

Sensing Botulinum Neurotoxin: Microfluidic Platforms Integrating Hydrogel- or SAM-based Elements

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A dominant homeland security concern is the ongoing development of increasingly sophisticated means of biological warfare – especially those centered on the deployment of a particular microbial toxin, botulinum neurotoxin type A (BoNT/A), which has been released previously as a bioweapon¹ and continues to rank high (next to anthrax) as a likely organism for bioterrorism². BoNT is a protease responsible for blocking the release of neurotransmitters in the synaptic gap between the nerve and the muscle, leading to flaccid paralysis and, in severe cases, death. Deliberate or accidental contamination of food or drink with microbial toxins like BoNT is not only a form of bioterrorism, but also a “global public health problem”³. Existing approved means of BoNT/A detection – the mouse bioassay and ELISA – are impractical with respect to the goal of on-site detection and analysis. As such, adequate preparation for imminent acts of bioterrorism warrants the development of *reliable microdevices that combine biochemical sensing and MEMS technologies* for on-site BoNT detection in, for example, milk supplies⁴. Toward this end, we have developed two separate platforms that rapidly detect BoNT enzymatic activity via (1) visual readout upon degradation (eradicates need for instrumentation) and (2) fluorescence upon cleavage (high sensitivity), whereupon each method boasts unique benefits over existing technologies. These biochemical sensing methods are currently being integrated into microfluidic devices, which offer the functional advantages of minimal sample handling and consumption (less than 1 μ L), increased flow control, and portability for potential on-site testing of contaminated samples.

A *BoNT/A-sensitive hydrogel (1)* was fabricated using a recombinant protein, rSNAP (derived from the BoNT/A SNAP-25 substrate), as the crosslinker. Heterobifunctionalized, linear poly(ethylene glycol) molecules linking an amine-reactive moiety to a photopolymerizable acrylate group were reacted with the rSNAP. Acrylated BoNT substrates were co-photopolymerized with acrylamide as “posts” within microchannels; because rSNAP functioned as the crosslinker within these hydrogels, the entire structure was degraded upon enzymatic cleavage by the toxin. Hydrogel posts have thus far been shown to degrade in response to nanomolar concentrations of BoNT/A. Furthermore, this microfluidic device can be interfaced with electrodes for signal transmission, indicating BoNT/A presence in a contaminated fluid. *BoNT-labile SAMs (2)* have also been constructed for sensing BoNT/A via a synthetic fluorophore-linked peptide (SNAPtide) derived from the minimal number of residues at the SNAP-25 cleavage site required for toxin recognition. The fluorescent SNAPtide was conjugated to gold surfaces using an aminoalkanethiol and a heterobifunctional amine- and thiol-reactive crosslinker. Upon cleavage by the toxin, the N-terminus fluorophore was released into solution for detection, demonstrating feasibility for

detecting trace amounts of BoNT/A. Future efforts for the peptide SAMs include optimizing and characterizing sensitivity as well as integration into microfluidic devices.

- (1) Arnon, S. S. "Botulinum Neurotoxin as a Biological Weapon: Medical and Public Health Management." *JAMA* **2001**, 285, 1059-1070.
- (2) Kennedy, D. "Beauty and the Beast." *Science* **2002**, 295, 1601.
- (3) Johnson, E. A. In *Food Safety: Contaminants and Toxins*; D'Mello, J. P. F., Ed.; CAB International, 2003, pp 25-45.
- (4) Wein, L. M., Liu, Y. "Analyzing a bioterror attack on the food supply: The case of botulinum toxin in milk." *PNAS* **2005**, 102, 9984-9989.

Related publications since beginning this project (overall UW-Madison contribution includes PIs Nicholas Abbott, David Beebe, Hongrui Jiang, and Eric Johnson):

Sridharamurthy, S.S., Agarwal, A.K., Beebe, D.J., Jiang, H. "Dissolvable membranes as sensing elements for microfluidics-based biological/chemical sensors." *Lab Chip* 2006, 6, 840-842.

Park, J.-S., Teren, S., Tepp, W.H., Beebe, D.J., Johnson, E.A., Abbott, N.L. "Formation of Oligopeptide-based polymeric membranes at interfaces between Aqueous Phases and Thermotropic Liquid Crystals." *Chem Mater*, 2006, 18(26), 6147-6151.

Sridharamurthy, S.S., Dong, L., Jiang, H. "A microfluidic chemical/biological sensing system based on membrane dissolution and optical absorption." *Meas. Sci. Technol.* 2007, 18, 201-207.

Frisk, M.L., Lin, G., Moorthy, J.M., Tepp, W.H., Johnson, E.A., Beebe, D.J. "Enzymatically Degradable Hydrogel 'Walls' for Microfluidic Detection of Botulinum Neurotoxin Type A." *In preparation*.

Frisk, M.L., Beebe, D.J. "Self-Assembled Fluorescent Peptide Monolayers for Sensing Botulinum Neurotoxin Type A." *In preparation*.