated in the same manner, with the plasma cleaner activated at the "low" setting for 30–40 seconds. Immediately after plasma treatment, 100 µL of the 1:10 bleb-to-POPC mixture was added to the chip and incubated for 1 hour. Following incubation, the well was washed three times with Tris buffer, and 180 µL of Tris buffer was added to the well for subsequent use.

Electrochemical Impedance Measurements:
The frequency of applied sinusoidal voltage was swept from 10^6 Hz to 1 Hz to capture the change in electrical signal at each step after the addition of biological materials. After measuring the bilayer of the POPC/bleb mixture with no agents added, PBS, verapamil and α-Hemolysin were added to each well and gently mixed. Resistance was measured every ~15 mins. It is important to note that the 25 µM α-Hemolysin concentration was prepared in a solution that also contained 30 µM verapamil due to limitations in the availability of wells. However, since the observed change in resistance is significant and opposite to the response elicited by verapamil alone, the presence of verapamil in this solution will not be accounted for or included in the results section. This ensures the analysis focuses solely on the α-Hemolysin response.

Results:
SLB change over time
Figure 2 shows the impedance magnitude (|Z|) as a function of frequency for a supported lipid bilayer (SLB) under different treatment conditions (Baseline, 1x PBS, 5 µM α-Hemolysin, 25 µM α-Hemolysin, and 30 µM Verapamil).

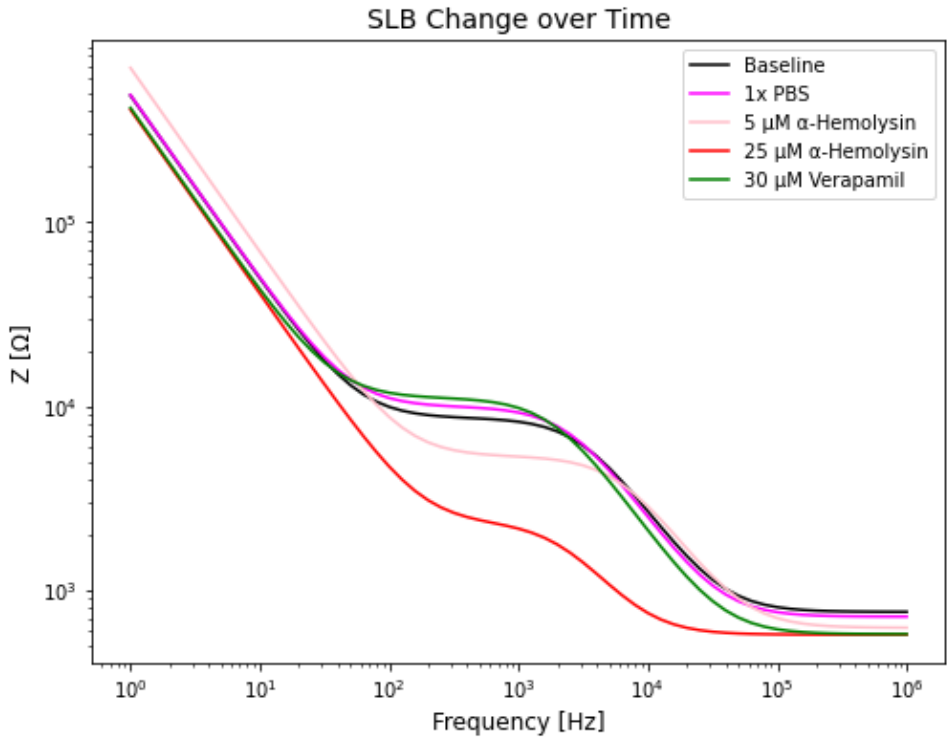

Figure 2. This graph shows the change in impedance magnitude Z (Ω) over a wide frequency range (100 to 10^6 Hz) for SLBs treated with various agents: Baseline (black line), 1x PBS (magenta), 5 µM α-Hemolysin (red), 25 µM α-Hemolysin (pink), and 30 µM Verapamil (green). The baseline condition (black) shows the impendence values of the bilayer in the absence of external agents. The addition of 1x PBS (magenta) causes a slight increase in resistance, suggesting minor changes to the bilayer's electrical properties. In contrast, α-Hemolysin treatments (5 µM and 25 µM) result in significant impedance reductions, with the effect being concentration-dependent. Overall, the figure illustrates the differential impacts of various agents on SLB integrity and ion permeability across the frequency range.

% Resistance Change

Percentage resistance change Δ*Rm* [%] was calculated to assess the dynamic behavior of the system over time. This metric quantifies how the resistance of the chip and bilayer changes relative to the baseline condition, where no agent is added.

$$\Delta Rm[\%] = \frac{R_t - R_0}{R_0} \times 100$$

Where:

R_t= resistance at final timepoint

R_0- initial resistance

This method allowed for the identification and characterization of dynamic changes in resistance over the course of 40 minutes of exposure to various analytes.

Analyte	Δ*Rm*
1x PBS	+1%
5 μM α-Hemolysin	-16%
25 μM α-Hemolysin	-83%
30 μM Verapamil	+12%

Table 1. Δ*Rm[%]* values observed after 40 minutes. Resistance changes greater than or less than 10% were flagged and are marked in green to highlight significant deviations from the baseline.

Table 1 shows the observed resistance changes for different analytes. The control group (1x PBS) exhibited minimal resistance change (1%), confirming system stability with no added agent. In contrast, the addition of α-Hemolysin caused strong resistance decreases, with −16% observed at 5 μM and −83% at 25 μM, indicating significant bilayer disruption after 40 mins of exposure. Verapamil (30 μM) caused a moderate resistance increase (+12%), suggesting interactions leading to increased resistance as expected. These results demonstrate that each analyte induces a distinct, measurable effect on bilayer resistance, likely due to varying mechanisms of action.

α-hemolysin's effect on bilayer stability over time

Figure 3 shows the box-and-whisker plot summarizing the % resistance change (ΔRm) observed at three different concentrations of α-Hemolysin (0 μM, 5 μM, 25 μM). Each box represents the distribution of resistance change values at three time intervals (~13 min, ~25 min, ~37 min).

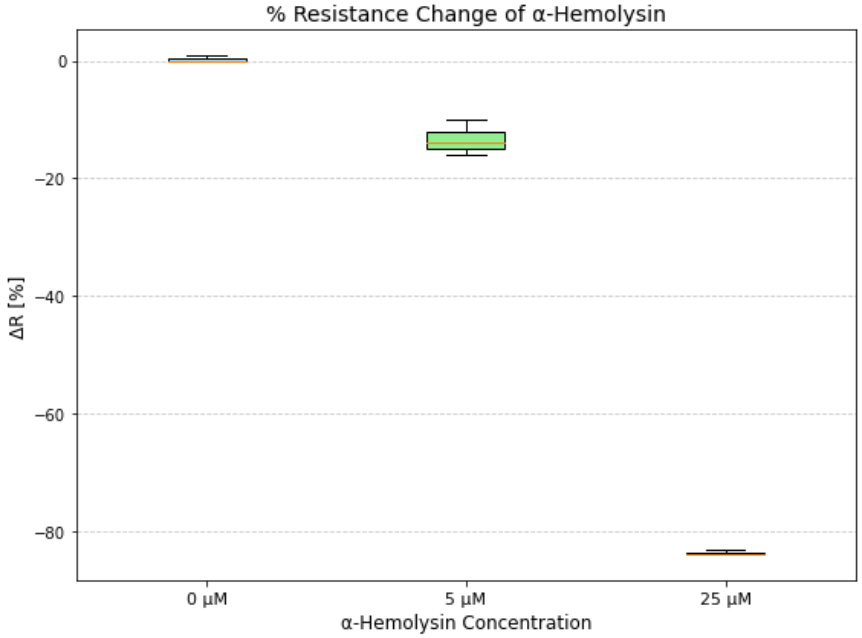

Figure 3. % resistance change (ΔR) observed at three different concentrations of α-Hemolysin (0 μM, 5 μM, 25 μM) over three time intervals.

The extent of bilayer disruption depends on the concentration of α-hemolysin, in which greater concentrations causes decreased resistance. At 0 μM (1x PBS) and 25 μM, the box is small and narrow, showing that we observe minimal variability over time. However, the 5 μM shows

moderate and consistent disruption over time. Overall, the results highlight the dose-dependent nature of α-hemolysin’s effect on bilayer stability over time.

3.0 References

Z. Lu, J. Treiber, K. Kallitsis, et al. “Automated Label-Free Assay for Viral Detection and Inhibitor Screening via Biomembrane-Functionalized Microelectrode Arrays.” Adv. Mater. 37, no. 51 (2025): e01985. https://doi.org/10.1002/adma.202501985

Appendix A – Future Directions

We intend to improve multiplexing using cell free expression on the chip devices as shown in Figure 4 below. We will have portable data acquisition that imports data to a standard Android device and interacts with a machine learning algorithm to identify host targets for threat agents.

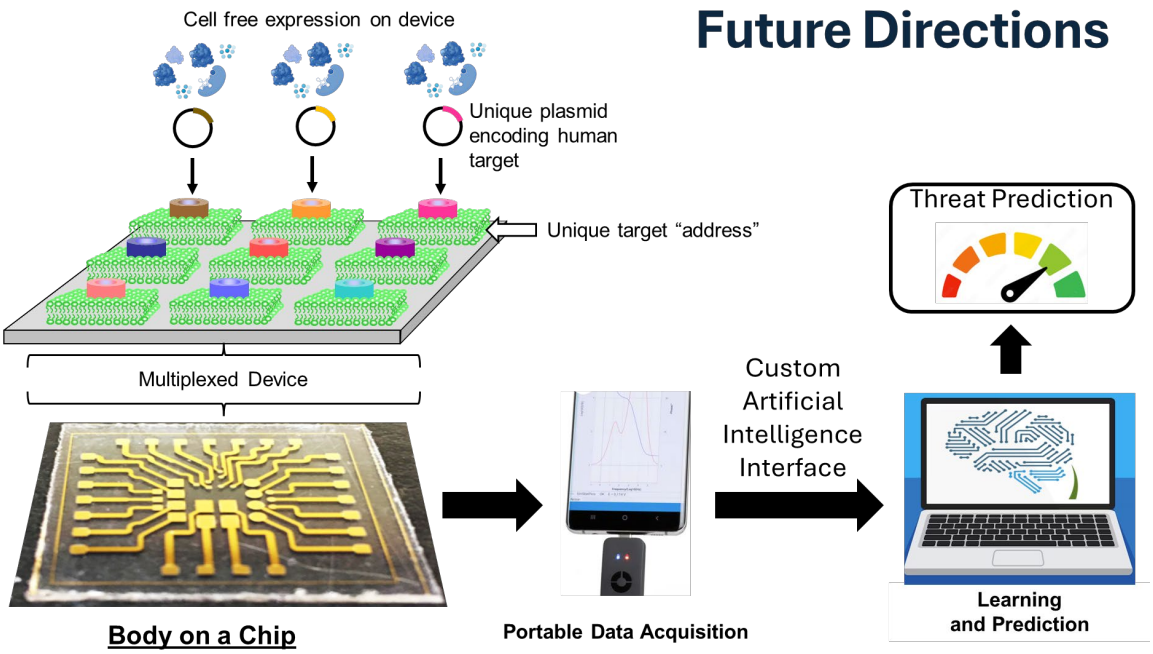

Figure 4. Body on a chip leverages cell free expression, data portability, and machine learning for threat agent identification.

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