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Nanopore sensor for botulinum toxin detection

(*Nanowerk Spotlight*) Botulinum neurotoxins (BoNTs) are the most poisonous substances known to humans, with a median lethal dose (LD50) of 1ng per kg of body weight and are the cause of the life-threatening neuroparalytic illness botulism. BoNTs are regarded as possible biological warfare agents that could be used for bioterrorism attacks on the food chain. Of the seven known serotypes of botulinum neurotoxins (BoNTs), designated A-G, BoNT-A, -B, -E and -F are toxic to humans. They are mainly produced by *Clostridium botulinum* and released from this bacillus as single polypeptide chains. After cleavage by bacterial or host proteases, these polypeptides adopt an active di-chain form of toxin, which consists of a 100 kDa heavy (H) chain linked to 50 kDa light (L) chain by a disulfide bond. H chains control cellular internalization of the toxins via receptor-mediated endocytosis, allowing them to preferentially attack the nervous system. Upon endocytosis of the holo-protein, the L chain is translocated from the vesicular lumen into the cytosol, where it acts as a Zn2+-endopeptidase to specifically cleave components of the soluble N-ethyl maleimide-sensitive fusion protein attachment protein receptor (SNARE) complex, and thus hampers exocytosis and neurotransmitter release. Currently, world-wide geographical distributions of BoNTs, which are dictated by the type of foods that serve as the toxins source, have started to blur owing to the ever increasing international trade of food products. Moreover, the majority of new cases of BoNT intoxication have a trend to be associated with drug use, in particular that of black tar heroin. Owing to the ease of distribution of BoNTs, along with their potency, the Center for Disease Control and Prevention (CDC) in Atlanta, Georgia, lists BoNTs as one of the six most dangerous bioterrorist threats. On the other hand, due to their specific actions, BoNTs are the first toxins licensed for human use in the United States to treat muscle dysfunctions/spasms and associated skin wrinkles. Both bioterrorism defense and medical attention require an early and rapid detection of BoNTs. Traditionally, this is accomplished by looking for signs of botulism in mice which receive an injection of human serum or stool samples. While this bioassay remains the most sensitive detection approach, it is costly, slow (takes days), associated with legal and ethical constraints, and is restricted to the CDC along with some state health department laboratories. Besides the use of living animals in detection, various biochemical assays, such as electrophoresis and immunoblot analysis have been used to detect the cleavage of specific SNARE proteins. These *in vitro* detections are faster (within a day), but with reduced sensitivity, compared to the mouse bioassay. The subsequent development of time-resolved fluorescence-based enzyme-linked immunosorbent assays (ELISA) allowed utilizing lanthanide (Eu3+)-tagged antibodies to detect BoNT-A and -B. ELISA sped up the outcome of BoNT assays to hours. Within a similar time-frame, the BoNT-A activity can also be monitored using the substrate SNAPtide™, a fluorescently conjugated synthetic peptide that contains the native cleavage site for BoNT-A. Recent developments in mechano- and fluorescence resonance energy transfer (FRET)-based sensors offer a reduced detection time and/or increased sensitivity. The micromechanosensor uses force spectroscopy to detect BoNT-B. This toxin cleaved synaptobrevin 2 (Sb2) is attached to a bead, which was suspended off a cantilever via Sb2 interaction with syntaxin 1A attached to the cantilever. The detachment of the bead from the cantilever marked the cleavage of Sb2, allowing detection of low nanomolar range of BoNT-B within 15 minutes. Additionally, *in vitro* detection using FRET probes that contain fragments of SNARE proteins as toxin substrates shows picomolar sensitivity within 4 and 16 hours. Although the two later assays are very promising for practical use, they require expensive and technically complex equipment. Overall, there is a need for further development of assays for detection of BoNTs. In recent work, Dr. Yong Wang and his collaborators described a nanopore-based assay for detection of BoNT-B.

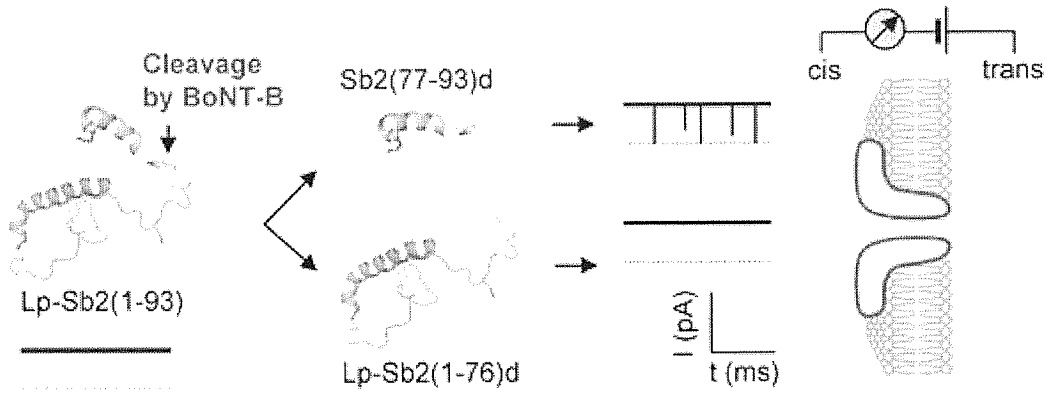


Illustration of the principle for nanopore BoNT-B detection. Lp-Sb2(1-93) (based on structure PDB ID code 2KOG in the Protein Data Bank (www.pdb.org)) would not affect the pore current, as shown by the hypothetical trace below the structure. After the cleavage of Lp-Sb2(1-93) by BoNT-B/Zn2+ (arrow), the short digested product Sb2(77-93)d would generate a distinct modulation of pore currents, as shown by downward transient blocks in the hypothetical trace, while the long product Lp-Sb2(1-76)d would not affect the nanopore current. Dotted lines are levels of zero current. Analytes would be applied from the cis (pore vestibule) side of the bilayer that partitions the recording chamber. (Reprinted with permission by American Chemical Society) "We utilized the emerging aerolysin pore to track the BoNT-B digestion of its substrate, a derivative of the synaptic protein Sb2 (also known as VAMP2). The dynamic change of the specific digestion product reported the existence of the toxin at sub-nanomolar (500pM) concentration within minutes," says Dr. Yong Wang. This system also demonstrates usefulness in investigating biophysical mechanisms for peptide translocation and interaction with the nanopore. The aerolysin protein pore can be used to elucidate the interaction of the nanopore with a synaptic protein Sb2 derivative and its BoNT-B cleavage products. "We determined that only the small C-terminal BoNT-B digest peptide in its native state can interact with the pore to generate current blocks, while other native peptides/proteins in the reaction mixture and serum cannot produce observable blocking events," notes Wang. "This research is motivated by the danger posed by BoNT which is one of the most potent bio-toxins," he adds. "Rapid and sensitive detection of this toxin, due to drug-use, food-poising or terrorism, is a priority in toxicology and biodefense. This is an area of future interest and a fertile ground for improvements of the present BoNT-B nanopore detection scheme. This approach could be adapted to the selective peptide detection for biosensing and investigating biological processes, including the detection of activity of *Clostridial* toxins, and biophysical mechanisms for biopolymers interaction and translocation through the nanopore. This important peer-reviewed scientific result was published in *ACS Applied Materials & Interfaces* online on December 16, 2014 ("Nanopore Sensing of Botulinum Toxin Type B by Discriminating an Enzymatically Cleaved Peptide from a Synaptic Protein Synaptobrevin 2 Derivative"). *By Dr. Yong Wang, Nanopore Single Molecule Sensing Group, Biological Engineering and Dalton Cardiovascular Research Center, University of Missouri-Columbia.*

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