



Pharmacological Countermeasures for Botulinum Toxin Exposures

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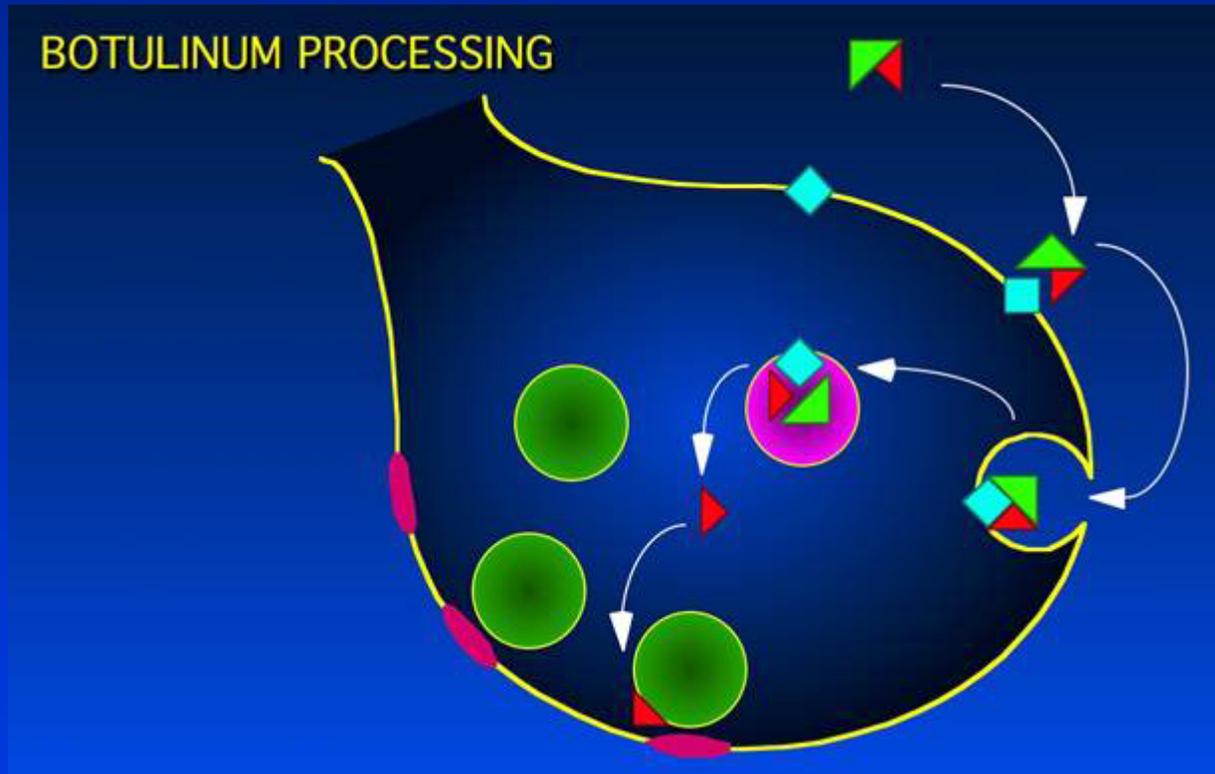
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Roles of Botulinum Neurotoxin

- Public health threat
- Weapon of mass destruction (WMD)
- Research tool for inhibition of synaptic activity
- Therapeutic agent for dystonia and movement disorders

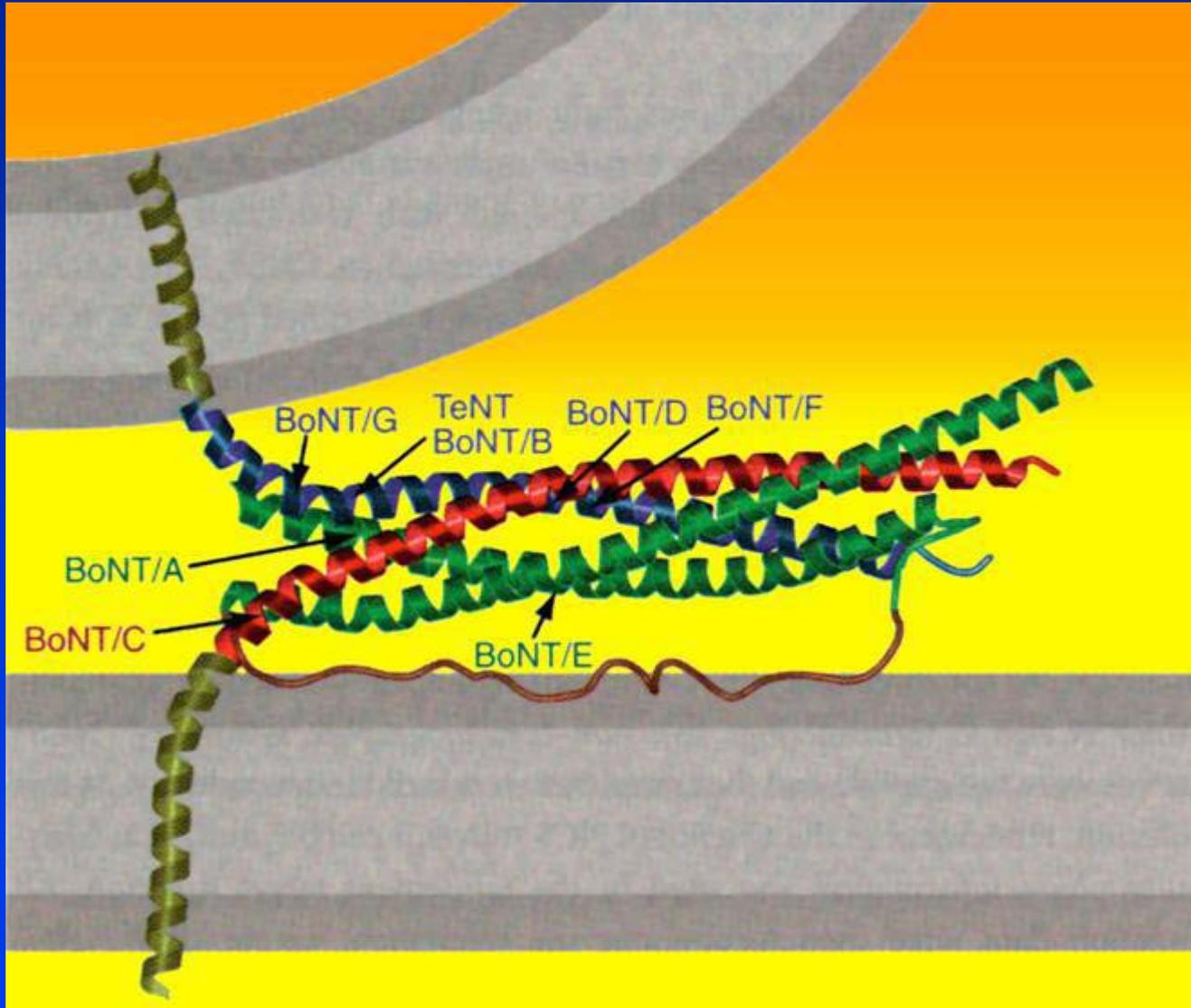
Each role has potential for human exposure and could benefit from a pharmacological therapy.

Mechanism of BoNT Action



- Toxin Binds to cell surface receptors (**exact structure unknown**).
- Toxin is internalized in an endosome which is acidified.
- Toxin dissociates and LC enters cytosol (**mechanism suspected**).
- Toxin enzymatically cleaves essential presynaptic proteins.

Mechanism of BoNT Action

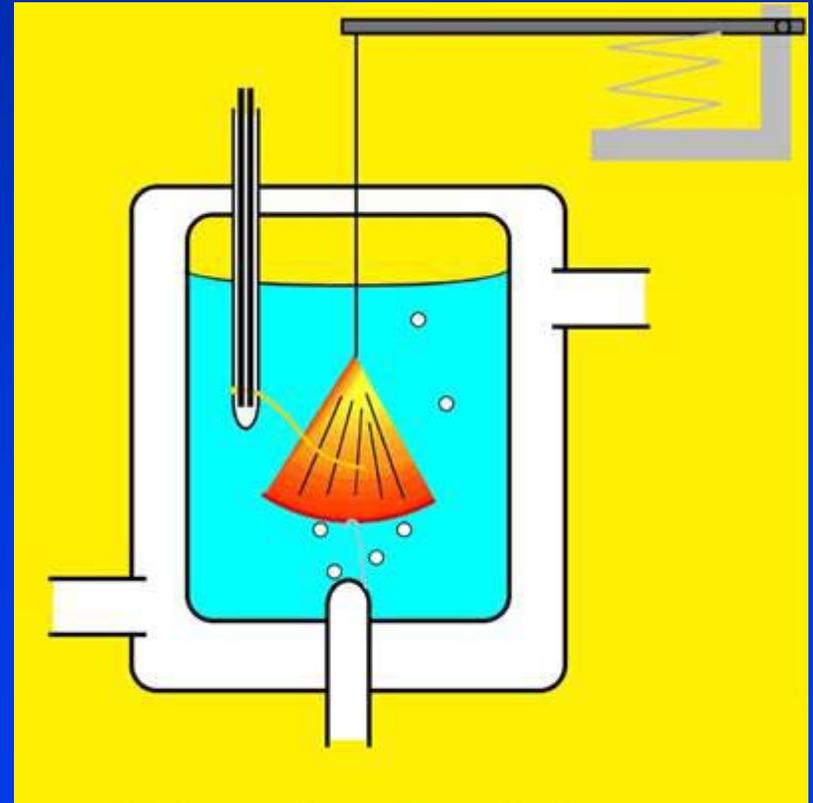


Strategies for Protection

- Vaccine (available now)
 - Needs to be applied before exposure (> 2 months)
 - Serotype selective (7 distinct serotype of toxin known)
- Antitoxin (available now)
 - Serotype selective
 - Ineffective after initial paralysis develops due to internalization of toxin
- Pharmacological (under development)
 - Potentially active against multiple toxin serotypes
 - Potential for benefit in late stages of paralysis

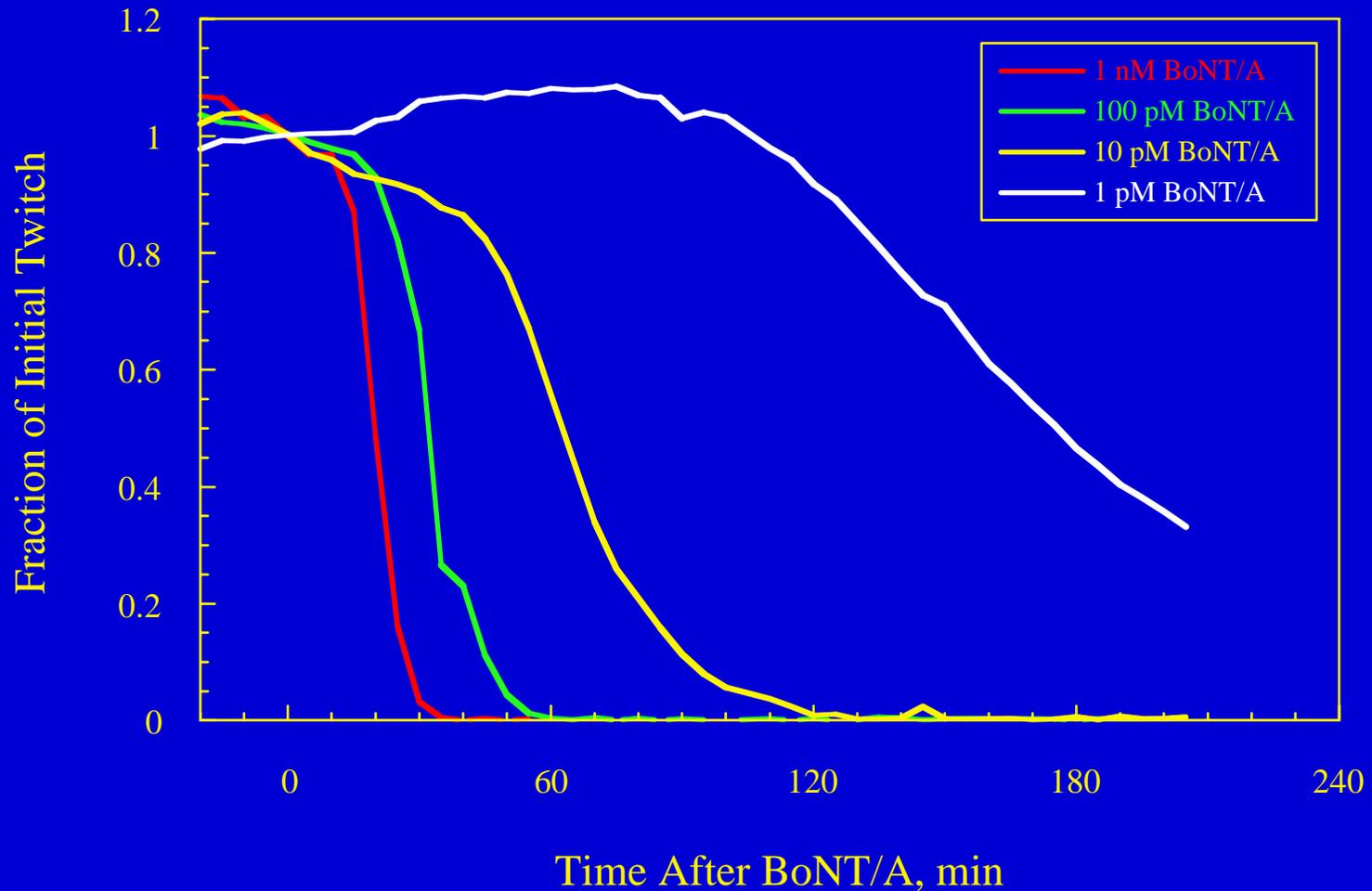
Methods Used : in vitro muscle

- Isolated mouse hemidiaphragm: isometric twitch tension in response to phrenic nerve stimulation.



In Vitro Muscle Paralysis with Botulinum Toxin

Variable is Time @ Fixed Paralysis Level



Methods Used: intramuscular injection

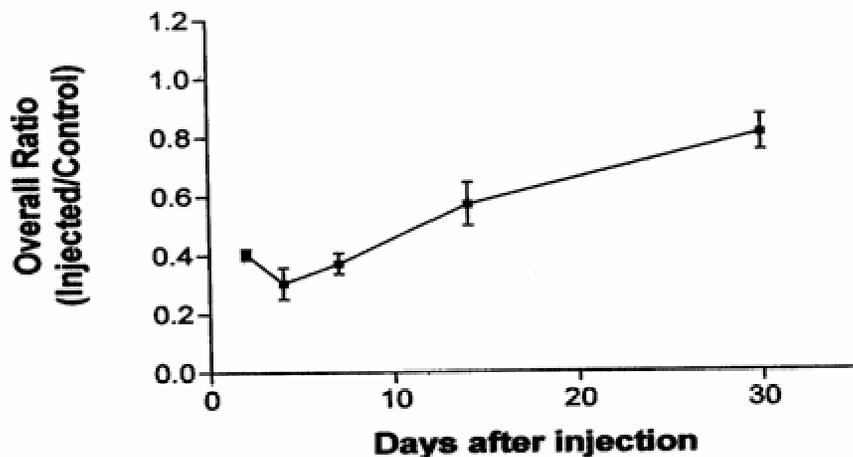
- Photographically monitor reflex toe spread over time.
- Confirm with muscle tension measurement on EDL.



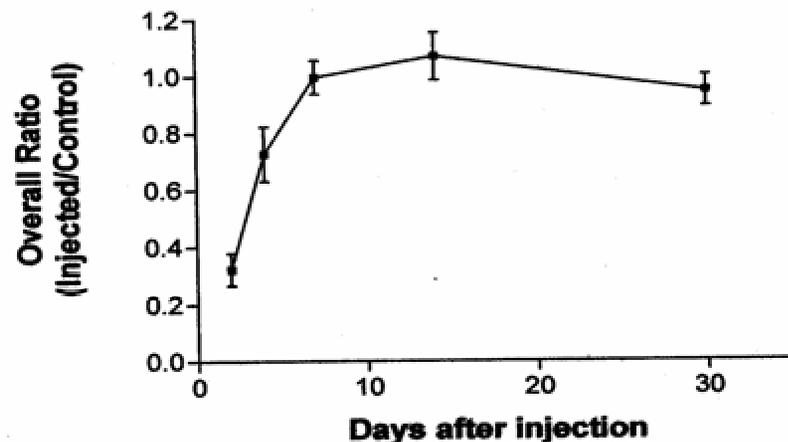
Toxin Inhibition is Monitored over Time

Variable is Normalized Toe Spread (toxin/control)

BoNT serotype A recovery (slow)

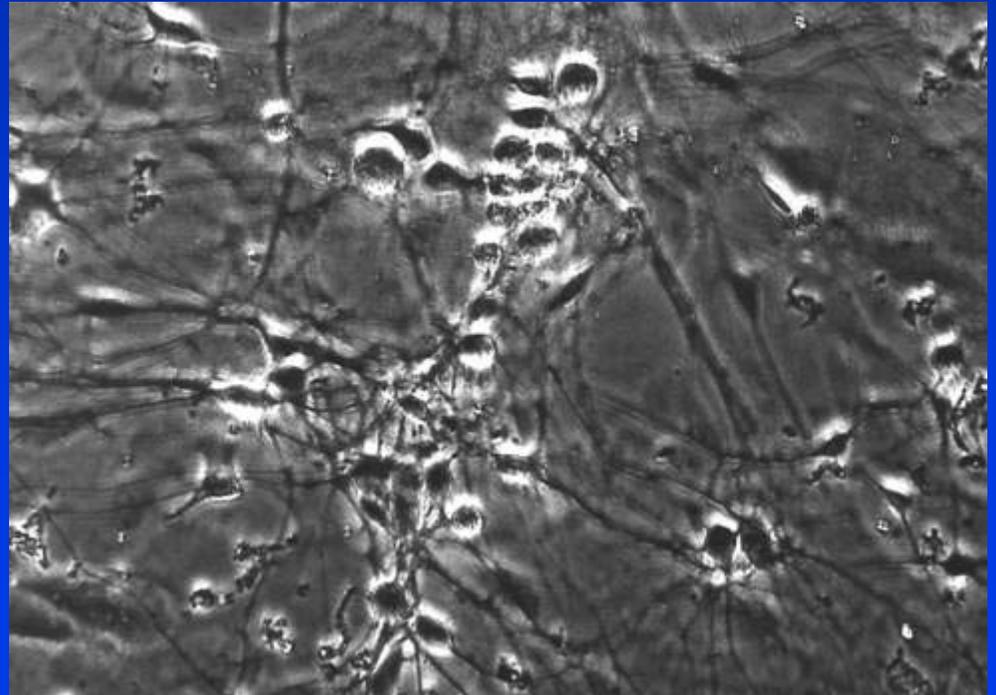


BoNT serotype E recovery (fast)

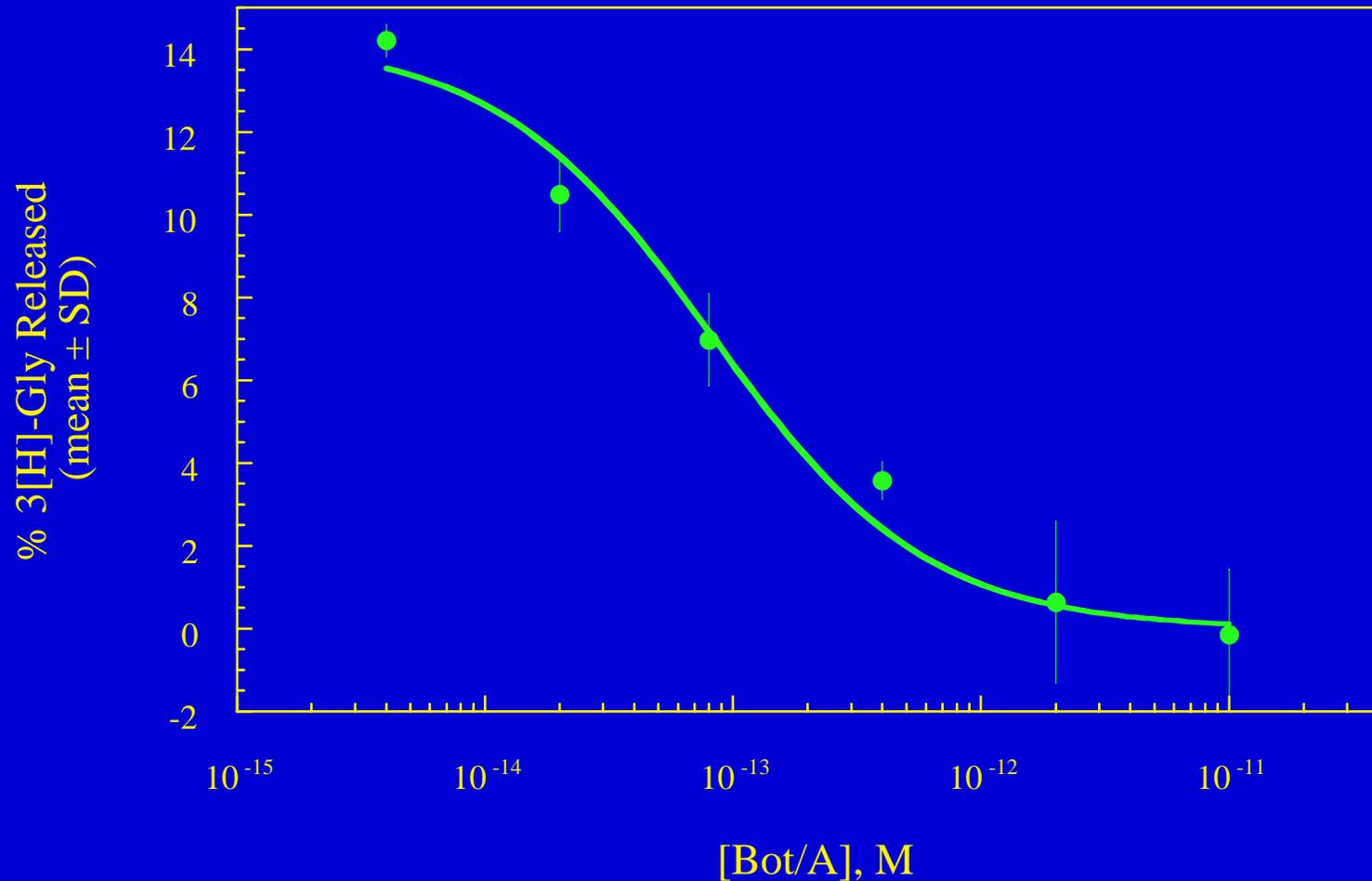


Methods Used: cultured neurons

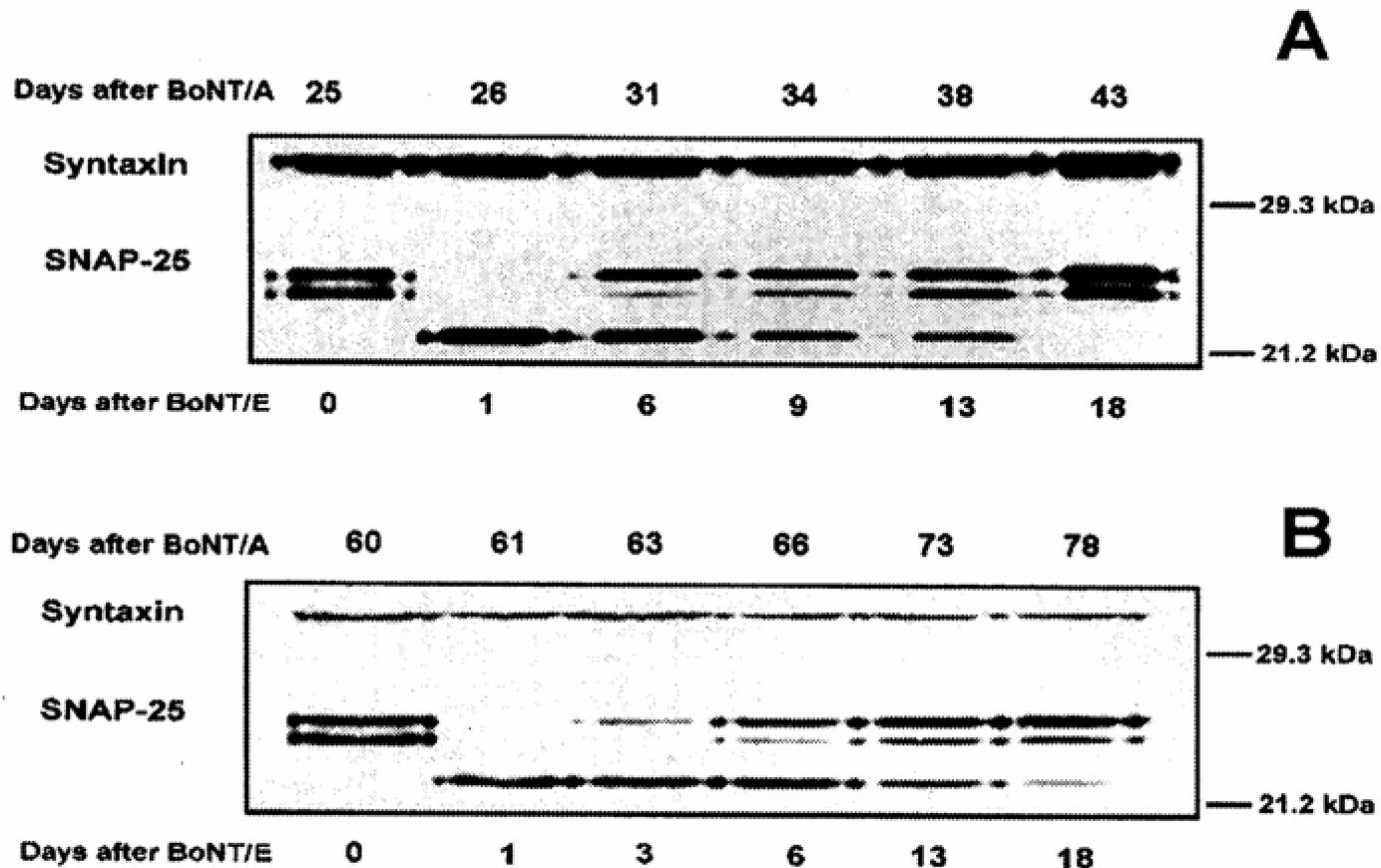
- Tissue cultured mouse spinal cord neurons: evoked release of radiolabeled neurotransmitter in elevated potassium and calcium.



Toxin Inhibition of Evoked Transmitter Release (Variable is "paralysis" @ Fixed Time)

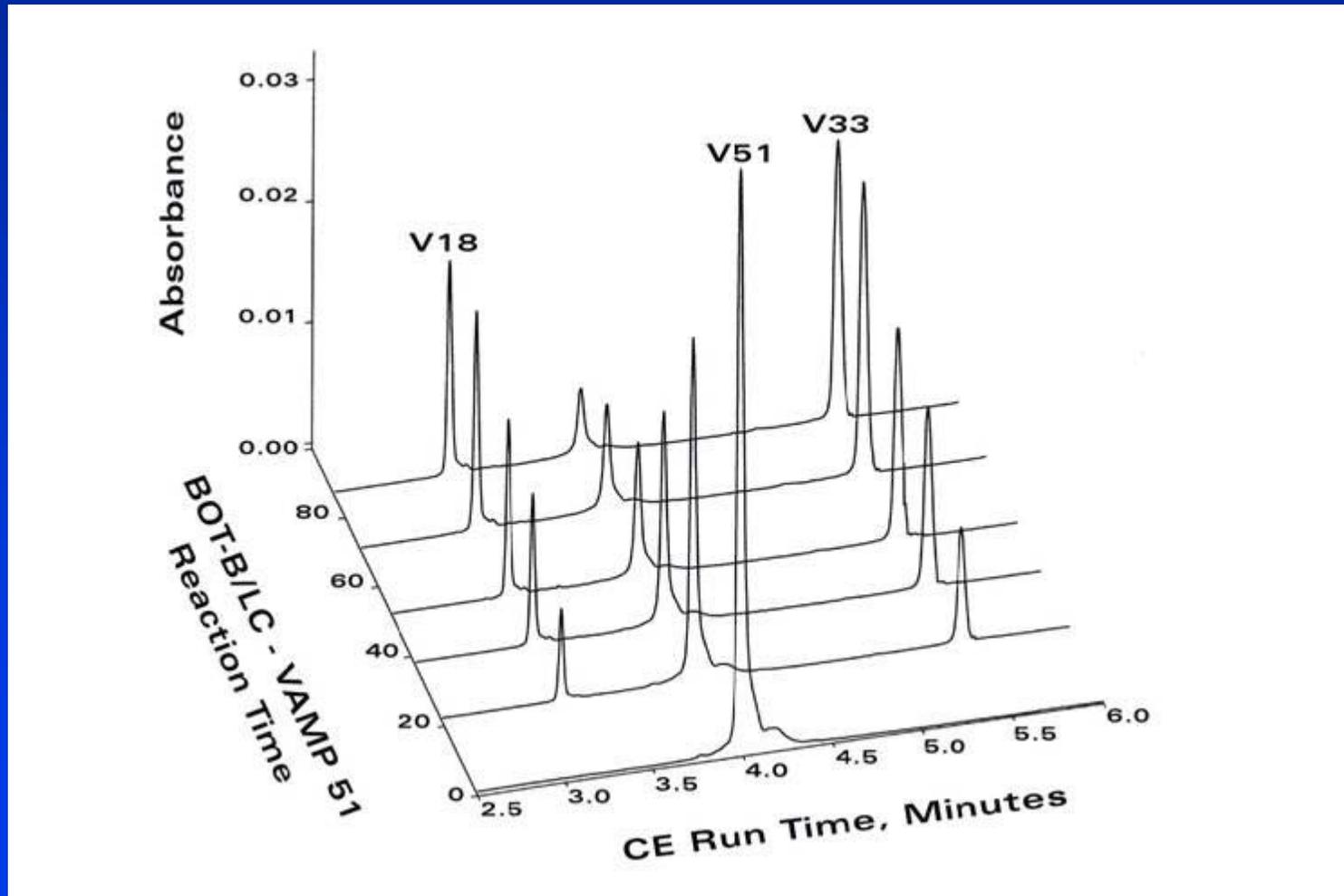


Monitor cleavage of target protein using quantitative mAb binding and gel separation of fragments.



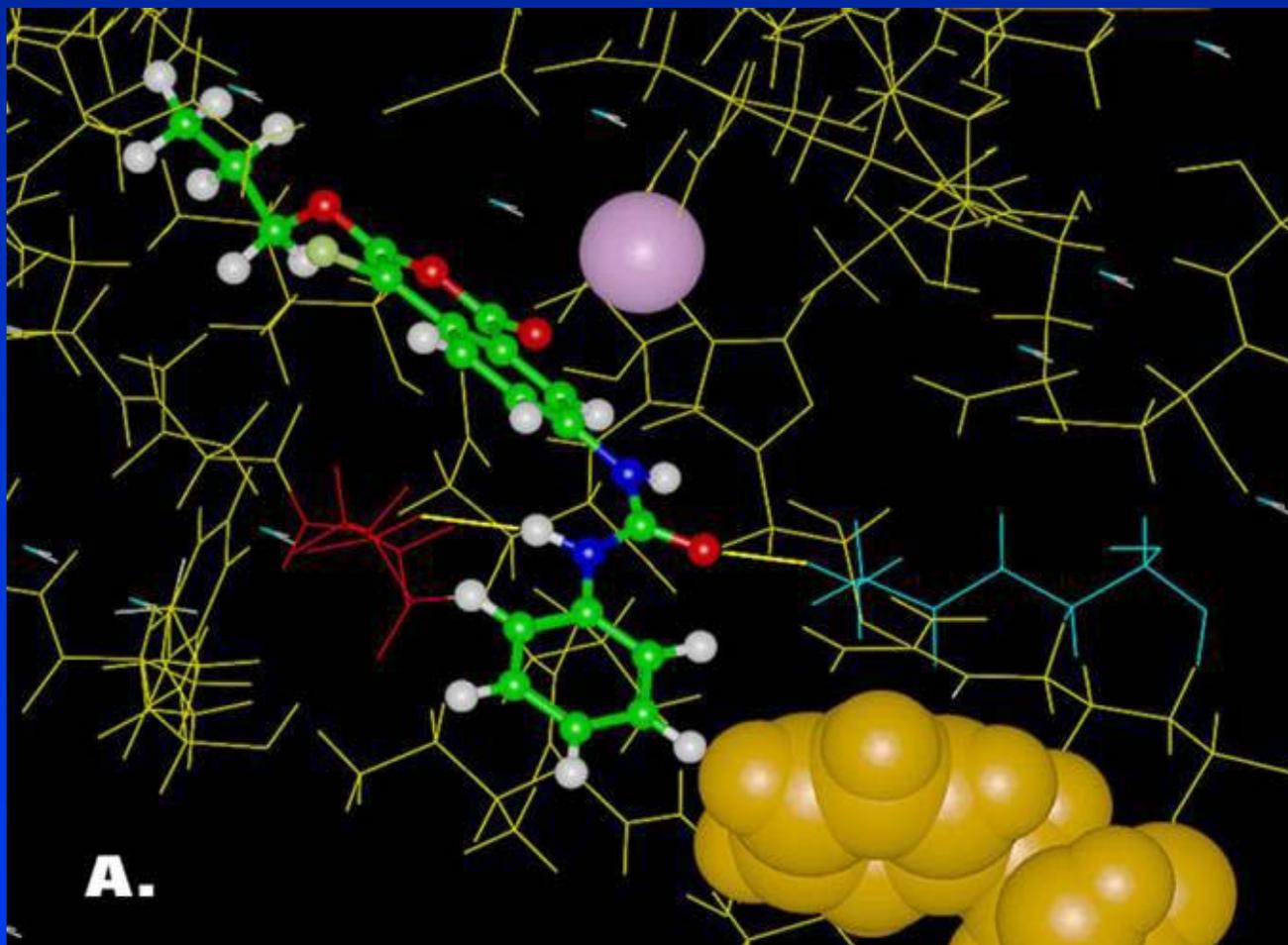
Methods Used: in vitro proteolysis

Monitor cleavage of synthetic VAMP51 (aa 44-94) with CE



Computer Modeling of Inhibitors

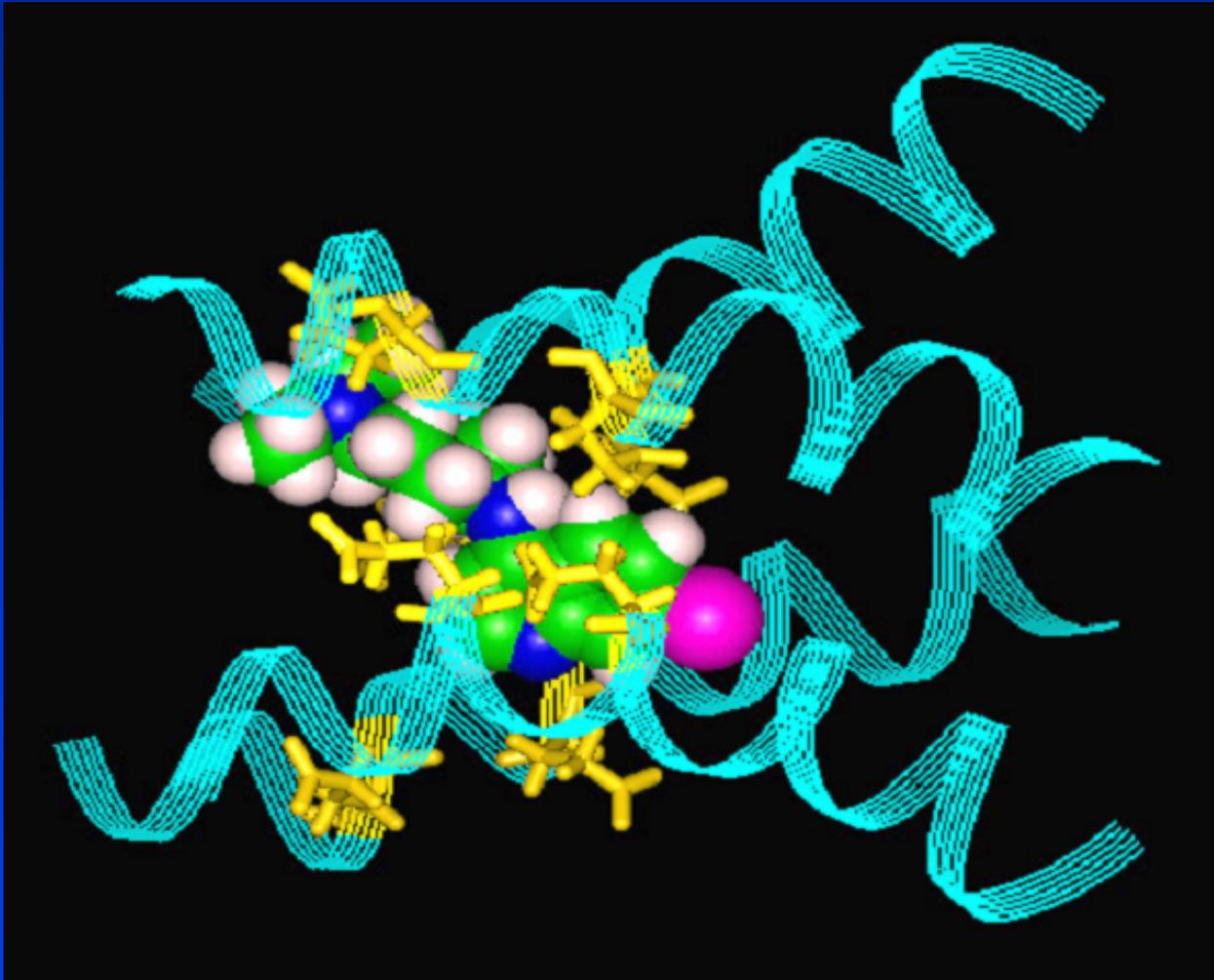
Optimize inhibitor drug design based on BoNT crystal structure(s)



ICD1578 in
BoNT/B active
zone.

Computer Modeling of Inhibitors

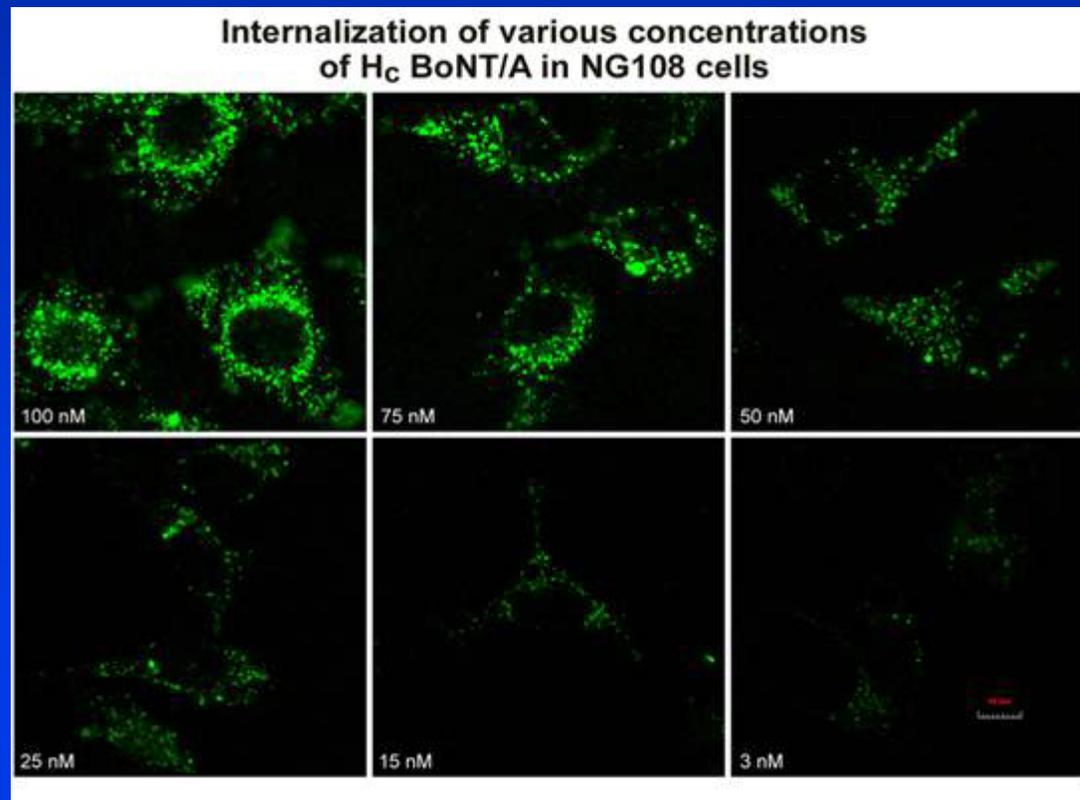
Inhibitor drug design based on theoretical BoNT structure and QSAR



Quinacrine and
BoNT Hn ion pore.

Targeted Drug Delivery

- Use isolated Hc of BoNT to carry antagonist into nerve terminal.
- Concentrates therapeutic drug in affected tissue/cells.
- Allows use of more hydrophilic antagonists/peptides.



Summary

- A pharmaceutical approach to post-exposure treatment of botulinum toxin offers advantages over existing therapies.
- Botulinum Toxin can be inhibited at several points in the toxin's mechanism of action.
- Activity of toxin antagonists can be monitored using several biochemical and/or physiological tests.
- Current testing systems are not “high-throughput” and could be improved.