Microfluidic Sensor for Botulinum Neurotoxin

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***Project Scope*:** Because of its unsurpassed potency, botulinum neurotoxin (BoNT) is recognized by the CDC as a "category A" bioterrorism agent – a high-priority risk to national security upon accidental or intentional dissemination.[1] Seven antigenic types of BoNT exist (A-G), each of which enzymatically cleaves a specific neuronal protein, leading to flaccid paralysis associated with botulism. Possible contamination of vulnerable food supplies (i.e. milk) with BoNT warrants the development of inexpensive, routine screening devices that quickly and confidently identify the toxin. To date, the only approved means of BoNT detection is the mouse bioassay – an assay that not only requires animal sacrifice, but also trained personnel, special facilities, and tedious protocols. Being able to prevent a widespread biological or chemical attack is a foremost concern of the DHS. In response, we are developing a microfluidic platform with high sensitivity, on-site portability, and multiplexing capabilities for reliable BoNT serotype A (BoNT/A) detection in solution.

***Recent Progress*:** Our sensor is based on the enzymatic activity inherent to botulinum toxin. Previously, we had focused on the use of hydrogels for sensing BoNT/A proteolytic activity.[2] While successful, the sensors did not possess a desired FBADS sensitivity of ≤ 40 pg/mL. The current sensor uses a fluorescent BoNT/A-recognizable peptide substrate – herein referred to as the SNAP peptide – to form self-assembled monolayers (SAMs) on Au surfaces or "pads." The Au pads are interfaced with straight PDMS microchannels that do not require complicated external components for fluid manipulation. Upon enzymatic cleavage by the toxin, a fluorescent fragment is released into solution, concentrated at an outlet port via evaporation for signal amplification, and imaged using fluorescence microscopy. Using arrayed microchannels, we can sense 40 suspect samples simultaneously; however, this assay is scalable to higher throughput upon interfacing with a robotic pipetter and/or on-chip fluorescence detectors. Over the past year, we have achieved an *LOD of ~3 pg BoNT/A per mL in less than 4 hours* with our SAM-based microfluidic approach, thus rivaling the sensitivity and time constraints of the mouse bioassay.

***Future Plans*:** With encouraging preliminary results, we aim to detect the toxin in complex matrices like milk and orange juice and also expand the platform to sense, in parallel, other toxic agents that operate via enzymatic cleavage such as BoNT serotypes B-G and tetanus toxin. Additionally, our data indicate that a lower LOD could be achieved through sensor optimization. Future work will also include the use of a FRET-based SNAP peptide for high-throughput BoNT detection in < 5 μL of contaminated sample within a microchannel.

***Relevance to Research Areas*:** The development of a multiplexed microfluidic platform for sensing toxins in food and drink would serve a two-fold purpose of *detecting biological threats* while simultaneously *ensuring food and agriculture security.*

***Publications*:**

[1] CDC, Emergency Preparedness & Response: Bioterrorism Agents/Diseases. In Dept. of Health and Human Services: 2007.

[2] Frisk, M. L., Tepp, W.H., Lin, G., Johnson, E.A., Beebe, D.J., "Substrate-modified hydrogels for autonomous sensing of botulinum neurotoxin type A", *Chem Mater* **2007,** 19, (24), 5842-5844.