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Botulinum Neurotoxin Sensing in Complex Matrices

Eric A. Johnson

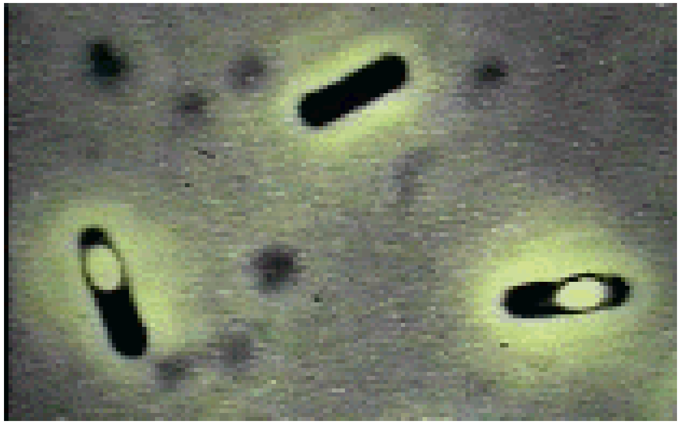
University of Wisconsin - Madison

eajohnso@wisc.edu

Collaborative Effort

- Eric Johnson - My laboratory has Select Agent Approval for <u>Clostridium</u> <u>botulinum</u> and BoNTs
- Nick Abbott - Liquid crystal detection systems
- Dave Beebe - Microfluidics and related platforms
- Hongrui Jiang - Transduction and sensing
- Hard working research specialists and students!
- University of Minnesota expertise.
- Interaction with botulinum community.

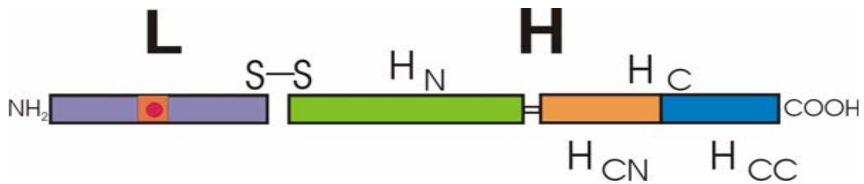
Clostridium botulinum

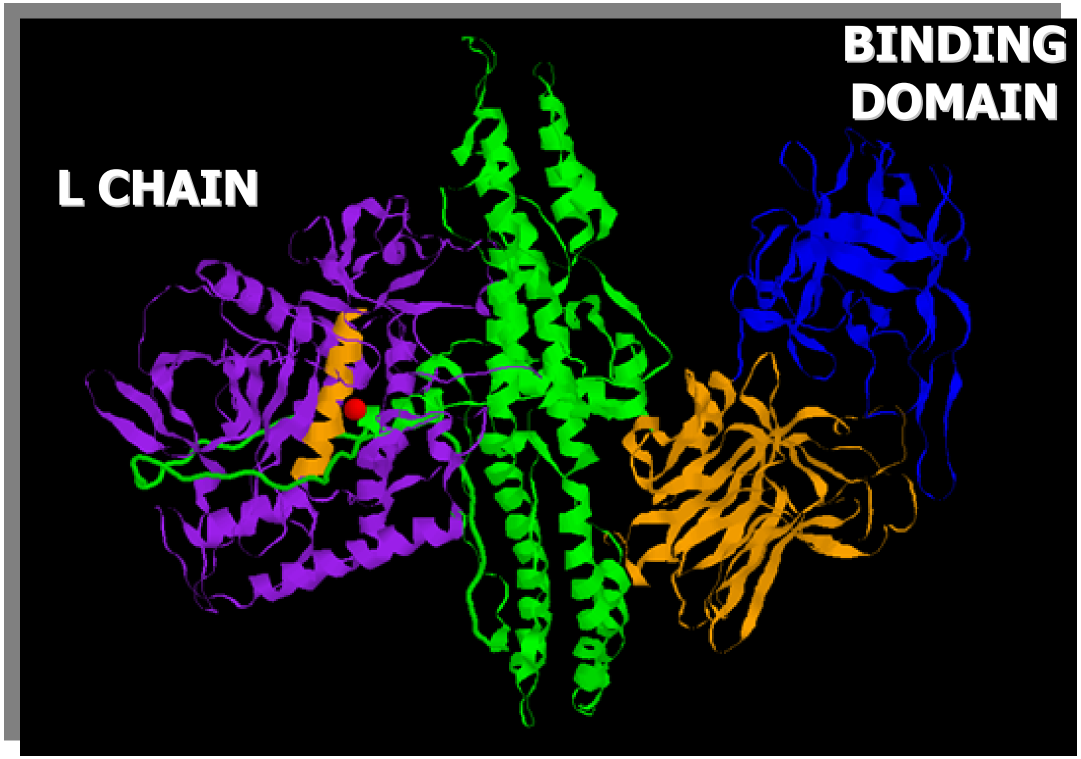


- Gram-positive, spore forming, anaerobic bacterium
- Produces the most poisonous toxin known - human lethal iv and inhalational doses have been estimated as 0.1-0.5 ng/kg. Oral lethal dose ~10- 70 micrograms for 70 kg human. Much less for infants!
- Causative organism of botulism
  - Severe neuroparalytic illness in humans and animals
  - Ingestion of botulinum neurotoxin or toxicoinfection of intestinal tracts or wounds

Humans and Livestock are Susceptible to Botulism

Structure of Botulinum Neurotoxins





TRANSLOCATION DOMAIN

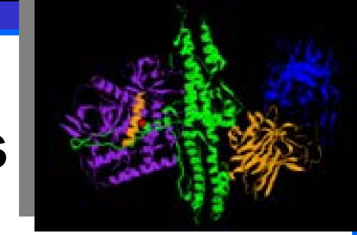
Considerations in Detection of BoNTs

- BoNTs are stable in many foods and clinical samples.
- Currently accepted detection method is mouse bioassay - relatively slow, requires animals, issues include nonspecific deaths, other. Sensitivity is exquisite - ca. 10 pg (1 MLD50) or aM to pM levels.
- Consequently detection systems ideally should be able to capture and concentrate samples from foods and clinical samples to reach sensitivity equivalent to mouse bioassay.

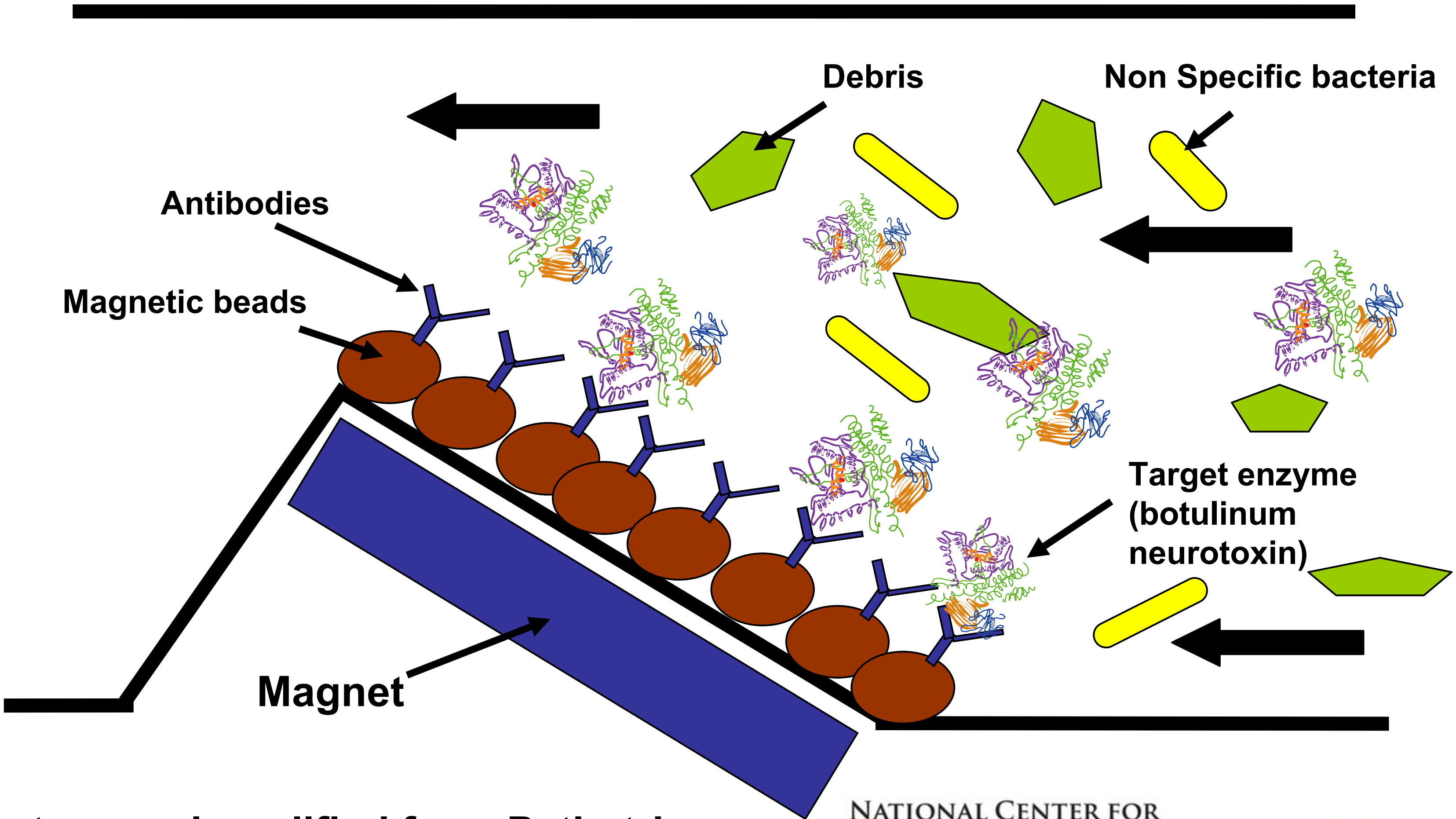
Testing Criteria from FBAD/HSARPA

- Testing cycle time of ≤20 min
- A bench-top or portable system
- LOD of 0.04 ng per ml of BoNT
- Pfp ≤ 1 in 1,000,000
- Pfn ≤1 in 1,000
- Final acquisition cost ≤$50,000 per unit
- Operation costs of ≤ $5.00 per sample test
- Yearly operation and maintenance costs not to exceed 10% of the cost of the instrument.

Capture of Target from Food or Clinical Samples



BoNT



Courtesy and modified from Pathatrix

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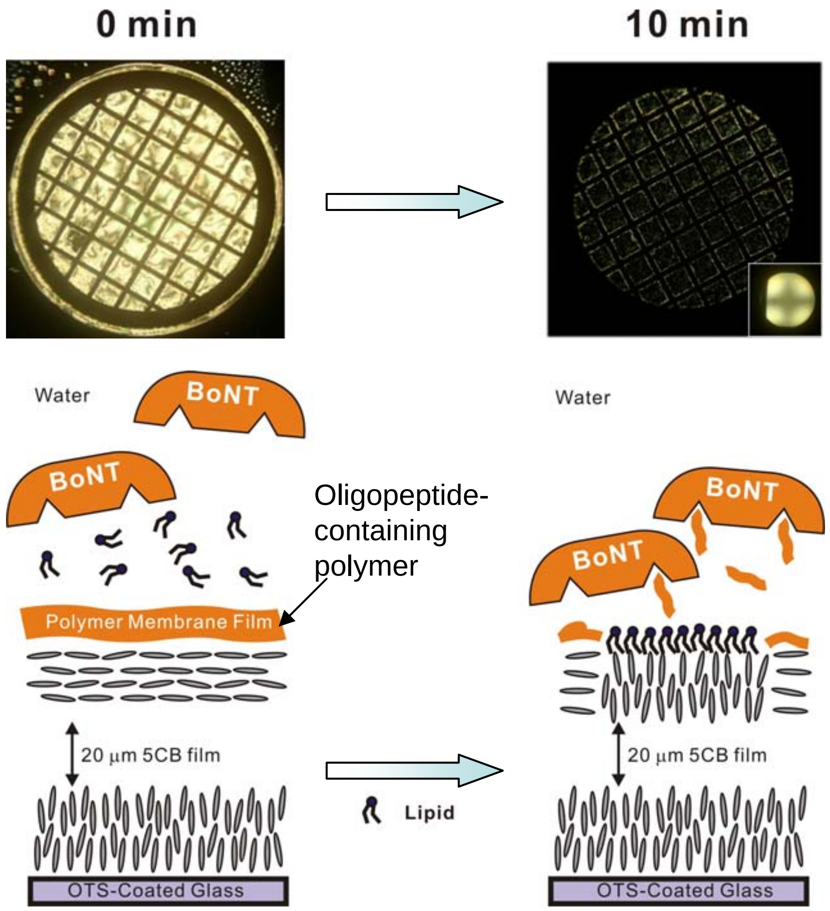
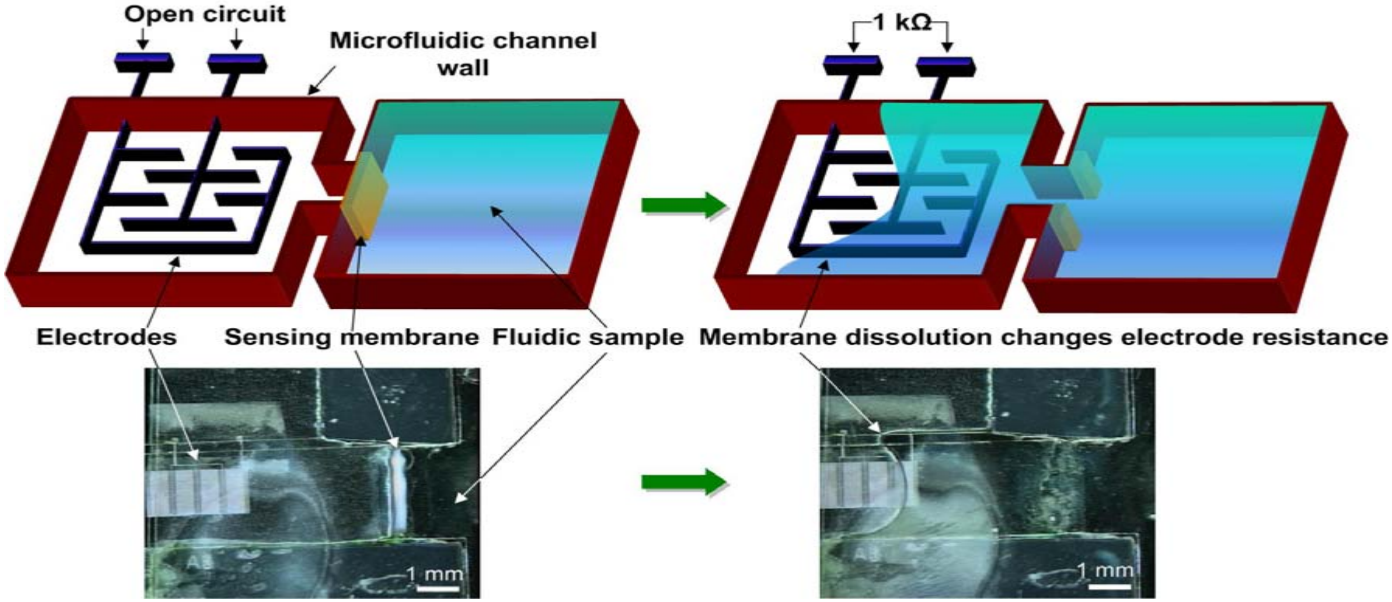
Oligopeptide-Based Polymeric Membrane



- ***Positive response*** is indicated by ***bright-to-dark*** transition.
- The polymeric membrane was generated by the *cross-linking of oligopeptides* at the aqueous-liquid crystal interface.
- ***Trypsin (a model)*** facilitates the transport of phospholipids through polymeric membrane *(color changes to black optical appearance)*.
- It was possible to detect trypsin *in low nanomolar range*.
- ***Oligopeptide-based polymeric membrane formed at the liquid crystal interfaces can also be used to detect BoNTs.***

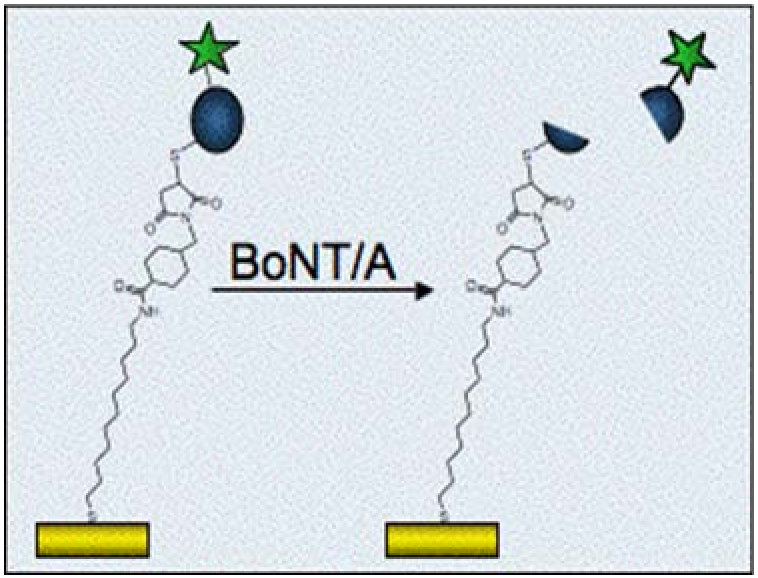
Electronic Detection of BoNT by Microfluidic Platforms - (a) Membrane Dissolution (Valves)

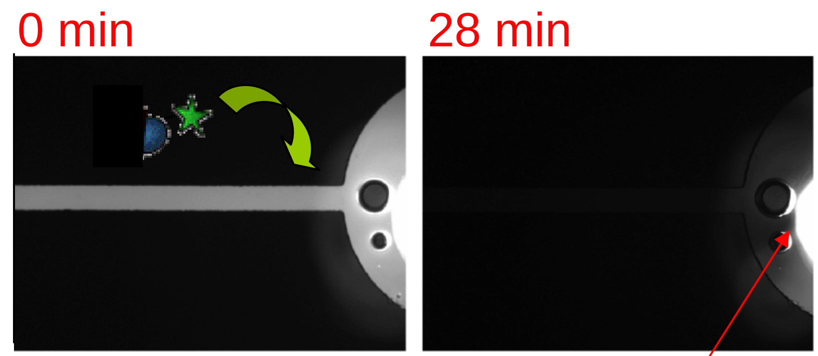


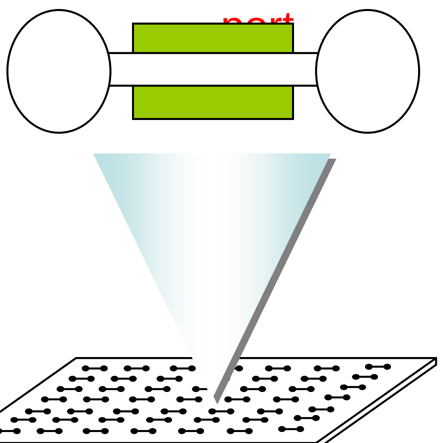
- Bio-recognition membrane separates fluidic sample from electrodes
- Specific target cleaves membrane (ex. protease activity)
- Sample flows into electrodes and changes resistance drastically
- Ideally ∞ transduction/amplification (enzyme activity)
- Zero power consumption until target detection
- Applicable widely to bio-sensing

S. S. Sridharamurthy, et al. *Lab on a Chip* 2006, vol. 7, pp. 840-842

Microfluidics: (b) Self-Assembled SNAP-derived Monolayers





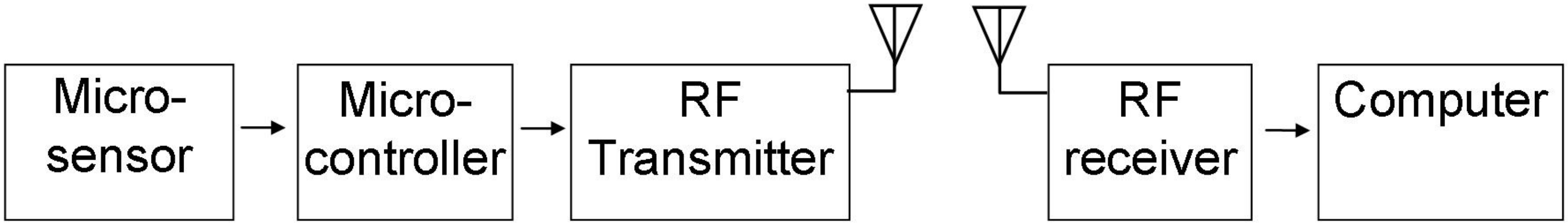


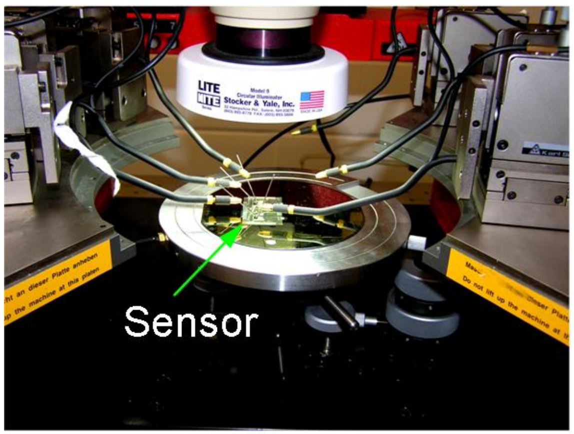
- BoNT/A cleaves peptide and releases fluorophore into solution (vol = 1 μL)
- *Evaporation* concentrates fluorophore at "collection port" for detection
- Scale up for *high-throughput* sensing

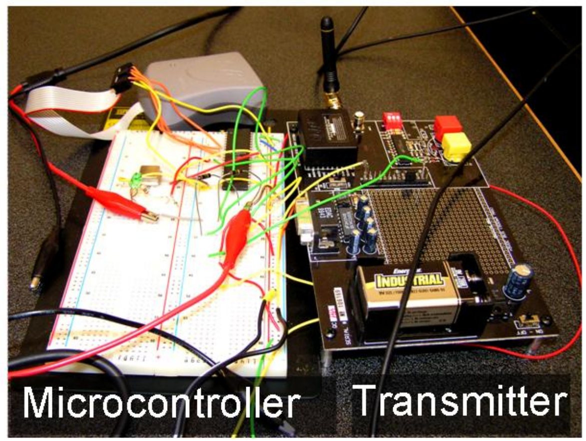
Transducing Chemical/Biological Events to Electrical and Optical Signals

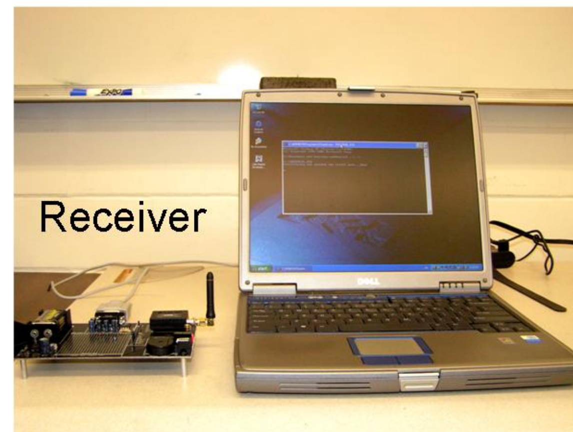
- Autonomous sensors to detect BoNT based on dissolvable membranes
- 2 detection schemes:
  - Electronic: Membrane dissolution causes sample fluid to flow into electrodes and changes resistance
  - Optical: Membrane dissolution or substrate cleavage causes change in absorbance of optical path between light source and detector
- Sensitive and specific – low false positive/negative
- Real-time
- Autonomous
- Microsensor:
  - Low cost
  - Low power
  - Distributed wireless network

Electronics Devices – Wireless Data Transmission









- Microcontroller (Atmel Corporation) directly driven by microsensor output
- Wireless transmission modules (Linx Technologies) runs at 900MHz
- Range of 150 feet

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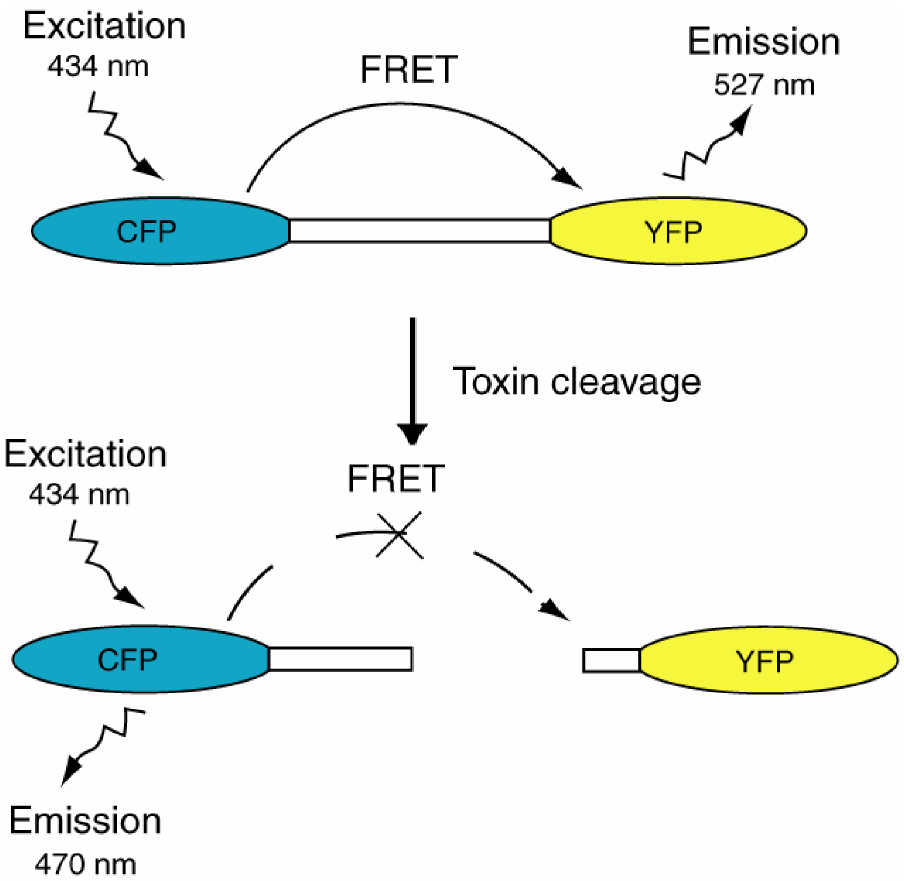
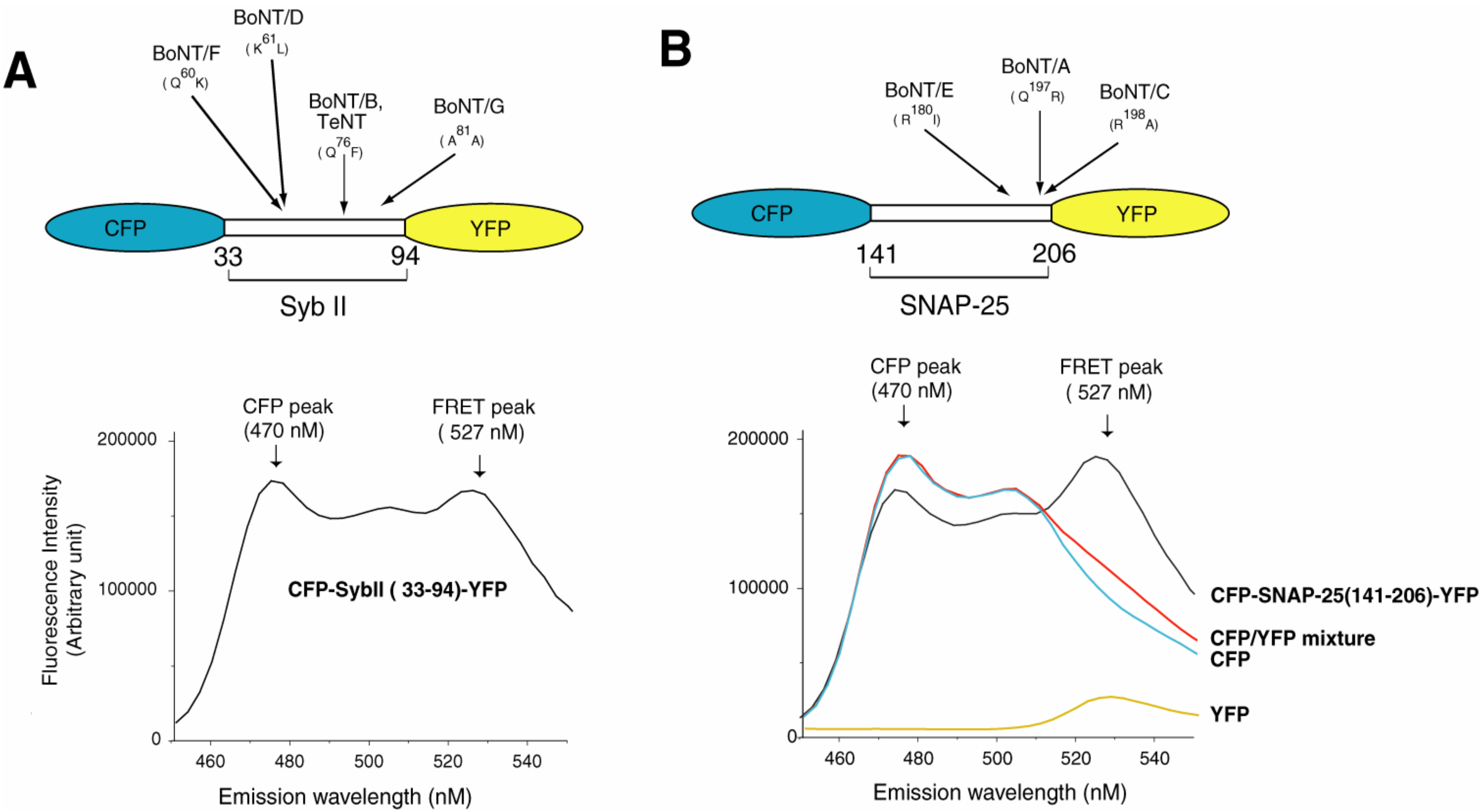
CFP/YFP FRET Based Toxin Sensors

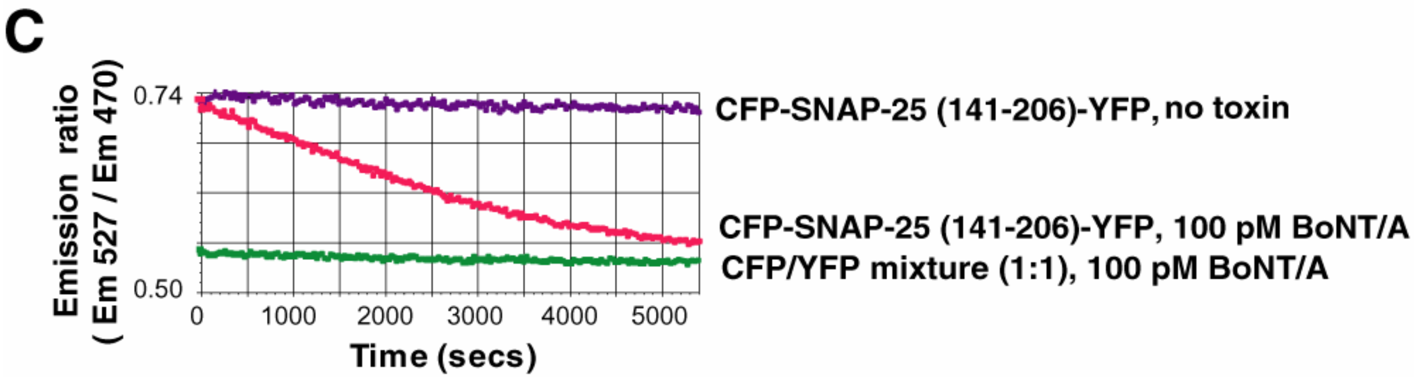


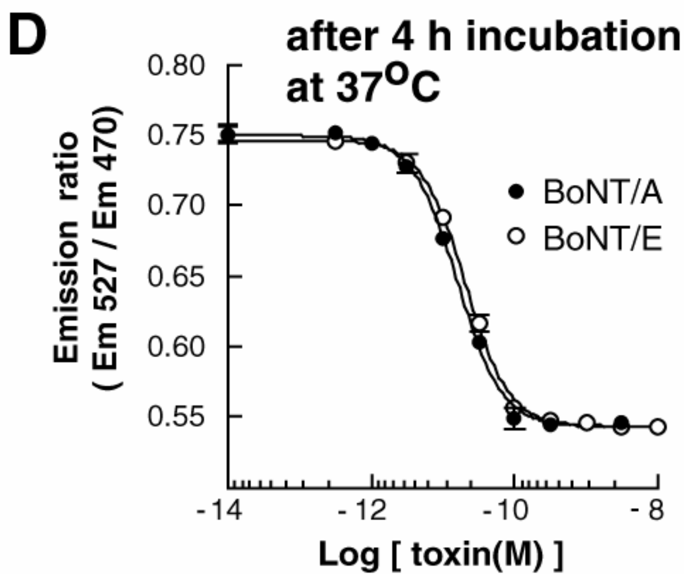
Dong et al. (2004) PNAS

Design Two Sensors that can Detect All 7 Botulinum Neurotoxins (plus Tetanus Toxin)



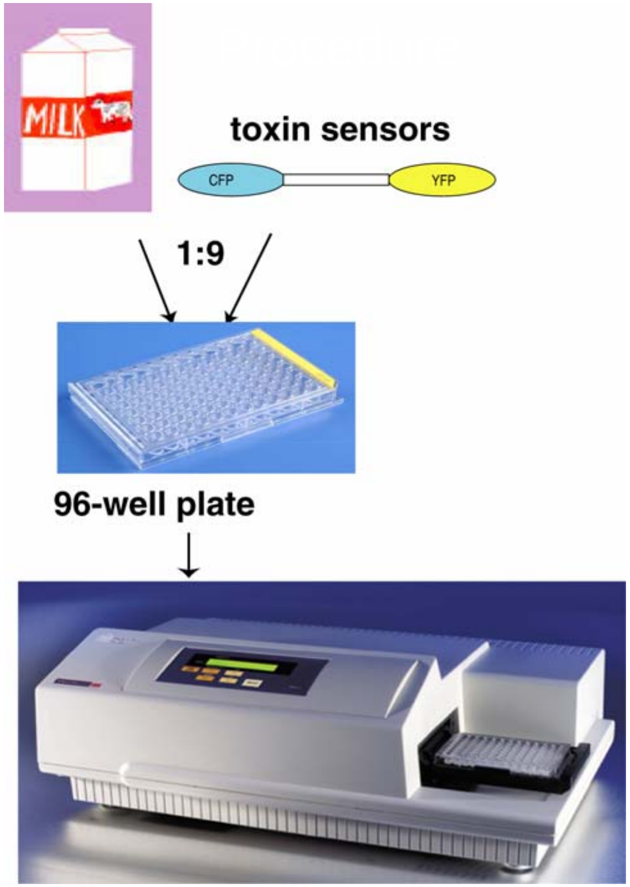
Detecting Botulinum Neurotoxin Activity in Real Time *In Vitro*

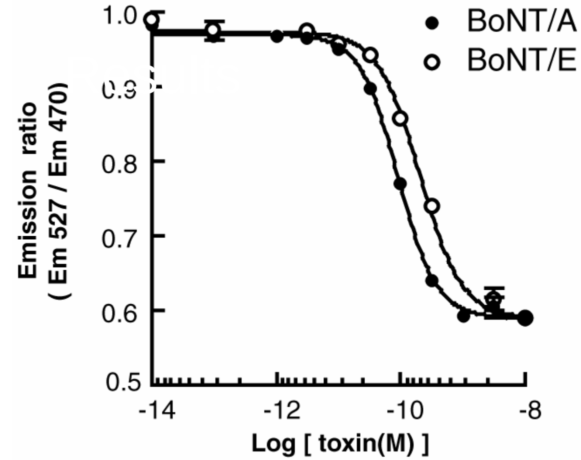




EC_{50} :	4 h	16 h
BoNT/A	15 pM	10 pM
BoNT/E	20 pM	6 pM
BoNT/B	242 pM	32 pM
BoNT/F	207 pM	98 pM

Screening Milk for Botulinum Neurotoxins





Toxin concentration in milk

EC_{50} :	4 h	16 h
BoNT/A	1 nM	0.5 nM
BoNT/E	2 nM	0.06 nM
BoNT/B	5 nM	1 nM
BoNT/F	4 nM	2 nM

Deliverables

- Sensitive detection systems for botulinum neurotoxin and potentially other toxins.
- Platform based on liquid crystal or microfluidic technologies.
- Utility with environmental, food, and clinical matrices.
- Rapid system with acceptable false-negative and false-positive results.

Acknowledgments

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