Ion Channel Drug Discovery – Recent Advances in Novel Non-Opioid Pain Research

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Eurofins DiscoverX®

Agenda

1. Ion Channels in Drug Discovery and Development
   - The Pain Pathway and Current Therapeutics
2. Case Study – “LTGO-33 Is a Novel Nav1.8 Inhibitor”
   - Presented by John Gilchrist – Latigo Biotherapeutics Inc.
3. Eurofins DiscoverX® Ion Channel Products
   - Capabilities, Stable Cell Lines, and Ready-to-assay Cells
4. Summary and Conclusions

Eurofins DiscoverX® a Global Leader in Cell-based Assays for Screening, and Profiling

Accelerating Breakthroughs from Discovery to Delivery

25+ Years of Experience Building Cell-based Assays

Fremont, California | Poitiers, France | Shanghai, China

Industry's Largest Cell-based Assay Portfolio

10+ Druggable Target Classes

60+ Ion Channel Assays

Target Discovery

Hit Screening

Lead Optimization

Safety Studies

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Pure & Native Full Length Membrane Proteins

Functional Kinases

GPCRs

Stable Cell Lines

Ready-to-Assay

Membrane Preps

Internally Validated

>30 Billion

Data Points

Ion Channels in Drug Discovery and Development

Ion channels represent a key target class for therapeutics to treat human disease

- Over 400 genes encode for ion channels and their accessory subunits in humans
  - They are ubiquitous proteins found in all cell types

- Ion channels are involved in normal physiological processes such as the transmission of pain signals in the central and peripheral nervous system

- Therapeutic intervention in normal or aberrant ion channel function can lead to effective treatment of human diseases, including the treatment of pain disorders

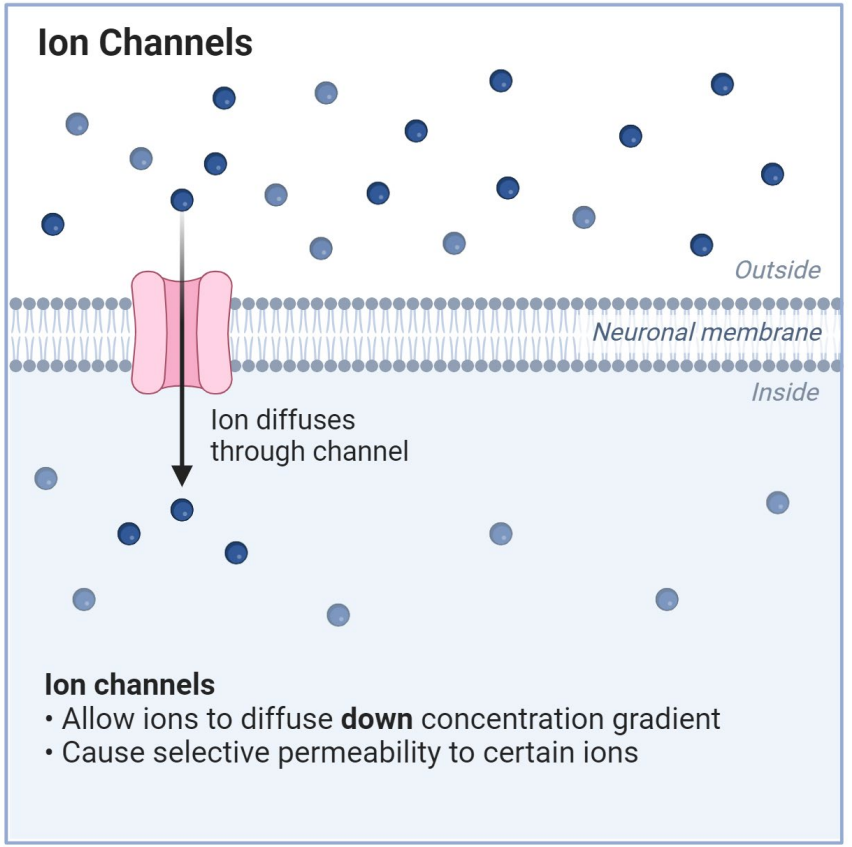


Image source: BioRender.com

Channelopathies

- Mutations in ion channel genes or their related proteins cause diseases (channelopathies) that range from mild to severe, affecting the nervous, cardiovascular and respiratory systems, as well as causing endocrine, kidney and immune-related diseases

CNS diseases	Potassium	Sodium	Calcium	Chloride
Epileptic syndromes	*KCNA1* *KCNA2* *KCNQ2/3* *KCNMA1* *KCNT1* *KCND2* *KCNH5* *KCNJ10* *KCNJ11*	*SCN1A* *SCN1B* *SCN2A* *SCN3A* *SCN8A*	*CACNA1H*	*CLCN2* *GLIALCAM*
Ataxia syndromes	*KCNA1* *KCNC3* *KCND3*		*CACNA1A*	
Familial Hemiplegic Migraine		*SCN1A*	*CACNA1A*	

Heart diseases	Potassium	Sodium	Calcium
Long QT and Short QT syndromes	*KCNQ1* *KCNH2* *KCNE1* *KCNJ2*	*SCN5A*	*CACNA1C*
Brugada syndromes	*KCNE3* *HCN4*	*SCN5A* *SCN1B* *SCN3B*	*CACNA1C*
Catecholaminergic polymorphic ventricular tachycardia			*RYR2*

Pancreas diseases	Potassium
Familial Congenital hyperinsulinism and neonatal diabetes mellitus	*KCNJ11* *ABCC8*

PNS diseases	Sodium	Potassium	Calcium
Pain syndromes and neuropathies	*SCN9A* *SCN10A* *SCN11A*	*KCNQ2*	*TRPA1*

Skeletal muscle diseases	Potassium	Sodium	Calcium	Chloride
Non dystrophic myotonias		*SCN4A*		*CLCN1*
Periodic paralysis	*KCNJ2* *KCNJ18*	*SCN4A*	*CACNA1S*	

Kidney diseases	Potassium	Chloride
Bartter's syndrome	*ROMK1*	*CLCNKB* *BSDN*
Dent disease		*CLCN5*
EAST/SESAME syndrome	*KCNJ11*	

Bone diseases	Chloride
Osteopetrosis	*CLCN7* *OSTM1*

Image source: Front. Pharmacol., 09 May 2016, Vol.7.
https://doi.org/10.3389/fphar.2016.00121

The pain pathway, opioids, and the need for non-addictive analgesics

The Pain Pathway

- Pain stimuli are detected by nociceptors in the peripheral nervous system
  - Signals are then transmitted to the Central Nervous System (CNS) via Dorsal Root Ganglion (DRG) neurons
- Sodium channels in DRG neurons initiate trains of action potentials in response to the incoming peripheral nerve signals
  - This transmits the pain signal to the brain where it is perceived

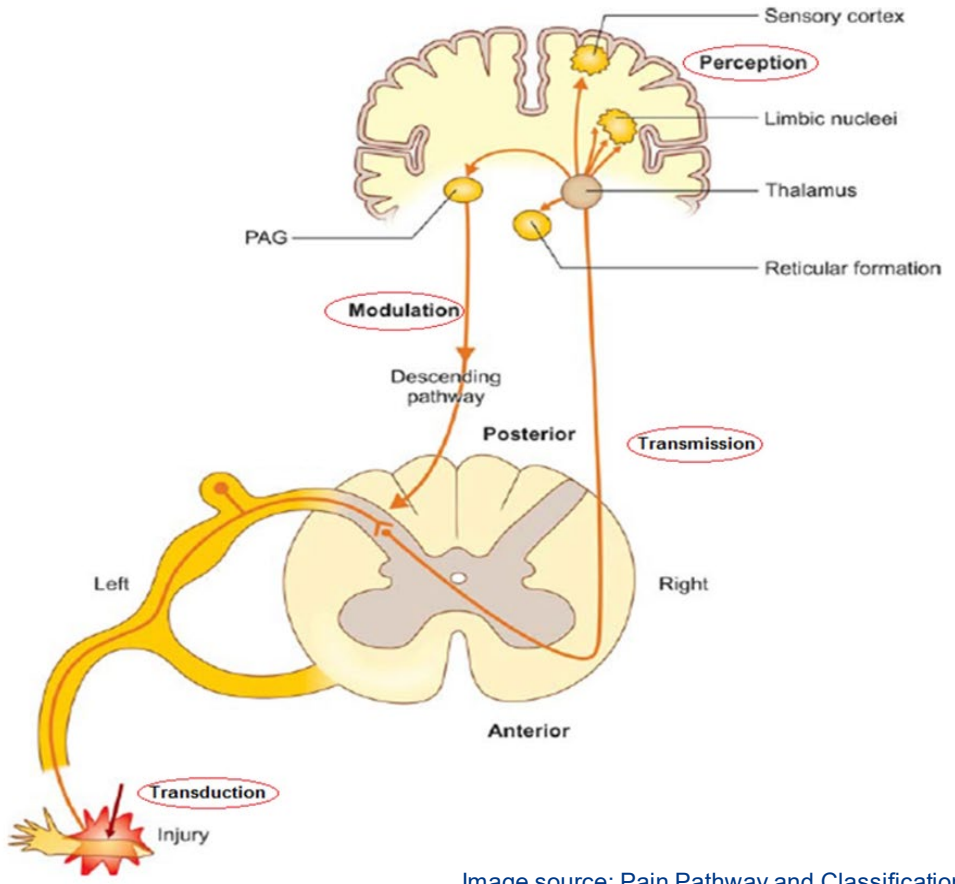
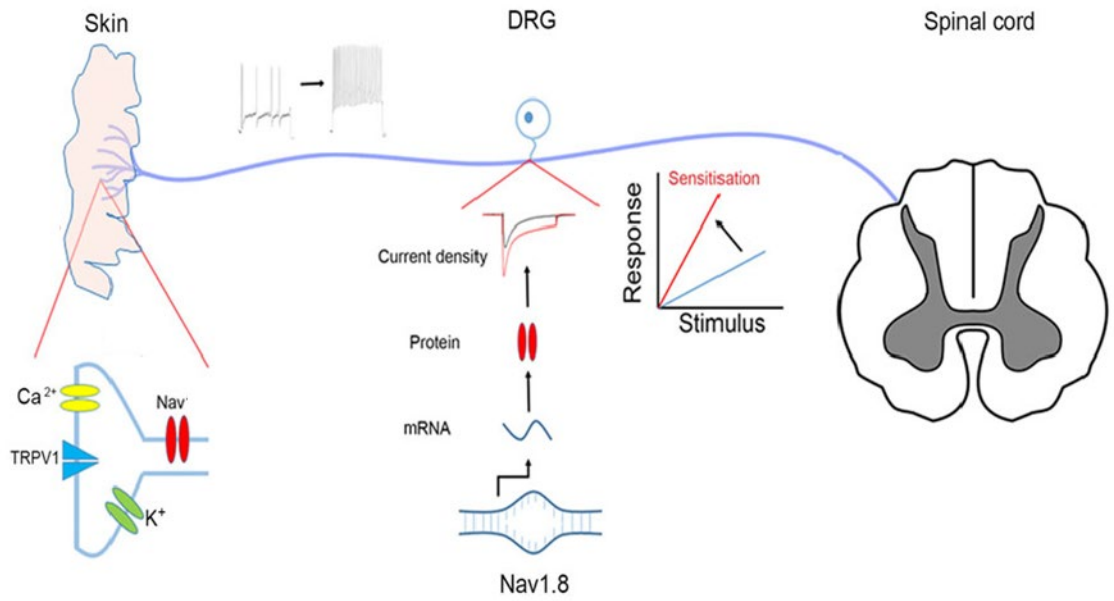


Image source: Pain Pathway and Classification of Pain (Daradia, The Pain Clinic https://daradia.com/pain-pathway/

The pain pathway, opioids, and the need for non-addictive analgesics

- Inhibition of Nav1.8 ion channels in DRG neurons should be an effective analgesic by reducing transmission of the pain signal from DRG neurons to the brain
- Highly addictive opioid drugs act by depressing neuronal excitability in the CNS by reducing the activity of Ca^{2+} channels and increasing the activity of K^+ channels
- Development of an effective inhibitor of Nav1.8 ion channels could lead to a non-addictive analgesic



Adapted from: *Acta Biochim Biophys Sin*, 2016, 48(2), 132–144. doi: 10.1093/abbs/gmv123

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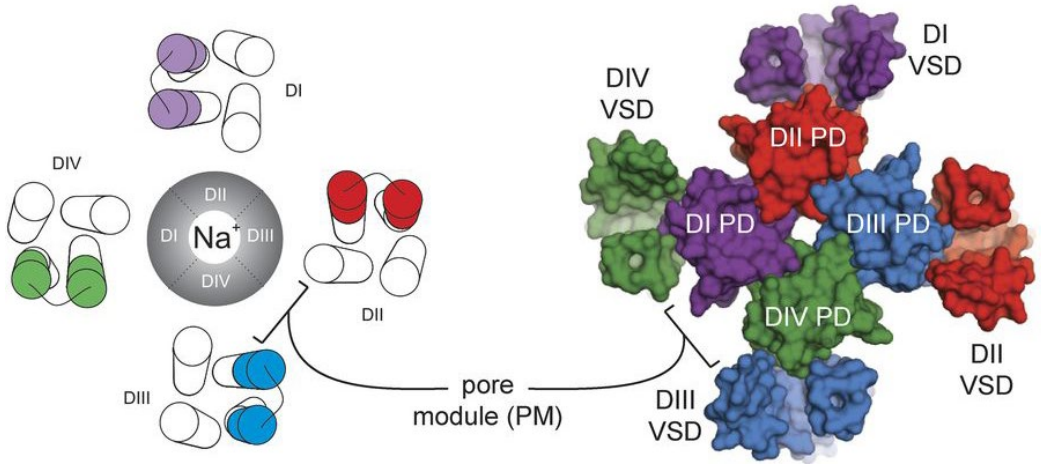
LTGO-33 Is a Novel $Na_V1.8$ Inhibitor

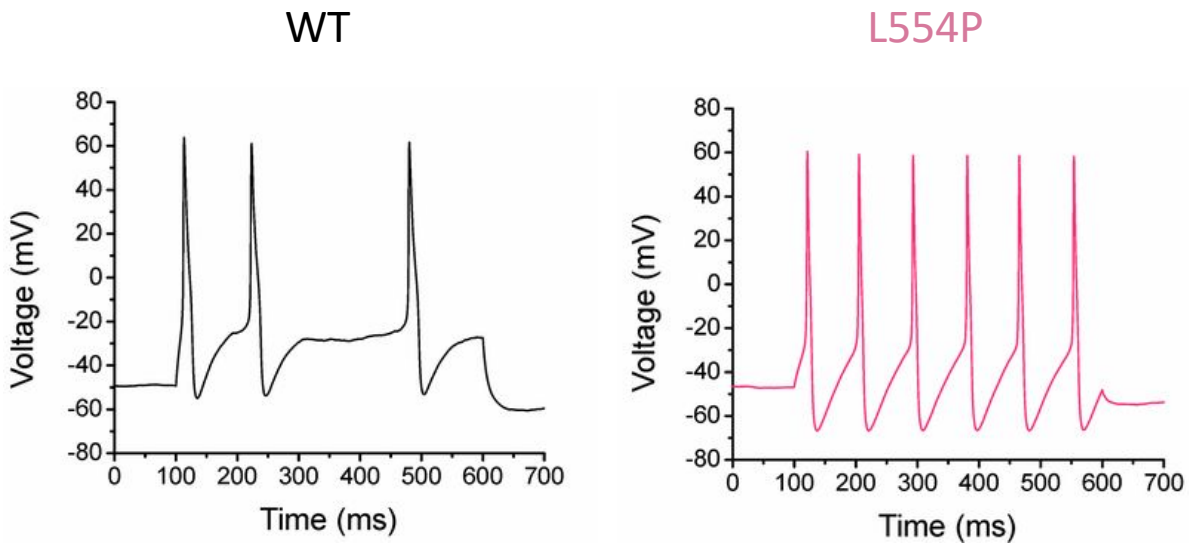
John Gilchrist, Ph.D.

Latigo Biotherapeutics Inc.

15 May 2024

$Na_V1.8$ Is a Validated Target for Analgesia





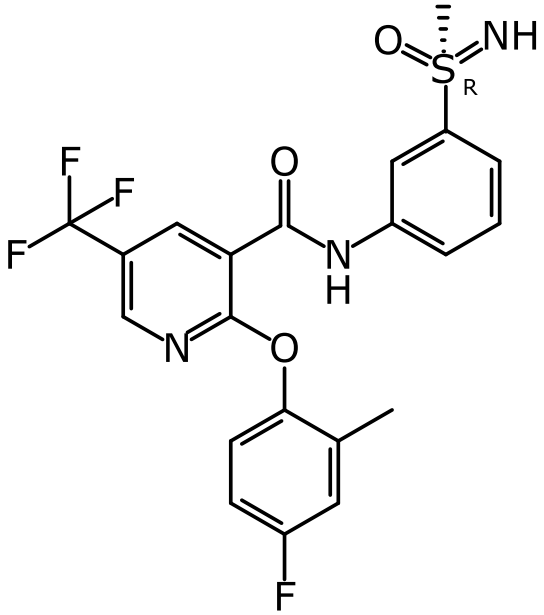
$Na_V1.8$ is a voltage-gated sodium channel found in nociceptive DRG sensory neurons

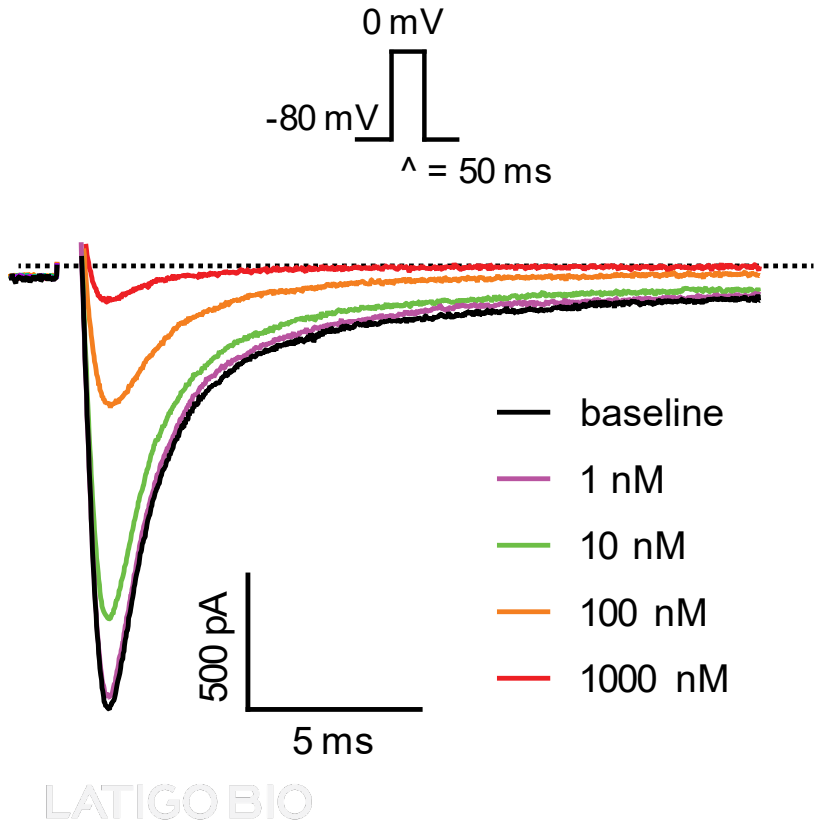
Genetically- and pharmacologically-validated as a target for pain

Ahern, Christopher A., et al. "The hitchhiker's guide to the voltage-gated sodium channel galaxy." *Journal of General Physiology* 147.1 (2016): 1-24.

Faber, Catharina G., et al. "Gain-of-function Nav1. 8 mutations in painful neuropathy." *Proceedings of the National Academy of Sciences* 109.47 (2012): 19444-19449.

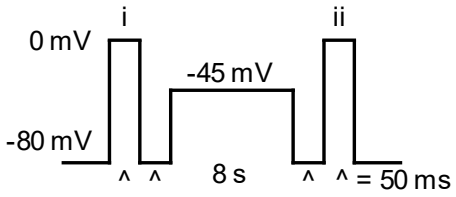
LTGO-33 Is a Potent $Na_V1.8$ Inhibitor

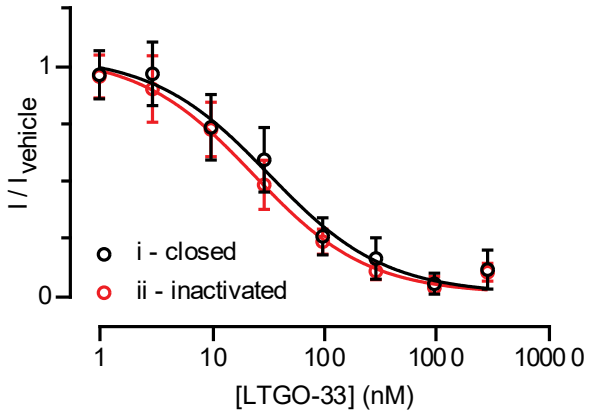




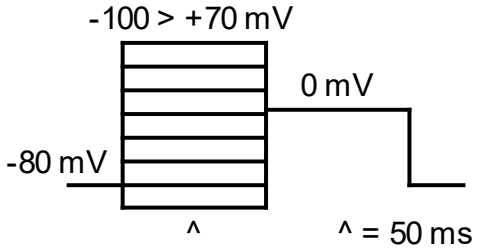
LTGO-33 inhibits $Na_V1.8$ with nM potency

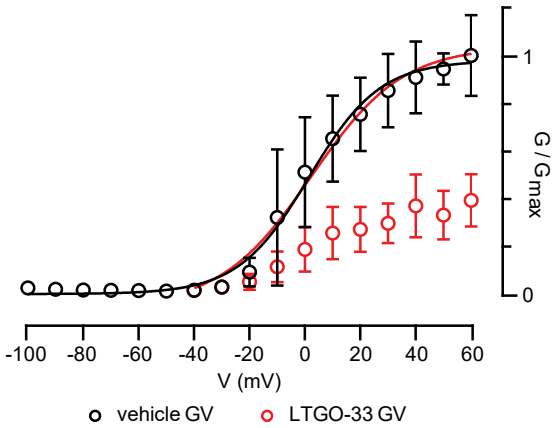
LTGO-33 Inhibition Is State-Independent



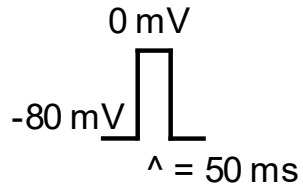


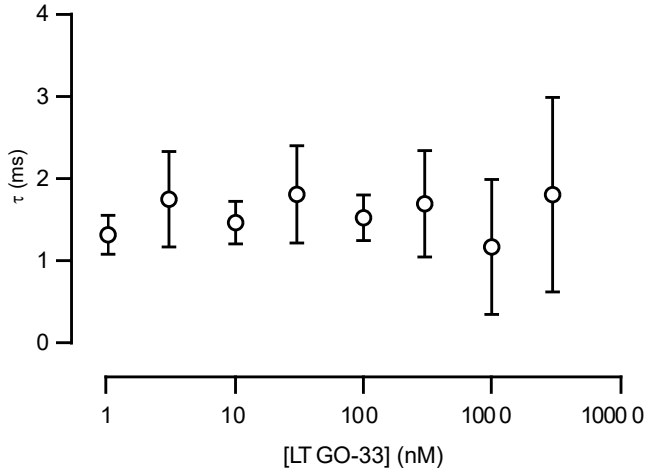
No preference for inhibition of resting vs inactivated state





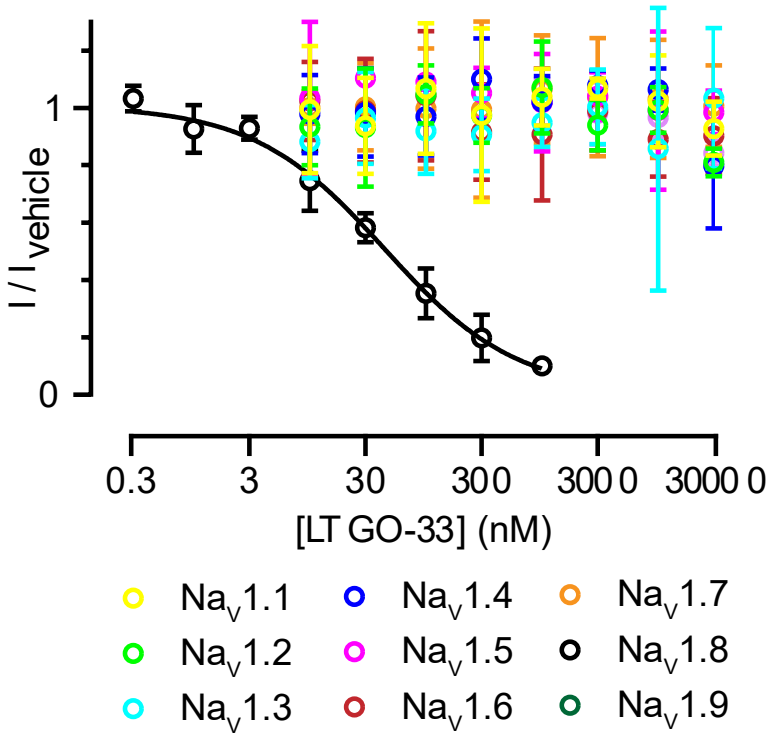
No shift in voltage-dependence of activation

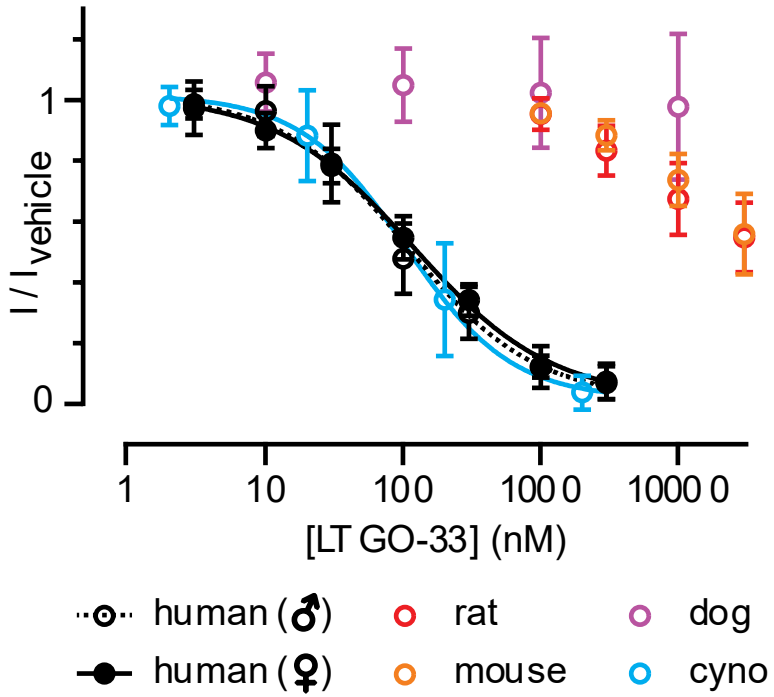




No change in rate of fast inactivation

LTGO-33 Is Isoform- and Species-selective

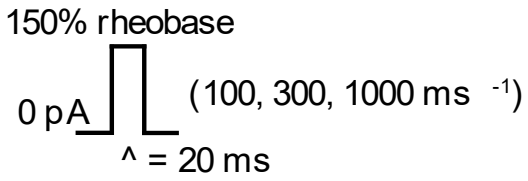




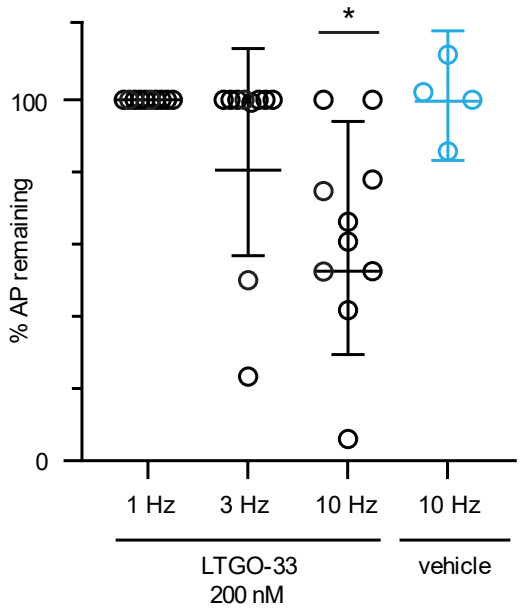
>600-fold selective for Na$_V$1.8 vs Na$_V$1.1-1.7 and Na$_V$1.9

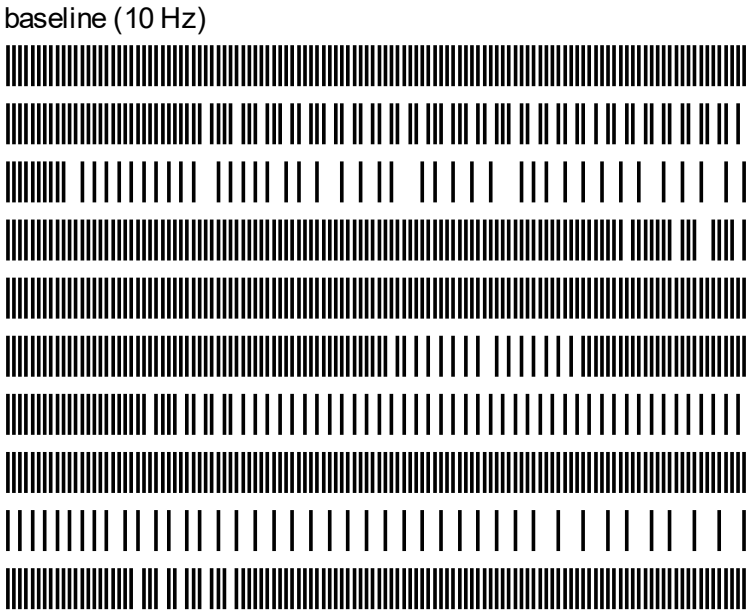
>250-fold selective for primate TTX-R vs rodent and dog TTX-R

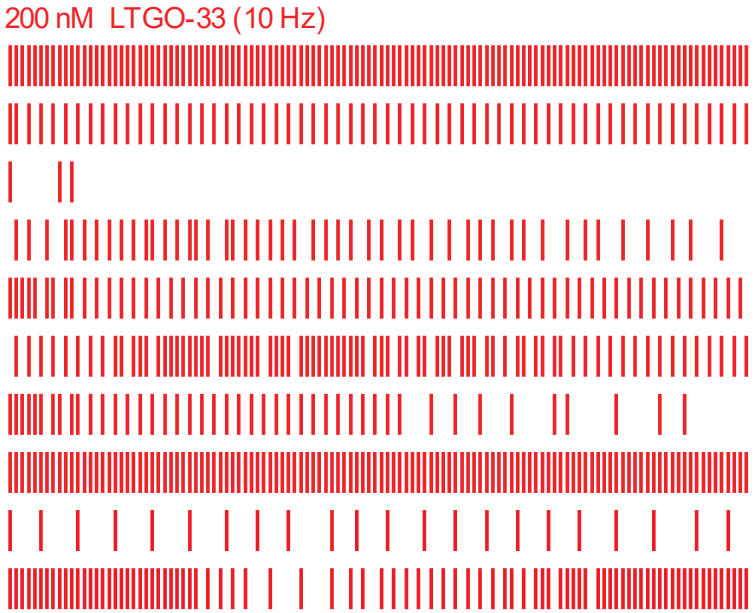
LTGO-33 Inhibits Action Potentials in hDRG Neurons



TTX-free to simulate physiological conditions

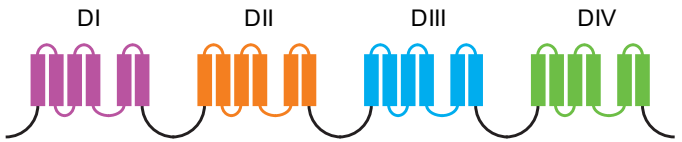


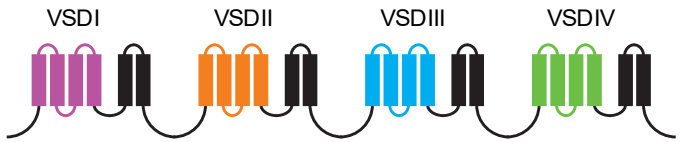




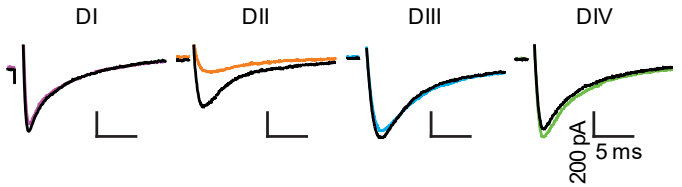
LTGO-33 is effective at decreasing AP firing at a higher frequency (10 Hz) at 2x hDRG IC_{50}

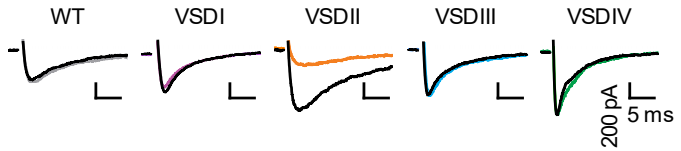
VSDII Is Required for LTGO-33 Activity



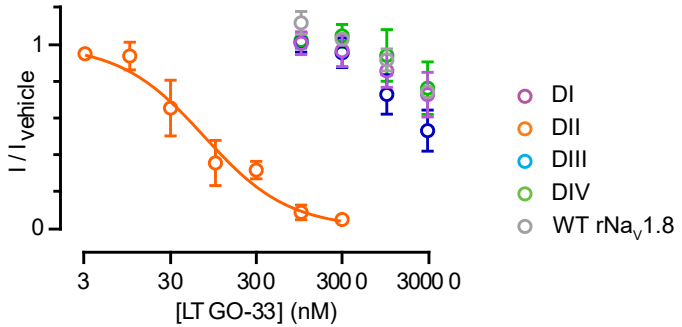


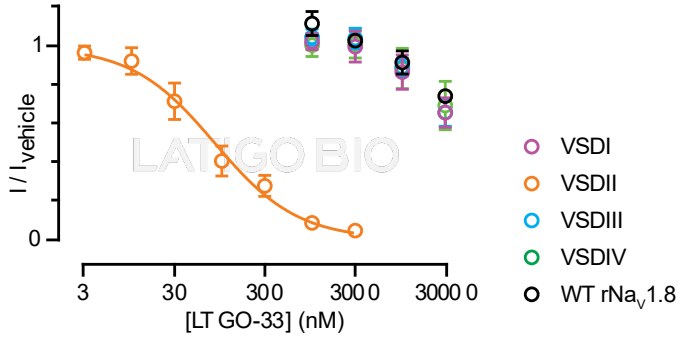
$rNa_V1.8$ (with reduced LTGO-33 sensitivity) used as host for gain-of-function domain chimeras





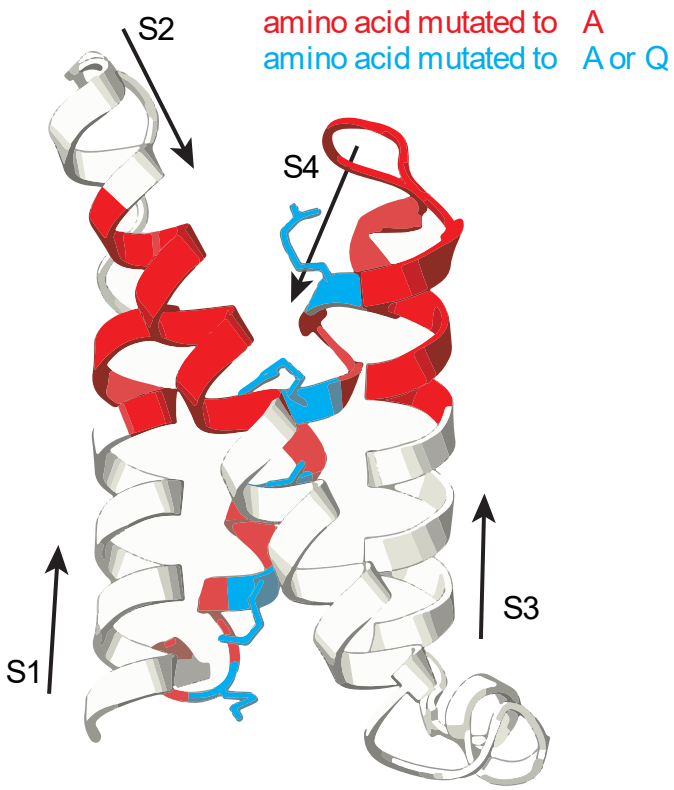
DII (IC_{50} 74 nM) and VSDII (IC_{50} 84 nM) chimeras inhibited by LTGO-33, all others IC_{50} >30 µM

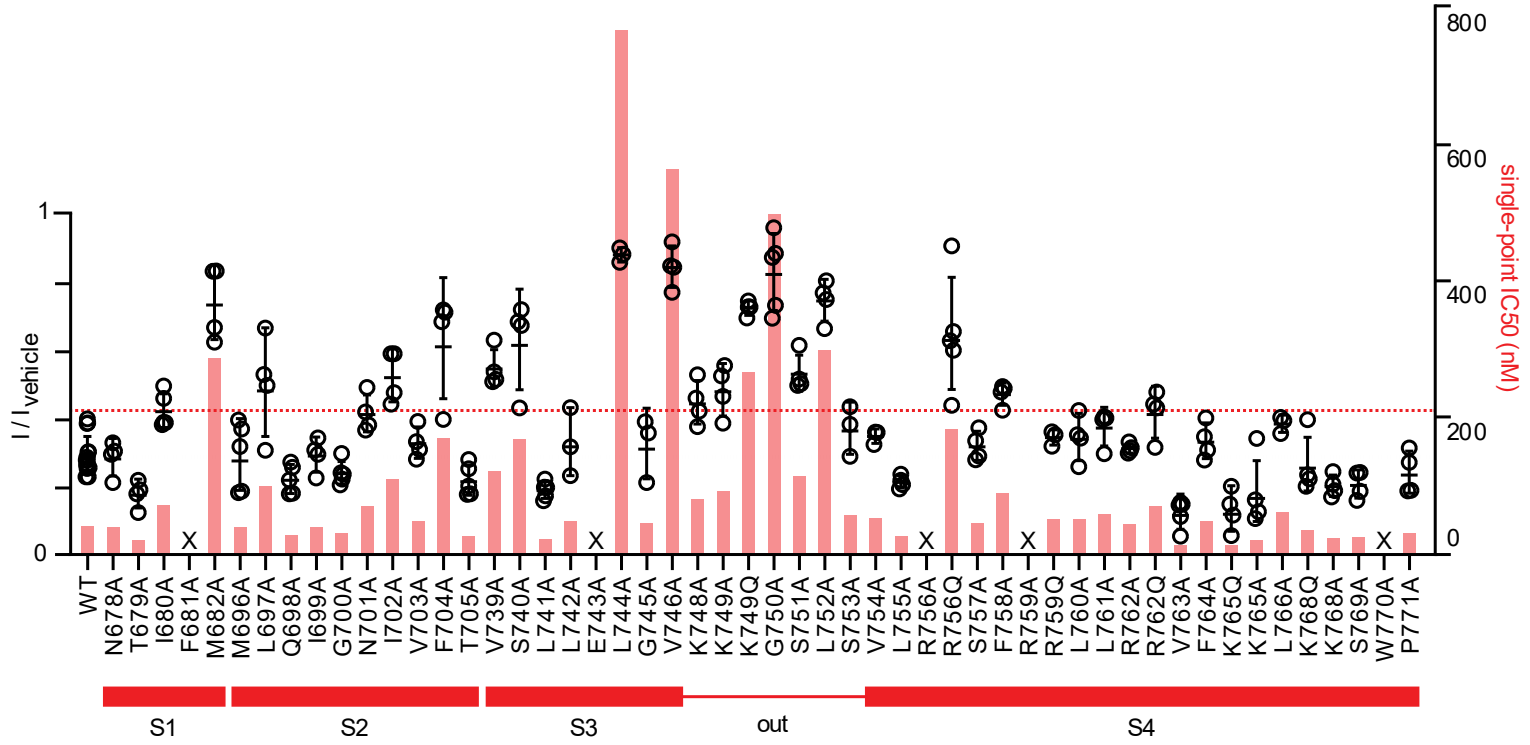




Comparable potency on DII and VSDII chimeras suggests VSD sufficient, pore unnecessary

Residues in S1, S3, and S4 Critical for LTGO-33 Activity

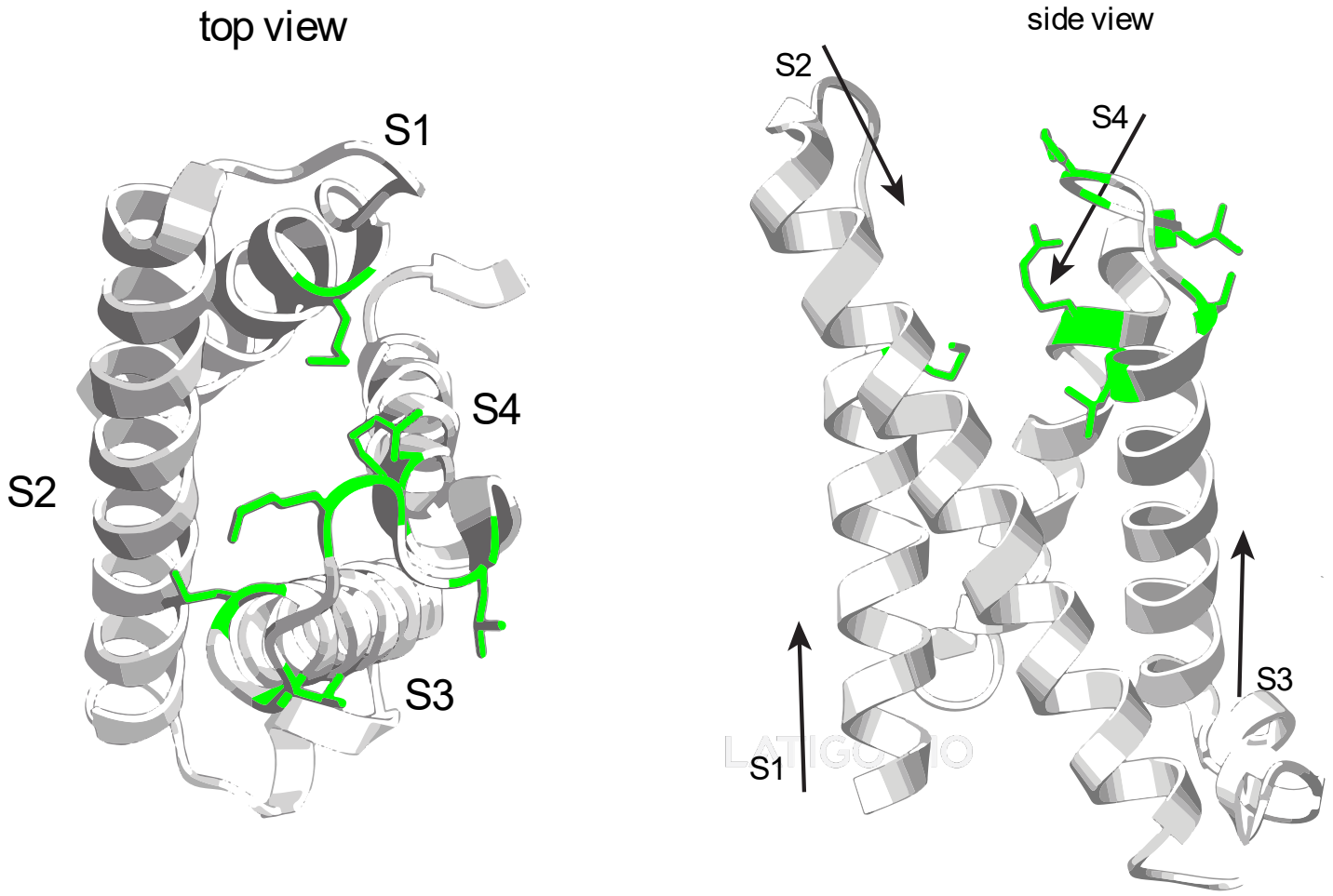




Alanine scan conducted through regions of dissimilarity between human and rat

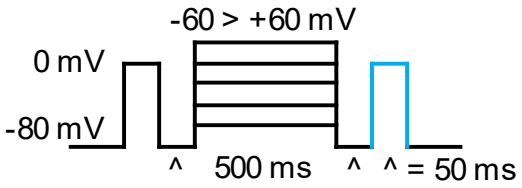
Amino acids showing >5x decrease in potency found in S1 and S3b-S4 loop

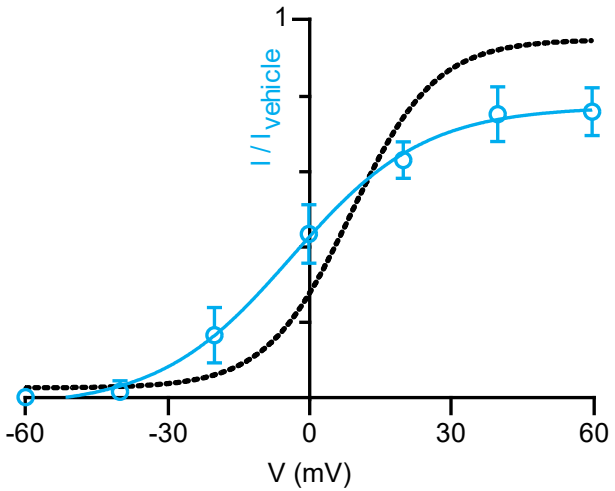
LTGO-33 Interaction Site in Extracellular VSDII

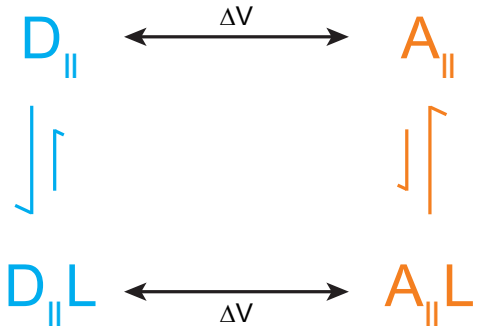


Important residues found in outer portion of VSDII – similar to ProTx-II binding site

Disinhibition and a Gating Model



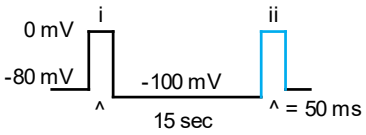


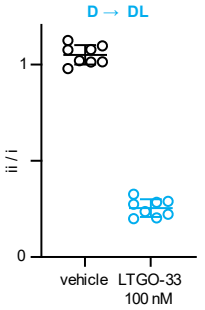


A basic model proposes LTGO-33 stabilizes the VSDII deactivated (down) state to prevent channel activation

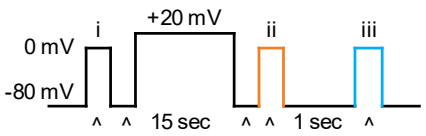
Depolarization can relieve $Na_V1.8$ inhibition by LTGO-33, akin to ProTx-II

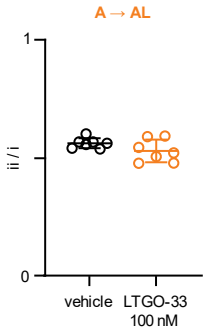
LTGO-33 Inhibits Closed but not Activated Channels



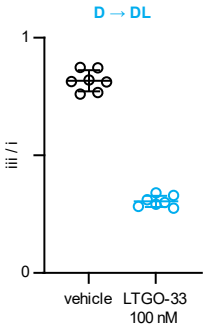


LTGO-33 can inhibit channels from the closed state (held at negative Vm)

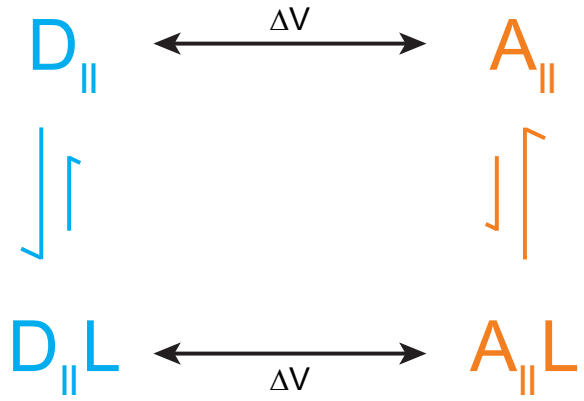




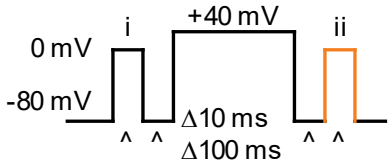
Similar inhibition in vehicle- and LTGO-33-treated cells due to slow inactivation, minimal further inhibition with LTGO-33

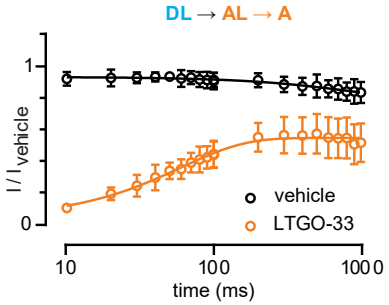


LTGO-33 inhibits channels after brief hold at negative Vm (closed state)

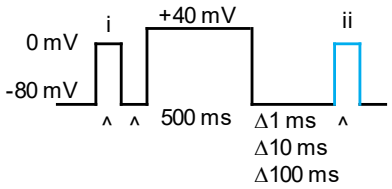


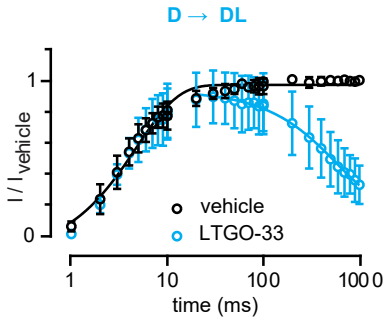
LTGO-33 Inhibition Can Be Relieved by Depolarization



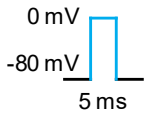


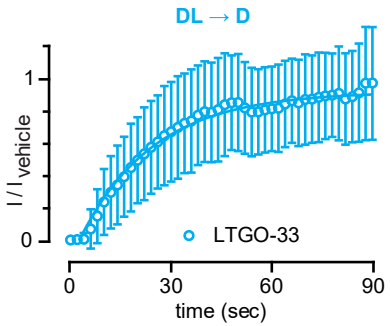
LTGO-33 disinhibition is complete within a 1 second depolarization



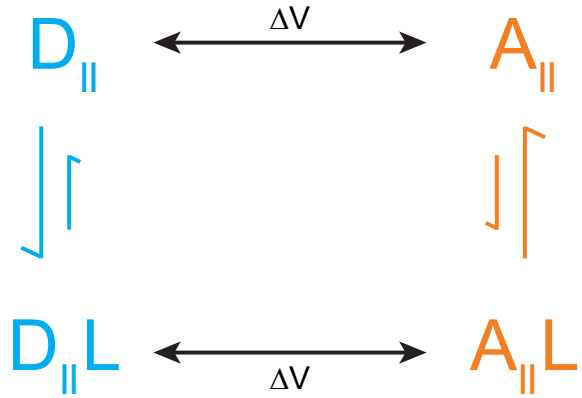


LTGO-33 reinhibition occurs rapidly in the deactivated state

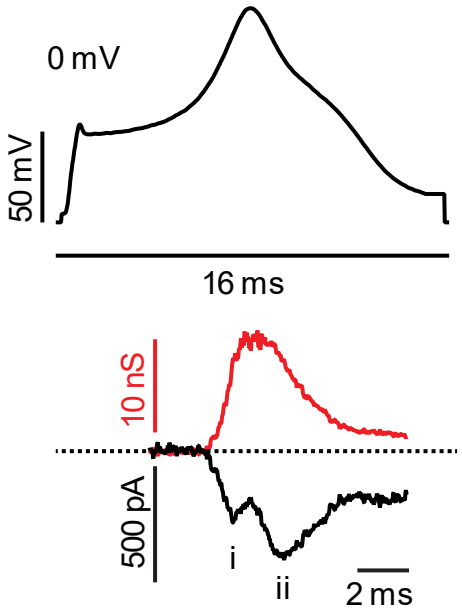




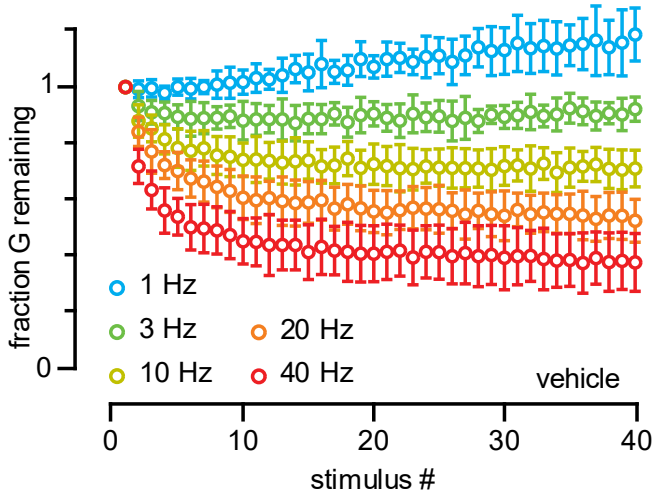
LTGO-33 is relatively slower to dissociate from deactivated VSDII

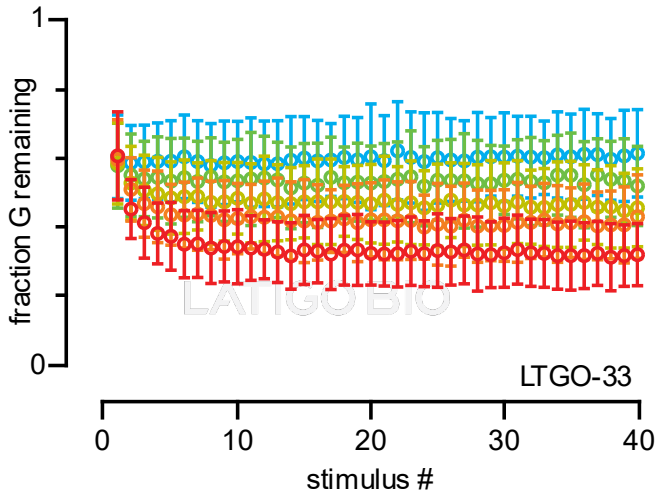


LTGO-33 Exhibits Minimal Reverse-use Dependence



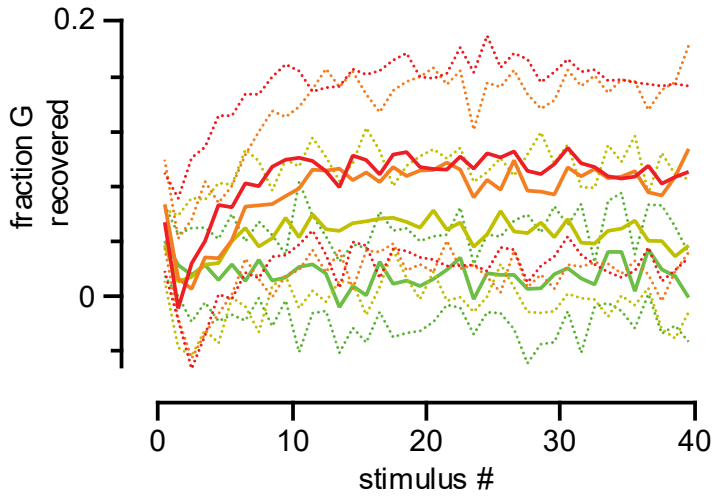
AP waveform applied to ND7/23 cells transfected with $hNa_V1.8$, conductance plotted



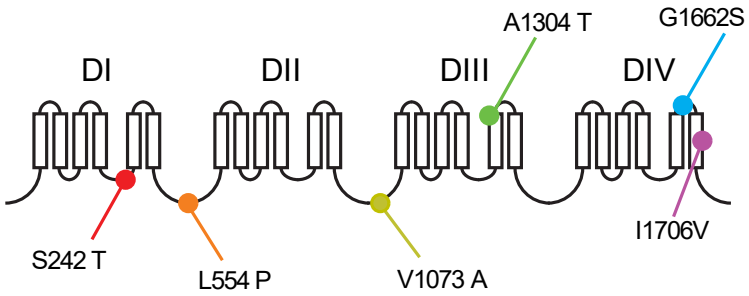


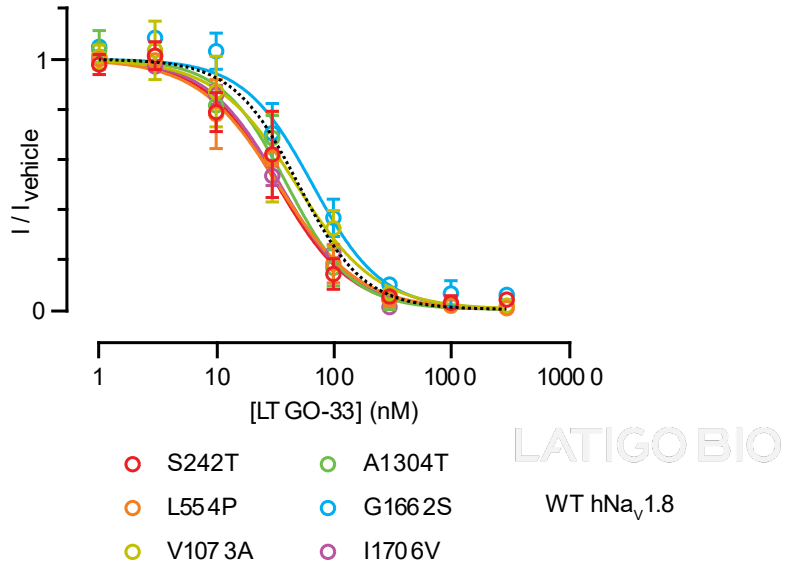
Use-dependent inhibition (UDI) observed at higher frequencies

Curve-fitting approach subtracted UDI from LTGO-33 treated cells – minimal reverse-use dependence observed up to 40 Hz



LTGO-33 Inhibits Nav1.8 Harboring Variants Associated with Pain Disorders





$Na_V1.8$ variants associated with pain disorders located outside of VSDII do not affect LTGO-33 pharmacology

Conclusions

- LTGO-33 is a potent, selective $Na_V1.8$ inhibitor that blocks AP firing in an *in vitro* human DRG neuronal model
- Unlike prior published $Na_V1.8$ inhibitors, LTGO-33 shows no preference for inhibiting from inactivated state
- LTGO-33 engages the channel at a novel site in VSDII similar to peptide spider toxins
- LTGO-33 is effective in inhibiting $Na_V1.8$ variants associated with pain disorders

Acknowledgements

Latigo Team

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Tina Holt

External Activities

AnaBios
Neuroservice USA
Charles River Labs
B'SYS
GenScript

Find the paper at *Molecular Pharmacology*!

The $hNa_V1.8/\beta1$ (CYL3025) and $rNa_V1.8$ (CYL3050) stable cell lines are products of Eurofins DiscoverX.

Agenda

1. Ion Channels in Drug Discovery and Development
   - The Pain Pathway and Current Therapeutics
2. Case Study – "LTGO-33 Is a Novel Nav1.8 Inhibitor"
   - Presented by John Gilchrist – Latigo Biotherapeutics Inc.
3. Eurofins DiscoverX® Ion Channel Products
   - Capabilities, Stable Cell Lines, and Ready-to-assay Cells
4. Summary and Conclusions

Eurofins DiscoverX® a Global Leader in Ion Channel Assays for Drug Discovery

Accelerating Breakthroughs from Discovery to Delivery

Ion Channel Stable Cell Lines Used for Drug Discovery for 15+ Years

Fremont, California | Lyon, France

eurofins | CALIXAR

Functional Kinases, GPCRs and Ion Channels

Reconstituted Full Length Membrane and Soluble Proteins

Nanodiscs, Proteoliposomes, and Amphipols

65+ Ion Channel Assays

Stable Cell Lines

Ready-to-Assay Frozen Cells

Membrane Preps

Membrane Trafficking Assays

Target Discovery

Hit Screening

Lead Optimization

Safety Studies

Validation Data by Electrophysiology Available for Cell Lines

Eurofins DiscoverX® Ion Channel Stable Cell Lines

PrecisION™ Ion Channel Cell Lines

- High-quality cell lines for target discovery, hit screening, lead optimization, and safety studies
- Suitable for manual and automated electrophysiology and fluorescence imaging studies
- Available as continuous culture and now in a ready-to-assay (RTA) format

Eurofins DiscoverX is now the home and exclusive provider of PrecisION Ion Channel cell lines

- Eurofins DiscoverX, part of Eurofins Discovery, the products company and the leading provider of cell lines, cell-based assays, and enzymes for drug discovery and development
- PrecisION ion channel stable cell lines portfolio was acquired through the acquisition of Millipore (EMD Millipore/Merck Millipore) after a series of smaller acquisitions

Eurofins DiscoverX® Automated Patch Clamp

Cell line production, marketing, and technical support

- Quality control (QC) validation of existing Eurofins DiscoverX PrecisION™ cell lines at the Fremont, CA lab facility
- Development and QC validation of frozen RTA PrecisION channel cell lines
- Validation of custom ion channel targets through custom development capabilities program
- Expansion of our Custom Development Capabilities program into rare disease targets
  - Channelopathy variants and wild type controls

Nanion SyncroPatch 384i

Eurofins DiscoverX® Ion Channel Products

PrecisION™ channel cell lines

hERG (K^+)

hERG-CHO
hERG-HEK

Na^+ Channels

$Na_V1.1$-HEK
$Na_V1.2$-CHO
$Na_V1.3$-CHO
$Na_V1.4$-HEK
$Na_V1.5$-HEK
$Na_V1.6$-HEK
$Na_V1.7$-HEK
$Na_V1.8/\beta1$-HEK
Rat $Na_V1.8$-ND7-23

K^+ Channels

$K_V1.1$-CHO
$K_V1.2$-CHO
$K_V1.3$-CHO
$K_V1.4$-CHO
$K_V1.5$-CHO
$K_V1.6$-CHO
$K_V1.7$-CHO
$K_V1.8$-CHO
$K_V12.2$-HEK
$K_V2.1$-CHO
$K_V2.1/K_V9.2$-CHO
$K_V3.1$-CHO
$K_V3.2$-CHO
$K_V3.3$-CHO
$K_V4.2$/KChIP2-CHO

K^+ Channels

$K_V4.3$/KChIP1-CHO
$K_V4.3$/KChIP2-HEK
$K_V7.2/K_V7.3$-CHO
$K_V7.3/K_V7.5$-CHO
$K_V7.4$-HEK
$K_V7.4/K_V7.5$-HEK
$K_{ir}2.1$-HEK
$K_{ir}6.2$/SUR2A-HEK
KNCQ1/hminK-CHO

HCN Channels

HCN1-HEK
HCN2-HEK
HCN3-HEK
HCN4-CHO

Ca^{2+} Channels

$Ca_V1.2$ $\alpha_{1C}/\beta_{2a}/\alpha_2\delta_1$
$Ca_V2.2$-HEK
$Ca_V3.2$-HEK

Other Channels

ASIC3-HEK
CFTR-HEK
TRPA1-HEK
TRPV1-HEK
TRPV3-HEK
TRPV4-HEK

Ligand–gated Channels

nAChR $\alpha_1/\beta_1/\delta/\varepsilon$-HEK
nAChR α_3/β_4-HEK
nAChR α_4/β_2-HEK
nAChR α_7/ric3-HEK
$GABA_A$ $\alpha_1/\beta_3/\gamma_2$-HEK
$GABA_A$ $\alpha_3/\beta_3/\gamma_2$-HEK
$GABA_A$ $\alpha_4/\beta_3/\gamma_2$-HEK
$GABA_A$ $\alpha_5/\beta_3/\gamma_2$-HEK
$GABA_A$ $\alpha_6/\beta_3/\gamma_2$-HEK
GlyRA1-HEK
GluR6-HEK

Human origin unless otherwise noted

CRO Services are performed on these cell lines by our sister company Eurofins-Discovery in St. Charles, MO

New PrecisION™ Ready-To-Assay Cells

Benefits of Ready to Assay (RTA) Cells

- RTA cells eliminate the need for continuous cell culture
- Eliminates inconsistencies that can be inherent in cell line cultures due to:
  - Different cell culture technicians
  - Different time windows of stable cell lines in culture
- RTA cells are frozen down at optimal expression
- Every production lot is QC validated

New PrecisION RTA cells *Coming soon!*

Cardiac Safety Panel:

- hERG-CHO (CYL3038RTA)
- Nav1.5-HEK (CYL3004RTA)
- Cav1.2 (CYL3051RTA)

Provided with a Certificate of Analysis that includes electrophysiology data and pharmacological characterization for each lot produced

Pain Targets:

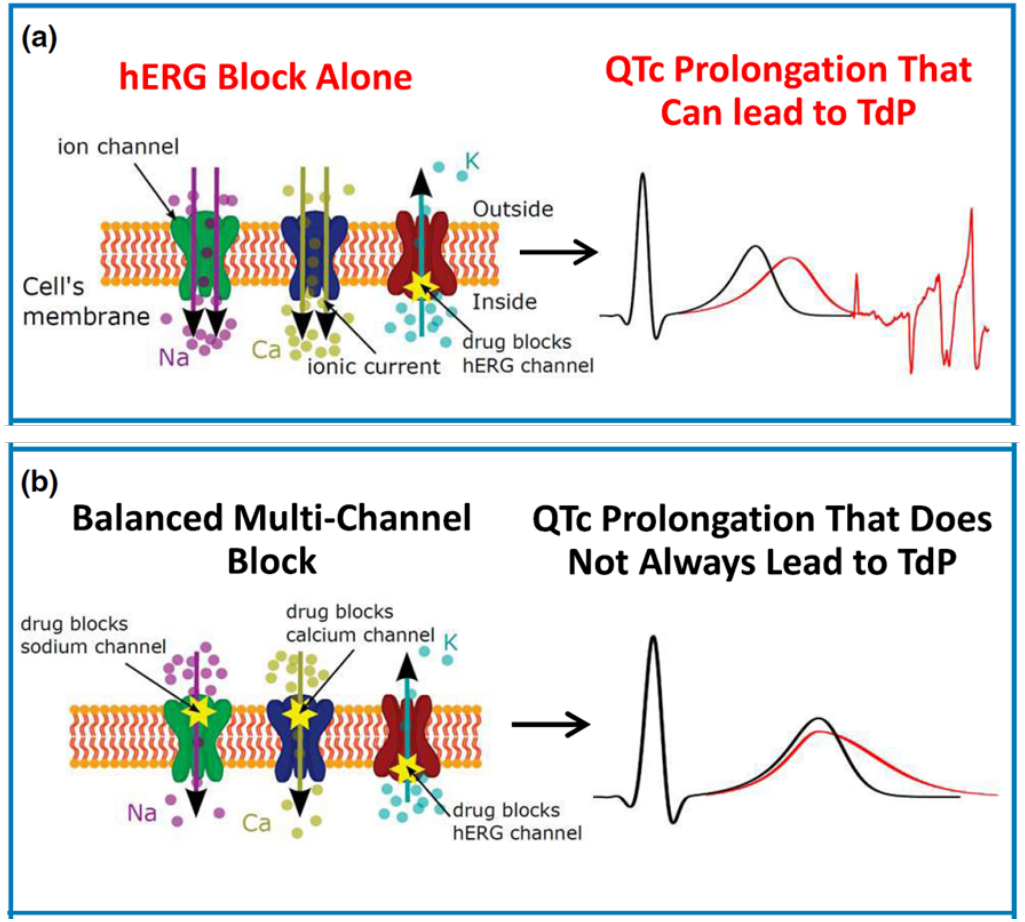
- Nav1.8 (CYL3025RTA)
- And other popular therapeutic pain targets

Let us know your favorites!

New PrecisION™ Ready-To-Assay Cells

In support of the new FDA CiPA guidelines, Eurofins DiscoverX® is launching the **Cardiac Safety Panel PrecisION stable cell lines in a frozen Ready-to-assay (RTA) format**

Blockade of Nav1.5 and/or Cav1.2 can offset hERG blockade and render the drug safe.



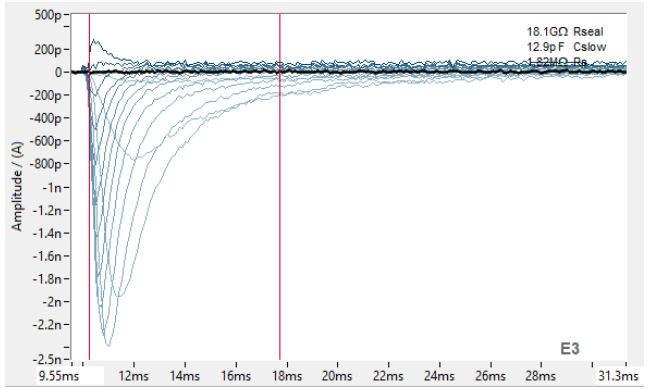
Translational Models and Tools to Reduce Clinical Trials and Improve Regulatory Decision Making for QTc and Proarrhythmia Risk (ICH E14/S7B Updates)

David G. Strauss[1,*], Wendy W. Wu[1], Zhihua Li[1], John Koerner[2] and Christine Garnett[3]

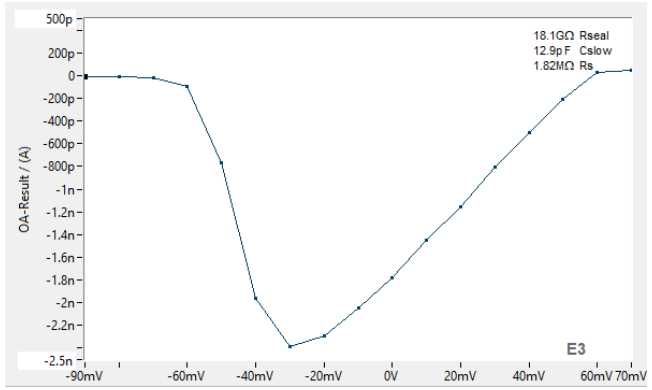
CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 109 NUMBER 2 | February 2021 doi:10.1002/cpt.2137

FDA = US Food and Drug Administration; CiPA = Comprehensive In Vitro Proarrhythmia Assay

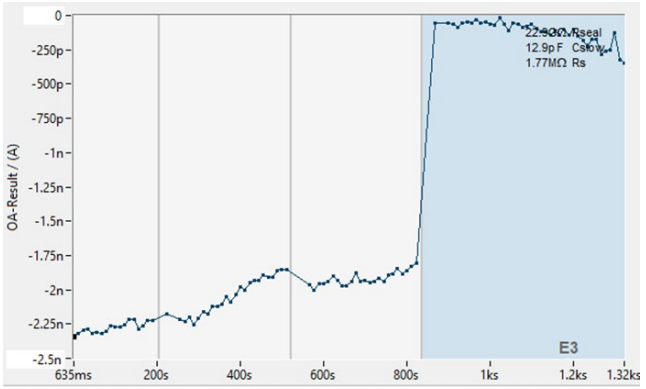
Current Voltage (I/V) Relationship and Tetracaine Pharmacology of PrecisION™ Nav1.5 Ready-To-Assay Cells



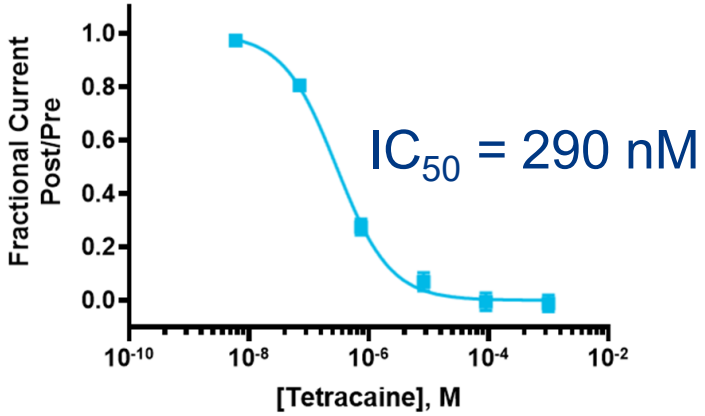
Raw Ionic Currents



I/V Plot



Peak Current over time (I-t plot)

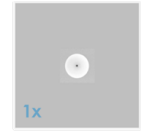


IC_{50} = 290 nM

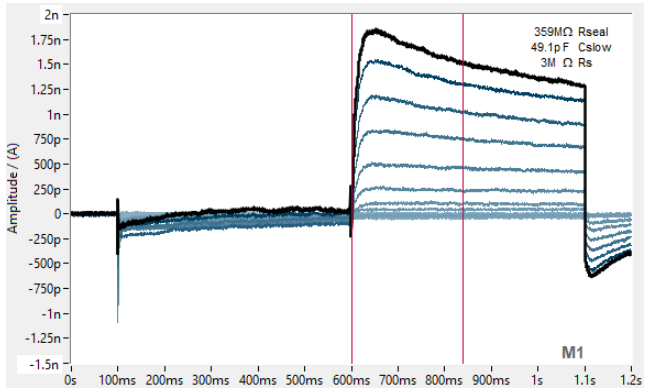
Nav1.5-HEK		
Part #	**Channel**	**Mean Success**
CYL3004	Nav1.5	74.0% (n = 2 runs)
CYL3004-RTA	Nav1.5-RTA	71.7% (n = 2 runs)

- Stable RTA currents are obtained that are sensitive to tetracaine
- Success rates are similar stable cell line

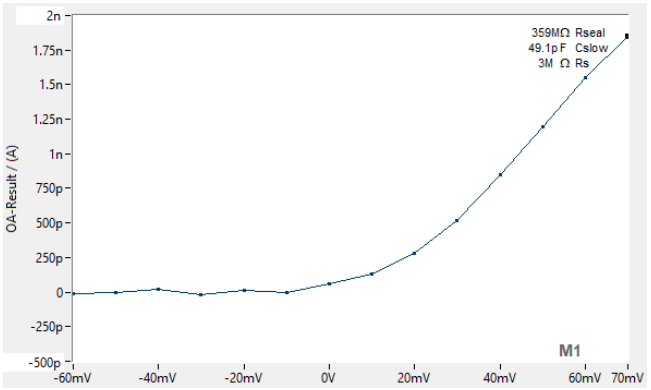
1-Hole/well Recording Plate



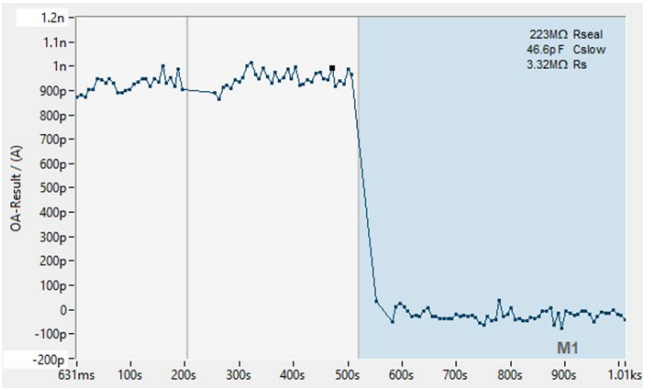
Current Voltage (I/V) Relationship and Cisapride Pharmacology of hERG-CHO Ready-To-Assay Cells



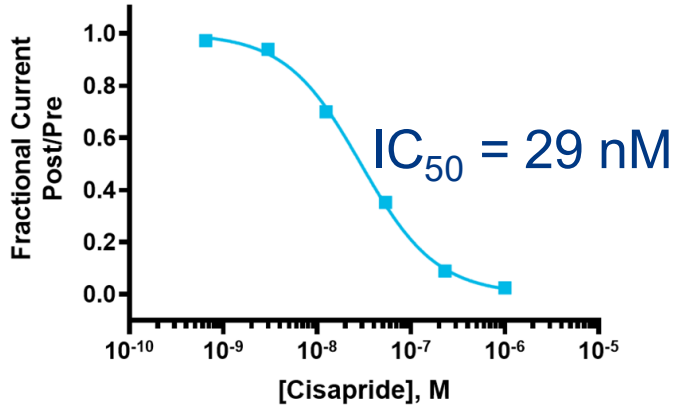
Raw Ionic Currents



I/V Plot



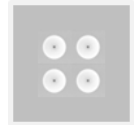
Peak Current over time (I-t plot)



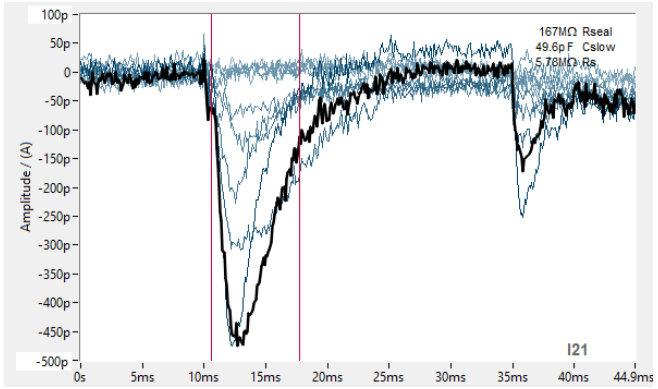
hERG-CHO		
Part #	**Channel**	**Mean Success**
CYL3038	hERG Stables	87.5% (n = 2 runs)
CYL3038-RTA	hERG RTA's	83.0% (n = 2 runs)

- Stable RTA currents are obtained that are sensitive to cisapride
- Success rates are similar stable cell line

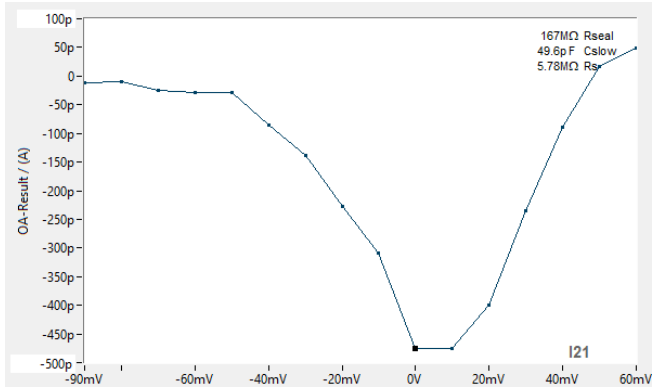
4-Holes/well Population Patch Plate



PrecisION™ Nav1.8 and Cav1.2 Ready-To-Assay Cells

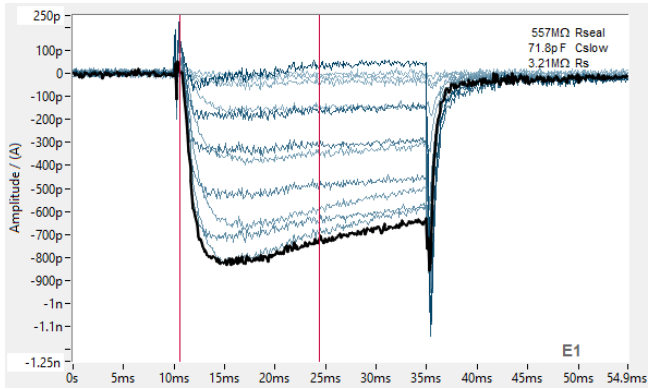


Raw Ionic Currents

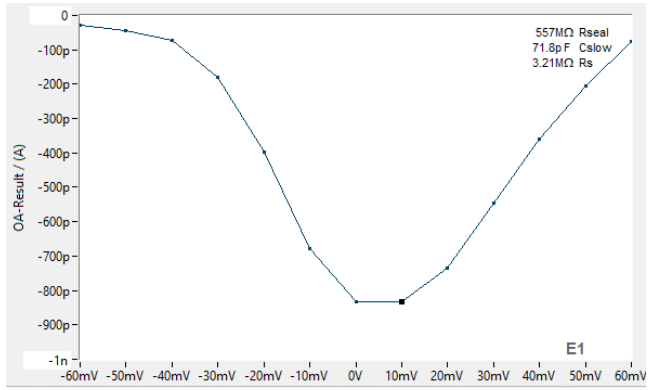


I/V Plot

Nav1.8-HEK		
Part #	**Channel**	**Mean Success**
CYL3025-RTA	**Nav1.8-RTA**	**73.2% (n = 2 lots)**



Raw Ionic Currents



I/V Plot

Cav1.2-HEK		
Part #	**Channel**	**Mean Success**
CYL3051-RTA	**Cav1.2-RTA**	**67.9% (n = 2 lots)**

Stable RTA currents are obtained and the success rates are similar stable cell line

Summary and Conclusions

- Analgesics designed to inhibit the activity of the Nav1.8 channel in the pain pathway are promising non-addictive alternatives to opioid analgesics
- Latigo has presented compelling evidence that LTGO-33 is a novel Nav1.8 inhibitor
- Eurofins DiscoverX® is in the process of converting selected stable cell lines into ready-to-assay format include cardiac safety and therapeutic pain targets

Learn more at discoverx.com/target-class/ion-channel/