Rapid and Low-Cost Field Toxin Analysis to Monitor Harmful Algal Blooms

Muhit Rana [1], Andrew Weber [1], Meredith Stewart [1], Avni A Argun [1*]

Abstract

Background: Harmful algal blooms (HABs) caused by the dinoflagellate Karenia brevis produce brevetoxins that represent significant threats to human health, marine ecology, and the economy. Detecting brevetoxin toxicity, which exists at very low levels, is challenging with current methods, limiting effective monitoring and management. Objective: To develop a sensitive, portable, and cost-effective method for the rapid, on-site detection of brevetoxins in seawater to support public safety, fisheries, and coastal management efforts. Methods: We designed a handheld portable device, the GinerSTAT, for electrochemical detection of brevetoxin-2 (PbTx-2) and brevetoxin-3 (PbTx-3) in seawater samples. The device utilizes specific electrodes to achieve high sensitivity and specificity, with detection limits reaching parts per billion. The entire sample collection and analysis process is completed within 10 minutes. Results: The GinerSTAT demonstrated robust performance in detecting brevetoxins in seawater, offering rapid results with high accuracy. The device is compact, user-friendly, and affordable, with an estimated cost below $500. These attributes make it a practical tool for field-based monitoring of HABs. Conclusion: The GinerSTAT offers a groundbreaking approach to brevetoxin detection, enabling real-time, low-cost monitoring of harmful marine toxins. This innovation has significant implications for enhancing public safety, preserving marine ecosystems, and supporting the marine economy.

Keywords: Harmful Algal Blooms, Brevetoxin Detection, Electrochemical Device, Marine Monitoring, Portable Sensor, Electrochemical Sensor.

Significance | The GinerSTAT enables rapid, cost-effective detection of brevetoxins in seawater, revolutionizing harmful algal bloom monitoring and mitigating health and economic impacts.

*Correspondence. Avni A Argun, Giner, Inc, 89 Rumford Avenue, Newton, MA 02466, USA.
Email: aargun@ginerinc.com

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1. Introduction

Harmful algal blooms (HABs) occur when microscopic marine algae produce compounds that are toxic to humans. Neurological and gastrointestinal illnesses can occur when the toxins become airborne and are inhaled or, when contaminated shellfish is consumed (Anderson, Reguera, Pitcher, & Enevoldsen, 2020; Hallengraeff, 1993). The toxin produced by K. brevis, brevetoxin (PbTx), binds to voltage-gated sodium channels in nerve cells leading to a disruption in neurological processes that cause neurotoxic shellfish poisoning (NSP)(Plakas & Dickey, 2010). NSP is especially prevalent in the Gulf of Mexico, where high density blooms of K. brevis, often referred to as “red tides” occur annually. Rising ocean temperatures in recent years, correlated with an increase in the number of toxic blooms, toxins and toxic species, have resulted in economic losses and growing environmental concern. Toxic levels of brevetoxin are as low as 1-15 parts be billion (ppb), making it difficult to detect (Pierce et al., 2005). The industry standard for algal toxin detection is enzyme-linked ELISA that not only requires sophisticated operation, but costs $200 per sample compared to the $5 per sample using Giner’s portable field-device. Another commonly used test for accurately identifying the toxicity of ocean samples, high performance liquid chromatography

Author Affiliation.
[1] Giner, Inc, 89 Rumford Avenue, Newton, MA 02466, USA.

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coupled with mass spectrometry (HPLC-MS), is also expensive and time consuming. Fully developed, Giner's portable device will be readily available to public health agencies for environmental toxin detection. The GinerSTAT will eliminate the significant time, effort and resources needed to collect and send samples to labs for testing. Additionally, lowering field detection limits will generate data that will be extremely useful in marine ecological research and monitoring.

1.1 A Rapid, On-Site Toxin Monitoring

This study reports the first of its kind electrochemical brevetoxin sensor prototype that uses aptamer modified screen-printed electrodes to demonstrate unprecedented specificity and sensitivity for field detection of brevetoxin-2 and -3. Figure 1 shows graphical representation of our technical approach where commercially available screen-printed electrodes (SPEs) are modified with Giner's aptamers with a one-time electrode treatment that is performed in bulk, resulting in disposable SPE test chips that are ready for use. Specific binding of the brevetoxin molecules to the aptamer-modified electrode and their electrochemical detection is carried out in the field as described in Figure 2. In addition, the aptamers are synthesized with a methylene blue (MeBlue) redox reporter molecule which improves electrochemical response. Target-aptamer binding initiates a conformal shift which alters the MeBlue distance from the electrode surface corresponding to a pronounced change in electrochemical signal. In our detection method the MeBlue molecule is shifted away from the electrode after aptamer binding with target, producing a signal-off measurement approach. The method is amenable for field use by non-technical personnel with minimal training. Each component (SPE modification, binding and electrolyte solutions, and detection algorithms) are optimized in this study. Upon validation of the assay performance with ocean water samples, a portable test kit that includes a battery-operated reader, aptamer-modified test chips, and binding and electrolyte solutions can be used for future field demonstration and validation studies.

1.2 Why Aptamers?

The decision to utilize aptamers as target recognition platform has stemmed from clear advantages, they provide over similar target recognition platforms such as antibodies.(Kashfia & Ajeet, 2023; Kumar et al., 2022; Rana et al., 2023) Specifically, the costly and time-consuming process associated with monoclonal antibody selection(Birch & Racher, 2006; Farid, 2007) has resulted in the use of polyclonal antibodies to detect the brevetoxin analog groups as a whole(Kreuzer, Pravda, O'Sullivan, & Guilbault, 2002; Naar, Branaa, Bottein-Dechraoui, Chinain, & Pauillac, 2001), compromising specificity. Table 1 lists key advantages aptamers provide when compared to antibodies in the context of development of field sensors.

1.3 Why Electrochemical Detection?

This label-free electrochemical approach to detect brevetoxin has distinct advantages over other methods that employ labeling steps, such as fluorophores for their detection platform. In general, direct electrochemical methods are faster and more viable as they do not require complex instrumentation or complicated sample preparation steps. Detection of the analyte binding event can be monitored in real time, (Iqbal et al., 2010; Xu et al., 2010) allowing continuous monitoring. Detection components are inexpensive and can be readily miniaturized into portable, low-cost devices. (Perumal & Hashim, 2014; Thévenot, Toth, Durst, & Wilson, 2001) Another advantage of the electrochemical transduction (rather than optical) is the ability to operate in turbid media of complex matrices.

The key components of the Giner brevetoxin sensing platform that advantageously set it apart from the competition and currently existing technologies are: 1) Utilization of synthetic aptamers (not antibodies) for target recognition; and 2) electrochemical detection with disposable electrodes to allow low cost (< $15 per test) and rapid turnaround time (< 5 min sample-to-result); 3) MeBlue redox signal enhancement that improves brevetoxin limit of detection. In this section, we highlight advantages of each key component of the assay and compare Giner's approach to the currently available laboratory-based brevetoxin detection technologies.

1.4 Advantages of Giner's Approach over Currently Available Technologies:

To date, concentration of brevetoxin has mostly been determined by transporting the samples from field to laboratories with a delay in sample processing. Laboratory-based brevetoxin measurements are currently made using two standardized techniques: Enzyme-linked immunosorbent assay (ELISA) and High-Performance Liquid Chromatography - Mass Spectrometry (HPLC-MS). Despite many advances in these methods, there are practical limitations owing to the requirement for intense skilled labor. It is still a challenge to find field-friendly approaches that could provide simplicity, selectivity, and sensitivity. (Vilarino, Fonfria, Louzao, & Botana, 2009)

Recent studies indicate different types of biosensor systems that use antibodies and labels as recognition elements for detection of brevetoxin, including Lateral Flow Immunoassays (LFIA), radioimmunoassay (RIA) and electrochemiluminescence-based immunoassay. Among these, LFIA is applied in resource poor or no-laboratory environments to provide semi-quantitative monitoring. Essential in the lateral flow format is the movement of a liquid sample along a strip of polymeric material, passing various zones where attached antibodies and labelled reporters interact with the analyte. (Posthuma-Trumpie, Korf, & van Amerongen, 2009) Tests are usually developed aiming at on/off results, the most successful use of this technique. When a quantitative result is required, precise evaluation of an optical signal is required by a

measuring device such as a flatbed scanner or a CCD camera and dedicated software, (Posthuma-Trumpie et al., 2009; Tisone & O'Farrell, 2009) increasing costs and analysis time. We believe that a, antibody-free electrochemical brevetoxin detection platform that does not require expensive instrumentation would be a viable and inexpensive alternative to current standard methods. Table 2 summarizes key advantages of Giner approach over currently available brevetoxin detection technologies.

2. Materials and Methods

2.1 Materials and Equipment

The new brevetoxin-3 aptamers were designed and produced by AM Biotechnologies (Houston, Texas) with the X-Aptamer selection kit. Aptamer attachments with 5' amino spacers were received from IDT Technology, USA (5'AMMC12-Aptamer). 100 µg stock solutions of brevetoxin (PBTx-2 and PBT-3) were purchased from Abcam, LLC. (Cambridge, Massachusetts). Our collaborator, Mote Marine Lab and Aquarium in Sarasota, Florida, supplied seawater samples. The seawater simulant was made using a sea salt mix, INSTANT-OCEAN™ (Blacksburg, VA) with a gravity of 1.026 and a salinity of 35 parts per thousand (ppt). The potentiostat purchased from PalmSens™, Netherlands, was paired with the Samsung™ Galaxy S6 Android Tablet. The custom fabrication test chips were printed on vinyl or non-conductive substances and purchased from Conductive Technologies in York, PA. For the lysis of K. brevis cells, a lysis buffer kit from Stratagene was used. The Florida strain of K. brevis used to spike sea water samples were obtained from Mote Marine Laboratory's Phytoplankton Culture Facility.

2.2 Methods

2.2.1 Synthesis and Nano-Plasmonic Test Design for Gold Nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) were synthesized using the standard citrate reduction method, as described in previous studies. (Muhit, Kashfia, & Dong-Jin, 2023; Rana et al., 2016; Rana et al., 2017) This nano-plasmonic test was designed according to the published articles.(Liu & Lu, 2006; Rana et al., 2016) Specifically, 2 mL of 50 mM $HAuCl_4$ was added to 98 mL of boiling deionized (DI) water in an Erlenmeyer flask. Subsequently, 10 mL of 38.8 mM sodium citrate solution was introduced, and the mixture was stirred until the solution's color transitioned to wine-red, indicating the formation of AuNPs. The resulting homogeneous gold nanoparticles were characterized using UV-Vis spectroscopy and stored at 4°C for subsequent experiments.

2.2.2 Aptamer Validation

Aptamer-modified screen-printed electrodes (SPEs), designed for specific binding to brevetoxin, were analyzed using voltammetric techniques to identify the most effective PbTx-2 aptamer candidates. (Eissa, Siaj, & Zourob, 2015) The aptamer sequences were initially discovered using the SELEX technique, and their dissociation constants with brevetoxin-2 were compared to evaluate binding efficiency.

Additional aptamer sequences were screened as potential candidates for PbTx-3 using a gold nanoparticle (AuNP)-based method. (Rana et al., 2016; Rana et al., 2017) In this approach, aptamer candidates were spiked into a solution containing AuNPs and buffer to assess their binding characteristics quantitatively by monitoring absorbance changes resulting from their interaction with the target molecule.

For aptamer validation, 100 nM of the aptamer was mixed with 100 µL of 13 nm AuNPs and incubated at room temperature (RT) for 5 minutes. Subsequently, 100 nM of the target molecule (PbTx-2 or PbTx-3) was added to the pre-incubated AuNP solution, followed by an additional 15–20 minutes of incubation at RT. Finally, ~30 mM NaCl was introduced to the 100 µL solution. The color change of the AuNP solution was observed and recorded photographically before and after the addition of salt. A color transition from red to purple signified aptamer binding to the target molecule, while no color change indicated an absence of binding. The optical density (OD) at 520 nm (Abs520) of the resulting nanoparticle assemblies was measured to determine the aggregation rate. UV-Vis spectra of each sample were obtained using a BioTek microplate reader in 96-well plates.

2.2.3 Control Experiments and Detection Procedure

Control experiments were performed in the absence of target molecules, containing only the aptamer, AuNPs, and the adjusted reaction buffer (1× PBS, 2 mM $MgCl_2$, pH 7.4). The same procedure was applied for the detection and validation of PbTx-2 and PbTx-3 in buffer solutions.

2.2.4 Aptamer Functionalization for Target Specificity

To improve target specificity, an electrochemically active molecule called a redox reporter was attached to the aptamer. The redox reporter produces a signal that can be used to measure the spatial shift in aptamer geometry that occurs during target binding. The current change between the bound and unbound aptamer indicates the concentration of target in the sample. The redox reporting molecules improves the current signal as well as stability of the molecule, by tuning out potentially interfering signals. Redox reporter, methylene blue (MeBlue) was attached to the 3' terminal end of the chosen aptamer sequence.

2.2.5 Method Development

Streptavidin coated carbon SPEs are washed with phosphate buffered saline (PBS). The biotinylated aptamer modified with MeBlue was diluted to 10 µM with TBS. The solution was added to the SPE working electrode, incubated for 30 minutes and then wash with DI water before performing square wave volumetry (SWV). A sample spiked with the brevetoxin target was added to the newly

functionalized electrode, incubated for one hour and analyzed once more with SWV. Both chosen aptamer sequences (BT10 and BT3) can be functionalized on the same electrode and analyzed under the same SWV parameters.

2.2.6 Field Instrument Construction

Enginasion (West Boyston, MA) fabricated a field portable potentiostat device capable of cyclic voltammetry, linear sweep voltammetry, and square wave voltammetry. The field ready prototype, GinerStat includes a Giner sensor test cell and a 3D printed housing that closes over the SPE with a rubber gasket seal. The device is compatible with Bluetooth connection to the custom GinerSens program on a Windows PC, allowing a real time transfer of data. Data files created by GinerStat and displayed by GinerSens are saved in .txt format allowing them to be imported into other programs for downstream data analysis.

2.2.7 Marine Sample Preparation

Mote Marine Laboratory supplied 17 samples of seawater with adjusted salinity and spiked with four different K. brevis concentrations, containing whole and lysed cells. Salinity levels can vary greatly from ocean samples to those from inner waterways, so two different salinities were tested: 35 PSU (Practical Salinity Unit) and 25 PSU. The lower salinity was achieved by diluting seawater samples with reverse-osmosis water. Samples of seawater from both PSU stocks were used to dilute the K. brevis culture to the highest cell count of 5 x 106 cells/L. An aliquot of each 5 x 106 cells/L culture was serial diluted, with the appropriate PSU stock, to produce final K. brevis test cellular concentration sets of 5,000; 50,000; 500,000; and 5,000,000 cells/L for each salinity. One sample set of each salinity was lysed by placing the culture in a freezer for approximately 1 hour to lyse the cells (without freezing). Controls were supplied using the same tap seawater for each salinity level contained within the samples. The K. Brevis and control samples were then analyzed by Mote using HPLC-MS-MS and a 20 mL aliquot was shipped to Giner overnight at 4 C. Samples were kept refrigerated, but never frozen, between the tests.

2.2.8 Marine Sample Analysis

The method of testing the Giner assay on spiked sea water samples was similar to the test performed for the aptamer validation. 100 µl of the spiked sea water sample was placed onto aptamer-functionalized SPE and incubated for one hour. Next, the SPE was washed with DI water and 100 µl of TBS for the testing electrolyte. MeBlue peak height was calculated and correlated with PbTx-2 concentration.

3. Results and Discussion

The electrochemical biosensor we developed uses aptamer functionalized screen-printed electrodes designed to detect brevetoxins, PbTx-2 and PbTx-3. The field ready device contains immobilized aptamers on the surface of the electrode, allowing all unbound sample to be washed away, thus removing the reading interference from other particles. The hand-held instrument, designed for in-situ processing of ocean samples, allows the direct detection of brevetoxins with high specificity in just four steps as shown in Figure 2.

3.1 Aptamer Selection/Screening

The brevetoxin aptamer sequence candidates were found by Eissa et al., using the SELEX technique (Table S1). From this list we chose Clone BT10 for our experiment given its low dissociation constant when bound to brevetoxin-2. Clone BT3 was chosen as the brevetoxin-3 aptamer for its similar structure to the BT10/PbTx-2 pair (Figure 3). The gold nanoparticle (AuNP) assay was used to screen both the BT3 and BT10 aptamers and to analyze the absorbance change observed upon aptamer/target binding. (Kumar et al., 2022) The decrease in absorbance indicates the successful binding of the BT3 aptamer and the PbTx-3 target, with readings compared to the already established BT10/PbTx-2 as a positive control (Figure 4).

3.2 Direct Brevetoxin Detection

Aptamer functionalization was necessary for target specificity in seawater samples due to the likely interference of other electroactive species in a complex sample matrix such as ocean water. The BT10 aptamers allows a wash step that removes any non-bound sample, ensuring that any subsequent electrochemical signal can be entirely attributed to the concentration of brevetoxin present in the solution.

We performed a direct electrochemical detection test of various concentrations of PbTx-2 in buffer using voltammetry (Figure 5A). The measured anodic current is due to the oxidation of brevetoxin functional groups. A similar test was performed using the aptamer modified SPE assay instead and produced a similar limit of detection for PbTx-2 (Figure 5B). Both detection methods showed successful and similar detection of PBTx-2, but in a solution that contained no non-target species.

3.3 Utilizing A Redox Reporter

The aptamer modified SPEs were not as successful in samples that contained non-target electrochemically active molecules, as it is likely that many species in the seawater mix could display electroactivity at low voltages. To combat this, we attached a redox reporter molecule to the aptamer, that can distinguish the signal from target and non-target through the change in peak current. We attached redox reporter, methylene blue (MeBlue), to the SPE working electrode surface via the 3' terminal end of the BT10 aptamer. Because aptamer loading between individual SPE sensors can vary, the raw methylene blue oxidation peak height does not accurately quantify the aptamer/target binding. Rather the percent change between pre- and post-scans provides a more reproducible value that remains consistent regardless of initial aptamer concentration. In this way the decrease in MeBlue oxidation current

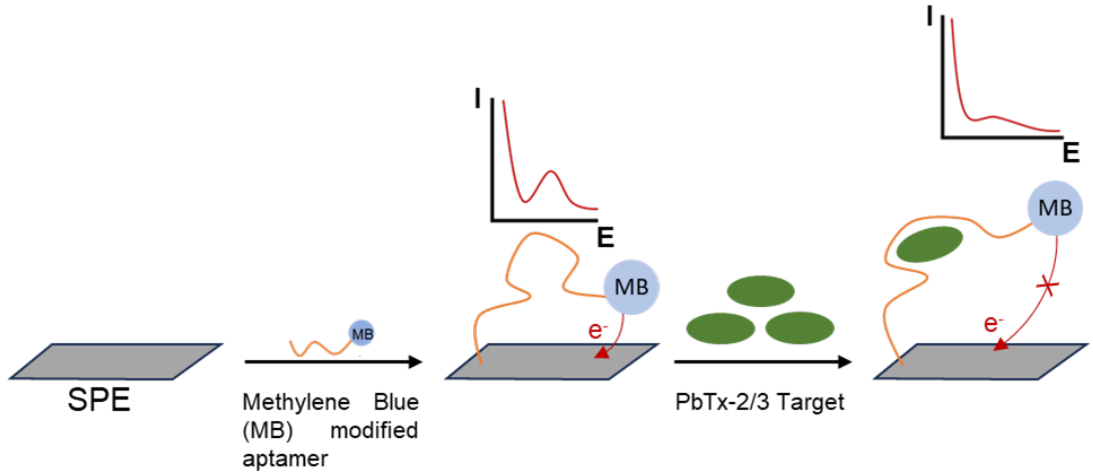

Figure 1. Graphical representation of our technical approach

Table 1. Key advantages of aptamers in sensors, over antibodies, as target binding platforms

	Antibody	Aptamer	Aptamer advantage
Development	Requires use of animals.	Identification via *in vitro* selection techniques.	Low cost and ethical
Production	*In vivo*; high batch-to-batch variation	Chemical synthesis; high reproducibility(Radom, Jurek, Mazurek, Otlewski, & Jeleń, 2013; Šmuc, Ahn, & Ulrich, 2013)	Rapid and cost effective
Modification	Modification at random positions.	Modification at defined positions (Kim & Gu, 2014)	Controlled immobilization
Recognition	Only at physiological conditions.	Tailored for desired/targeted conditions.	Operation versatility
Specificity	No opportunity for cross reactivity isolation.	Cross reactivity can be isolated by a negative selection step(Sharma & Shukla, 2014)	High specificity
Operation	Unstable in harsh chemical, humidity and temperature conditions	Stable at high temperature, extremes of pH, organic solvents, and detergents(Li, Zhao, & Qiu, 2013)	Longer shelf life Reliable field operation

Table 2. Advantages of proposed sensing approach over currently available competing technologies

Metrics	**HPLC-MS**	**ELISA & other antibody sensors**	**Giner's Sensor**
Sensitivity	< 0.1 ppb	0.1 ppb to 1 ppb	0.25 ppb
Selectivity	Selective for each analog	Not selective for analogs	Selective for brevetoxin-2, -3
Equipment cost	> $100 K	~ $5K to $10 K	$500
Per-test cost	> $200	$100 to $200	< $15
Development costs	Not Applicable	> $10K antibody selection process	~ $2K aptamer selection process
Labor requirement	Intensive, skilled labor	Skilled labor for biology kits	Easy-to-use, minimal training
On-site detection	Not portable	Depends on the kit requirements	Handheld, portable equipment
Assay time	Hours to days	Several hours	< 5 minutes
Sample processing	Complex sample prep	Antibody labeling	Simple mixing
Sample composition	Known MS interferences	Major issues in complex matrices	Minor matrix dependence

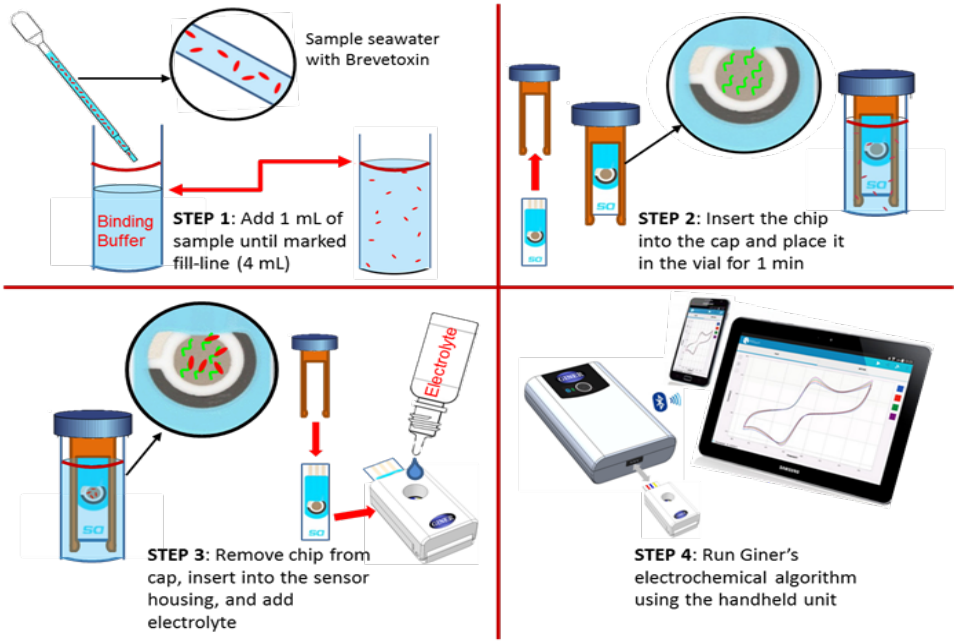

Figure 2. Schematic illustration of Giner's electrochemical assay for four-step detection of PbTx-2, -3 in seawater samples.

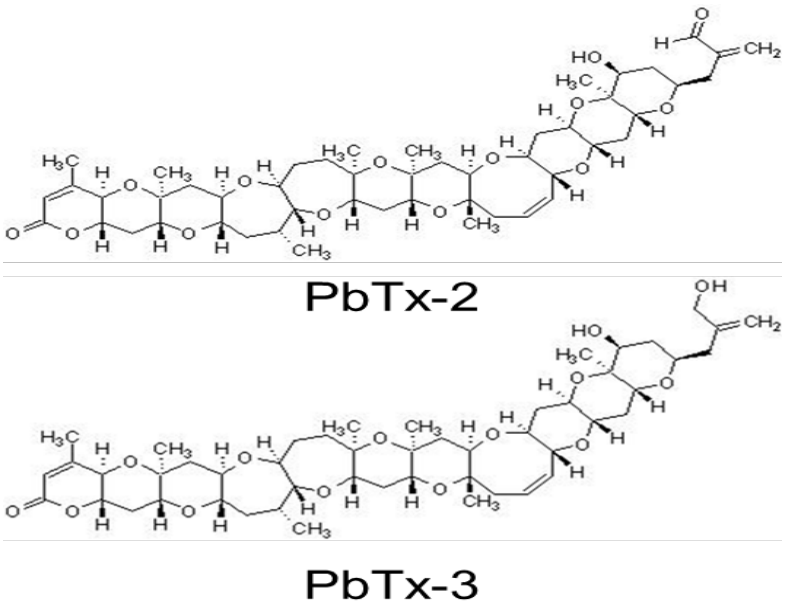

Figure 1. A) Schematic of the gold nanoparticle assay used to confirm proper aptamer/target binding. The inset shows the red to purple change in solution absorbance on successful binding. B) Gold nanoparticle assay results confirming successful binding between the BT3 aptamer and PbTx-3 (blue) along with the already established BT10/PbTx-2 binding (green).

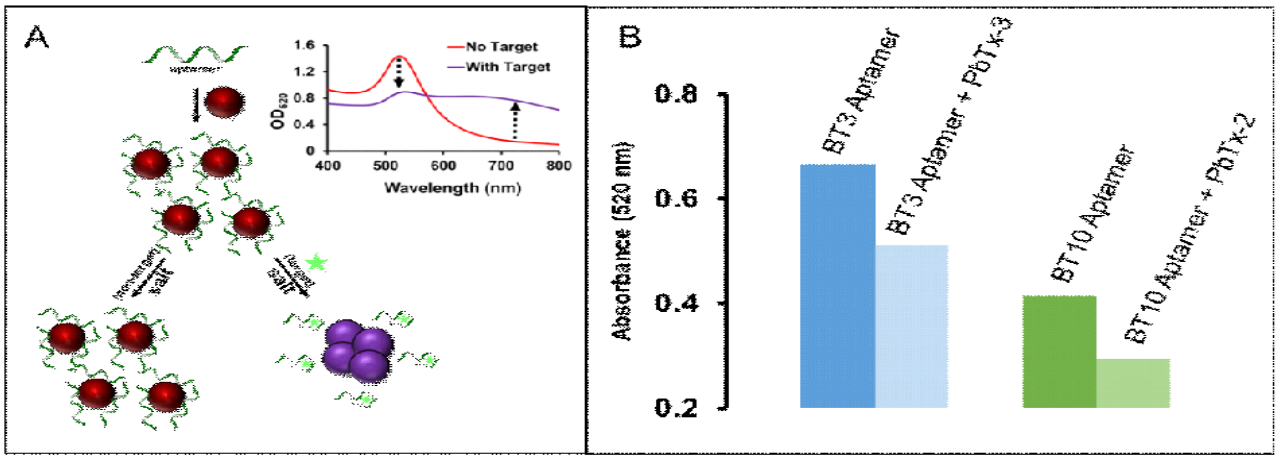

Figure 4. A) Schematic of the gold nanoparticle assay used to confirm proper aptamer/target binding. The inset shows the red to purple change in solution absorbance on successful binding. B) Gold nanoparticle assay results confirming successful binding between the BT3 aptamer and PbTx-3 (blue) along with the already established BT10/PbTx-2 binding (green).

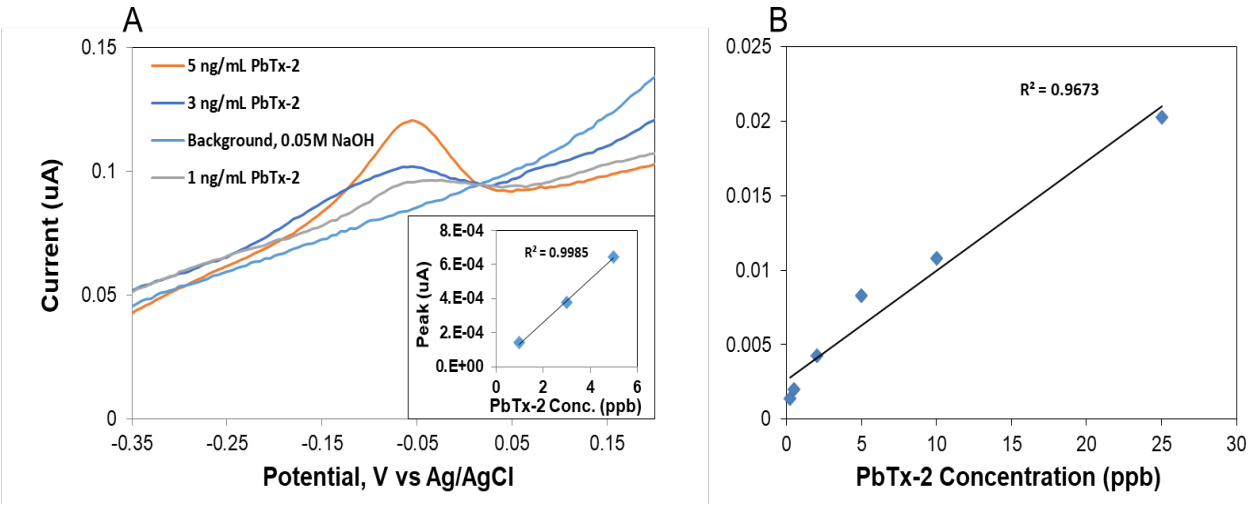

Figure 5. Selected results for the direct electrochemical detection of PbTx-2. Left, direct detection raw voltammetry data measured at various PbTx-2 concentrations in buffer solution, the resulting calibration curve is inserted. Right, a calibration curve for PbT.

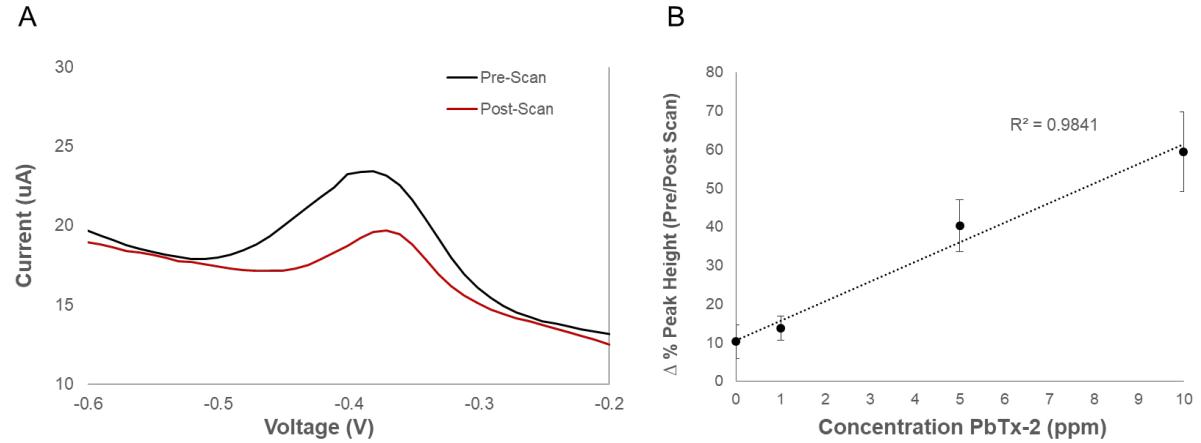

Figure 6. A) Raw SWV data of methylene blue tagged BT10 pre- and post-scans. The decreased peak current is indicative of successful PbTx-2 capture. B) Calibration curve for similar aptamer modified SPEs at various PbTx-2 concentrations.

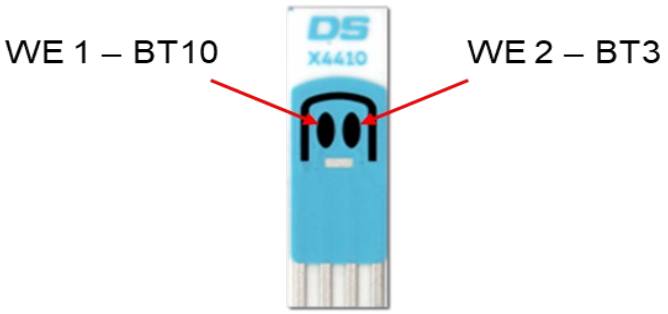

Figure 7. A commercially available SPE with two working electrodes that can each be functionalized with a different aptamer, allowing for simultaneous detection of PbTx-2.

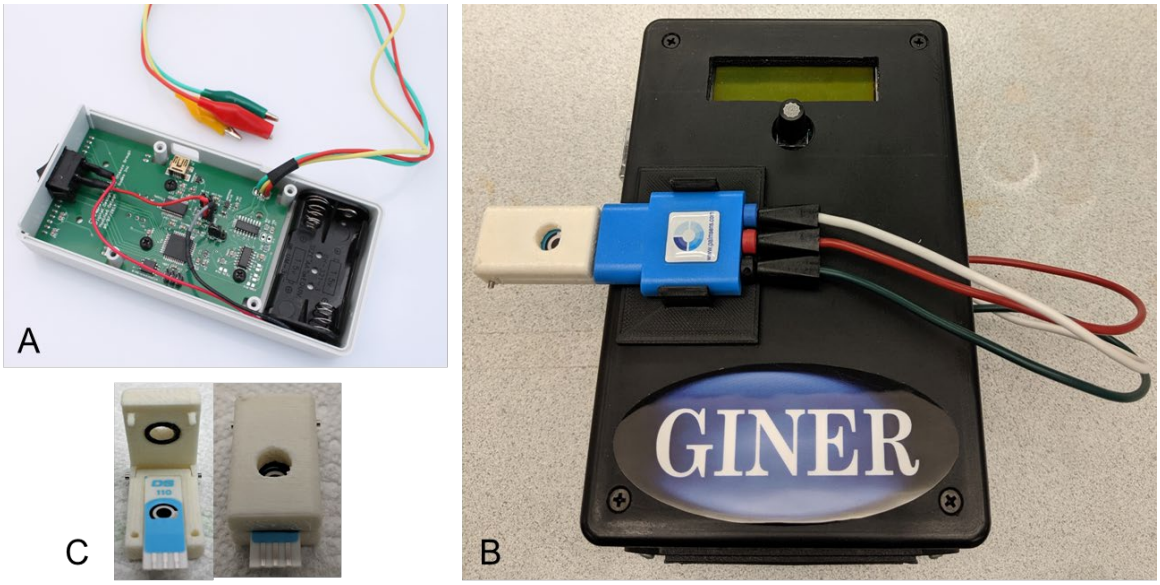

Figure 8. Giner's prototype device developed in Phase II. A) The unmodified CheapStat potentiostat. B) Giner's field-ready prototype, GinerStat. C) Sensor cell holds the SPE and allows the operator to easily add sample solution.

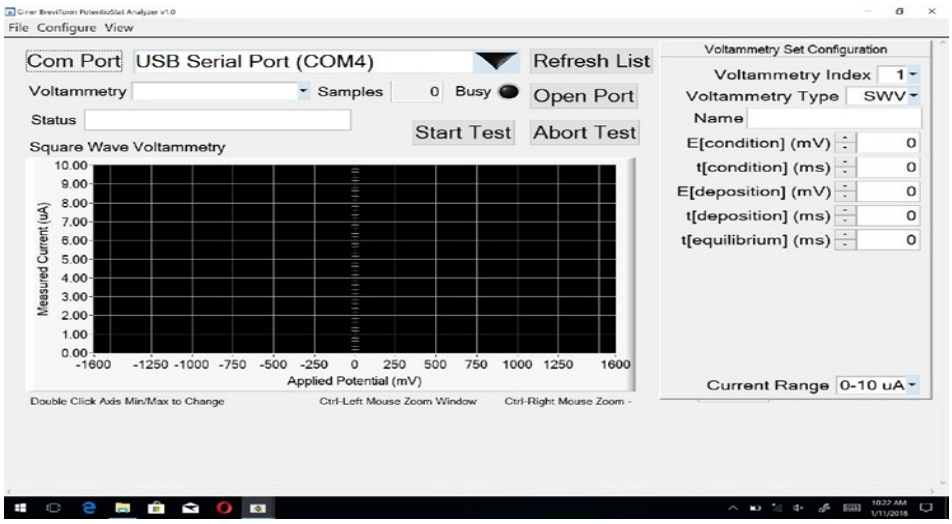

Figure 9. The GinerSens graphical analysis software package that is also used to control the GinerStat device.

between the two scans can be related to the brevetoxin concentration in the tested sample. Figure 6A is an exemplary voltammagram of this pre/post-scan method. Figure 6B is a calibration curve for PbTx-2 spiked into PBS samples showing the percent change in the MeBlue signal before and after target incubation at varying concentrations.

3.4 MeBlue Detection Scheme

The incorporation of the MeBlue redox reporter allowed both the BT10 and BT3 aptamers to be placed on the same electrode and analyzed under the same SWV parameters (Figure 7). Pre-functionalization of the SPE allows the sample to be processed in 20 seconds after a 30-minute incubation period. The brevetoxin assay we developed is inexpensive, capable of high throughput sample analysis and easy to perform in the field.

3.5 Hardware Development

The completed prototype uses a potentiostat device along with a custom Windows application (GinerSens) for brevetoxin detection in field samples. The device is programmed to run cyclic voltammetry (CV), linear sweep voltammetry (LSV) and square wave voltammetry (SWV). The potentiostat has been modified so that all its internal hardware is located in separate custom housing (Figure 8B). The Giner sensor test cell is a 3D printed housing that closes over the SPE with a rubber gasket seal, allowing the sample to be easily added for analysis (Figure 8C). The modified units allow up to 10 hours of operation between recharge with nickel hydride batteries and Bluetooth for real-time wireless transmission of voltametric data to a nearby device.

3.6 Software Development

The custom software package, GinerSens, is modeled after LabView programming, and allows SWV scans to be performed remotely (Figure 9). Six separate voltammetry procedures can be saved and loaded onto the instrument at any given time (denoted by the 'Voltammetry Index'). As stated above these six procedures can then be initialized through the device itself but remotely from a computer. Data files created by GinerStat and displayed by GinerSens are saved in .txt format allowing them to be imported into other programs for downstream data analysis. The LabView run-time application programmed for the device is free of charge and can be installed on any computer with a Windows operating system.

3.7 Sample Analysis

The Giner assay was evaluated with a SWV scan before and after K. brevis was added to the SPE. The change in the MeBlue peak height was calculated and correlated with PbTx-2 concentration. Due to unforeseen complications, we were not able to establish a calibration curve of brevetoxin concentrations in various salt matrices. However, we were able to correlate aptamer signal changes with various brevetoxin concentrations based on the HPLC-MS-MS results (Table S3). The oxidation current of MeBlue was measured before and after K. brevis addition and incubation. The percent decrease in these two current signals was correlated with the concentration of brevetoxin in the sample (more brevetoxin results in a larger decrease). When compared to the control, the signal for the 5x106 lysed sample is 35 PSU seawater showed a 14% decrease indicating successful aptamer binding to PbTx-2. The 25 PSU lysed sample which contained the highest levels of brevetoxin produced a 12% change when compared to the control. The whole cell samples showed no significant difference in signal between the pre and post scans. This result can be explained by the fact that whole cell samples prevented any PbTx-2 from being accessed by the aptamers and therefore no signal change was measured. This does not minimize the field usefulness of this assay, because sonication of algal samples for five minutes proved successful in lysing over 95% of the algal cells.

4. Conclusion

During this study, we developed an inexpensive and rapid electrochemical assay for the detection of brevetoxin in seawater samples. The first of its kind assay utilizes aptamer modified screen printed electrodes and custom test cells to deliver sample analysis in under 10 minutes. The field ready prototype, GinerStat uses Bluetooth to transmit data to a nearby computer for onsite analysis. Our device will allow for rapid response to HAB events, mitigating health and economic consequences to the affected areas. The complex nature of seawater required extensive development of our assay and its sensitivity to brevetoxin concentrations. The current LOD for the assay is 500,000 cells/L, but continued refinement to the assay should yield detection limits closer to that of HPLC-MS. While the sensitivity can still be improved, our electrochemical assay's field deployment will be an invaluable resource for screening HAB conditions and analysis of seawater toxins.

Author contributions

A.A.A., A.W. and M.R. conceived the study and designed the experiments. M.R. performed the gold nanoparticles-based experiments for aptamer characterization and aptamer and target binding study. A.W. performed electrochemical experiments and hardware development. M.S. reviewed the data and gave scientific feedback. M.R., A.W., M.S., and A.A.A. wrote the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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