# Bombing Botox Out of the Body

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## What is Botulinum Neurotoxin?

USATODAY reports that the most toxic biological compound is used by 5.6 million people annually. The bacterium, *Clostridium botulinum* makes a toxin called botulinum neurotoxin (BoNT, or Botox A). While research suggests Botox may remedy certain ailments; excess Botox can cause nerve damage and even death. There is an urgent need to create a drug that can block BoNT's effects in the event of its misuse. BoNT enters motor neurons and interrupts nerve impulses, causing paralysis. The toxin consists of a heavy chain that is the targeting infiltration system, and a light chain as the warhead. When the light chain enters a motor neuron, it cleaves one of several SNARE proteins, preventing the nerve's ability to release neurotransmitters. For example, serotype A of the toxin cleaves SNAP-25 at a Gln-Arg peptide bond. Vital for the toxin's catalysis, key amino acids include His223, His227, and Glu262, which bind the Zn(II) ion. The Glu224 side chain joins in the BoNT catalytic machinery. Asp370 is essential for interacting with the Arg residue in the substrate's scissile peptide bond. The BoNT/A active site can alter its structure to bind to unlike molecules: arginine and a hydrophobic cinnamic acid derivative. This flexibility in the active site provides an opportunity for the development of more effective inhibitors of the toxin. The West Bend SMART (Students Modeling A Research Topic) Team made a model showing how BoNT houses polar and hydrophobic molecules using 3D printing.

## Mechanism of Botulinum Intoxication

**A 4-step process:**

1) BoNT is ingested and enters the blood stream. 2) The heavy chain targets motor neurons via receptor mediated endocytosis. 3) While in the acidic endosome, the heavy chain changes conformation, releasing the light chain into the cytoplasm. 4) The light chain cleaves SNARE proteins, leading to paralysis.

Key:
A - Light Chain
B - N-Terminal half of Heavy Chain (Translocation Domain)
C - C-Terminal half of Heavy Chain (Binding Domain)
D - Receptor on Neuronal Cell Surface
E - Cell Membrane
F - Cytoplasm
G - SNARE proteins
H - Vesicle containing neurotransmitters

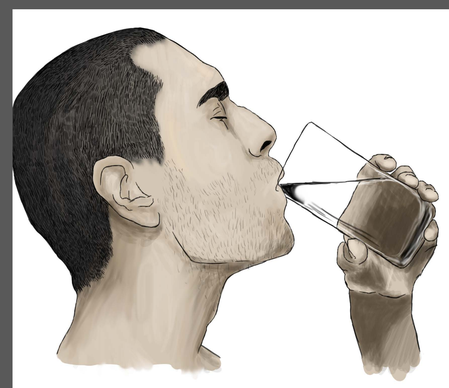


Figure 1

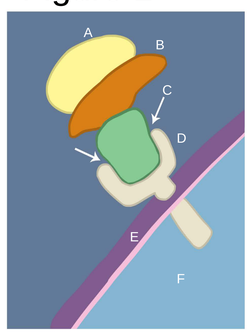



Figure 2

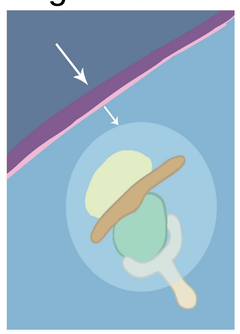
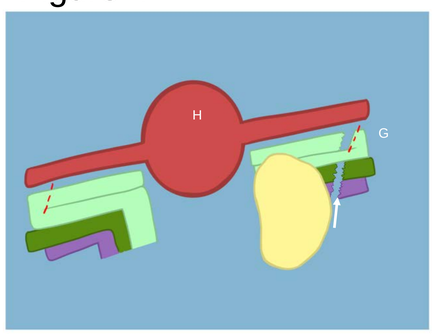


Figure 3

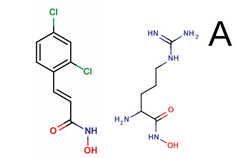
Figure 4

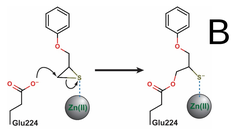


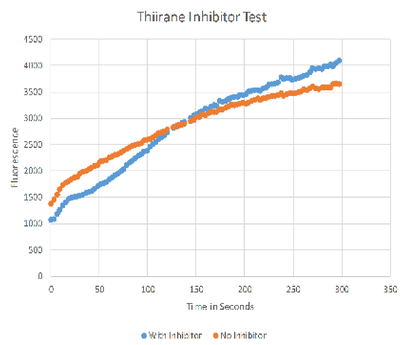

BoNT Diagram © Erin Richards (Photoshop CS8)

**Figure 1**
**Heavy chain binds to receptor on nerve membrane**
**Figure 2**
**BoNT molecule enters the nerve cell through receptor-mediated endocytosis (inside a protective vesicle)**
**Figure 3**
**Light chain is released into cytoplasm of the nerve**
**Figure 4**
**Light chain cleaves SNARE proteins, inhibiting neurotransmitter release and therefore muscle contraction**

## Botulinum Neurotoxin Data



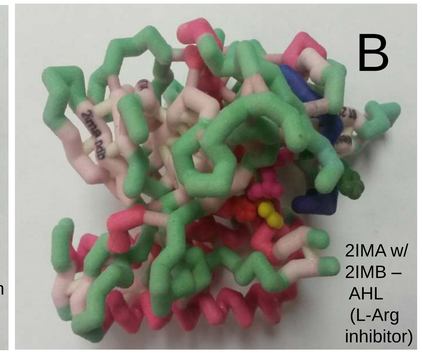




Dr. Silvaggi utilizes X-Ray diffraction and steady state enzyme kinetics to identify new molecules that can inhibit the BoNT protease. The molecule depicted in this graph is not a Thiirane. The blue line represents the fluorescence with the inhibitor present, and the orange line represents the fluorescence without the inhibitor present. The proof-of-concept thiirane did not inhibit the light chain protease. More work is required to determine if this is because the thiirane does not react with the enzyme like Dr. Silvaggi thought it would, or if this particular compound just does not fit into the active site. Dr. Silvaggi would like to create a covalent inhibitor of the BoNT light chain, like the one shown in Figure B above.

## Focus of Future Research

With the widespread use and availability of BoNT it is plausible that BoNT may be usable as a bioterrorism weapon, that we could not control. BoNT is extremely dangerous because it is not detectable until the symptoms becomes fatal. Therefore, it is necessary to find a way to stop BoNT once it has entered the body and prevent paralysis. Through research hopefully a inhibitor is found that will bond to the BoNT/A active site and prevent the symptoms of the Botulinum Neurotoxin.

## Botulinum Neurotoxin Serotype A


Our Jmol model utilizes magnets to show how different inhibitors can be interchanged. The GB4 inhibitor (Picture B) is the most effective inhibitor known to this day. The important amino acids involved in the binding site are histidine 223 (chartreuse), glutamic acid 224 (khaki), histidine 227 (red), glutamic acid 262 (yellow), and zinc 500 (orange).

**Works Cited**

Silvaggi, Nicholas, Grant Boldt, Mark Hixon, Jack Kennedy, Saul Tzipori, Kim Janda, and Karen Allen. "Structures of Clostridium Botulinum Neurotoxin Serotype A Light Chain Complexed with Small-Molecule Inhibitors Highlight Active-Site Flexibility." Chemistry and Biology (2007): 533-42. Print.
MedAnim. "BOTOXÂ® Mechanism of Action Video." YouTube. YouTube, 12 July 2009. Web. 02 Mar. 2015.

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