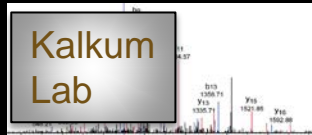
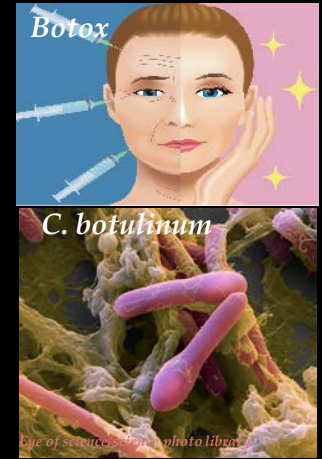


# Botulinum Neurotoxin Activity in Complex Biological Samples



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City of Hope National Medical Center, Duarte CA



Skyline Users Meeting - 2020

# Botulism Disease

## Categories of Human Botulism:

Classic or food-borne botulism



Wound botulism



Prevent botulism by first cleaning the wound

Infant botulism



Honey may quiet them, but botulism can kill them



The estimated human median lethal dose, LD50 of type A botulinum neurotoxin (BoNT):

- 1.3–2.1 ng/kg intravenously/intramuscularly
- 10–13 ng/kg when inhaled
- 1000 ng/kg when taken by mouth (Arnon *et al*, 2001).

# Functional Detection of Botulinum Neurotoxin



## Advantages

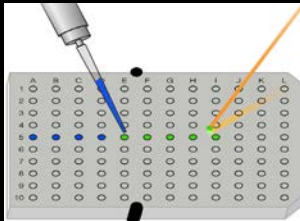
Exquisitely sensitive:  
~5 pg/mouse (~33 amol)

## Disadvantages

- Costly
- Lasts for a long period (up to 5 days)
- Many mice suffer from botulism and die painfully, ~600,000 annually
- Only 17 test sites in the US

Endopep-Mass Spectrometry (MS)  
Assay (CDC)

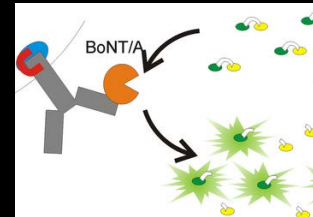
Assay with a Large-Immunosorbent  
Surface Area, ALISSA (COH)



Quantitative, do not require  
animals

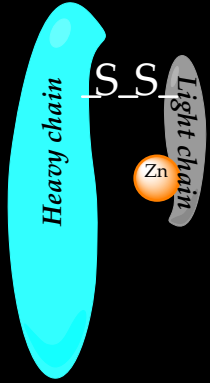


Rely on antibody-based  
capturing of the toxin

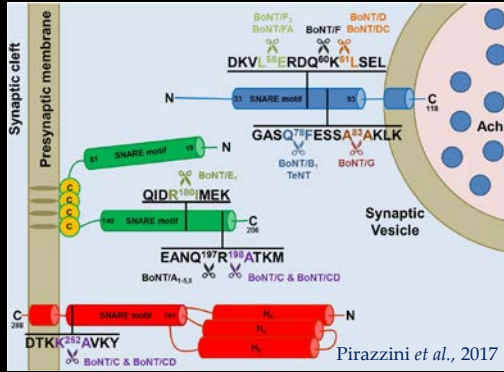


# Multimeric Substrates upon BoNT Cleavage Produce an Amplified Signal

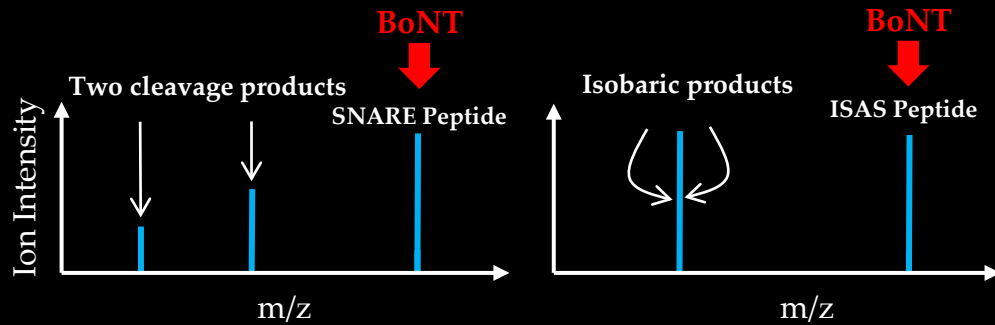
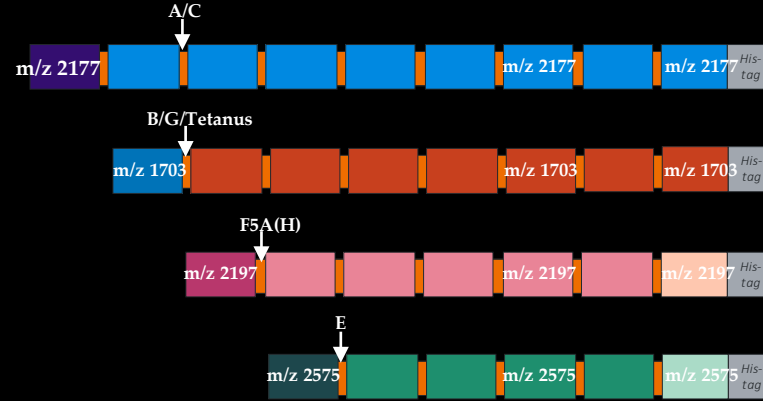
BoNT is a Zn-metalloprotease



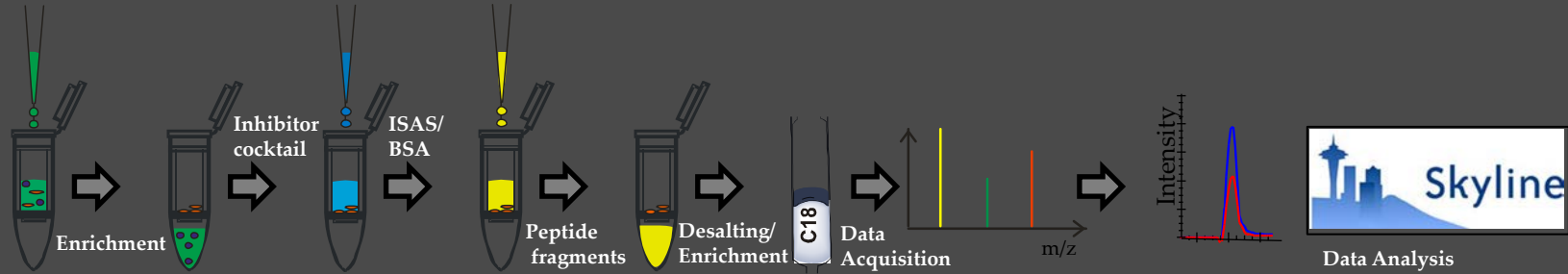
Proteins in nerve terminals



ISAS proteins contain multiple BoNT cleavage sites



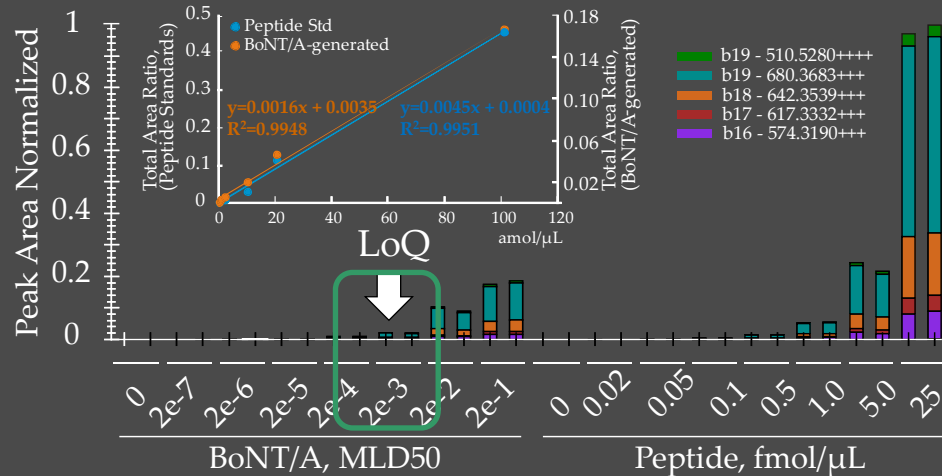
# Enrichment of BoNT/A from Human Serum without Use of Antibodies



Dual trapping columns set up to avoid:

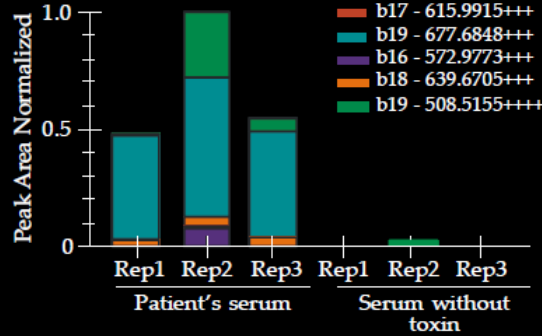
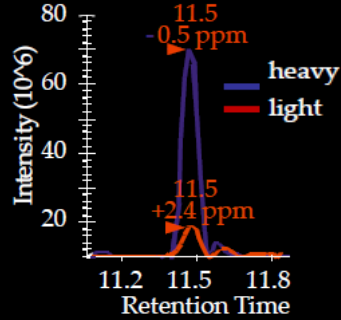
- Column overload
- Carry-over from the previous sample
- Peak broadening
- Poor separation performance.

## Analytical Performance of the PRM Assay

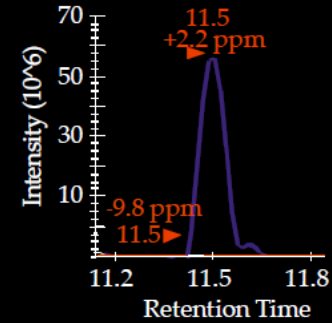


# Detection of Toxin in Serum from Suspected Case of Food-borne Botulism

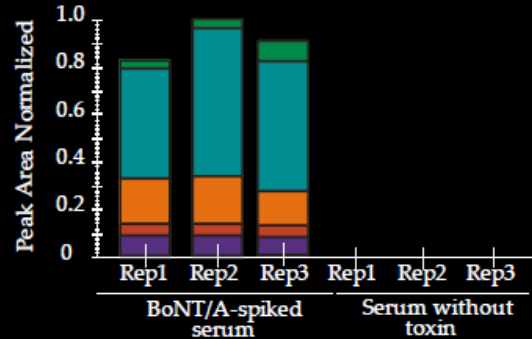
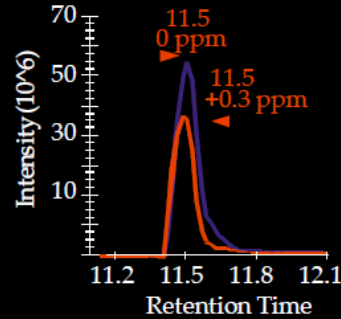
Patient's serum



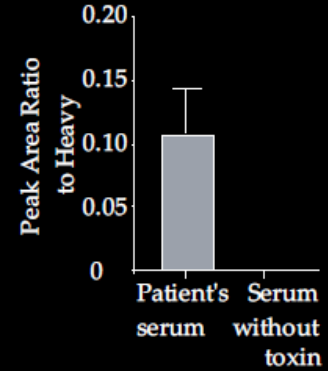
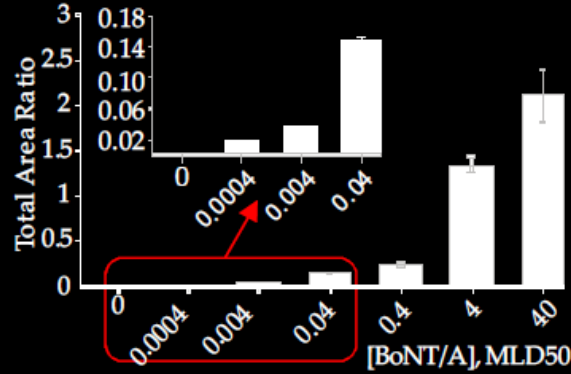
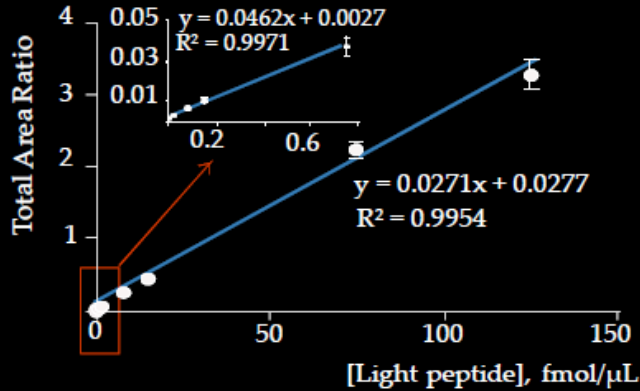
Serum w/o toxin



Serum spiked w/toxin



# Quantification of Toxin in Serum from Suspected Case of Food-borne Botulism



**LoQ  $\leq$  0.04 MLD50 of BoNT/A in 200  $\mu$ L serum**

Mouse assay: 0.5 mL injected, corresponding to 10% MLD50  $\leftarrow$  below LoD!

# Thank you!

## Prof. Markus Kalkum, PhD

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- Jason R. Barash
- Ruth Motter

## Loma Linda University Medical Center

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**Skyline Team!**

Antelope Valley, CA Poppy Reserve

