ICCAV/NICEATM/ECAM Scientific Workshop
on Alternative Methods to the Mouse Bioassay
for Botulinal Neurotoxin

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Essential Characteristics of Potential Test Methods to Replace or Supplement the Mouse LD_{50} Assay

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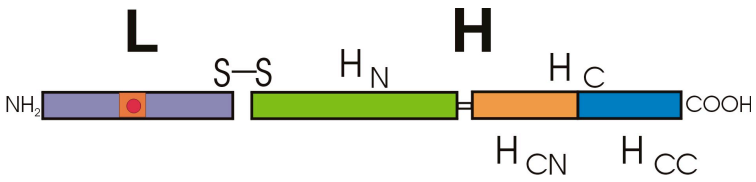
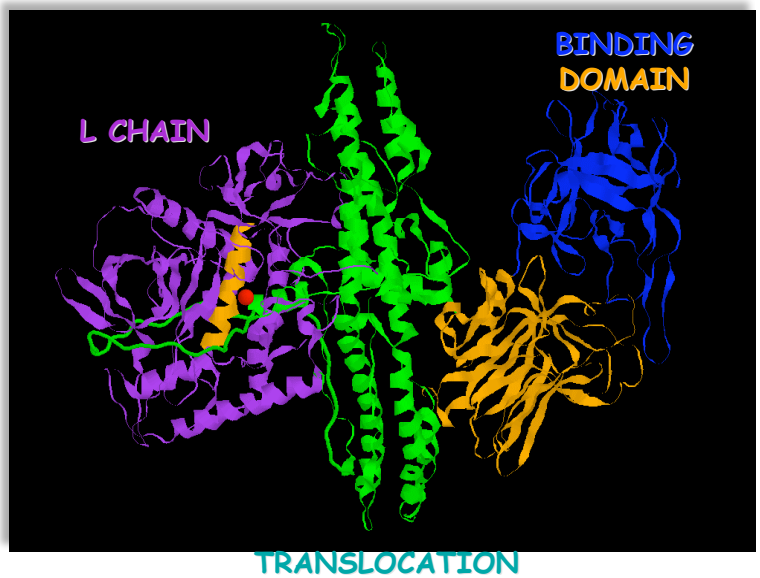
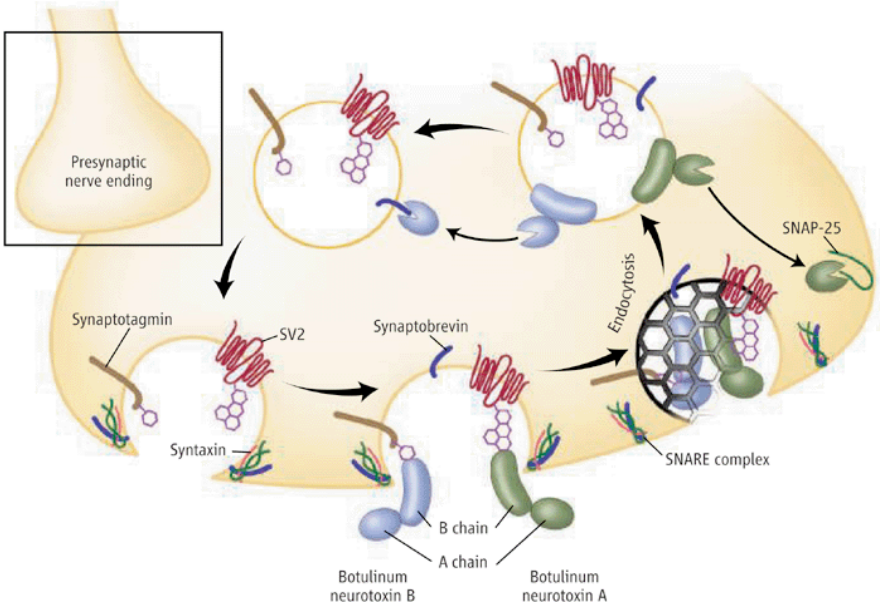
STRUCTURE OF BOTULINUM NEUROTOXINS







Schematic of BoNT Action.
ILLUSTRATION: C. CAIN/*SCIENCE*

Ideal Characteristics of a Non-Animal Assay: It Should Evaluate Each Step of the In Vivo Intoxication Process

- Binding to receptors including gangliosides and protein(s) receptors of the NMJ.
- Endocytosis of the BoNT in a manner analogous to that occurring In Vivo.
- Entry of the catalytic light chain into a suitable compartment with neuronal substrate (e.g. cytosol).
- Cleavage of SNARE protein substrates (SNAP-25, syntaxin, VAMP/synaptobrevin).
- Currently, most in vitro methods measure individual steps.

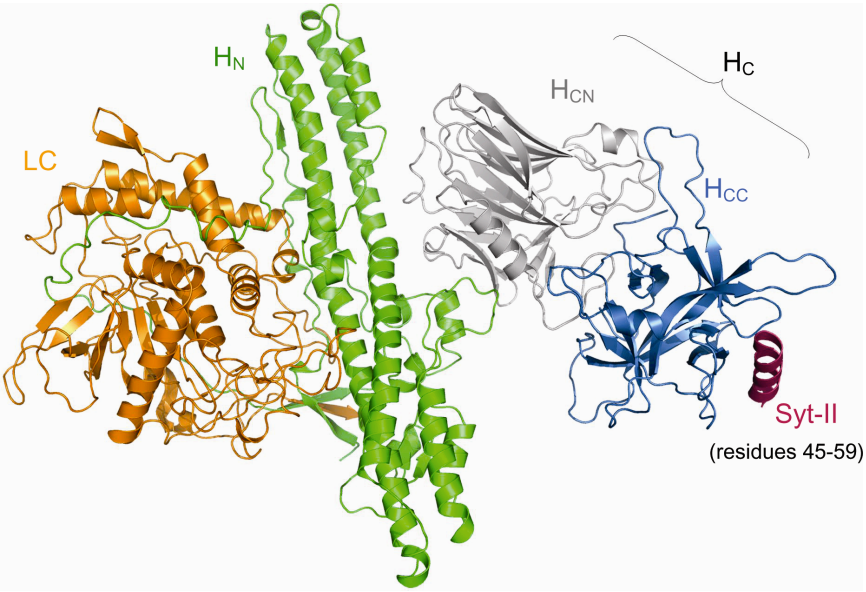
Ideal Characteristics of a Non-Animal Assay (cont.)

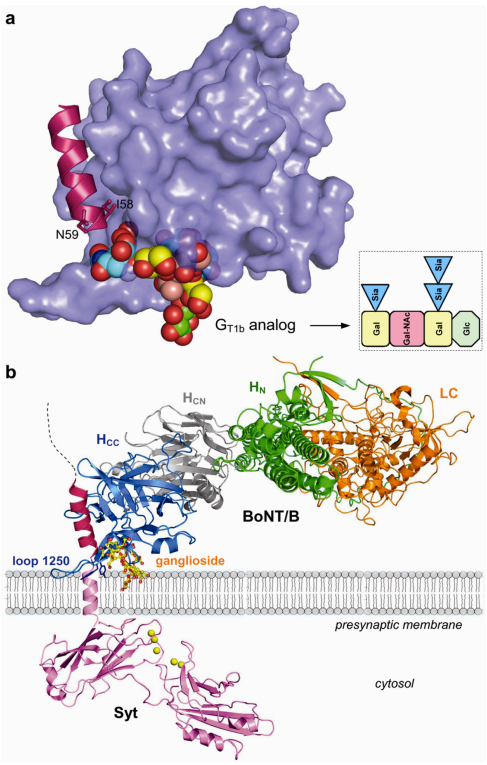
- Sensitivity analogous to the mouse LD50 (1-2 LD50 per ml = ca. 7-15 pg per ml).
- Determination of all seven serotypes and subtypes.
- Assay capable of distinguishing toxin serotypes.
- Multiplexing desired.
- Desired false positive: 1 in 1,000,000.
- Desired false negative: 1 in 1000.
- Rapidity is desired depending on intended use.

Ideal Characteristics of a Non-Animal Assay (cont.)

- Robustness: capable of detecting BoNT in complex food and clinical matrices.
- Relatively simple platform adaptable to wide-range of laboratory and field situations.
- Inter-laboratory consistency and statistical validation of results.
- Assay can contribute to understanding of biological characteristics of BoNT.

Receptor Binding of BoNT/B to Syt-II




a
I58
N59
GT1b analog
Sia
Sia
Sia
Gal
Gal-NAc
Gal
Glc
b
HCN
HN
LC
HCC
BoNT/B
loop 1250
ganglioside
presynaptic membrane
cytosol
Syt