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 Harmful cyanobacterial/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.

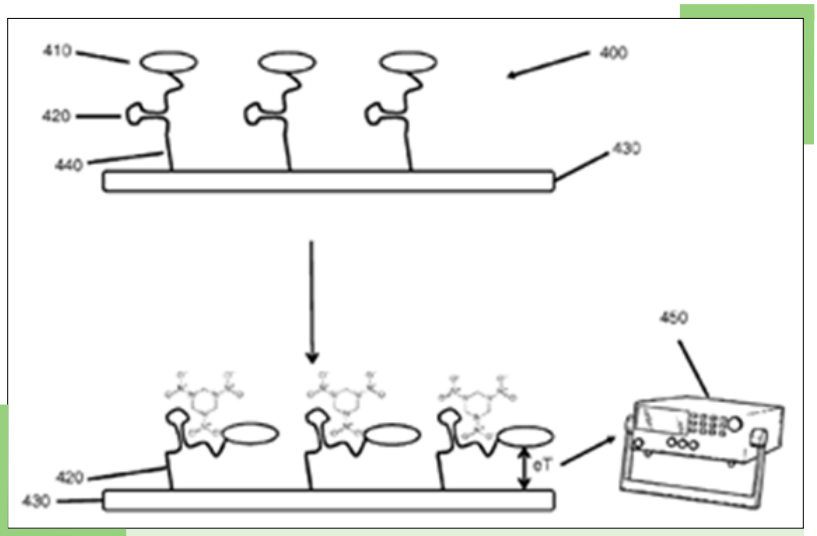

Figure 1: *Conceptual illustration from US Patent 20170107515 A1 illustrating the integration of DNA based aptamer sensing elements with an impedance based detection platform.*

Objective To develop a field portable technology based on DNA aptamer sensing elements integrated into an electrochemical based platform able to rapidly assay for 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 4 DNA aptamers across 3-4 log toxin concentrations using custom sensor/electrode via impedance spectroscopy. Assess aptamer specificity against target and non-target toxins of custom sensor/electrode as well as sensor performance after repeated use. Assess performance parameters of electrode sensors using square wave voltammetry. Aptasensor electrochemical platform, able to monitor specific algal toxins, will be evaluated for sensitivity/specificity and compared to standard methods to determine efficacy.

Assessment of sensitivity of two published microcystin-LR aptamers (AN6 and HC1) was performed using traditional electrodes and square wave voltammetry.

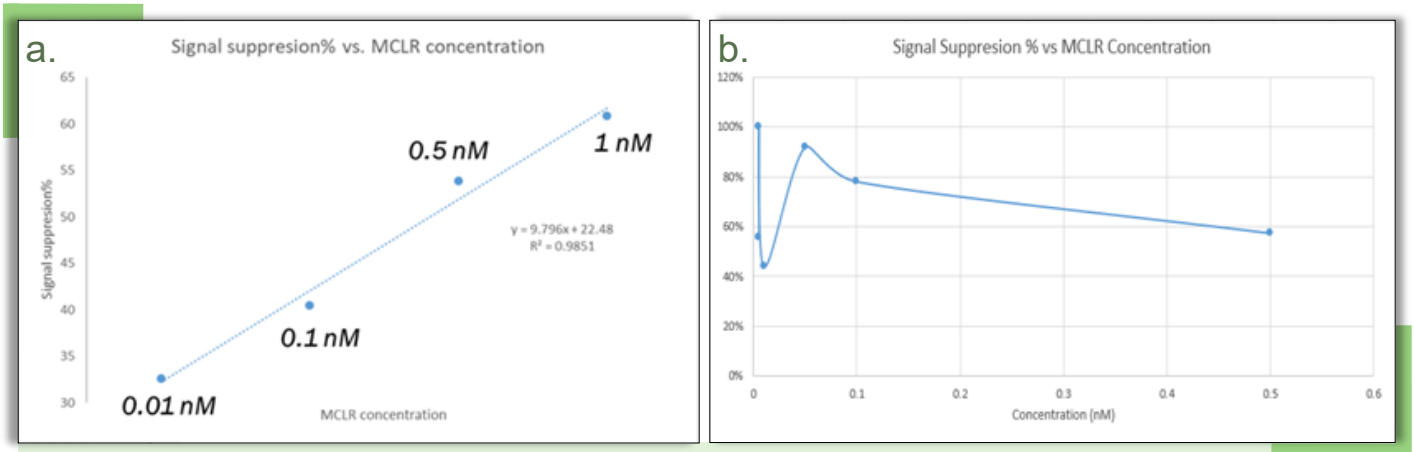

Figure 1: *Initial Traditional Electrode Square Wave Voltammetry sensitivity trial for a) AN6 and b) HC1.*

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Results

Initial trials indicated a dose-dependent response 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. While this does fall within the EPA standards for recreational water for current bloom events, aptasensor technology, with LLDs at the picomolar scale would allow for early detection of bloom events.

Major Milestones

Date	Milestone
FY21-Q1-Q3	Establish the lower limit of detection (LLD) for Aptasensor via EIS/SWV
FY21, Q3-Q4	Assess aptamer specificity against target and non-target toxins
FY22, Q3	Go-No Go Point: Aptasensor platform achieves sensitivity/specificity meets EPA regs
FY22-Q2-Q4	Technical Report-Methods for optimizing and integrating DNA aptamers onto an electrochemical based platform and their utility for rapid HAB toxin quantification
FY22, Q2-Q3	Assess aptasensor sensitivity/specificity with spiked samples in complex aqueous milieu
FY22, Q4	Aptasensor evaluated with field collected bloom samples
Costs	**FY21:**$200k **FY22:**$226K **TOTAL:**$426K

Partnership/Leveraging Opportunities

This work leverages findings from a previous ERDC Army Basic research project that identified RNA aptamers capable of binding nitroaromatic compounds that 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 will 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 will demonstrate potential scalability of the process or technology to encompass a field relevant HAB event.

USACE District Liaison: Eric Glisch of New Orleans District