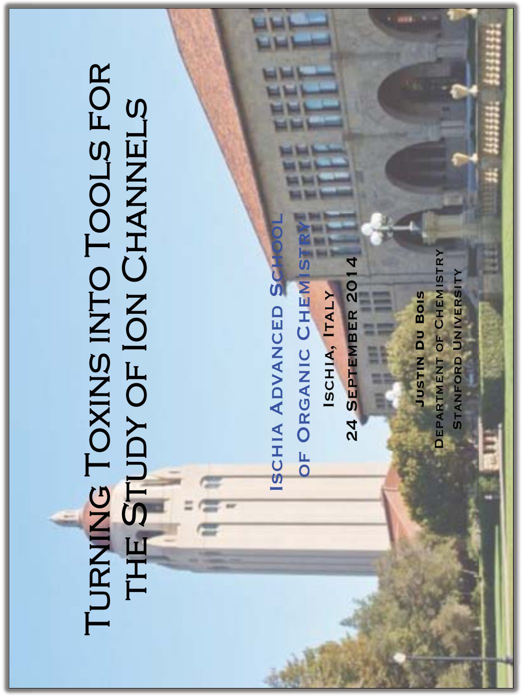
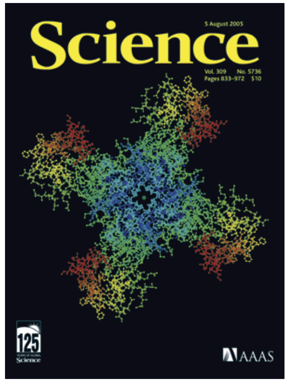
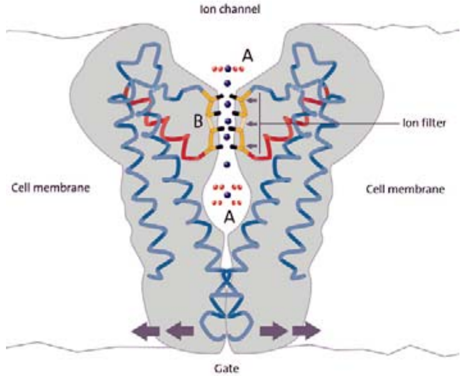


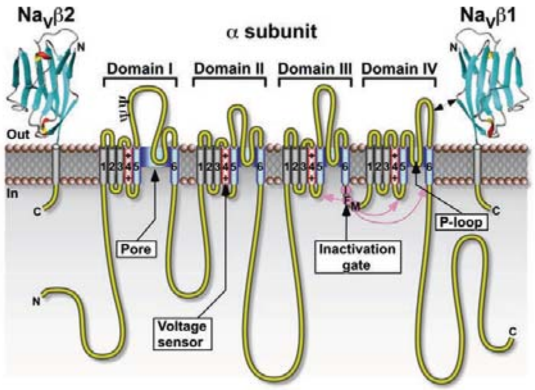
Voltage-Gated Ion Channels: Remarkable Molecular Machines

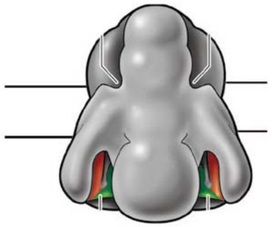




- transmembrane proteins that function to transport charged ions across lipid bilayer
- found in all electrically excitable cells; respond to changes in membrane voltage
- selective for K^+, Na^+, Ca^{2+}, Cl^-; ions pass at rates on the order of 10^7 per second!
- crystallographic analysis of bacterial and mammalian K^+ ion channels

The Voltage-Gated Sodium Channel





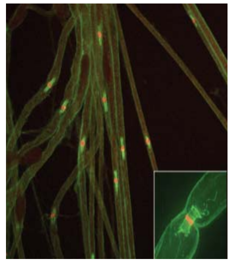
19Å cryo-EM image
Sato *Nature* **2001**

taken from King *Channels* **2008**, *2*, 100

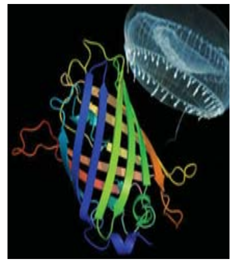
- ~260 kDa α-subunit; functional expression in heterologous vectors
- α-subunit comprised of four repeat domains, each with 6 TM helices
- 10 gene loci encoding α-subunit identified in mammalian cells

Tools of the Channelologist

electrophysiology



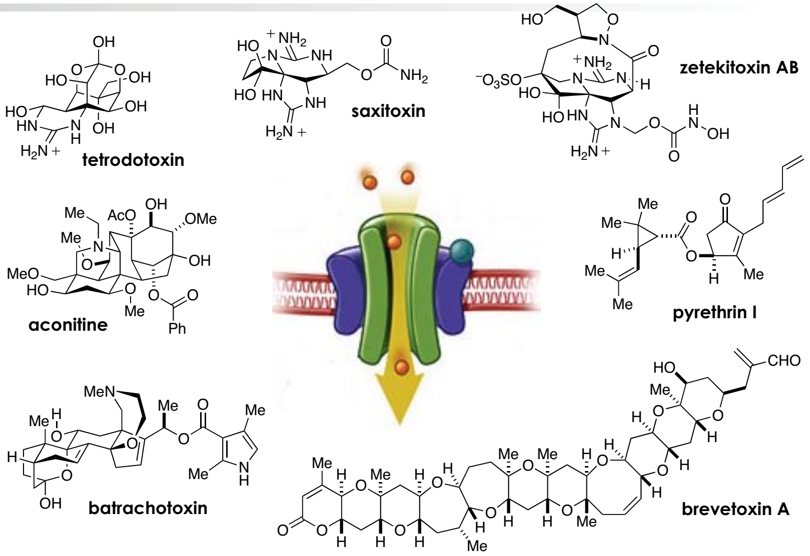
immuno -cytology & histology



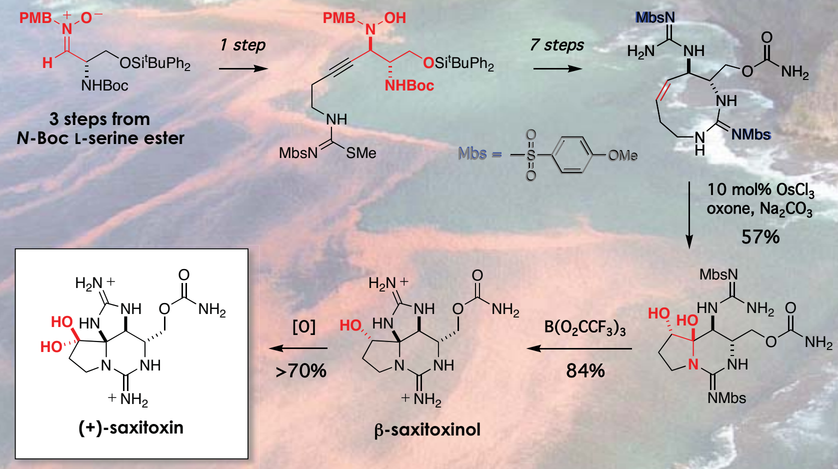
fluorescent protein labeling

fast and dynamic nature of neuronal processes necessitates methods that afford rapid temporal response

Sodium Ion Channel Modulators

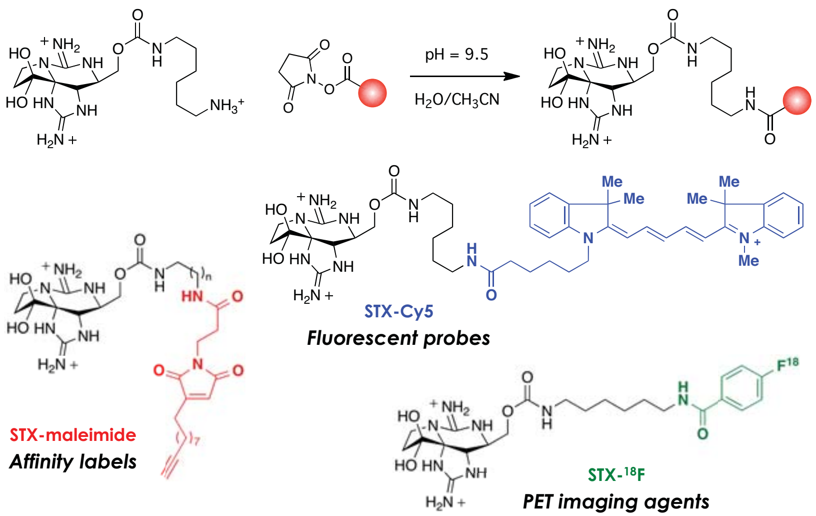


A Total Synthesis of (+)-Saxitoxin – The Cliffs Notes Version

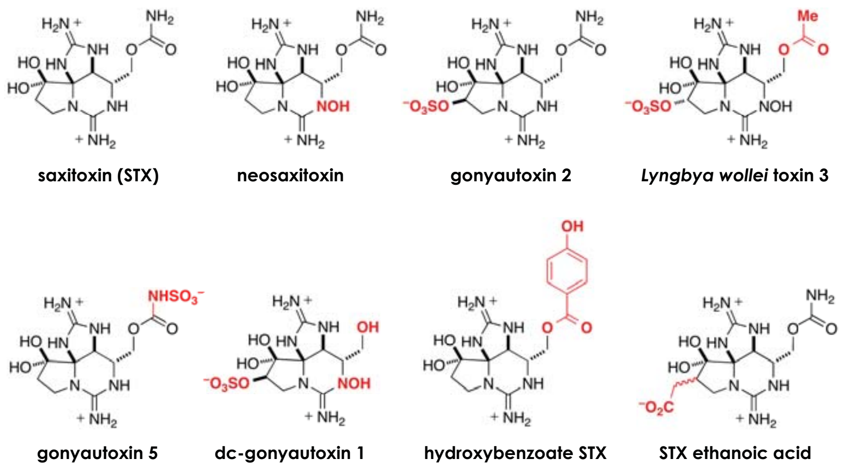


- *desired target assembled in 14 linear steps from commercial material*
- *de novo construction provides access to a unique chemical probe*

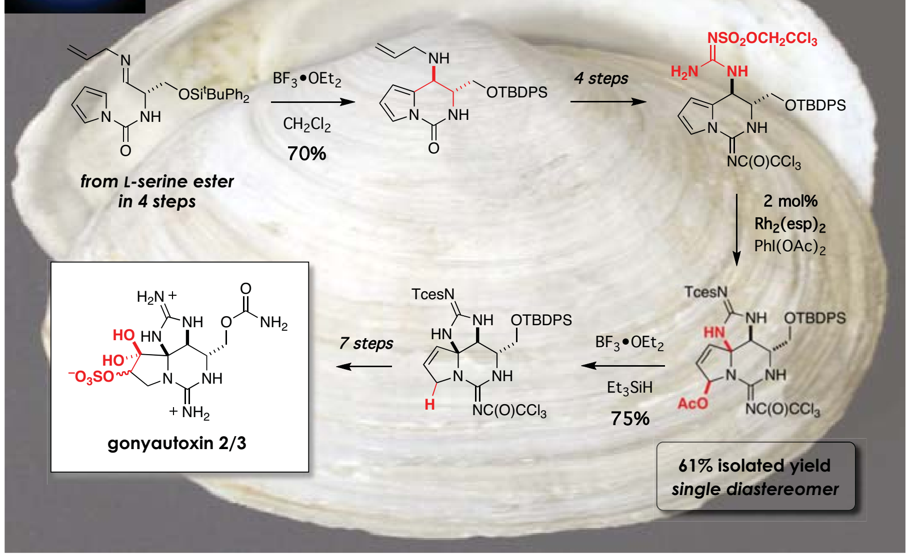
Toxin Conjugates as Molecular Probes of Na_V Function



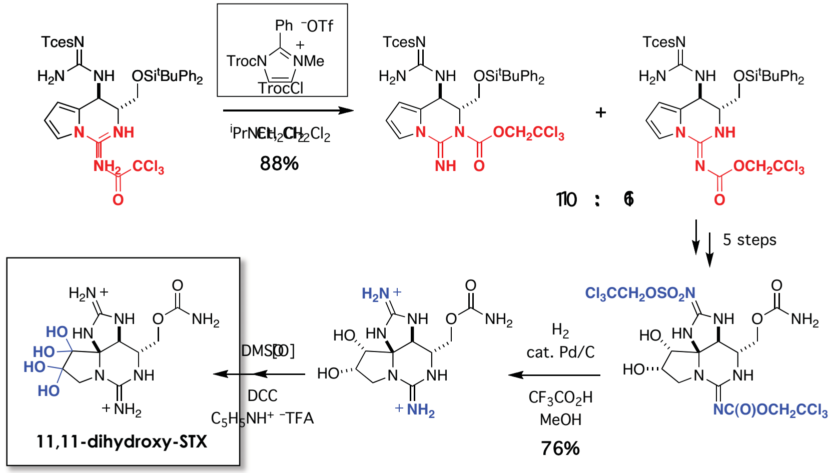
A 'Library' of Bis-Guanidinium Toxins



An Asymmetric Synthesis of (+)-Gonyautoxin – Abridged

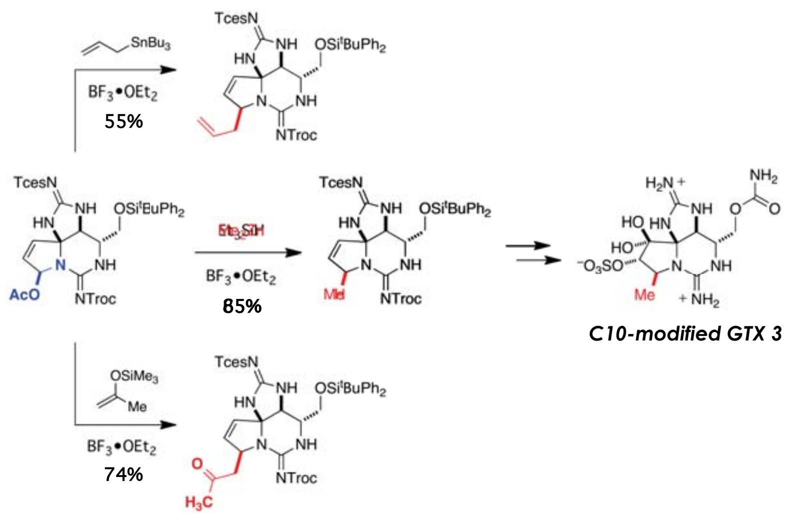


Second Generation Improvements to Streamline Synthesis

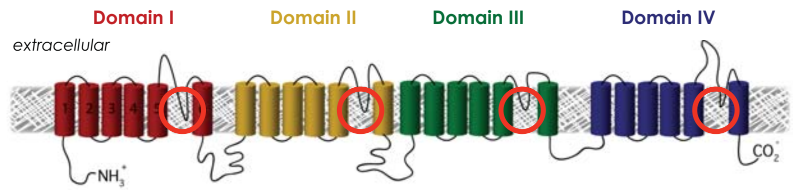


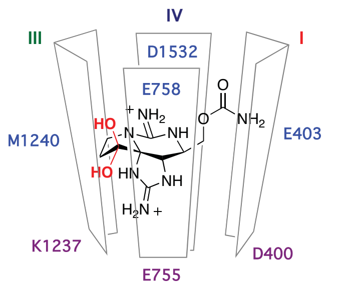
recently isolated PSP from *Alexandrium tamerense* – Quilliam *J. Nat. Prod.* 2008, *71*, 1518

'Unnatural' Toxins: C10-Substitution



A Highly Evolved "Cork" Stoppers Ion Flux



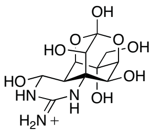


outer vestibule: E403 E758 M1240 D1532
selectivity filter: D400 E755 K1237 A1529

name	gene locus	localization	TTX/STX sensitivity
$Na_V1.1$	SCN1A	CNS	✓
$Na_V1.2$	SCN2A	CNS, PNS	✓
$Na_V1.3$	SCN3A	Brain, DRG, PNS	✓
$Na_V1.4$	SCN4A	Skeletal muscle	✓
$Na_V1.5$	SCN5A	Cardiac	
$Na_V1.6$	SCN8A	CNS, DRG, PNS	✓
$Na_V1.7$	SCN9A	DRG, SN, spinal cord	✓
$Na_V1.8$	SCN10A	DRG	
$Na_V1.9$	SCN11A	DRG	
Na_X	SCN7A	Glial cells, DRG	?

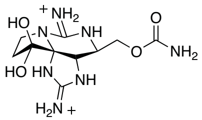
Novakovic *Trends Neurosci* **2001**, *24*, 473
Llewellyn *Nat. Prod. Rep.* **2006**, *23*, 200

A Comparison of Toxin Volume and Surface Potentials



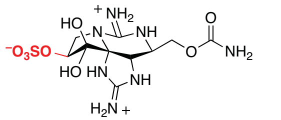
(–)-tetrodotoxin

252 Å³



(+)-saxitoxin

242 Å³

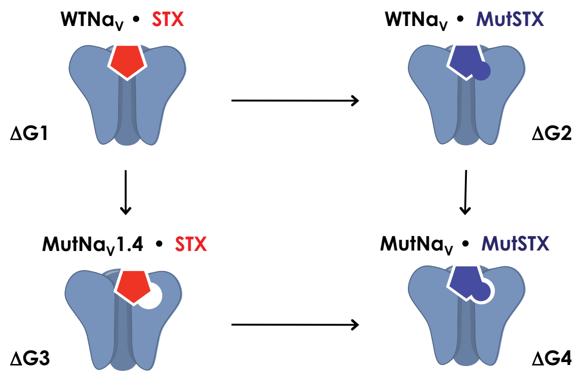


(+)-gonyautoxin III

305 Å³

electrostatic surface potential **–4 kT** to **+4 kT**

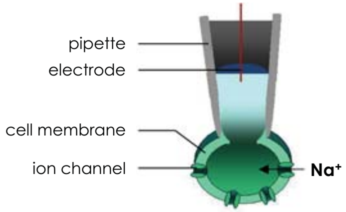
Double Mutant Cycle Analysis to Study Protein Structure

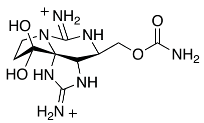


$$\Delta\Delta G_{int} = \Delta G1 - \Delta G2 - (\Delta G3 - \Delta G4)$$

- **Magnitude of $\Delta\Delta G_{int}$ varies with distance between two groups**
- **Quantitative assessment of localized interactions**
- **Previous studies limited by structural diversity of natural toxins used**

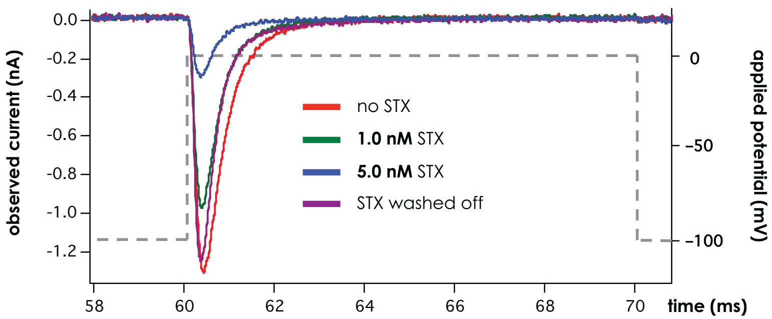
Evaluating STX Block: Whole-cell Voltage-Clamp Recordings





(+)-saxitoxin

IC_{50} = 2.9 ± 0.1 nM
against Na_V1.4 (CHO)



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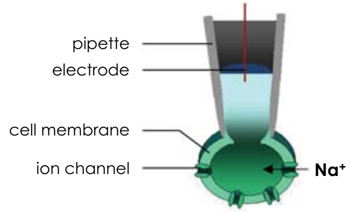

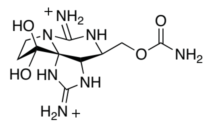

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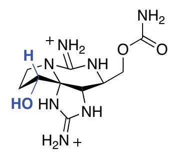
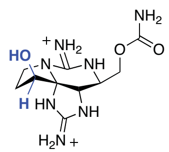
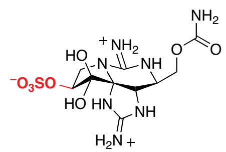


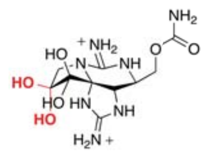
α-saxitoxinol
IC_{50} = 559 nM



β-saxitoxinol
IC_{50} = 32 μM



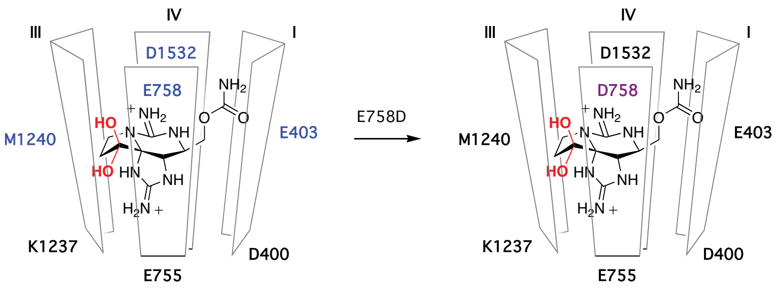
gonyautoxin III
IC_{50} = 15 nM

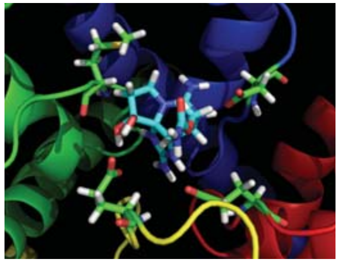


11,11-dihydroxysaxitoxin
IC_{50} = 2.25 μM

structure-activity data for C11- and C12-derivatives challenge binding model

A Single Point Mutation Gives a Dramatic Result



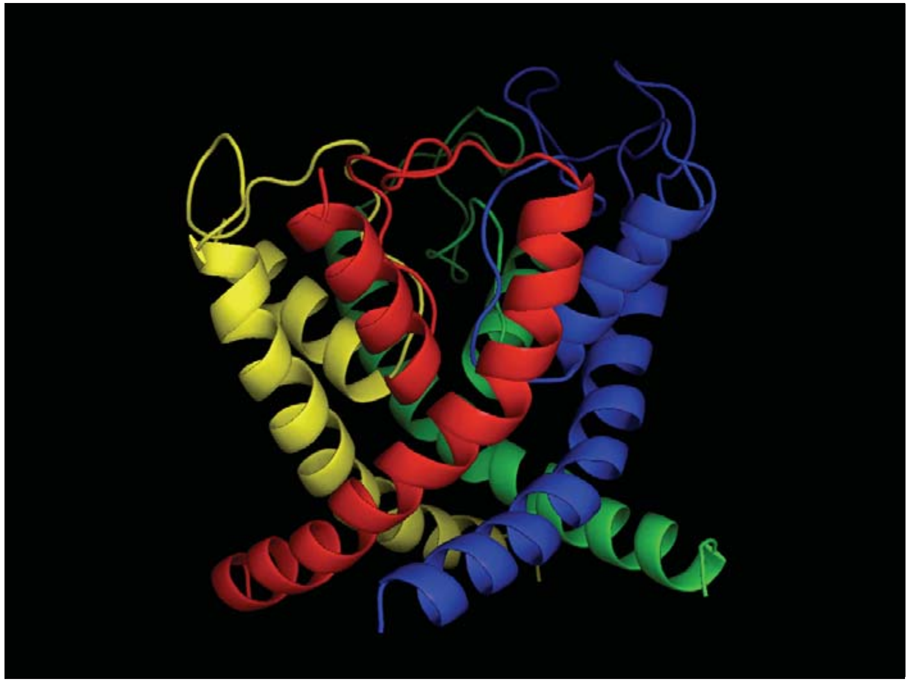


IC_{50} = 2.9 nM for wild type $Na_V1.4$

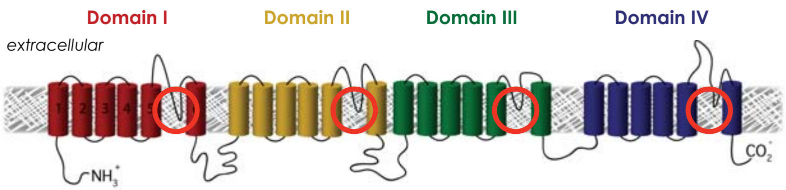
IC_{50} = 3.2 μM for E758D mutant

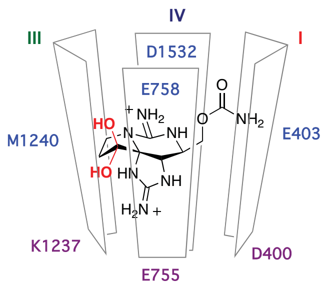
STX resistance in *Mya arenaria* ascribed to E→D mutation

Catterall & Trainer, *Nature* **2005**



A Highly Evolved "Cork" Stoppers Ion Flux





outer vestibule: E403 E758 M1240 D1532

selectivity filter: D400 E755 K1237 A1529

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$Na_V1.9$	SCN11A	DRG	
Na_X	SCN7A	Glial cells, DRG	?

Novakovic *Trends Neurosci* **2001**, 24, 473

Llewellyn *Nat. Prod. Rep.* **2006**, 23, 200

Human Pain Conditions linked to Sodium Channel Gene Mutations

Erythermalgia

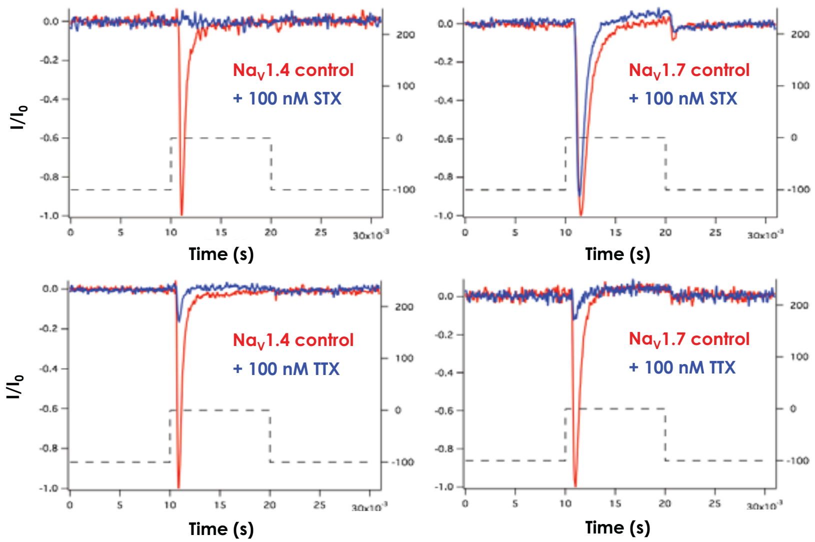
Two autosomal dominant mutations in the $Na_V1.7$ gene which cause a hyperpolarizing shift in activation resulting in redness, hyperalgesia, and burning pain

Congenital Pain Insensitivity

$Na_V1.7$ gene functional deletion - inability to sense pain.

Heckert, J. "The Hazards of Growing Up Painlessly," *The New York Times Magazine* Nov. 15, 2012

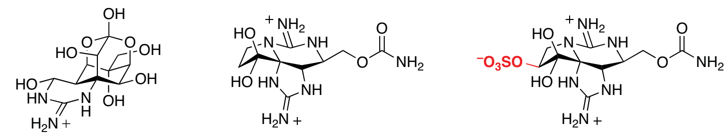
Human $Na_V1.7$ is TTX 'Sensitive' and STX 'Resistant'



A Possible Explanation For Differences in Toxin Affinity?

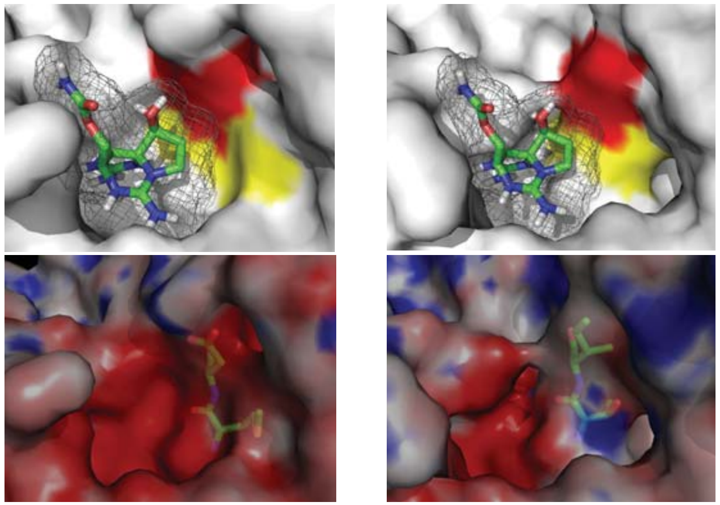
Na_V	Domain I	Domain II	Domain III	Domain IV
1.1	L M T Q **D** F W E N	V L C G **E** W I E T	A T F **K** G W M D I	T T S **A** G W D G L
1.2	L M T Q **D** F W E N	V L C G **E** W I E T	A T F **K** G W M D I	T T S **A** G W D G L
1.3	L M T Q **D** Y W E N	I L C G **E** W I E T	A T F **K** G W M D I	T T S **A** G W D G L
1.4	L M T Q **D** Y W E N	V L C G **E** W I E T	A T F **K** G W M D I	T T S **A** G W D G L
1.6	L M T Q **D** Y W E N	V L C G **E** W I E T	A T F **K** G W M D I	T T S **A** G W D G L
1.7	L M T Q **D** Y W E N	V L C G **E** W I E T	A T F **K** G W T I I	T T S **A** G W D G L

toxin	$Na_V1.4$	$Na_V1.4$ M1240T	$Na_V1.4$ D1241I	$Na_V1.4$ M1240T D1241I	$Na_V1.7$	$Na_V1.7$ T1398M I1399D
TTX	17.1 nM	466	8.7	90	18.6	5.0
STX	2.8	73	53	1153	702	2.3
GTX-III	14.9	228	38	1084	1513	22



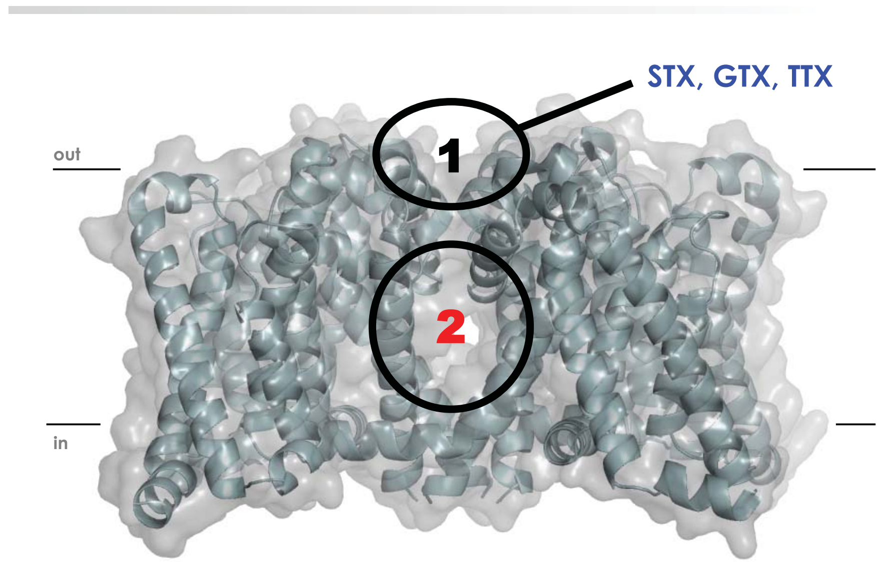
(–)-tetrodotoxin (+)-saxitoxin (+)-gonyautoxin III

Domain III Amino Acids Alter Pore Dimensions & Electrostatic Surface

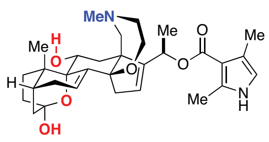


$Na_V1.4$ $Na_V1.7$

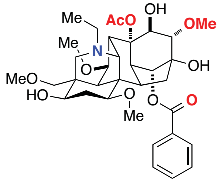
Site II – The Inner Pore



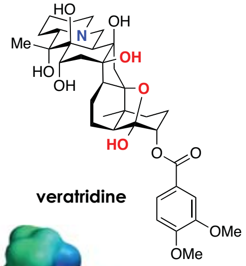
A Common Na_V Receptor Site for all three Lipid-Soluble Neurotoxins?



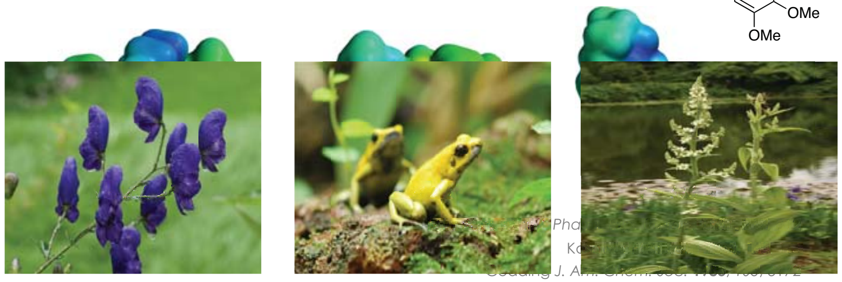
batrachotoxin



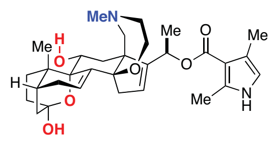
aconitine



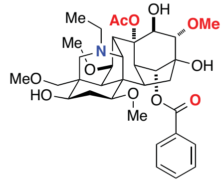
veratridine



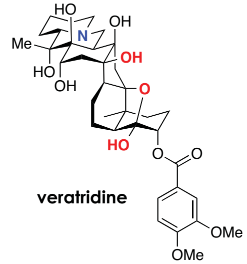
A Common Na_V Receptor Site for all three Lipid-Soluble Neurotoxins?



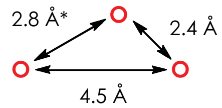
batrachotoxin



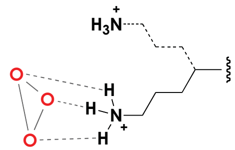
aconitine



veratridine



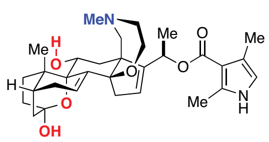
avg. distance to **N** *~6.0 Å*



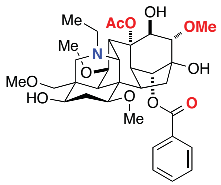
holding open a lysine gate?

Musutani, et al *J. Pharm. Exp. Therap.* **1981**, *217*, 812
Kosower *FEBS Lett.* **1983**, *163*, 161
Codding *J. Am. Chem. Soc.* **1983**, *105*, 3172

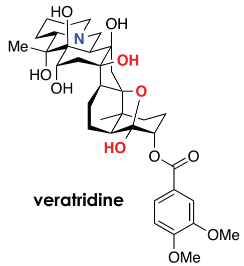
A Common Na_V Receptor Site for all three Lipid-Soluble Neurotoxins?



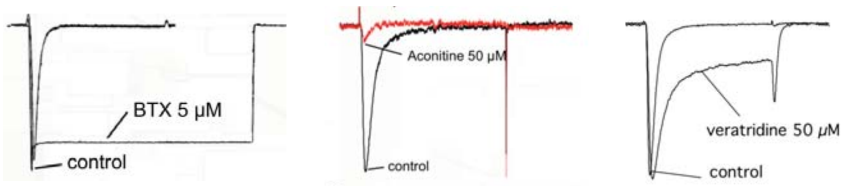
batrachotoxin



aconitine



veratridine



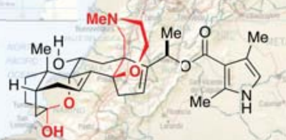
electrophysiology recordings (current vs time) reflect differences in mode of action

The Venom of the Columbian Arrow Poison Frog

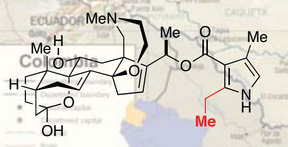
Phyllobates terribilis

Pitohui dichrous
(Papua New Guinea)

- first isolated from phyllobates aurotaenia (**1965**) (family *Dendrobatidae*)
- 5000 frogs delivered 27 mg of pure toxin
- structural elucidation/pharmacological evaluation by Daly and Witkop (NIH)
- mouse LD_{50} (subcutaneous) = 2 μg/kg



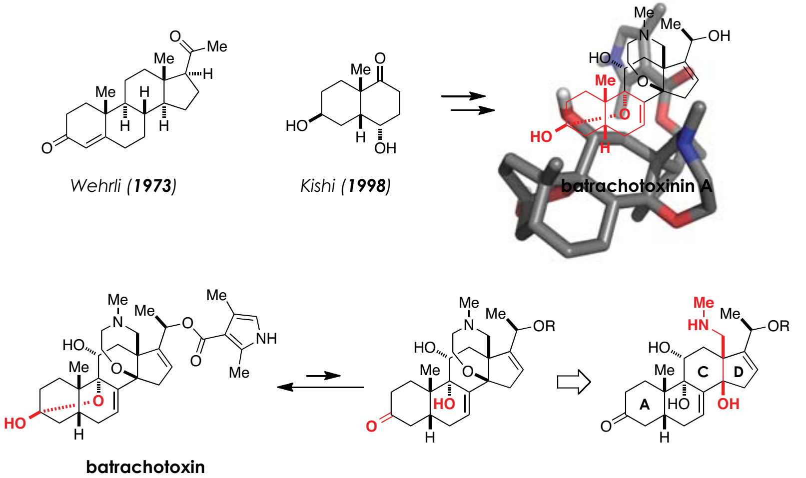
batrachotoxin (BTX)



homobatrachotoxin

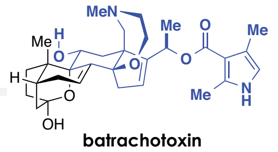
Daly & Witkop *Science* **1971**, *172*, 995

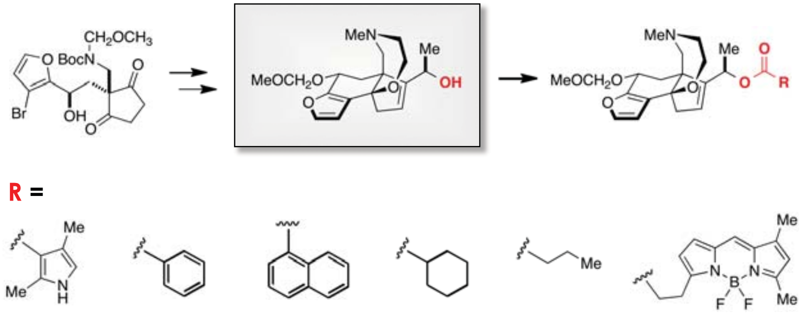
Designing A Synthesis of Batrachotoxin – Prior Art



Kishi *J. Am. Chem. Soc.* **1998**, *120*, 6627
Wehrli *Helv. Chim. Acta* **1973**, *56*, 139

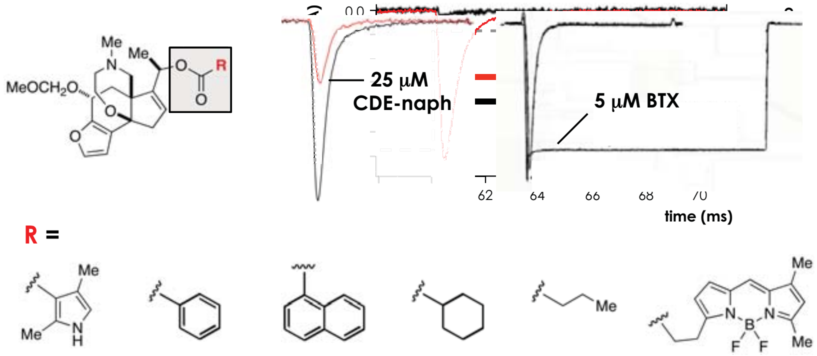
A Truncated Form of Batrachotoxin





final step esterification affords C/D/E ring analogue structures

Electrophysiological Analysis of BTX C/D/E Analogues

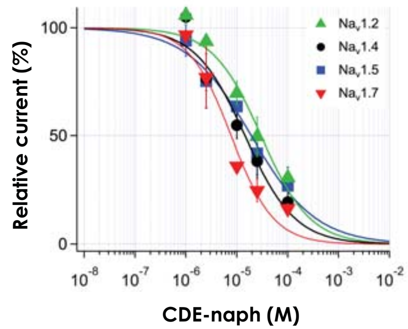


11.1 ± 0.3	81.4 ± 4.8	17.4 ± 0.4	38.8 ± 1.0	41.4 ± 1.0	19.2 ± 2.4

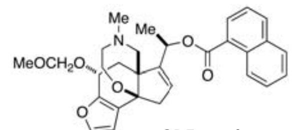
IC_{50}(μM)

Relative Potency of CDE-naph Against Na_V Subtypes

Minimal differences in IC_{50} between subtypes
S6 Residues that line inner pore > 96% conserved

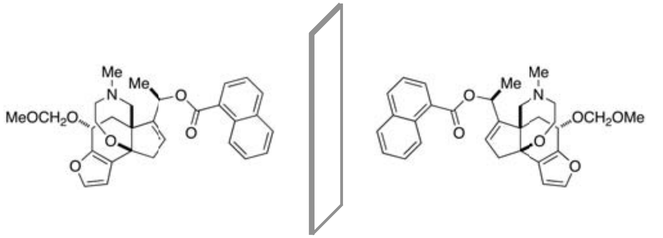


Na_V isoform	IC_{50} (μM)	Stdev
rNav1.2	**30.1**	± 6.9
rNav1.4	**17.4**	± 0.4
hNav1.5	**19.3**	± 3.5
hNav1.7	**7.8**	± 1.7



CDE-naph

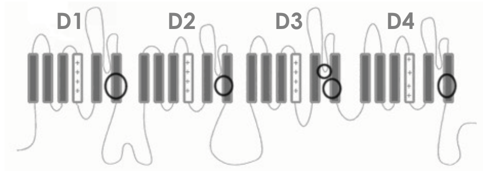
Through the 'Looking Glass'

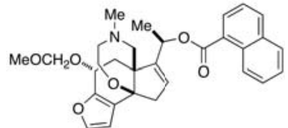


IC$_{50}$(μM) **13.8 ± 0.7** **15.4 ± 0.6**

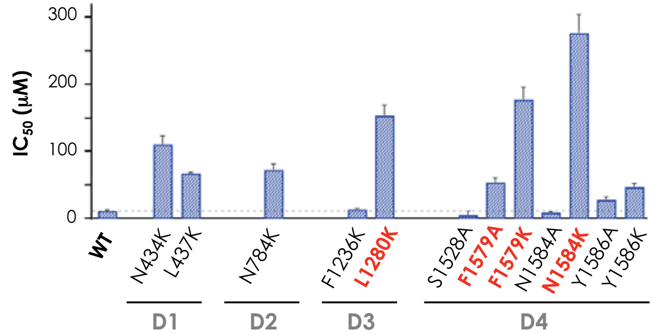
Mirror image isomers display nearly identical potency

Single Point Mutagenesis Experiments with Na$_V$1.4

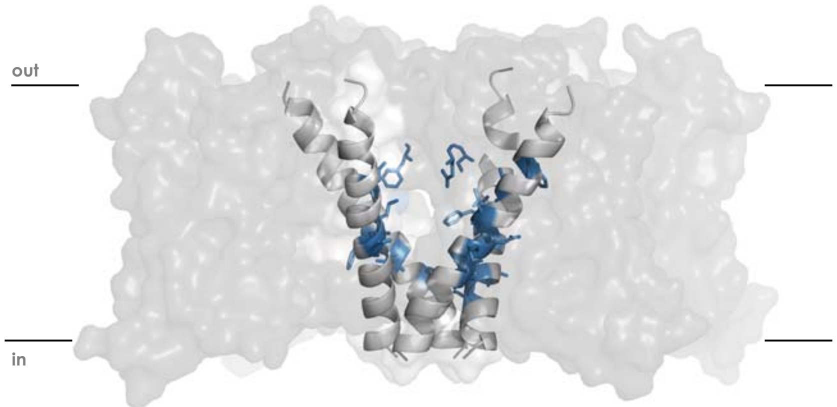




CDE-naph



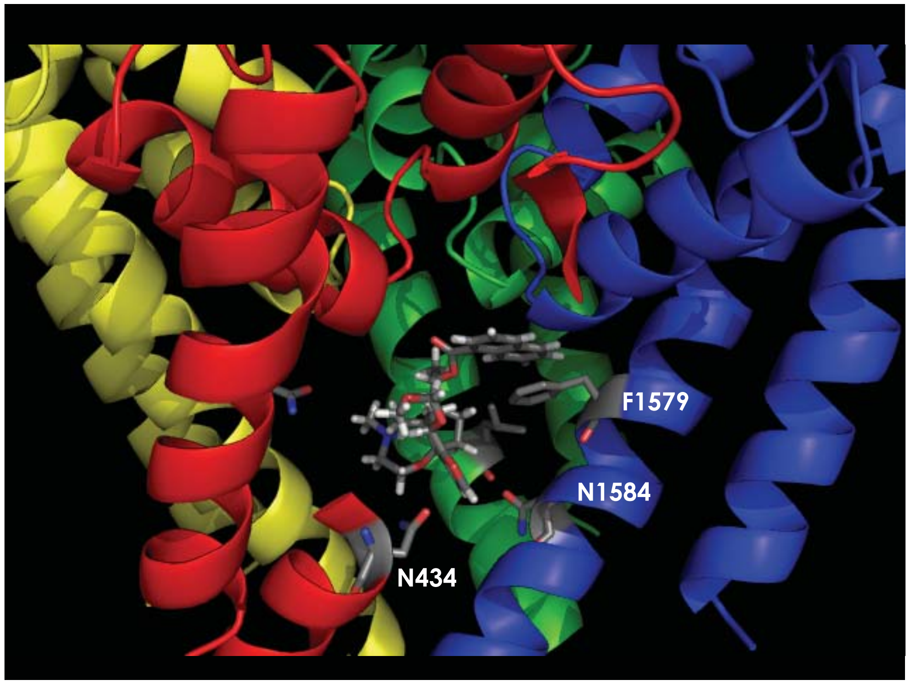
Homology Model of the Inner Pore (Na$_V$Ms)



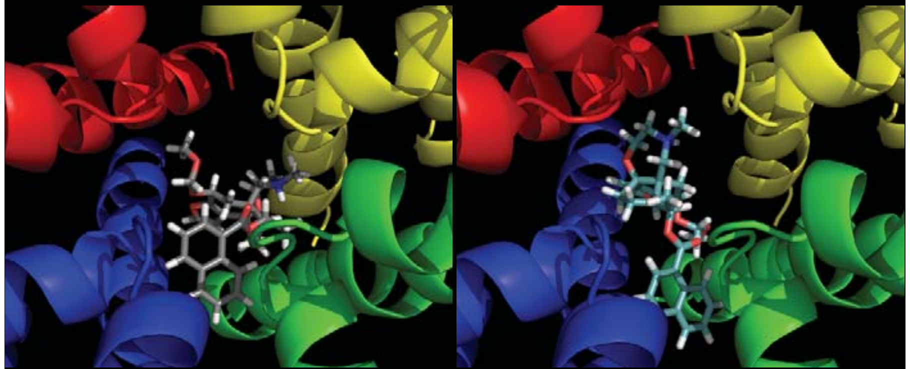
Minimal differences in IC$_{50}$ between subtypes

S6 Residues that line inner pore > 96% conserved

Catterall *et al. Nature* **2011**
Clapham, Yan *Nature* **2012**
Wallace *et al. Nature* **2013**

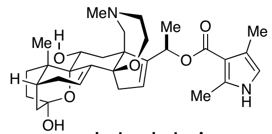


Rationalizing the Lack of Enantiospecificity

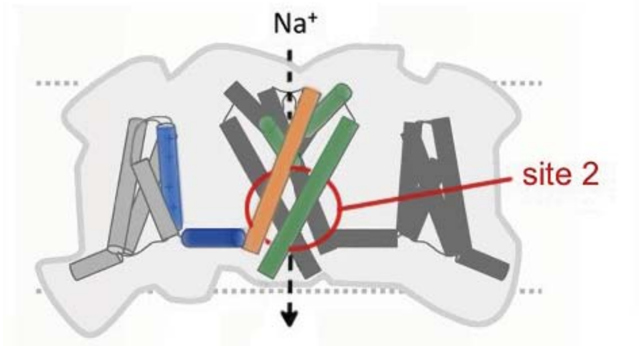


Many Outstanding Questions Remain

- binding is state-dependent to open channel
- **hyperpolarizes threshold activation by 30–50 mV**
- **eliminates inactivation gating**
- reduces single channel conductance by ~60%
- modifies Na^+ ion selectivity



batrachotoxin



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