WG 1 (Chemical, Biological, Radiological and Nuclear materials)

Development of a Microfluidic Device for Automated Biosensing

Doron Gerber.
Bar Ilan University.

Field-deployable bio/chemical sensor devices are essential for real-time detection of chemical and biological threats across diverse, non-laboratory settings. These portable “labs-on-a-chip” enable rapid on-site identification of contaminants, addressing critical needs in environmental monitoring, public safety, defense, and smart city infrastructure. Modern biosensors leverage miniaturized microfluidic systems and sensitive transducers to deliver fast, reliable, and automated detection. In this work, we present our efforts to overcome three key challenges in developing an integrated, field-ready biosensing platform:

1. Bioassay Development – We designed a microfluidic bioassay for the detection of toxins and pathogens, enabling rapid and specific identification of biological threats.
2. Automated Sample Handling – We engineered microfluidic tools for critical sample preparation functions, including filtration, mixing, and dilution, ensuring consistent and reliable fluid processing.
3. Electrochemical Sensor Integration – We initiated the incorporation of an electrochemical biosensor into the system to provide sensitive, real-time signal transduction for enhanced detection accuracy.

By combining automated sample processing with high-sensitivity biosensing, our platform aims to bridge the gap between laboratory-based analysis and real-world deployment. This development represents a step toward a fully autonomous, high-performance biosensing device for broad applications in environmental and biological threat detection.

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Elucidating the Activation Mechanism of Botulinum Neurotoxin A: Role of α-Clostripain and NTNH

Eran Diamant.
Department of Biotechnology, Israel Institute for Biological Research.

Botulinum neurotoxin A (BoNT/A), produced by Clostridium botulinum, is one of the most potent toxins known. Understanding the mechanism of its activation is crucial, as BoNT/A has significant medical and cosmetic applications, in addition to its role in botulism. Identifying the endogenous protease responsible for BoNT/A activation is particularly important, as it sheds light on the toxin's biosynthesis and regulation, providing insights that could influence therapeutic developments and safety protocols. BoNT/A is initially synthesized as a 150-kDa polypeptide with limited potency and requires proteolytic cleavage to become fully active. Surprisingly, the endogenous protease that transforms this polypeptide into an activated, full-potency toxin, consisting of a 100-kDa heavy chain (HC) connected to a 50-kDa light chain (LC) by a disulfide bond, is still unknown. This study aimed to identify the BoNT/A-activating protease. We screened cation-exchange chromatography fractions of C. botulinum A culture supernatant for activity using a toxin-simulating substrate comprising the LC and the translocation domain (HN). Proteomic analysis of the active fraction identified α-clostripain as a candidate BoNT/A-activating protease. Recombinant α-clostripain cleaved the simulating substrate between the toxin LC and HN. However, incubation of recombinant α-clostripain with recombinant inactivated BoNT/A (rBoNT/Ai) resulted in non-specific digestion of the toxin. Since similar non-specific digestion was observed also by C. botulinum A culture supernatant, we hypothesized that the toxin should be protected by an accessory protein to prevent non-specific cleavage. Indeed, incubation of rBoNT/Ai with α-clostripain or culture supernatant in the presence of recombinant non-toxic non-hemagglutinin (NTNH) resulted in specific cleavage of the toxin into 100- and 50-kDa fragments. Functional validation showed a 77-fold increase in toxicity after α-clostripain-mediated activation in the presence of NTNH, as confirmed in a mouse model. These findings elucidate for the first time the activation mechanism of BoNT/A, highlighting the coordinated interaction between α-clostripain and NTNH.