Research Topics Seminar

Presenter: Jared Hammill

Advisor: Dr. Peter Wipf

August 1st, 2009

Overview

- Part 1: Solid Phase Peptide Synthesis
  - Background
  - Scotophobin: a storied past
  - Design and synthesis of a BoNT A inhibitor

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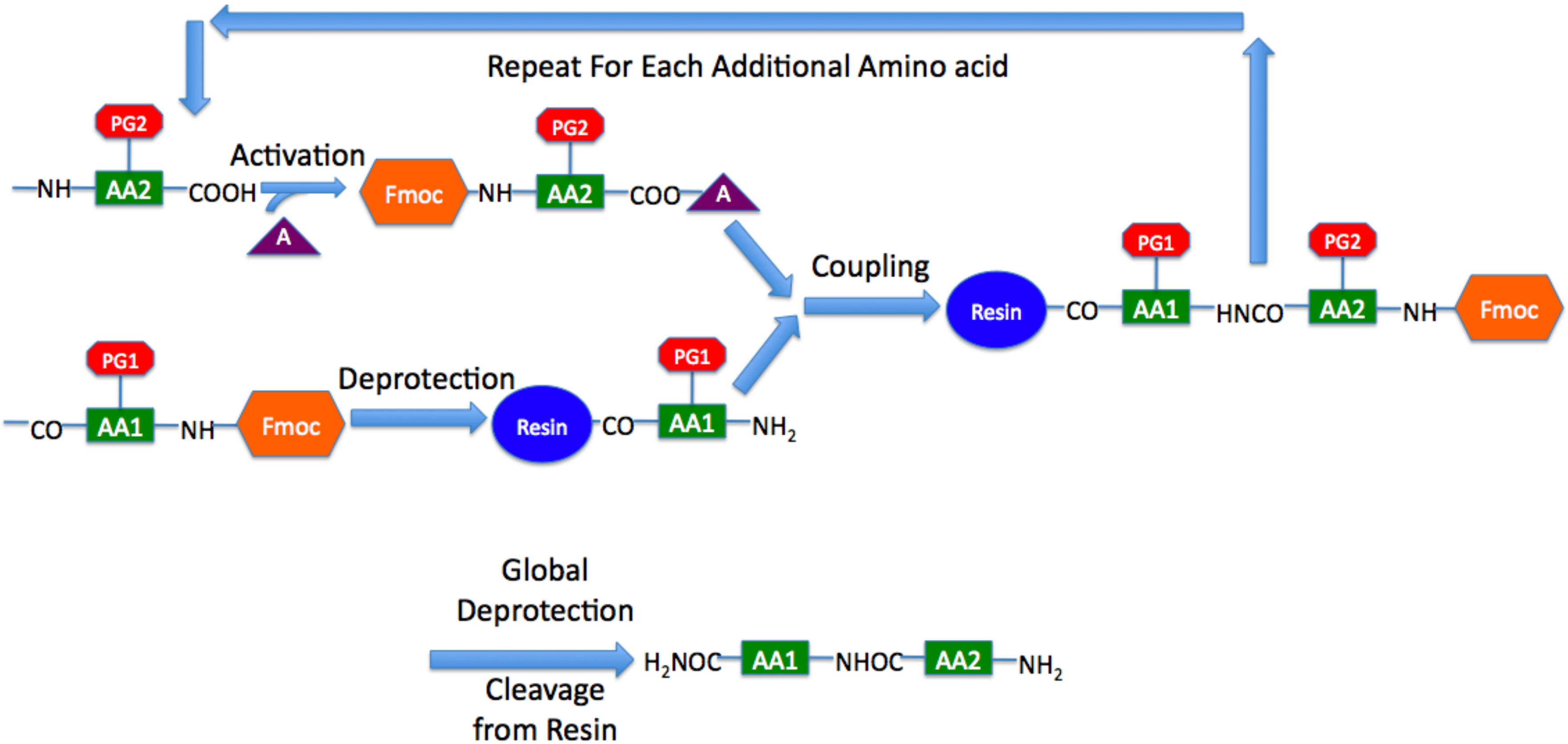
  - Background
  - Scotophobin: a storied past
  - Design and synthesis of a BoNT A inhibitor
- Part 2: Accessing the Bicyclo[3.3.1]nonane scaffold
  - Background
  - Progress
  - Future work

Solid Phase Peptide Synthesis

Introduced in 1963 by Merrifield

-Insoluble solid supports allow excess reagents and unwanted by-products to be dissolved and washed away



Merrifield, R. B.1963. J. Am. Chem.Soc. , 85, 2149-2154

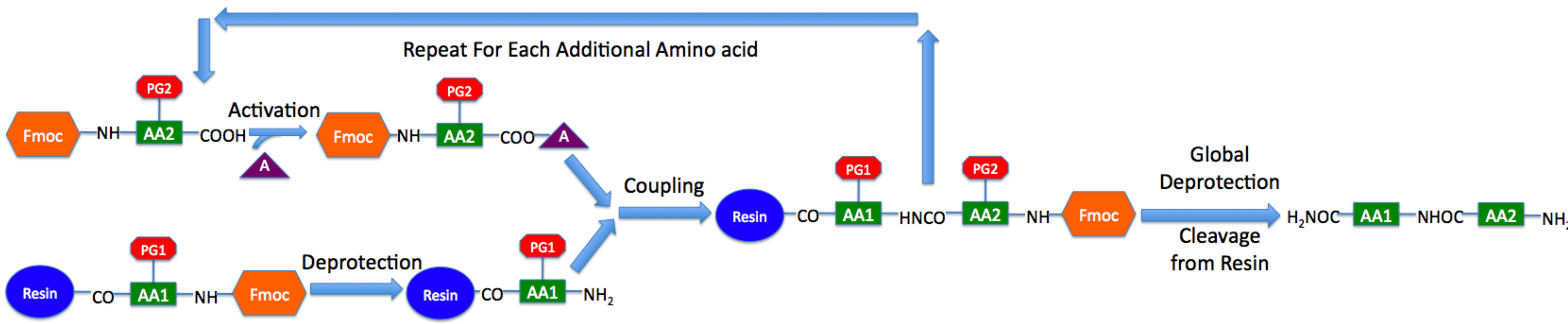
Solid Phase Peptide Synthesis

Boc SPPS

-TFA to deprotect intermediates and HF for cleavage from resin

Fmoc SPPS

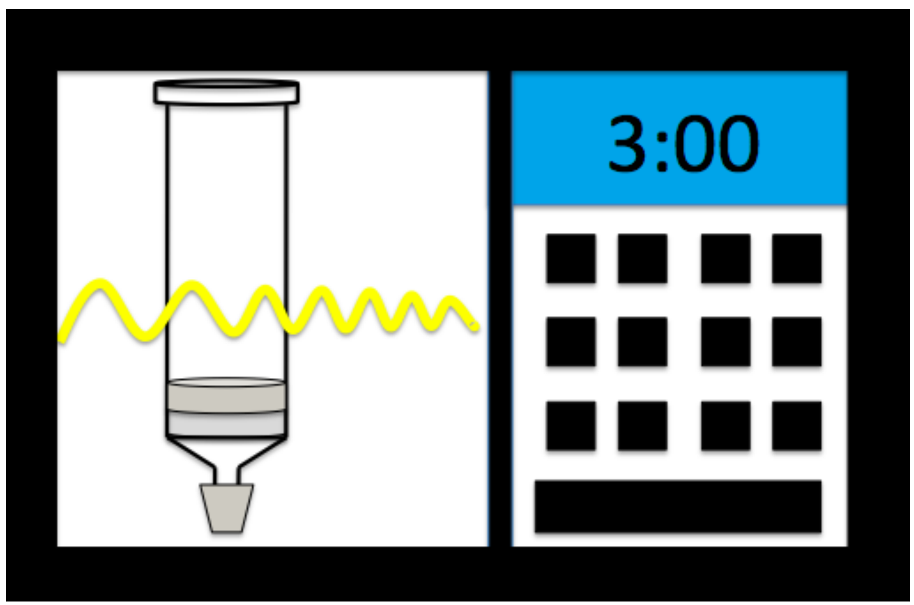
-Piperidine to deprotect intermediates and TFA for cleavage from resin



Merrifield, R. B.1963. J. Am. Chem.Soc. , 85, 2149-2154

Microwave Assisted SPPS

- First reported in the 1940's
- Appears in organic synthesis literature in 1980's
- Pioneered by authors like Gedye, Giguere, and Majetich
- Microwave irradiation allows for reduced reaction times



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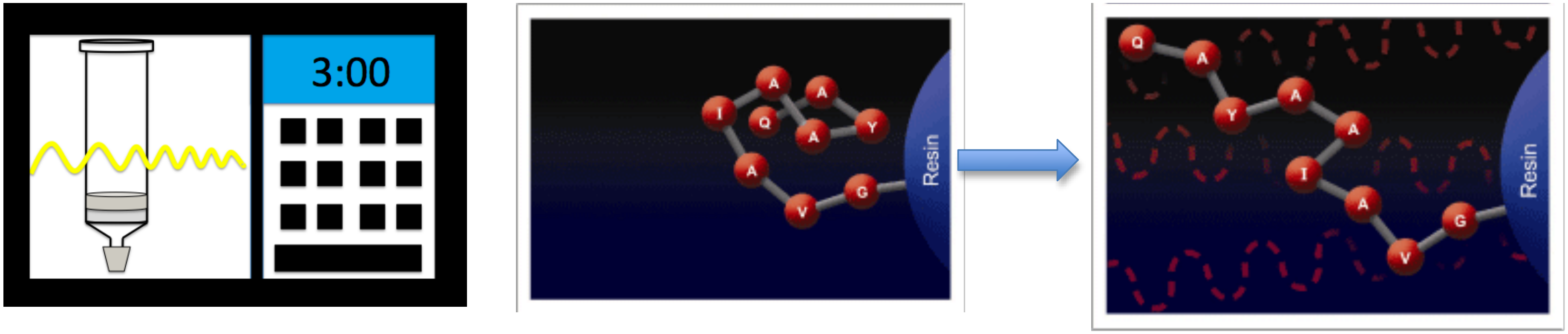

Major advantages of microwave irradiation:

1)more efficient energy transfer to the reaction mixture instead of the vessel

2)allows for homogenous heating

3)decreases peptide aggregation on solid support

2009 CEM corporation. “Microwave Chemistry: How it all Works.” <http://cem.com/page130.html>

Scotophobin
Chasing a Memory

Early Evidence

- 1965: Four groups showed memory transfer in mammals
  - Three groups hypothesized RNA to be the transfer unit
  - George Unger & Oceguera-Navarro hypothesized a small peptide as the transfer unit

Setlow, B,(1997): George Unger and Memory Transfer *J. His. Nuer.*, 6: 181-192

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- Unger showed transference effect was elimanated upon incubation of the brain extract with chymotrypsin, but not with ribonuclease
- Several negative results attempting to repeat the memory transference experiments caused a controversy to emerge
  - However, these experiments assumed RNA was the transfer unit

Setlow, B,(1997): George Unger and Memory Transfer *J. His. Nuer.*, 6: 181-192

George Unger

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Theory

Unger proposed that when a pre-synaptic neuron fired, it released a connector peptide. This peptide could be taken up by a nearby post-synaptic neuron, thereby establishing a new neural network. Repeated connections could strengthen the signaling pathway.

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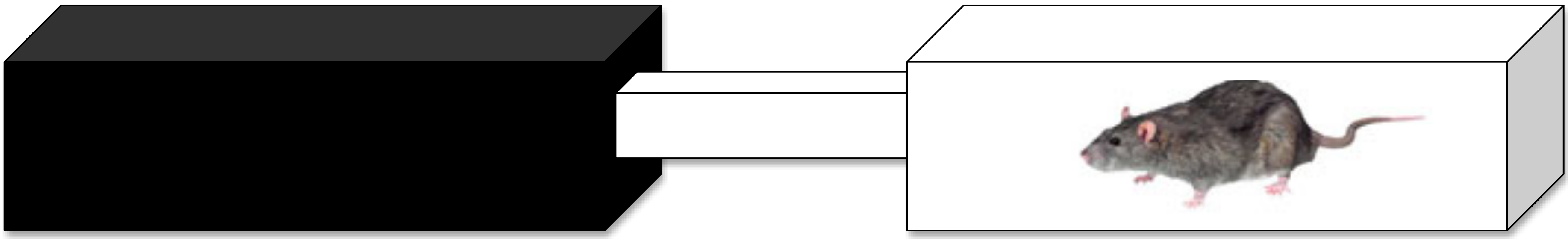
- 1968
  - Unger published a study which received much attention in both the public and scientific communities
  - The study concluded that a learned response was transferred from rats to mice and a single peptide was responsible

Setlow, B,(1997): George Unger and Memory Transfer *J. His. Nuer.*, 6: 181-192

The Experiment



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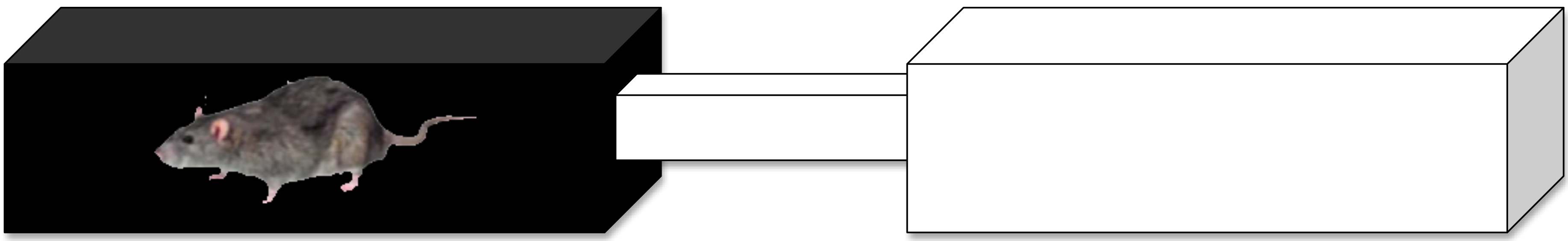

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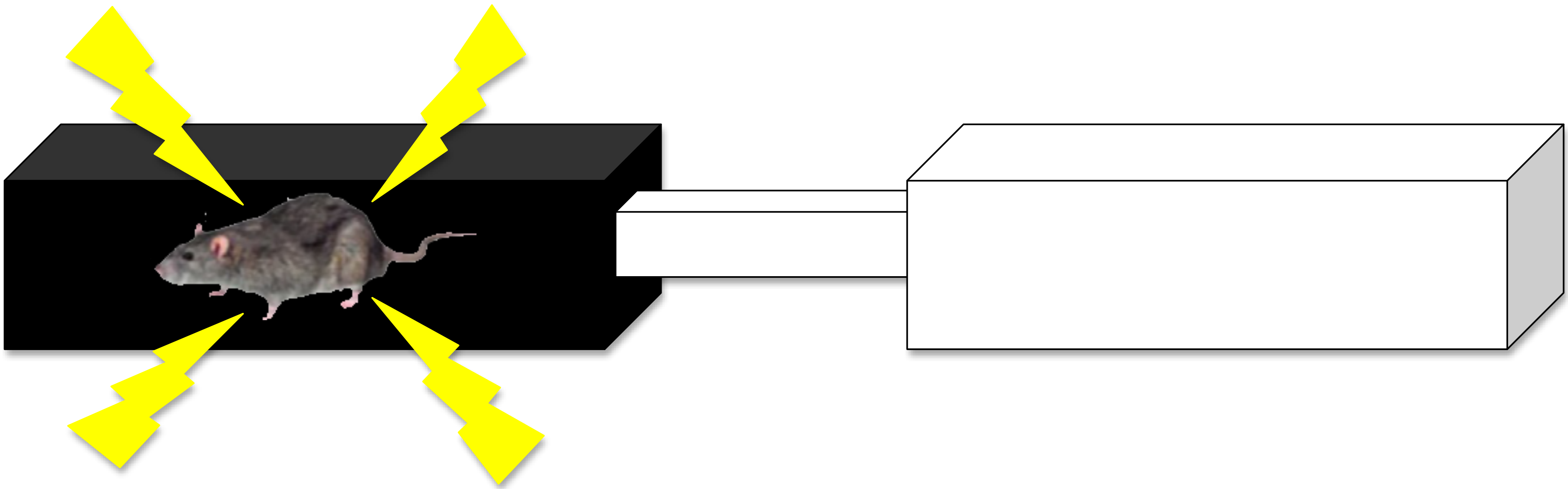

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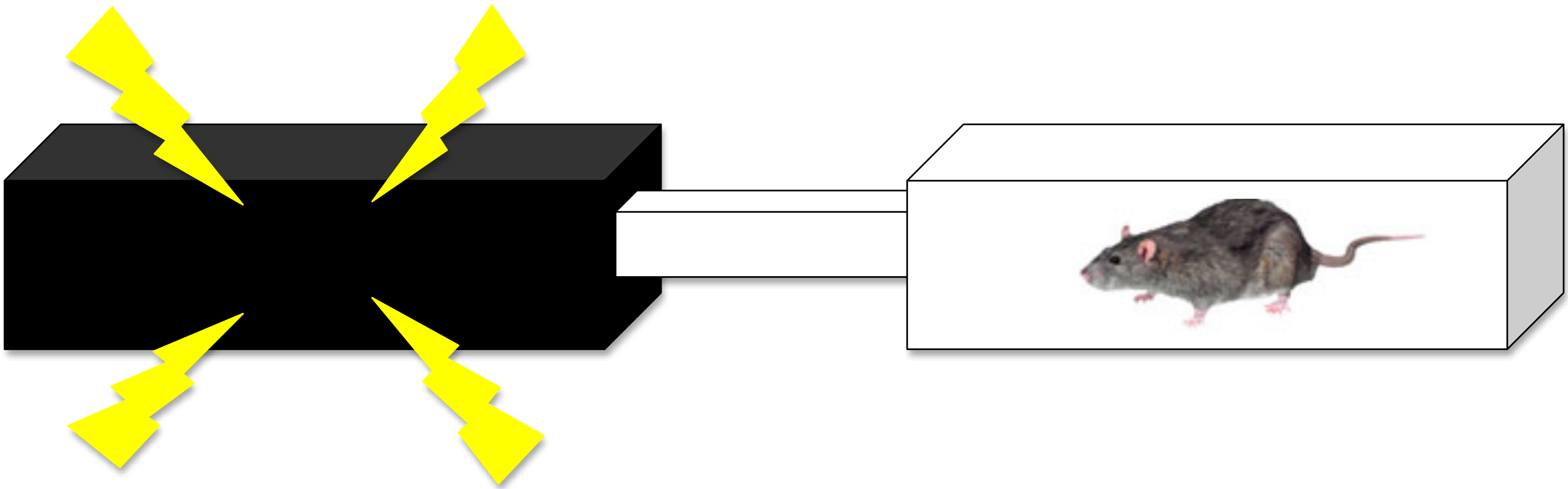

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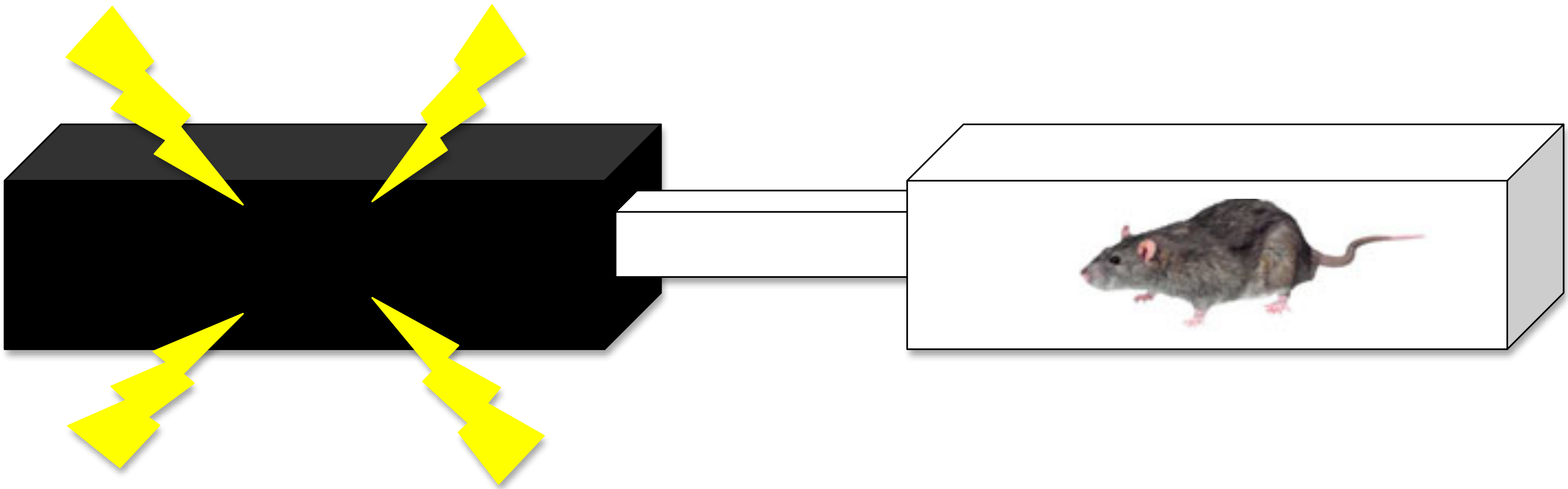
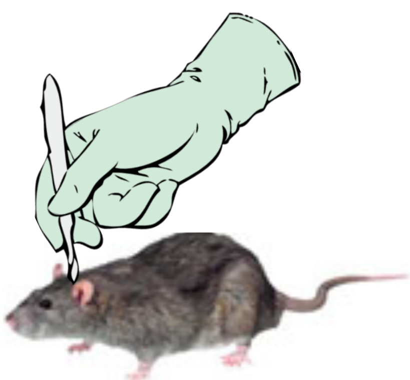
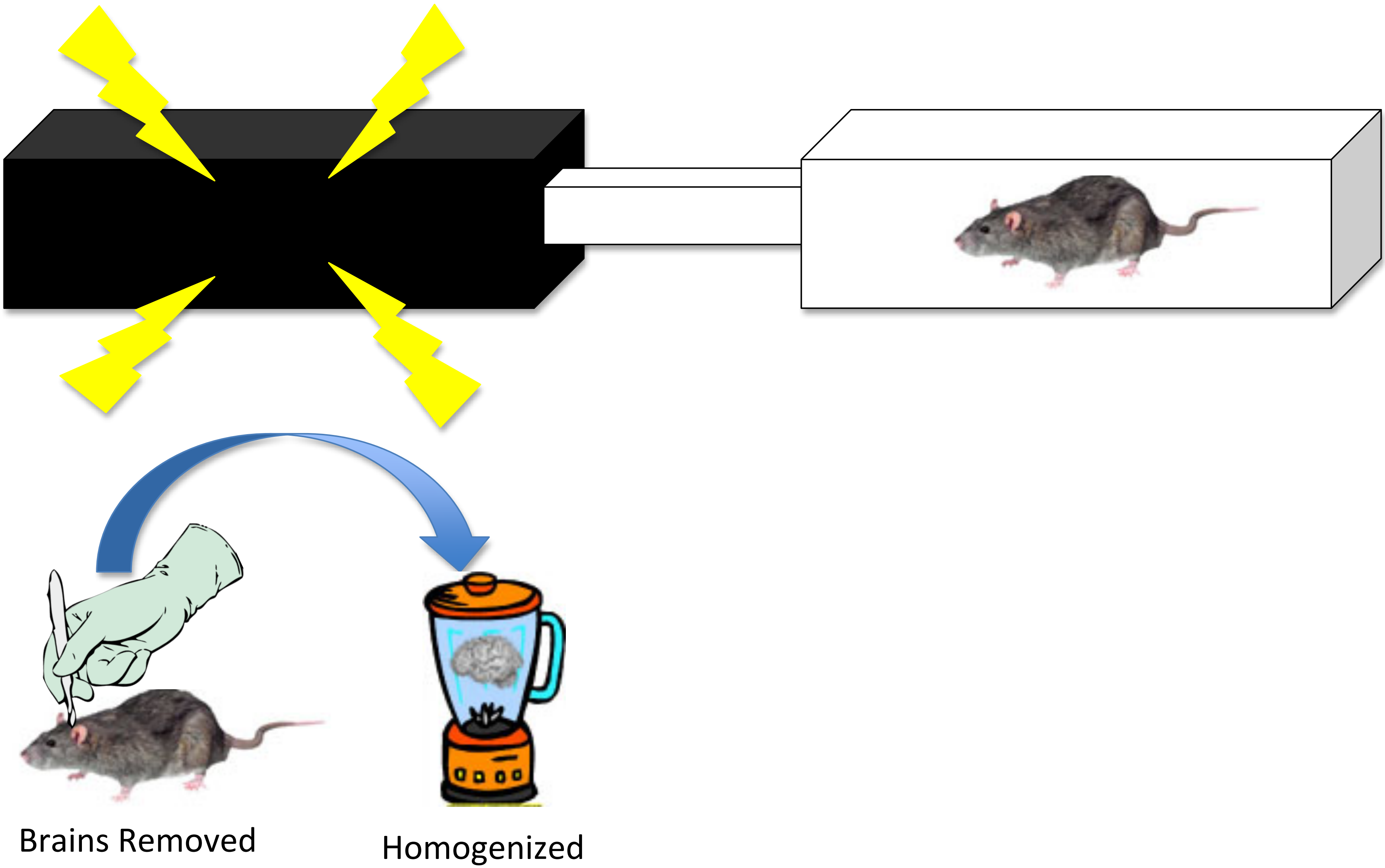




Brains Removed

The Experiment



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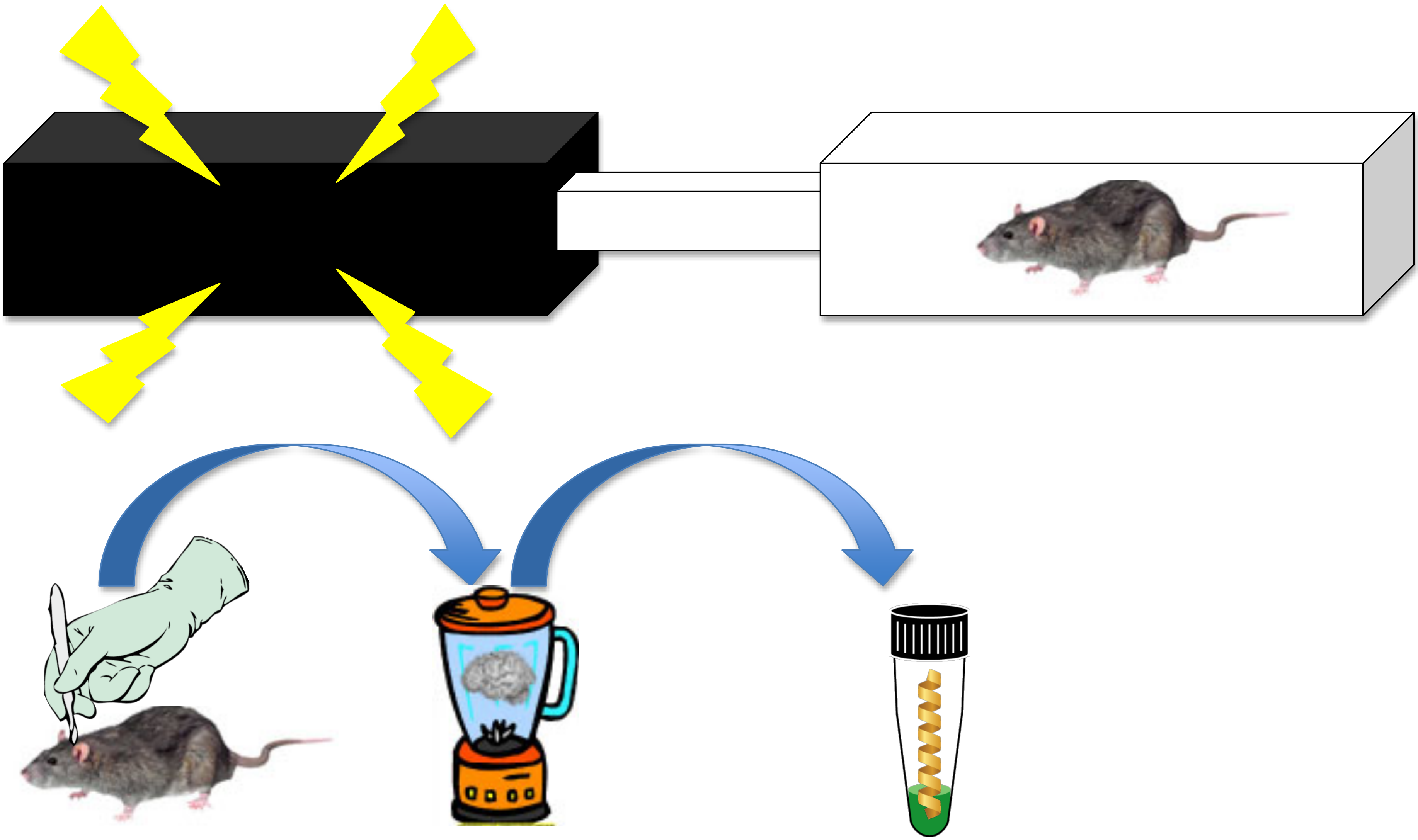

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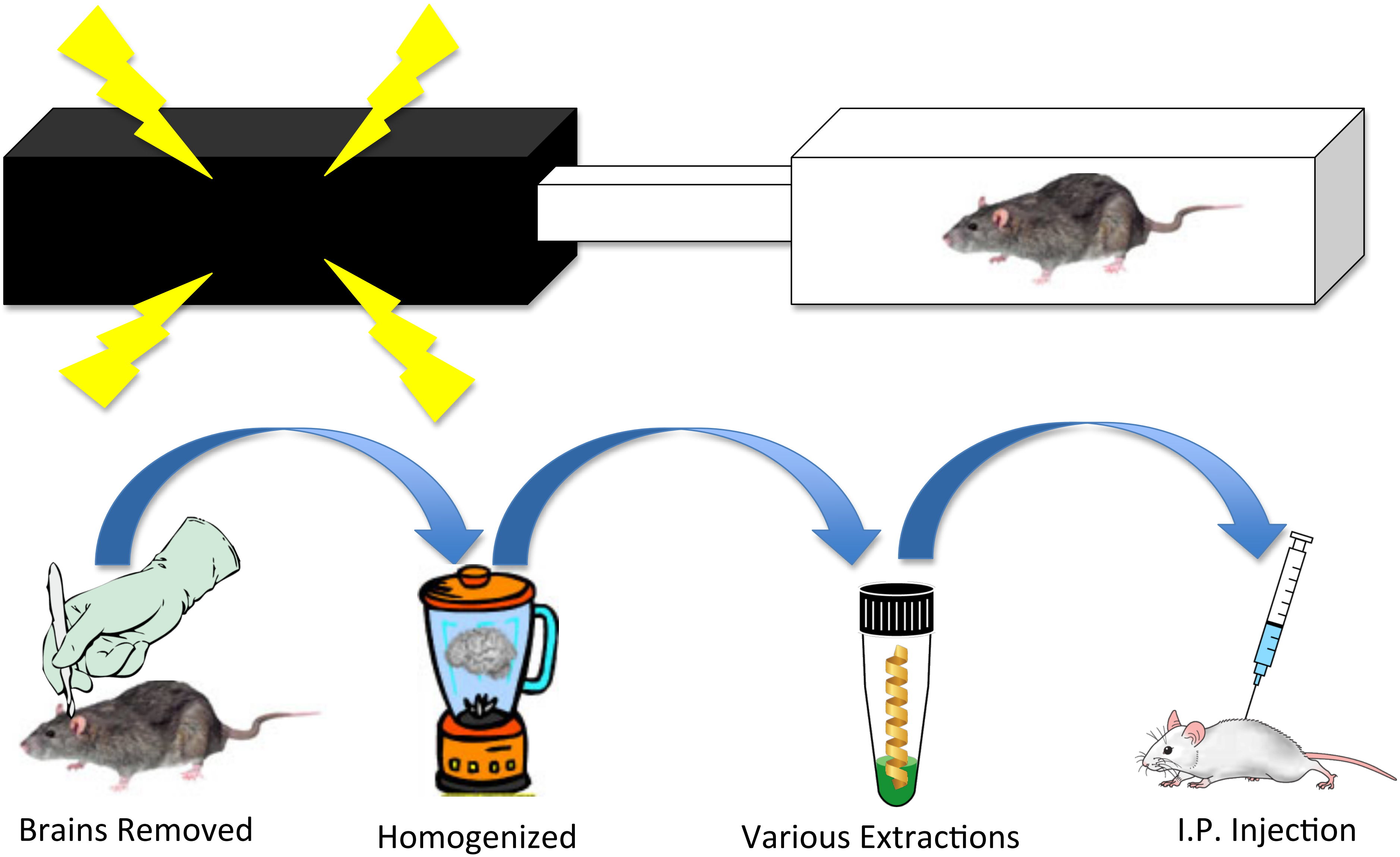
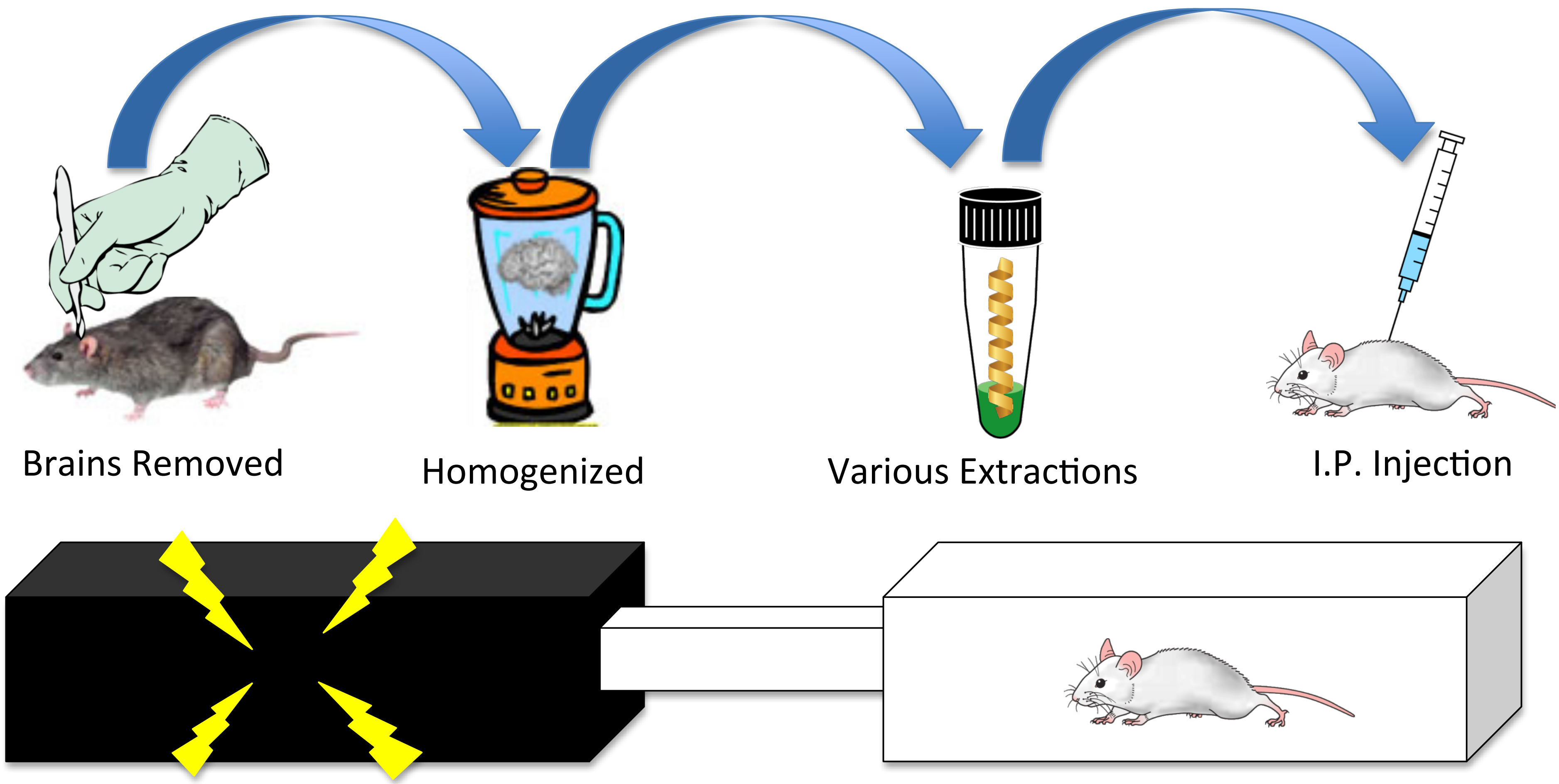


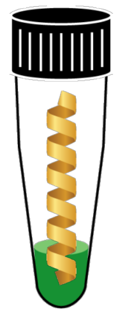
The Experiment



Mice receiving extracts from trained rats spent significantly less time in the dark box as compared to mice injected with untrained rat brains

The Experiment

Homogenized

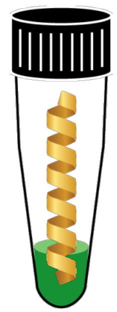


Various Extractions

Active ingredient:
peptide

The Experiment

Homogenized



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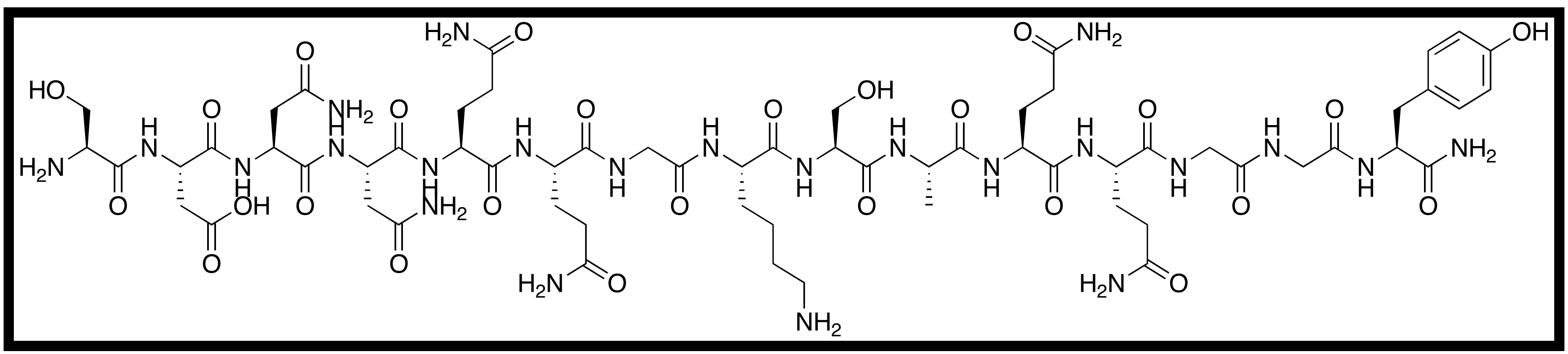
Isolation & Characterization Studies



Scotophobin

- Past work
  - Several isolation and characterization studies led to confirmation of the final pentadecamer structure
  - Unger willingly provided the peptide to a variety of groups for testing with mixed results in animal models
  - Scotophobin has been previously synthesized via traditional organic chemistry utilizing Merrifield's SPPS techniques
  - Last study published 1979, one year after Unger's death

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Our contribution

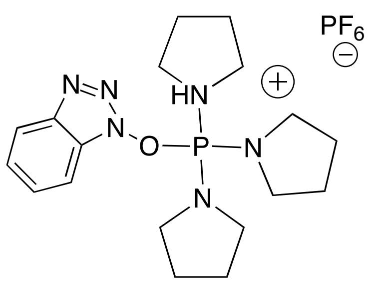
-Since 1979, advancement in peptide synthesis and characterization should allow us to provide a sample of superior quality

-Through collaborations with UNC, we will avoid animal models which have shown problems with reproducibility by screening scotophobin and other neuroactive peptides against an array of GPCRs

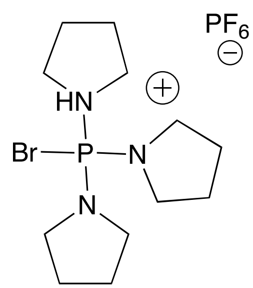
Setlow, B,(1997): George Unger and Memory Transfer *J. His. Nuer.*, 6: 181-192

Optimization

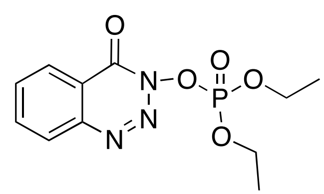
Coupling Agent	Additive	Mass of Product	Overall Yield (%)
PyBop	HOBt	5 mg	3 %
PyBrop	HOBt	15 mg	10 %
DEPBT	None	39 mg	25 %



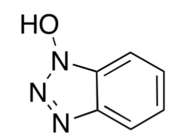
PyBop



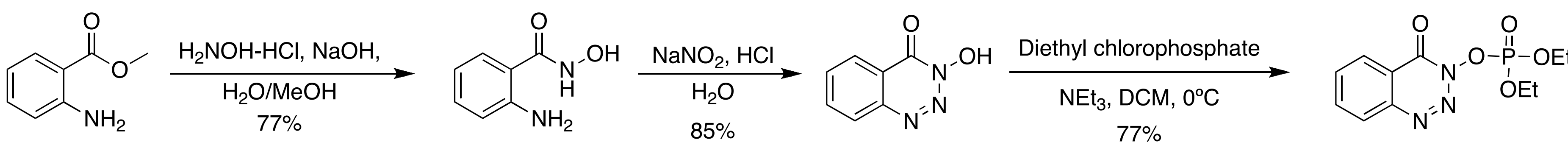
PyBrop



DEPBT



HOBt

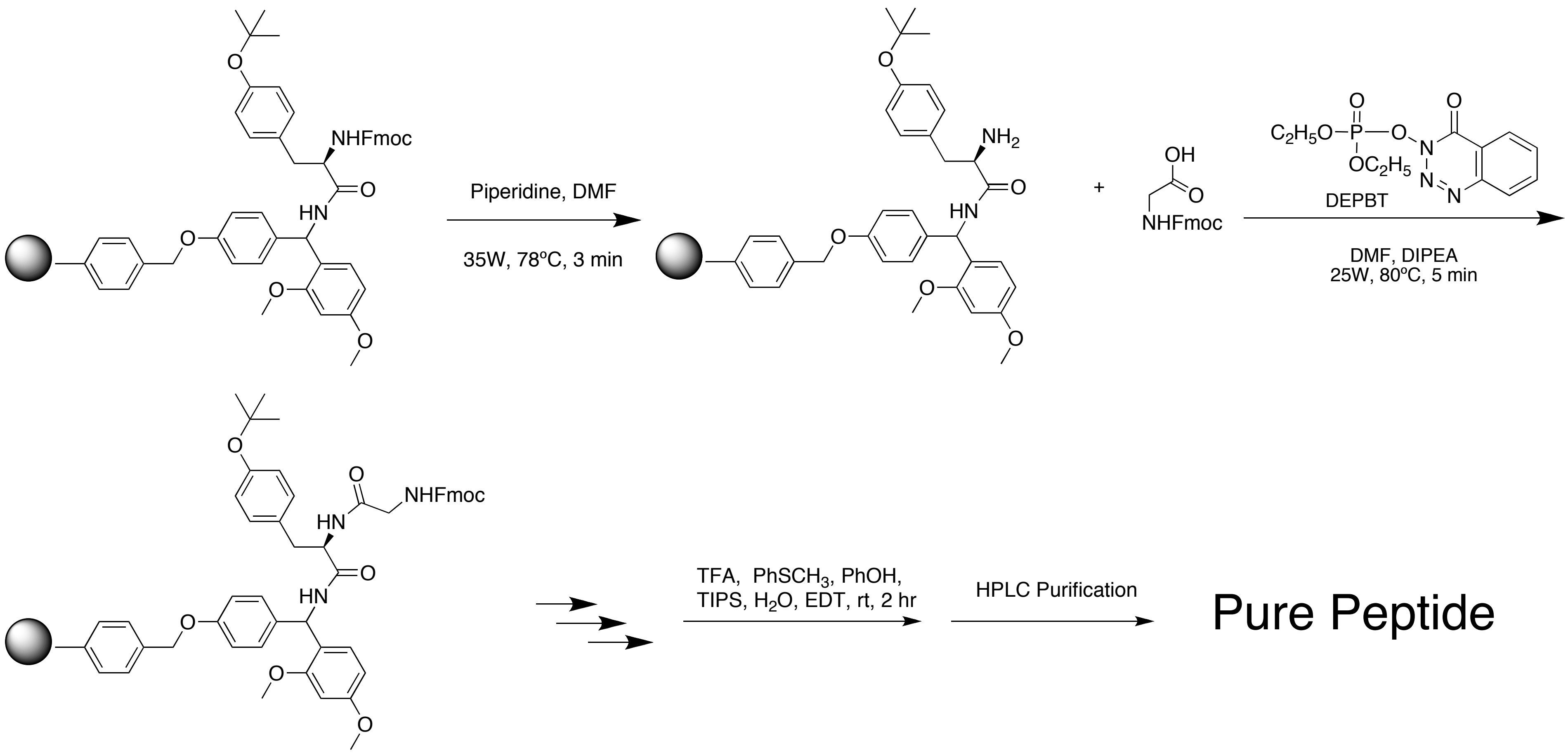


Optimization

Cocktail:	Overall	TFA	$PhSCH_3$	$PhOCH_3$	1,2-EDT	PhOH	H_2O	DMS	NH_4I	TIS
Reagent R	3.4%	90	5	2	3	-	-	-	-	-
Reagent K	2.6%	82.5	5	-	2.5	5	5	-	-	-
Reagent H	3.3%	82	5	-	2.5	5	3	2	1.5	-
Reagent *	23%	89.	3	-	-	2.5	0.5	-	-	4
Reagent J*	22%	87.5	3.5	-	2	3.5	2	-	-	1

Reagent A corresponds to the cocktail developed by Dr. Banerjee, Reagent J corresponds to the cocktail optimized by myself. TFA (trifluoroacetic acid), 1,2-EDT (1,2-dithioethane), DMS (dimethyl sulfide), TIS (triisopropyl silane)

Final SPPS Conditions



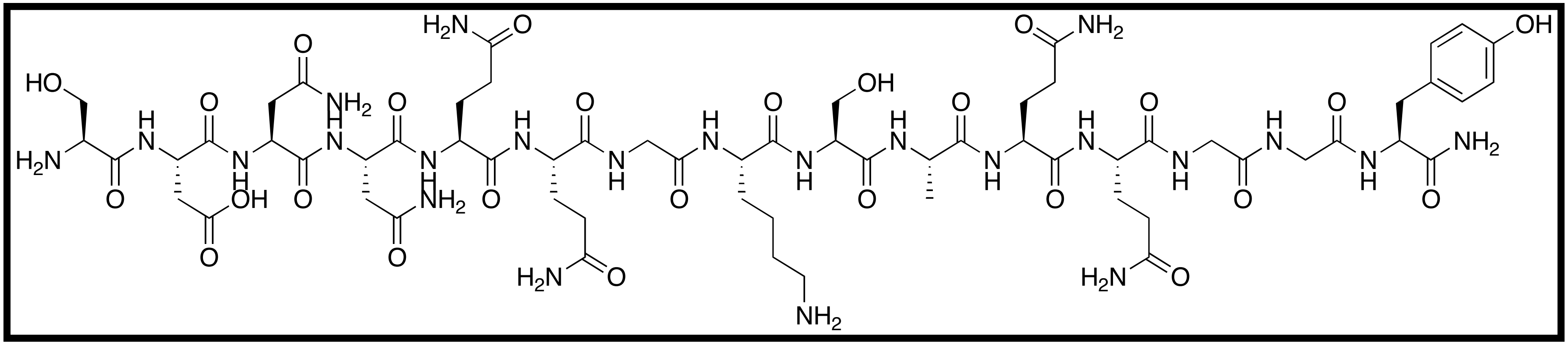
Initial Conditions:

Coupling: Amino acid, PyBOP, HOBt, DIPEA, in DMF (40W, 70ºC, 5 min)

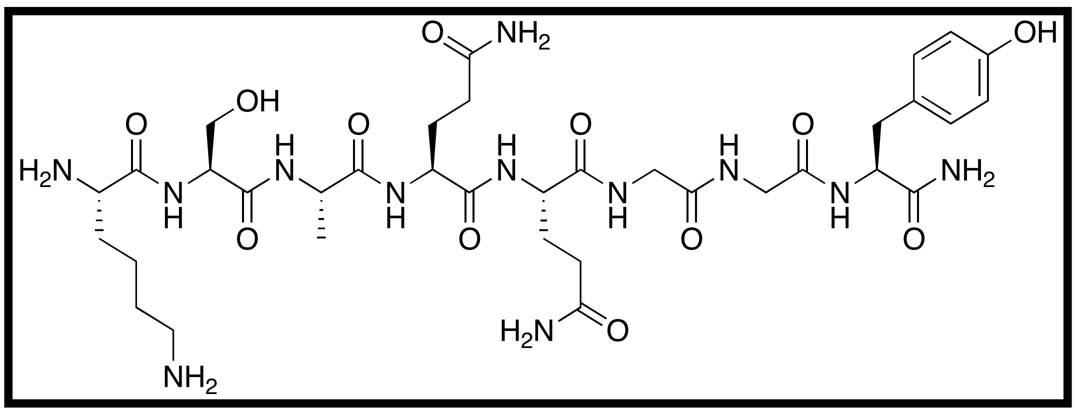
Deprotection: 20% piperidine, HOBt, in DMF (50W, 50ºC, 3 min)

Cleavage: TFA, $PhSCH_3$, PhOH, TIPS, H_2O

Results

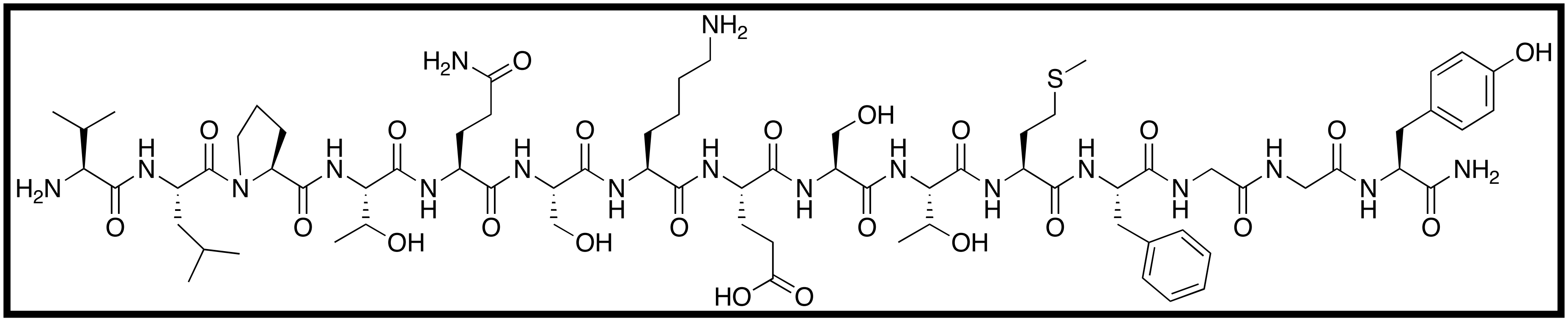


Scotophobin (34 mg, 22% Yield) was successfully synthesized
as confirmed by ^{1}H, ^{13}C, DEPT135, COSY, HMBC, HMQC, and Edmann degradation

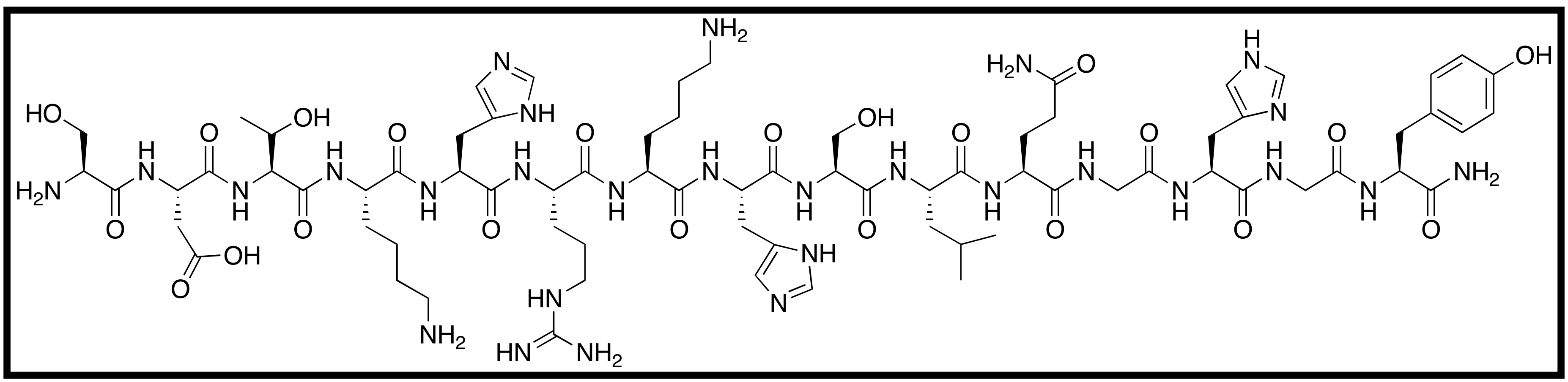


Scotophobin 8-15 analogue (46.5 mg, 55% Yield) was successfully synthesized
as confirmed by ^{1}H, ^{13}C, DEPT135, HMBC, HMQC, and COSY

Results



B-lipotropin (19.1 mg, 11% Yield) was successfully synthesized
as confirmed by ^{1}H, ^{13}C, DEPT135, HMBC, HMQC, and COSY



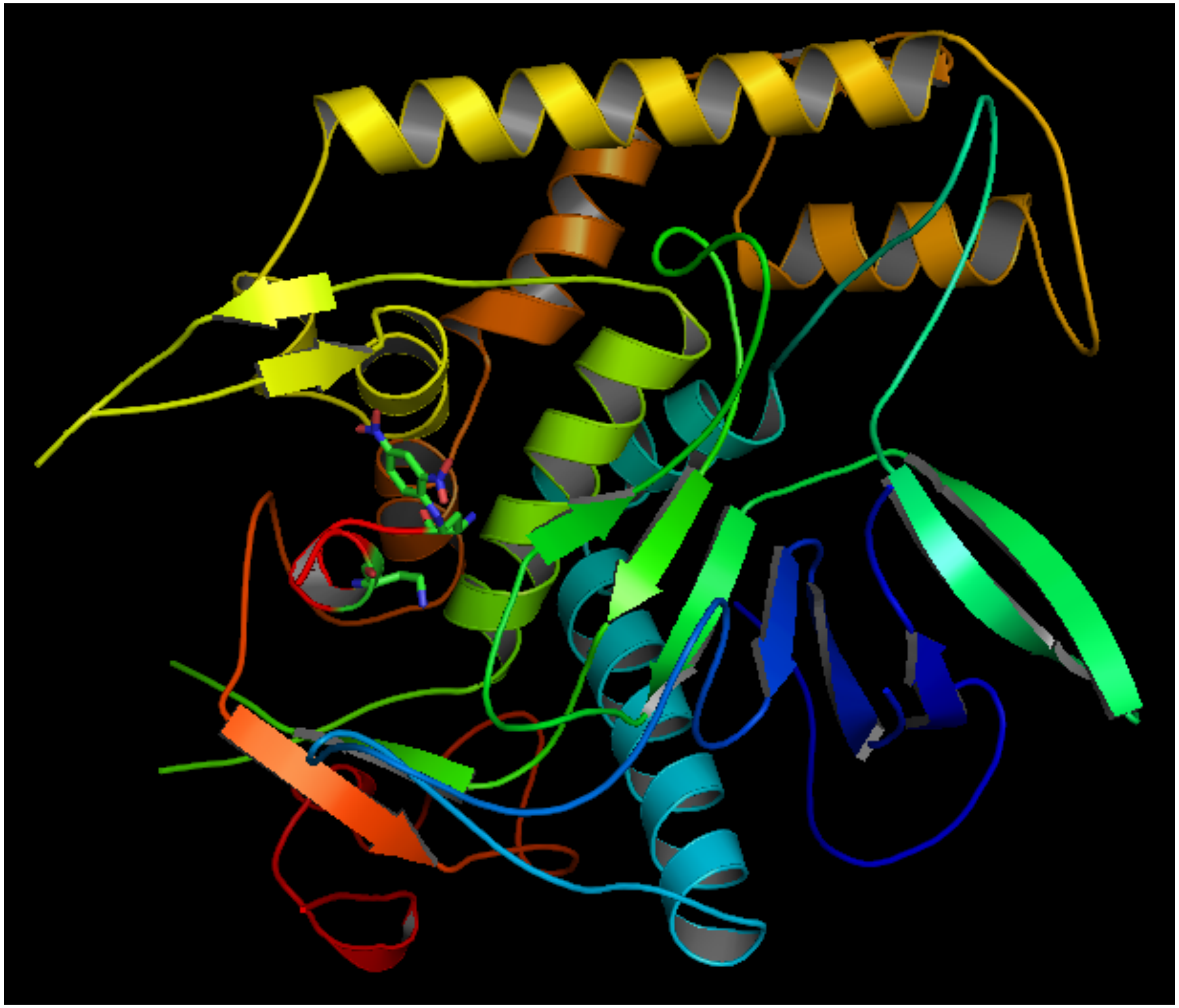
Substance P Precursor (14 mg, 9% Yield) was successfully synthesized
as confirmed by ^{1}H, ^{13}C, DEPT135, HMBC, HMQC, and COSY

Future Work

We are currently awaiting the results of the full GPCR screen before deciding on a future direction of this project

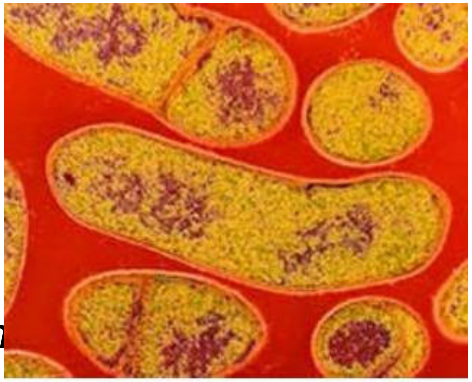
Botulinum Neurotoxin

Design and Synthesis of Peptidic Inhibitor



Botulinum Neurotoxin (BoNT)

a neurotoxic protein


Clostridium botulinum

•First described in the 1820's as a "sausage poison," botulinum is derived from the latin word botulus meaning sausage

•1895 Emile van Ermengem first isolated the bacterium

•BoNT's are produced by an obligate anaerobe of the genus *Clostridium*

•Clostridium botulinum is a soil bacterium whose spores can survive a variety of climates and is found all over the world

•Seven different types identified: BoNT (A-G), with BoNT A being the most toxic

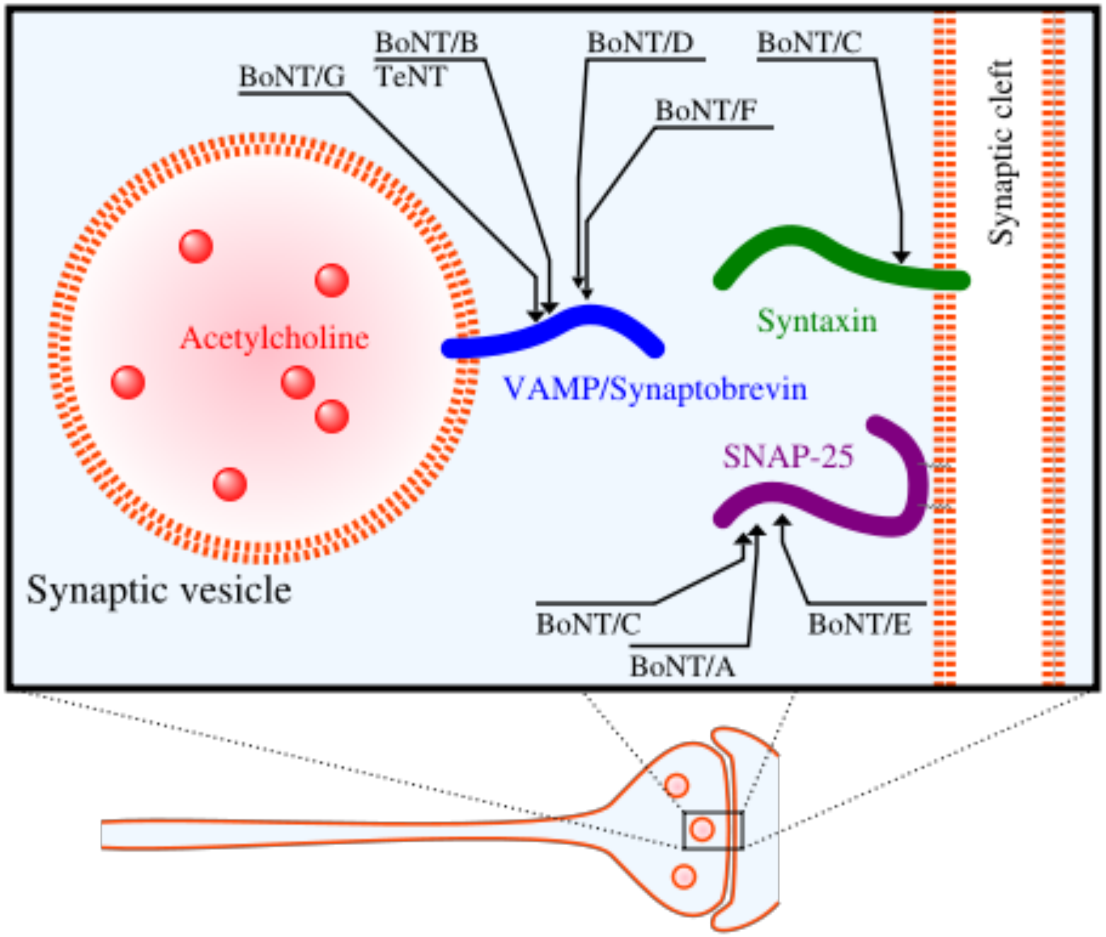
•BoNT's are one of the most poisonous naturally occurring substances, with mouse LD_{50} values in the range of 1–5 ng/kg

•Making this readily available, very potent potential bio-warfare agent, with **no post-exposure intervention available**

•BoNT's are composed of two chains: a 100-kDa heavy chain joined by a disulfide bond to a 50-kDa light chain

Montecucco C, Molgó J (2005). "Botulinal neurotoxins: revival of an old killer". Current opinion in pharmacology 5 (3): 274–9.

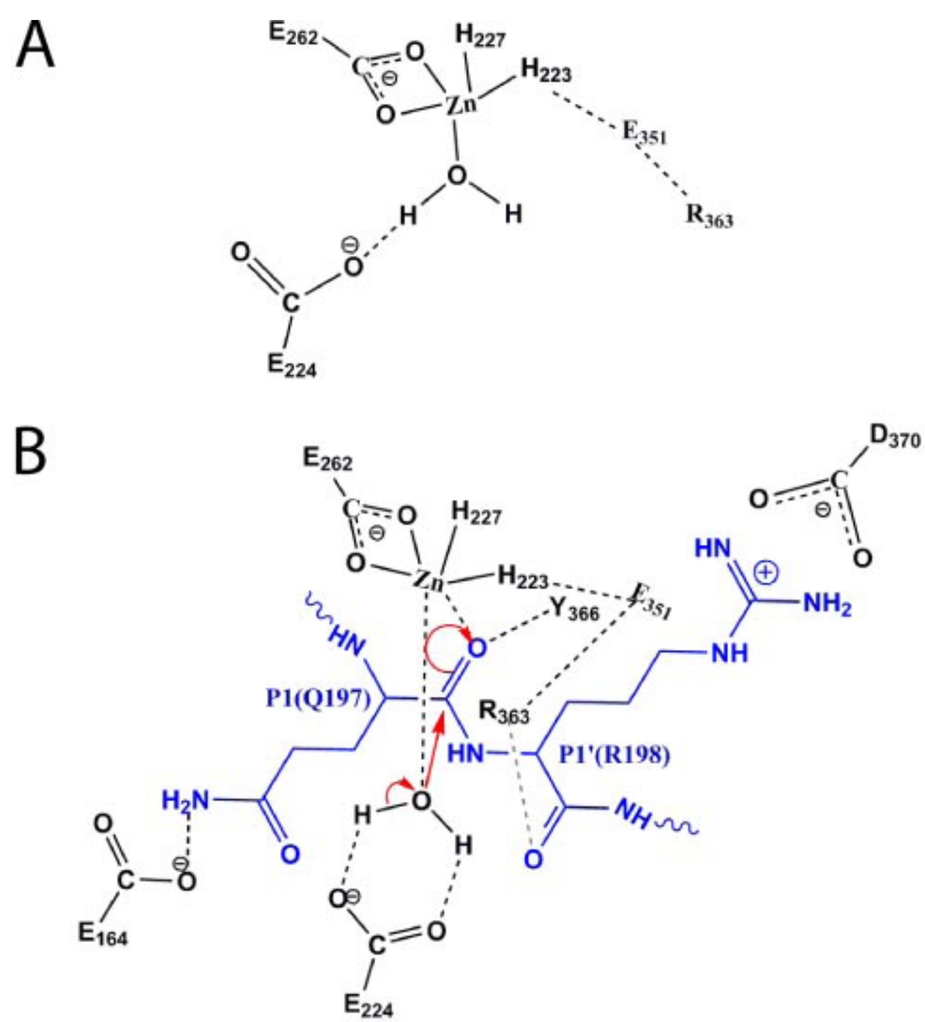
Mode of Action

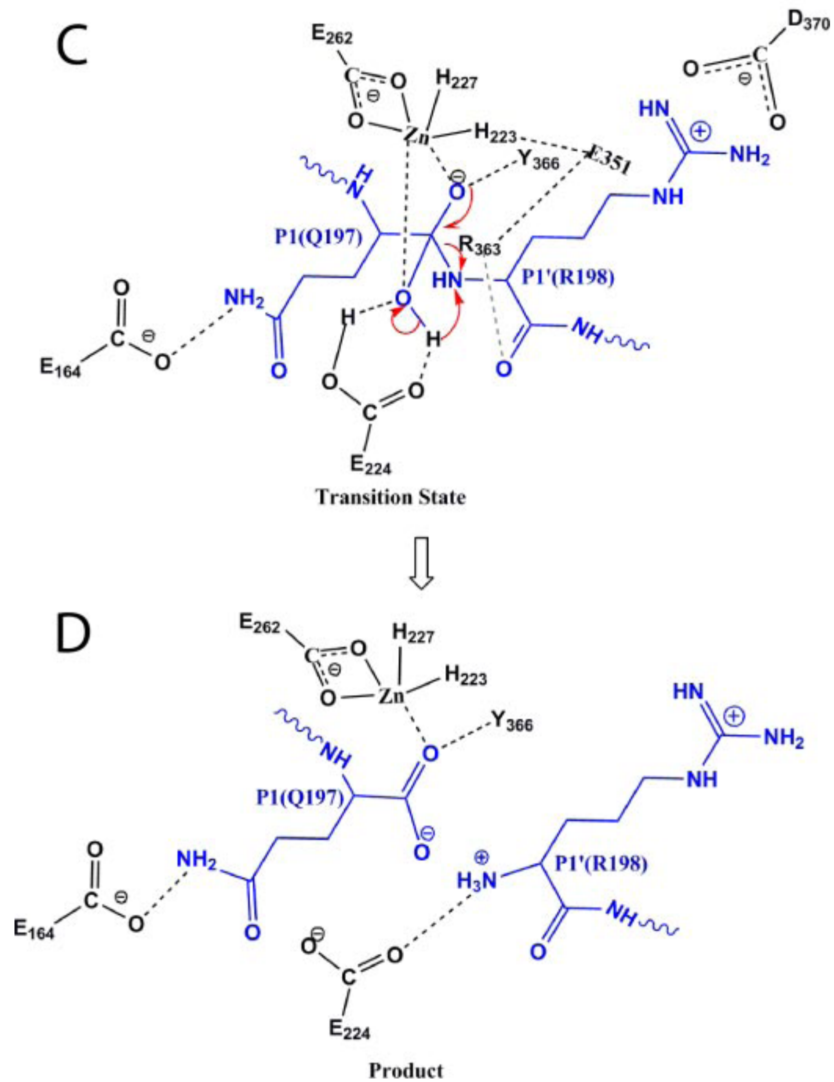


- Proteolytically cleaves SNAP-25, syntaxin or synaptobrevin at a neuromuscular junction, preventing vesicles from anchoring to the membrane to release acetylcholine
- By inhibiting acetylcholine release, the toxin interferes with nerve impulses and causes flaccid (sagging) paralysis of muscles

http://en.wikipedia.org/wiki/Botulinum_toxin

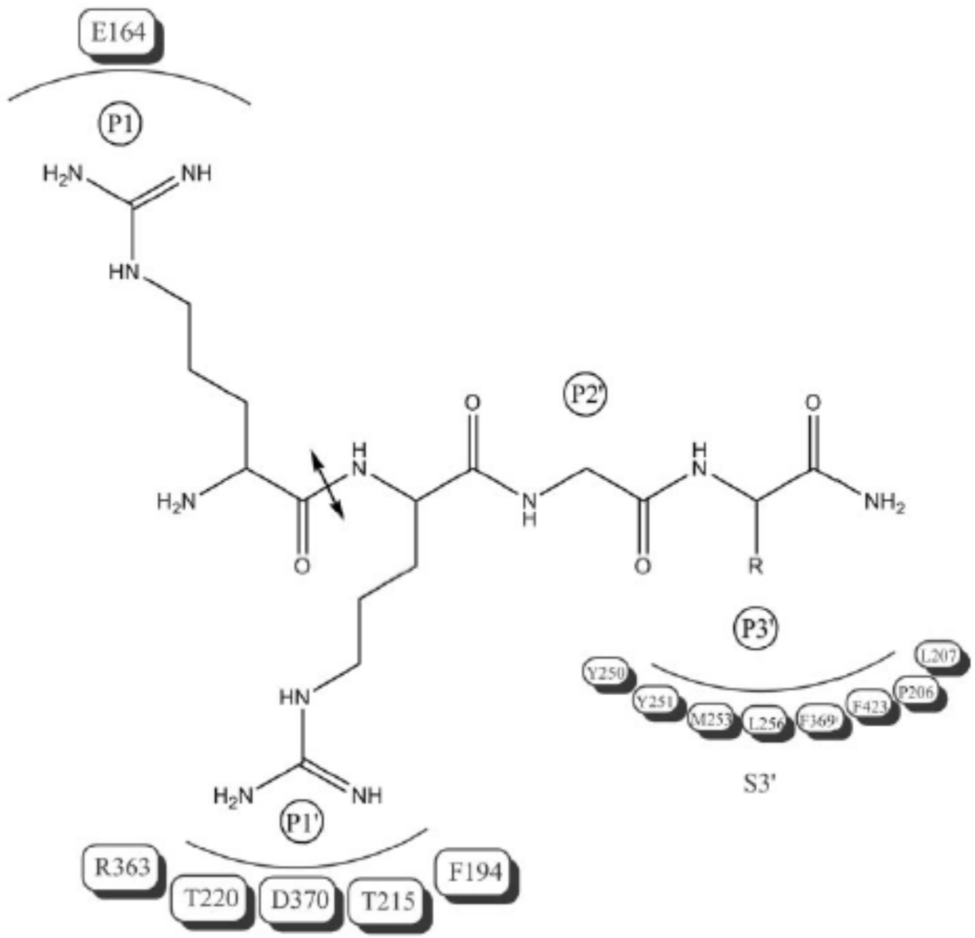
Proposed Mechanism of Action





Kumaran et al., J. Biol. Chem., 283 (2008), 27, 18883-18891

Inhibitor Design

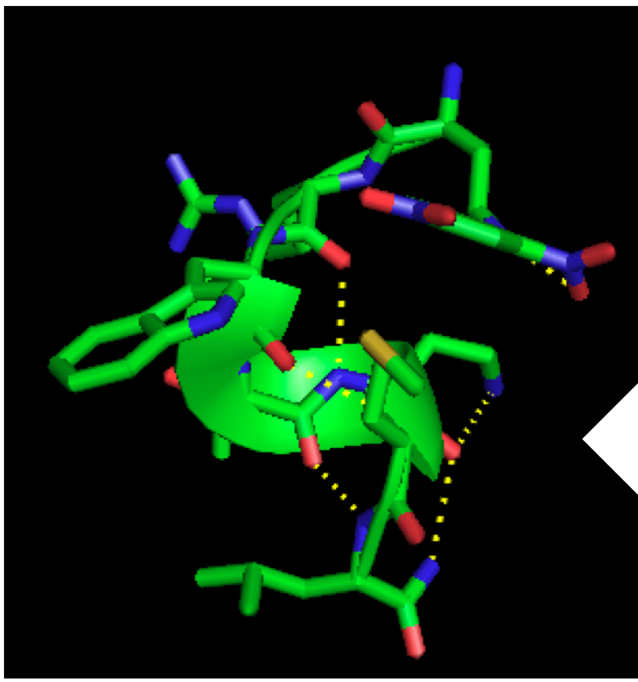


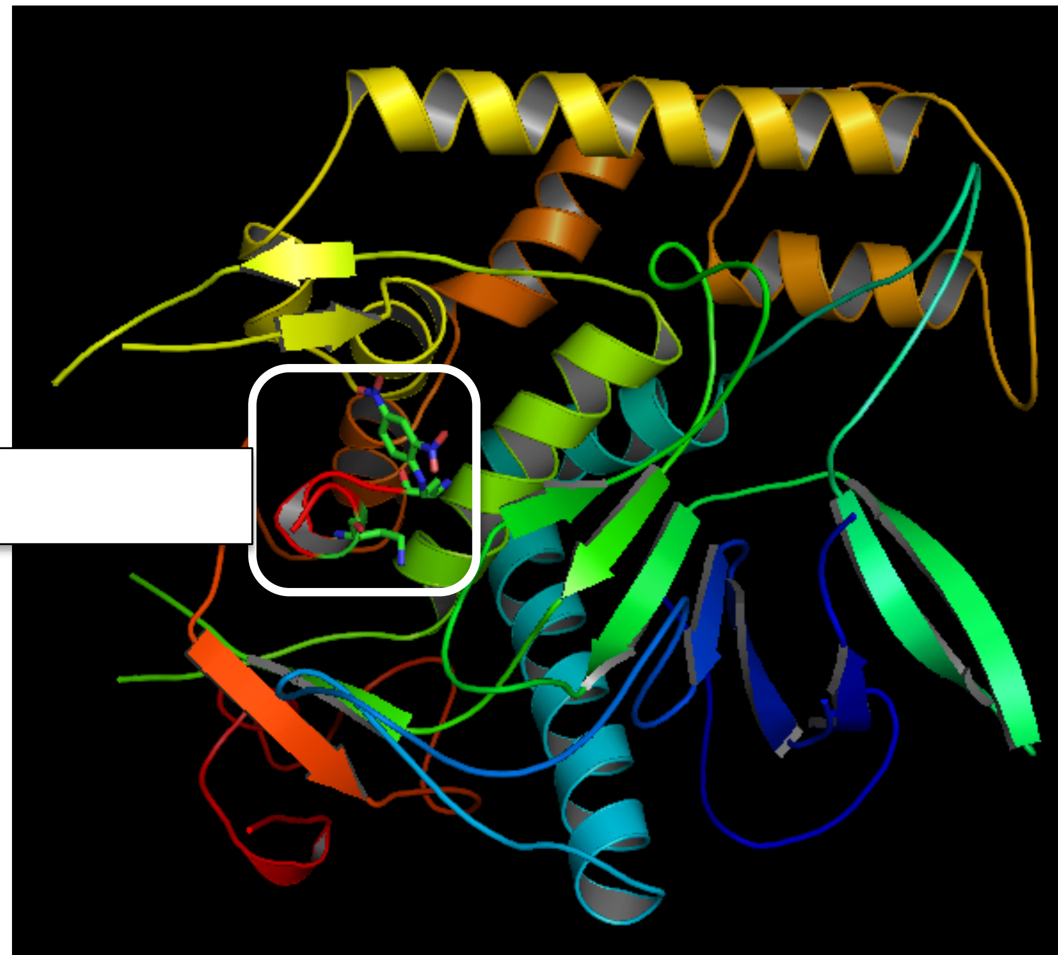
In addition, Desigan Kumaran et al., found that tetramers containing RR have a high binding affinity

Kumaran et al., J. Biol. Chem., 283 (2008), 27, 18883-18891

Inhibitor Design

"If I have seen further, it is by standing on the shoulders of giants."~ Sir Isaac Newton

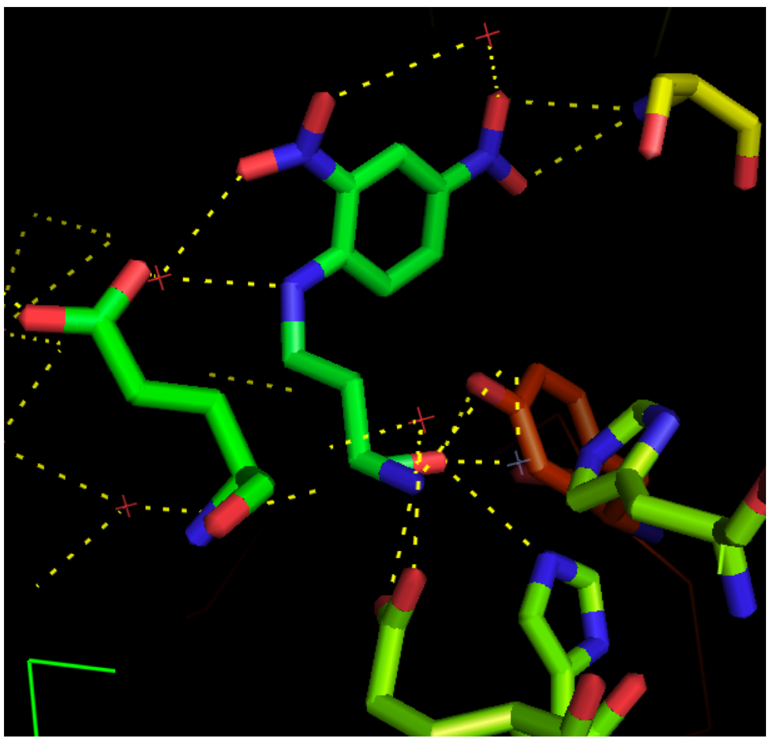


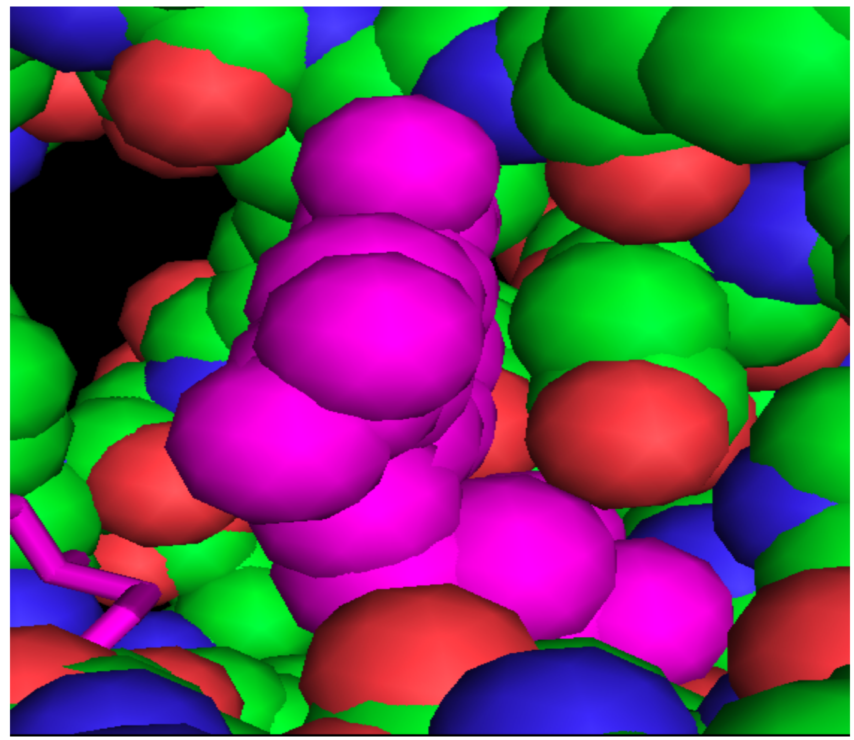


Previous inhibitor exists as 3_{10} Helix

Interactions of Dab(DNP)

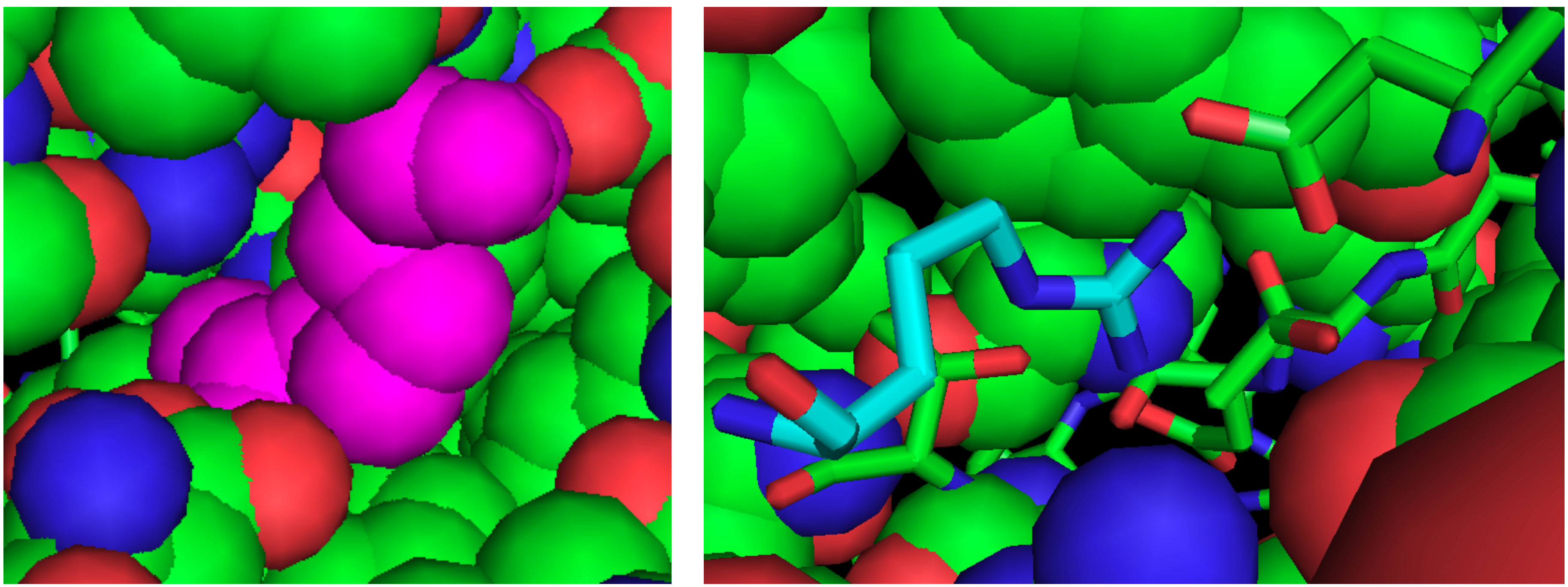
- H-bonding interactions through water with Ser259, GLu164, His227, Tyr366, Glu224, His223
- Moderately favorable steric interactions with binding pocket





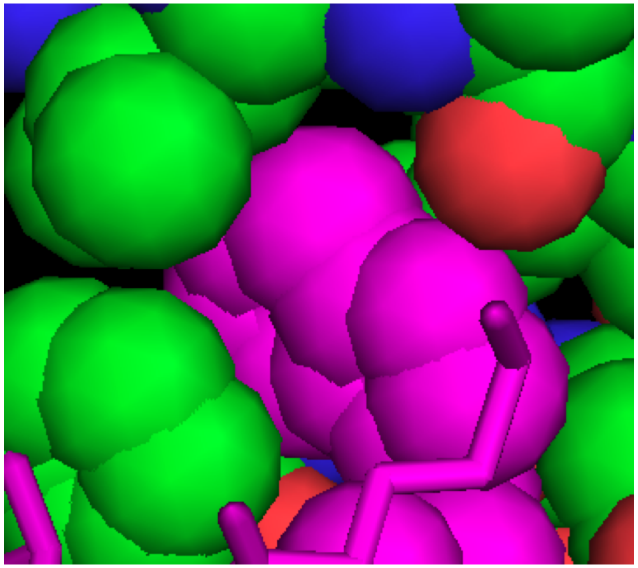
Interactions of Arginine

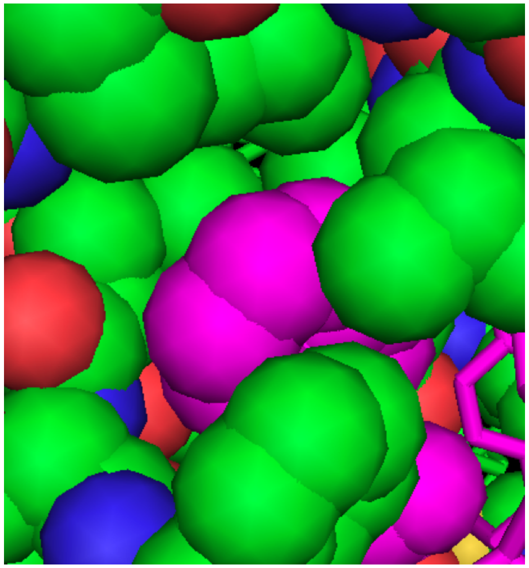
- Potential electrostatic interactions with Thr220, Thr215, Asp216, Asp37
- Favorable steric interactions with binding pocket

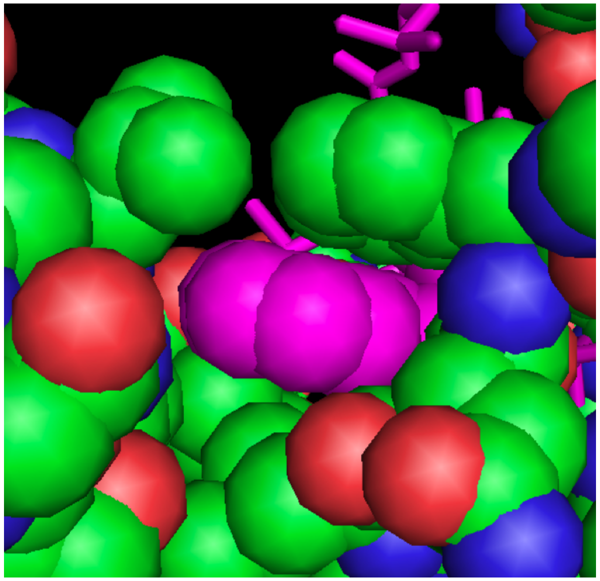


Interactions of Tryptophan

- Fits perfectly in binding pocket, locking inhibitor in binding site
- An aromatic residue is commonly found directly preceding the N-terminus of a 3_{10} helix

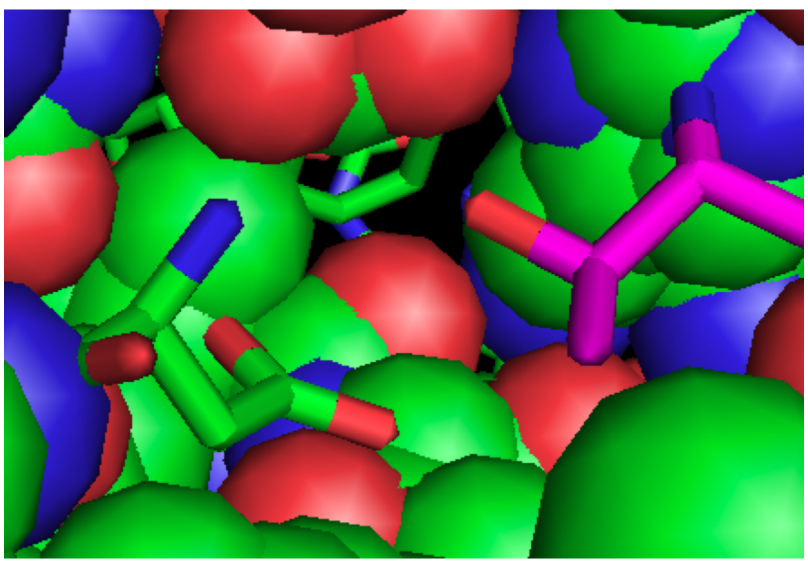


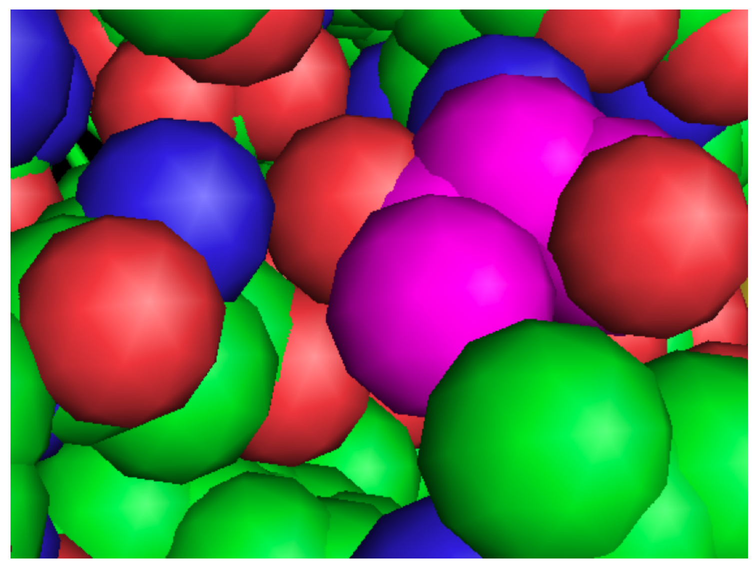


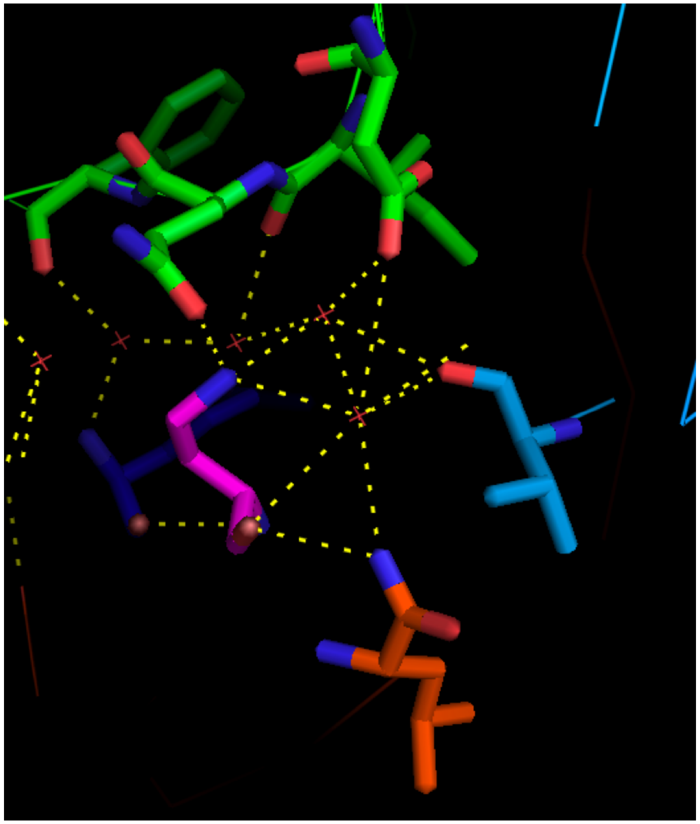


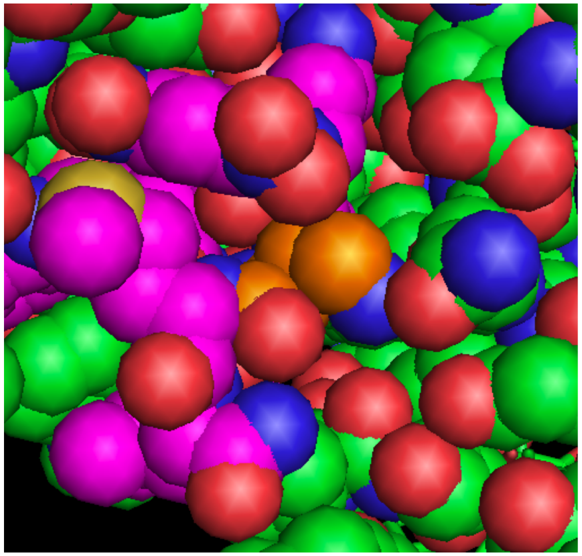
Interactions of Threonine

- Favorable electrostatic interaction of the backbone carbonyl of ASP307 with free hydroxy
- Negative interaction of free hydroxy with side chain of ASP307
- Ala is more commonly found in this position of a 3_{10} helix than Thr





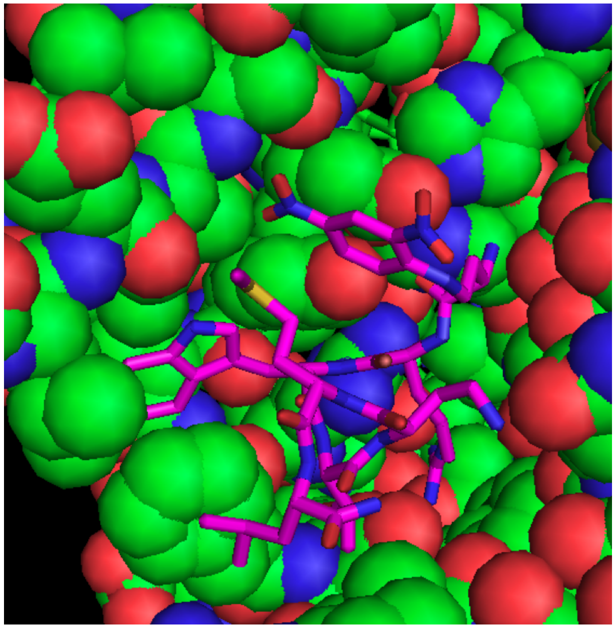


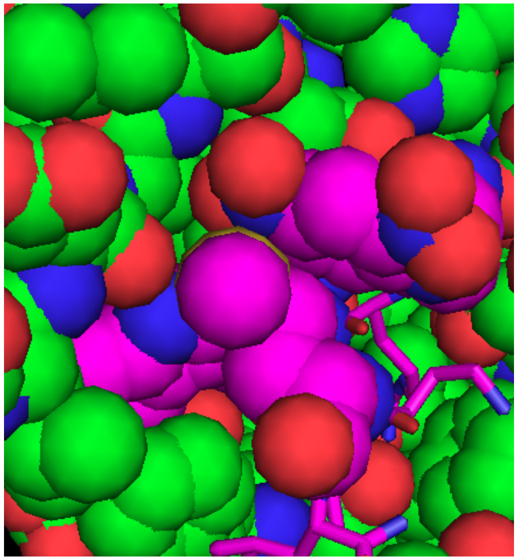


Interactions of DAB

- Direct electrostatic stabilization to Gln163
- Electrostatic interaction deriving from the backbone of Lue203, Val70, Ile161, the side chain of Asp159, and several water molecules
- May be further stabilized by the potential for H-bonding with its own carbonyl
- Good steric fit with Dab(DNP) residue

Interactions of Methionine

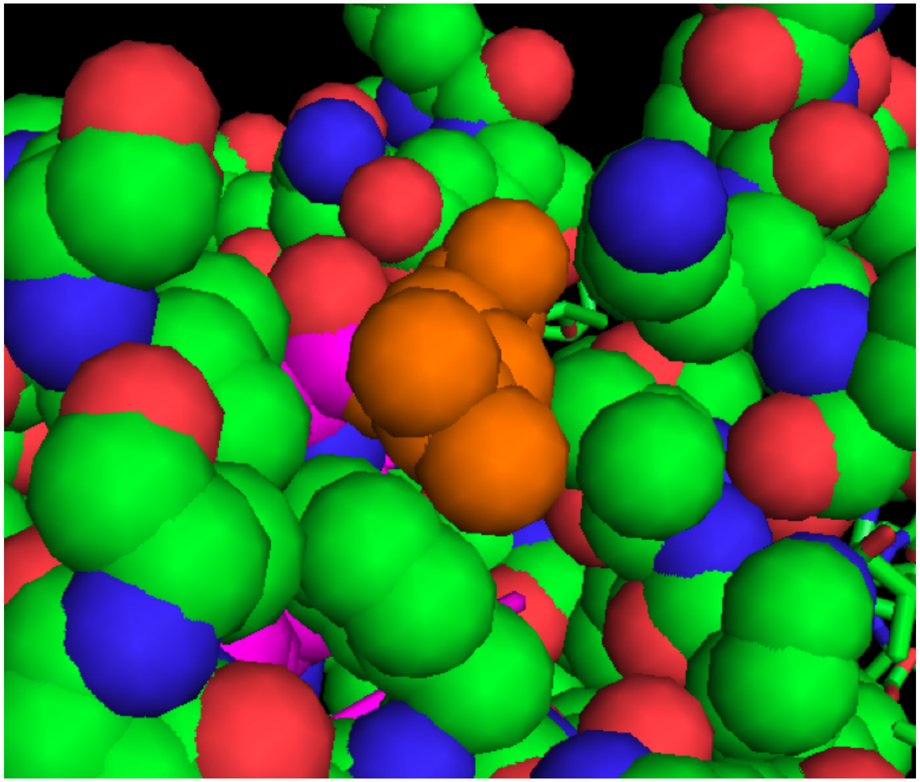




- Potential electrostatic interaction with backbone of Glu257
- Sterically fits in hydrophobic region between the Trp and the Dab(DNP) residues
- Methionine, although usually a helix-promoting residue, is underrepresented in 3_{10} helices and Asp is more commonly found at this residue

Interaction of Leucine

- No direct interactions of side chain
- Leu is generally a helix-promoting residue. It is commonly underrepresented in 3_{10} helices and Ile may be a better substitution



Future directions

- Optimization of reduction
- Testing proline catalyzed Aldol
- Synthesis of more highly functionalized bicyclo[3.3.1]nonane analogues

Aknowledgements

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- Dr. Billy Day
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- The Wipf Group

Solid Phase Peptide Synthesis:

- Dr. Abhisek Banerjee
- Kazi Islam
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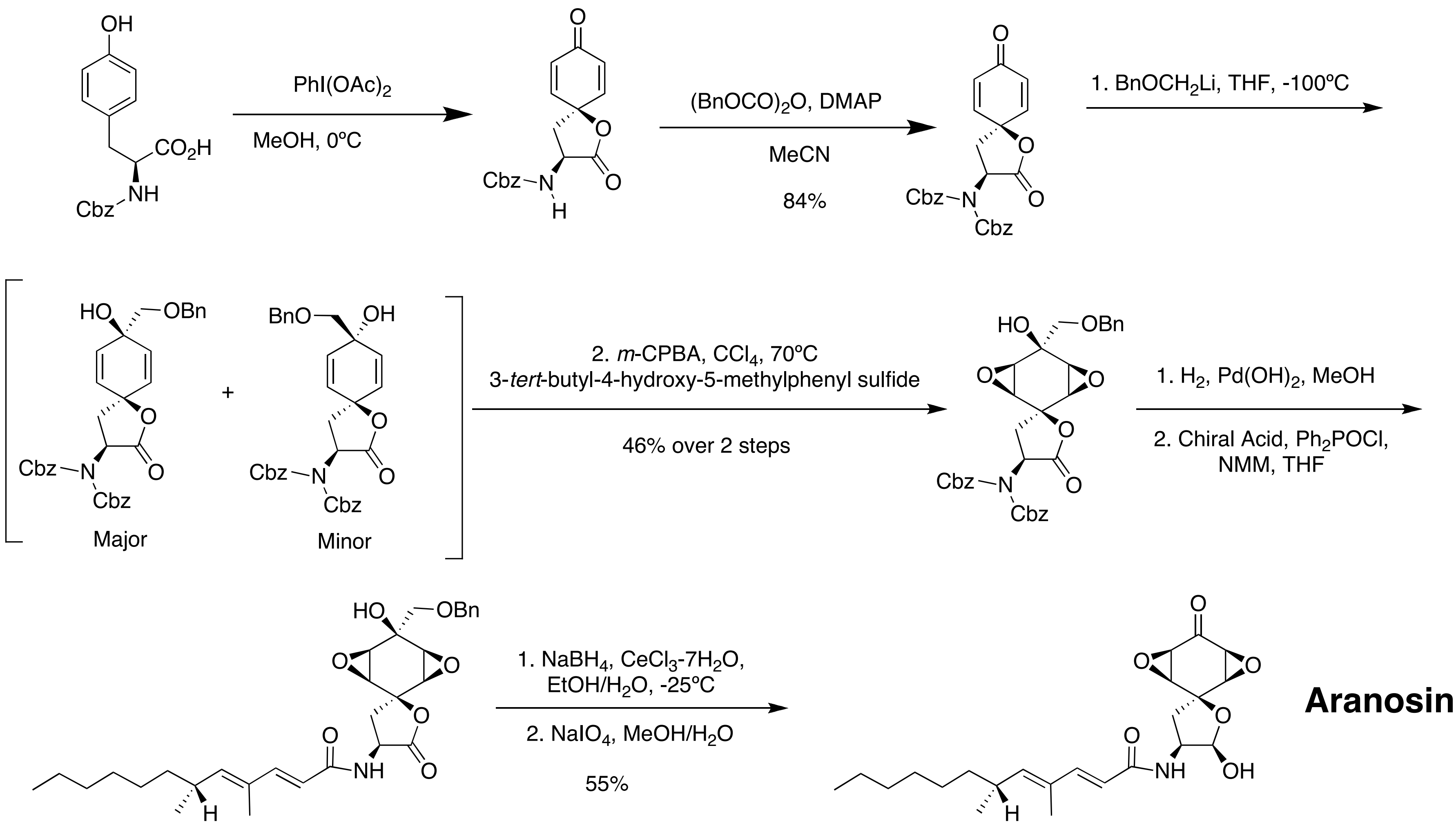
OH
CO_2H
Cbz
NH
$PhI(OAc)_2$
MeOH, 0ºC
O
Cbz
N
H
O
O
$(BnOCO)_2O$, DMAP
MeCN
84%
Cbz
N
Cbz
1. $BnOCH_2Li$, THF, -100ºC
HO
OBn
BnO
OH
Major
Minor
2. *m*-CPBA, CCl_4, 70ºC
3-*tert*-butyl-4-hydroxy-5-methylphenyl sulfide
46% over 2 steps
1. H_2, $Pd(OH)_2$, MeOH
2. Chiral Acid, Ph_2POCl,
NMM, THF
NH
H
1. $NaBH_4$, $CeCl_3$-$7H_2O$,
EtOH/H_2O, -25ºC
2. $NaIO_4$, MeOH/H_2O
55%
Aranosin