Integrated Aptamer-Based Electrochemical Biosensor Platform for Rapid Detection of Freshwater Cyanotoxins

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USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Cyanobacteria harmful algal blooms (HABs) are a worldwide problem and cyanotoxins are a priority concern for US inland waterways. Microcystins (MCs) are the most reported freshwater toxins and are potent hepatotoxins. A key tool in the effective management of HABs and their toxins is the ability to rapidly determine the presence/concentration of toxins in waterways.

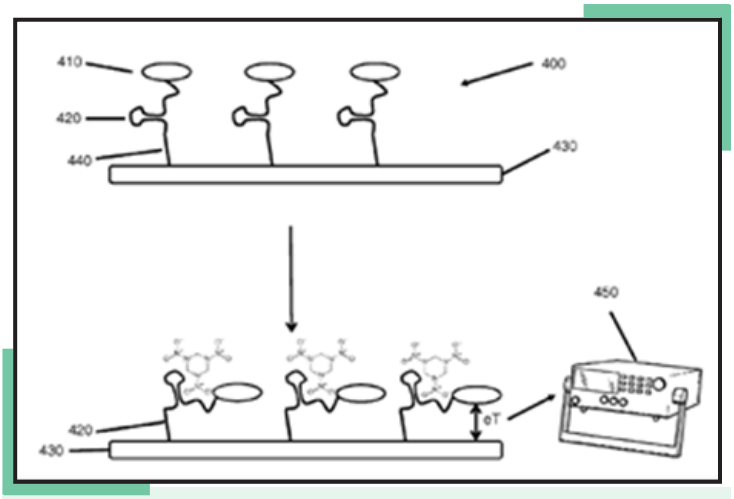

Figure 1. *Conceptual illustration from US Patent 20170107515 A1 illustrating the integration of DNA based aptamer sensing elements with an impedance based detection platform.*

Objectiv

This project aimed to develop a field portable technology based on DNA aptamer sensing elements integrated into an electrochemical based platform (Aptasensor) to enable rapid assays to detect the presence of multiple freshwater cyanobacterial toxins in a single water sample. Current methods rely on complex procedures require time-consuming sample preparation and laboratory instrumentation, which result in delays in critical management and mitigation strategies.

Approach

Establish the lower limit of detection (LLD) for multiple DNA aptamers across 3–4 log toxin concentrations using a custom electrochemical platform consisting of DNA aptamers covalently attached to a gold electrode (Aptasensor). Assess Aptasensor specificity against target and non-target toxins as well as sensor performance after repeated use. Assess performance parameters of Aptasensor using impedance spectroscopy and square wave voltammetry. Evaluate aptasensor electrochemical platform compared to standard methods to determine efficacy. Assessment of sensitivity of two published MC-LR aptamers (AN6 and HC1) was performed using traditional electrodes and square wave voltammetry.

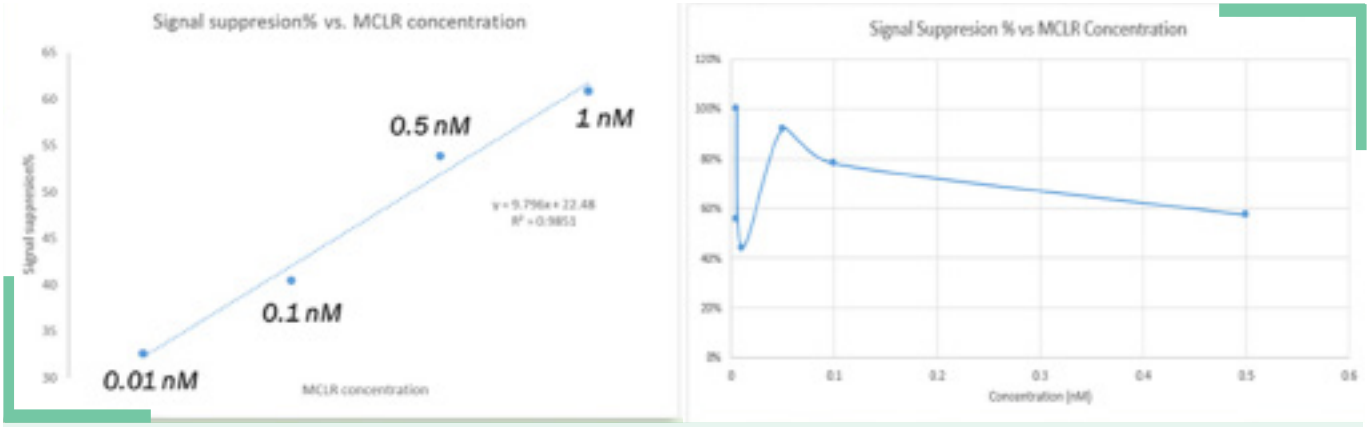

Figure 2. *Initial traditional electrode square wave voltammetry sensitivity trial for (a) AN6 and (b) HC1.*

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Results

Initial trials indicated a dose-dependent response of the Aptasensor using known toxin standards with sensitivity in the picomolar range, effectively reaching lower than the LLDs for standard cyanotoxin detection methods. However, variability was observed in repeated trials. Additionally, methodology for these trials proved to be time intensive with a completion time of three days. These obstacles may be overcome with further method development in future funded efforts. There are relatively new technologies available to rapidly assess the presence of cyanotoxins within water using fluorescently labeled antibodies on chip. These technologies have been developed to detect specific cyanotoxins on the nanomolar scale, which fall within the EPA standards for recreational water for current bloom events and offer a viable alternative to DNA aptamer based sensing technologies.

Major Milestones

Deliverable	Description
Publications	**Tech Report:** Fernando et al. In review. "Evaluation and Investigation of an Integrated Aptamer-Based Electrochemical Biosensor (Aptasensor) Platform for Rapid Detection of HAB Toxins." ERDC TR.

Partnership/Leveraging Opportunities

Project work leverages findings from a previous ERDC Army Basic research project that identified RNA aptamers capable of binding nitroaromatic compounds and resulted in US patent 20170107515A1. In addition, this work leverages research on testing and evaluating DNA aptamers integrated into electrochemical detection systems for their ability to bind small chemicals.

Value to USACE Mission

This research aimed to develop and demonstrate a process or technology for the reduction of HAB events through early and rapid detection of cyanotoxins. The technology developed in this project demonstrates potential scalability of the process or technology to encompass a field relevant HAB event.