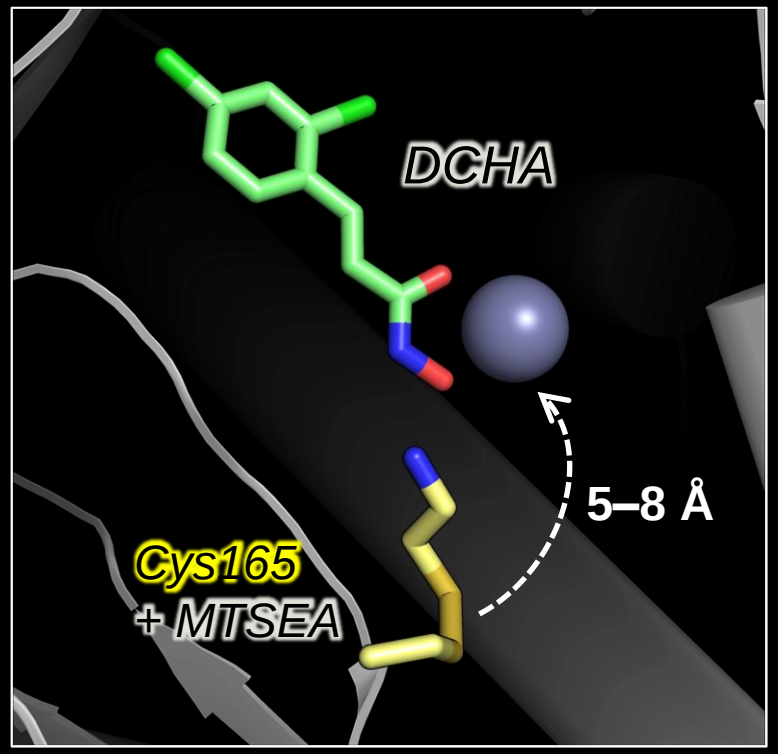
Covalent Inhibition of Botulinum Neurotoxin A



Exploration of Warhead Reactivity and Function Using a Bifunctional Approach

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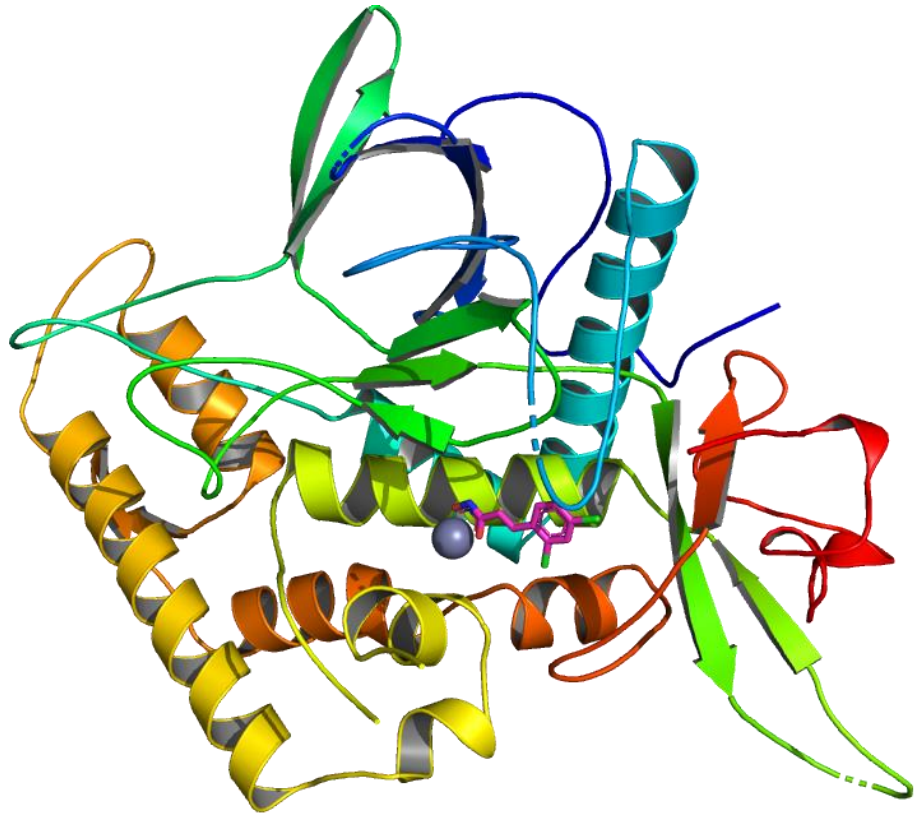
(Janda Lab)

Background

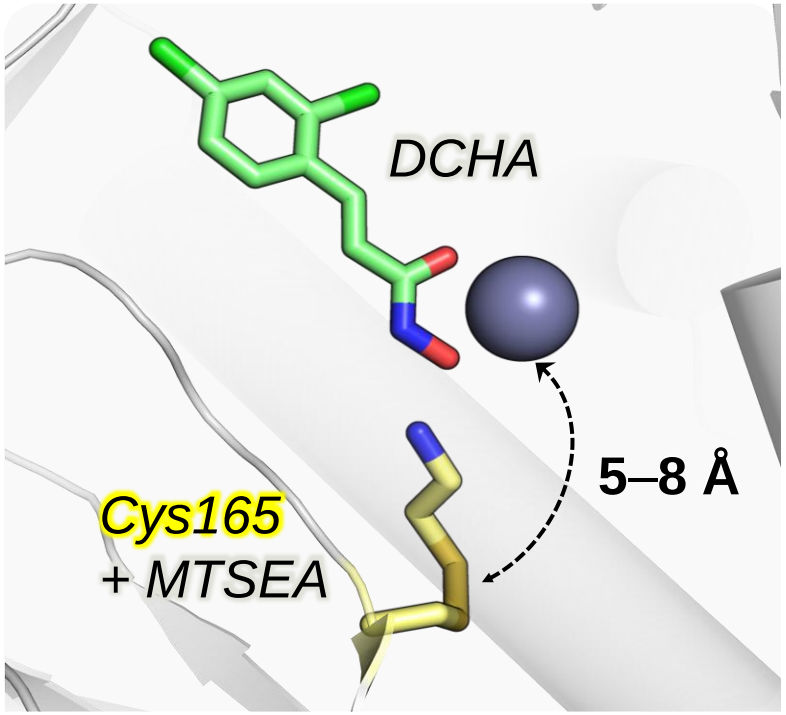
- Botulinum neurotoxins (BoNTs), produced by *Clostridium Botulinum,* are the most potent known toxins.
- BoNT/A has an intravenous LD_{50} of 1–2 ng/kg and has an extremely long half-life (months)
- Used for a plethora of therapeutics but also high potential for bioterrorism
- Catalytic activity stems from the light chain (LC) through its selective cleavage of SNAP-25. The LC is a zinc metalloprotease and is a target for metal-binding small-molecule inhibitors
- Currently, no known small-molecule therapeutics exist for the treatment of BoNT/A intoxication



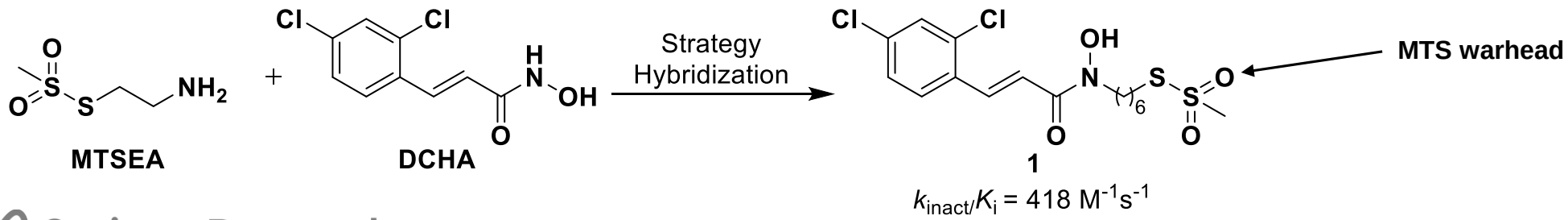
BoNT/A LC crystallised with small molecule inhibitor DCHA (PDB: 2IMA)

Hypothesis and Previous Work

- Covalent inhibition has potential to abrogate BoNT/As innate longevity and remove need for long-term dosing
- Previous work[1] demonstrated that Cys165 can be covalently modified by a small electrophilic molecule (MTSEA)
- Current best-in-class inhibitor DCHA binds zinc through hydroxamate in a bidentate fashion
- Hybridization of both strategies[2] resulted in **1** that was shown to inhibit BoNT/A LC in a bifunctional manner
- Methanethiosulfonate (MTS) warhead is highly reactive and unsuitable for therapeutic use



Overlay of DCHA (PDB: 2IMA) and MTSEA (PDB: 4ELC) BoNT/A LC crystal structures



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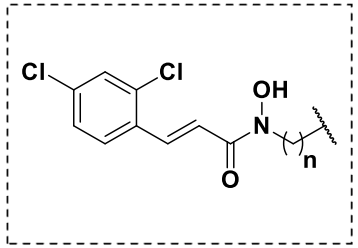
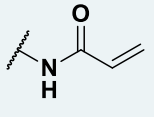
[1]Stura *et al*, *J Biol Chem*, **2012**, 287, 33607–33614
[2]Lin *et al*, *J Med Chem*, **2020**, 63, 11100–11120

Chemistry and Biological Evaluation

Warhead screen



Acrylamides



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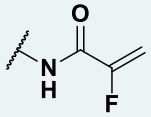
n = 4, **4**
n = 5, **5**
n = 6, **6**



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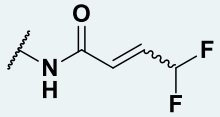

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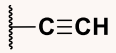
Alkynes



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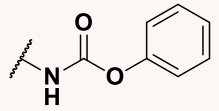
***O*-phenyl carbamate**



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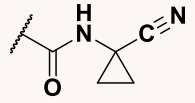
Cyanocyclopropyl



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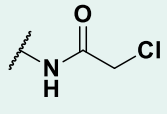
Alkyl halides



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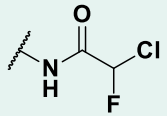

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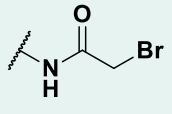

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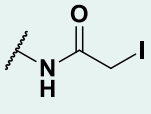
n = 4, **28**



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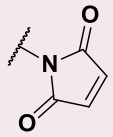
n = 4, **31**

Maleimides



n = 3, **32**
n = 4, **33**

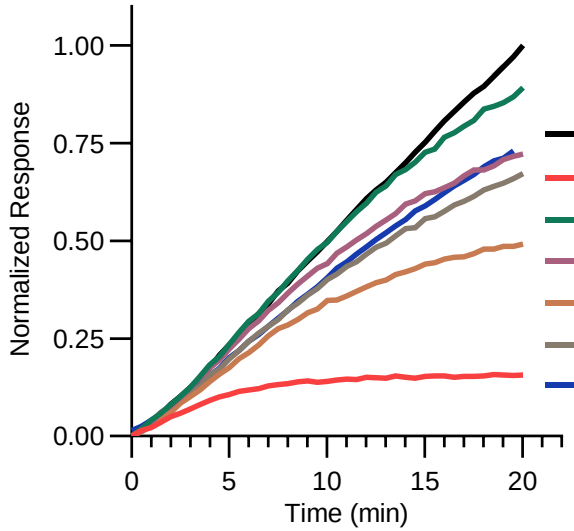
Epoxides

n = 3, **34**
n = 4, **35**
n = 5, **36**
n = 6, **37**

Aldehyde

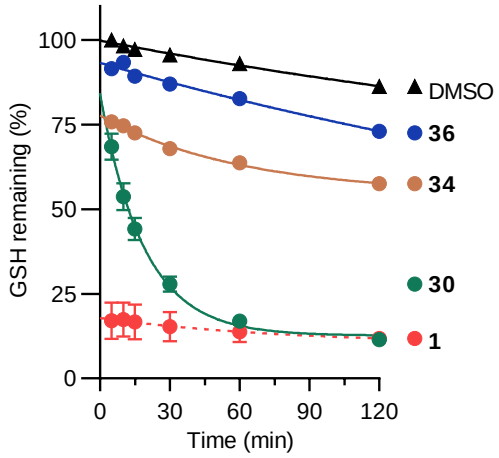
n = 5, **38**

Continuous SNAPtide FRET Assay

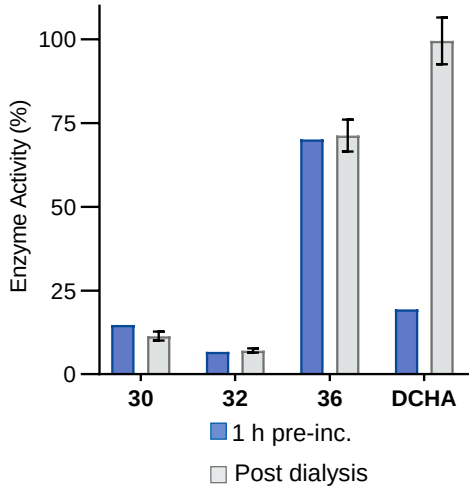


	k_{inact}/K_i ($M^{-1}s^{-1}$)
No Inhib.	
1	514 ± 17
30	17.2 ± 4.0
31	12.4 ± 2.0
32	43.0 ± 8.4
33	46.3 ± 4.9
36	25.4 ± 1.7

GSH Reactivity

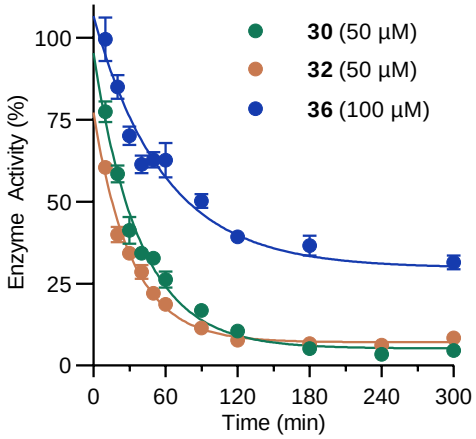


Exhaustive Dialysis

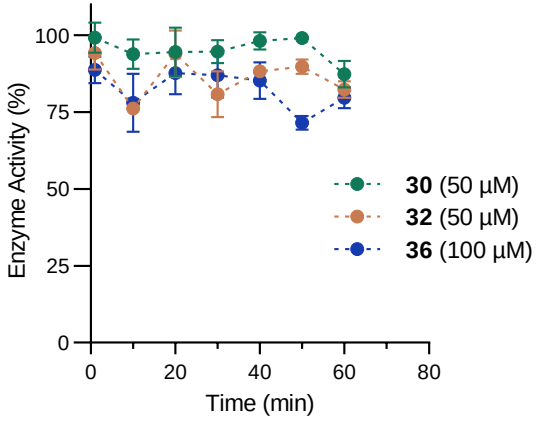


Pre-incubation

WT



C165A



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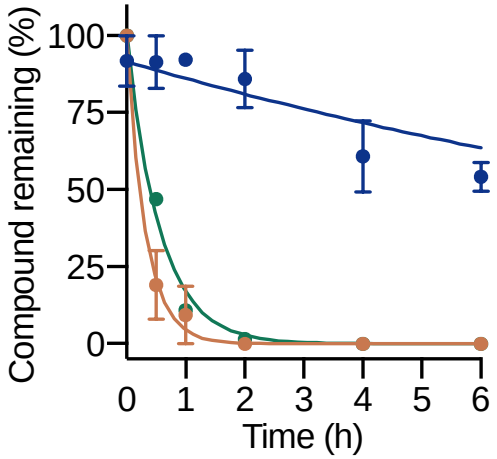
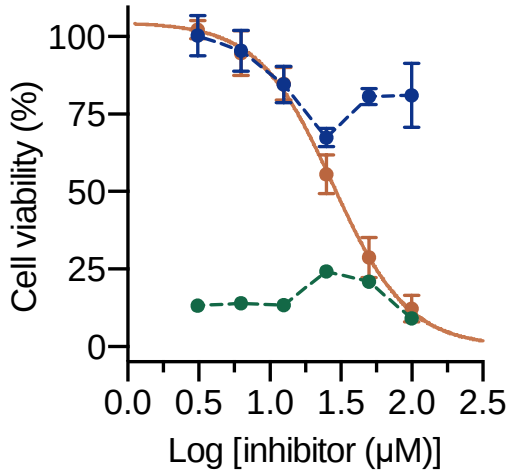
Stability and Cellular Evaluation

Serum Stability

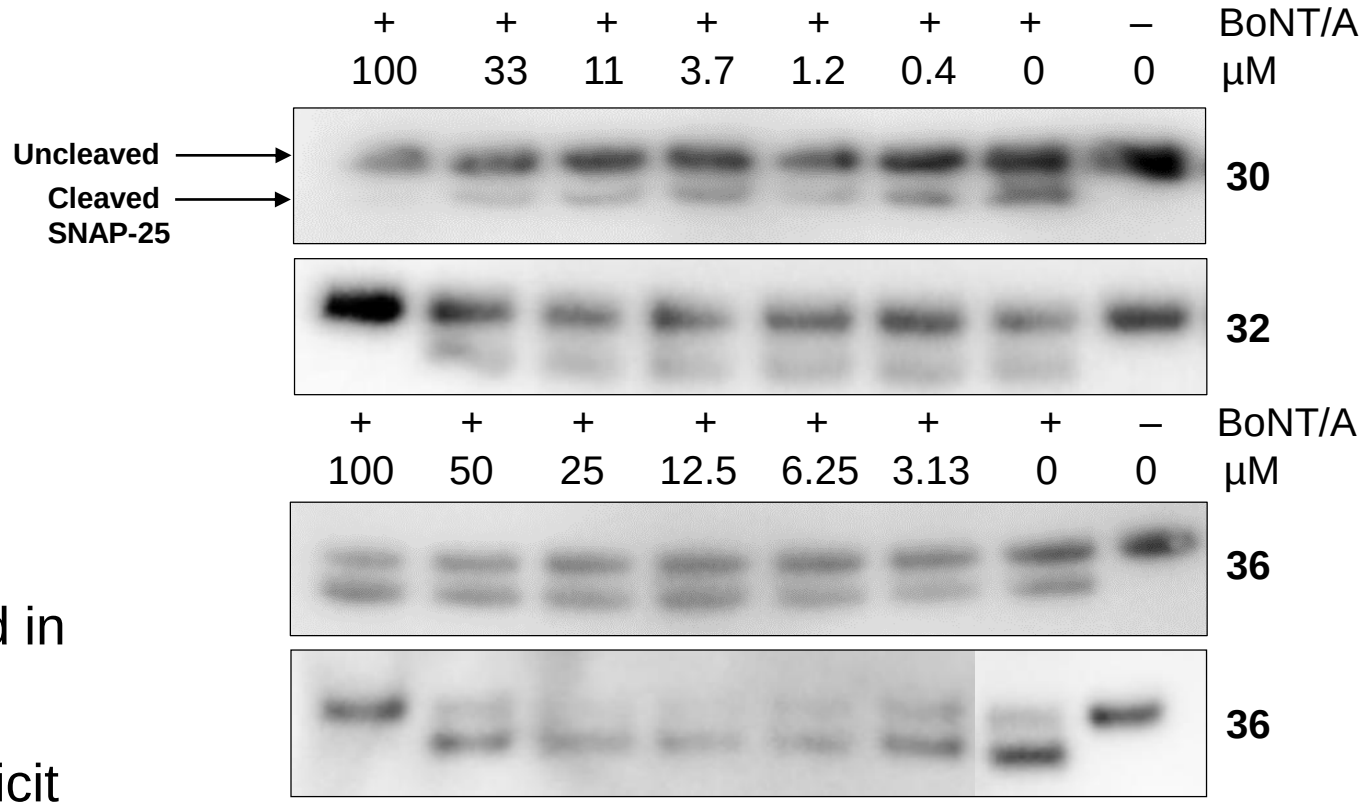


HEK 293



30 **32** **36**

hiPSC Assay



RSC Lysate Assay

- Trend in warhead reactivity was reflected in compound stability and toxicity
- Moderate response in cells but did not illicit the desired potency
- Poor cellular potency attributed to poor permeability of hydroxamate-based compounds

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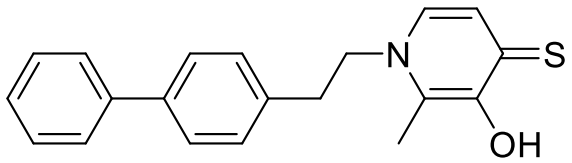
Conclusion, Future Work and Acknowledgements

- Several warhead types were shown to bind covalently to BoNT/A LC with epoxide-containing **36** forming a proximity-based covalent bond.
- A trend in compound reactivity reflected stability and toxicity profiles
- Hydroxamate-based compounds did not furnish the desired cellular potency, however, a fundamental framework for the bifunctional covalent inhibition of BoNT/A LC has been established.
- This strategy will be incorporated into other metal-chelating pharmacophores for the inhibition of BoNT/A:



3,4 - HOPTO

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Prof. Kim Janda

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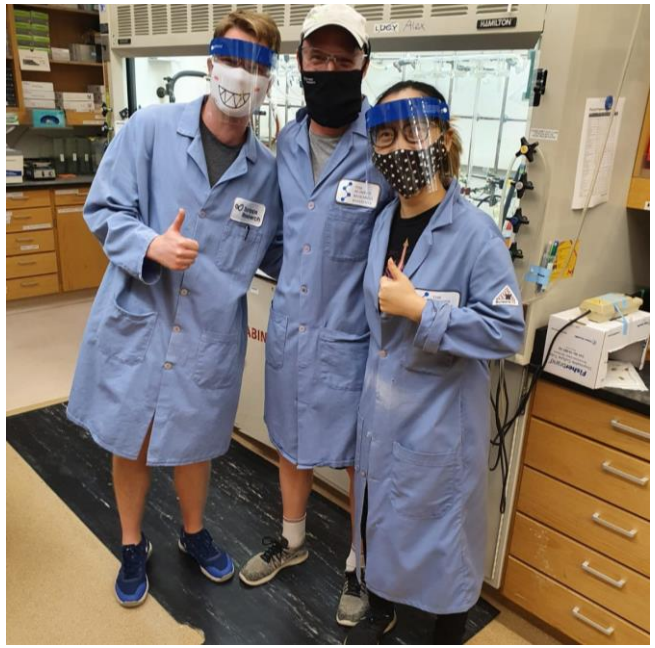

Myself, Alex and Lucy aka 'The BoNT Bros'

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Lin *et al*, *RSC Med. Chem.*, **2020**, Advance Article