ICAGEN™

ADVANCING ION CHANNEL & TRANSPORTER DISCOVERY

Douglas Krafte, CSO
Nathan Zahler, Product Manager

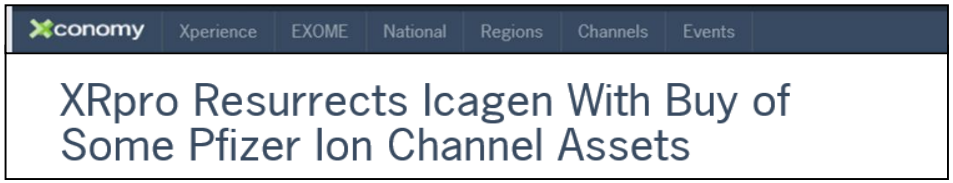
Aurora's 13th Annual Ion Channel Retreat
July 7-9, 2015
Vancouver BC, Canada

Why Icagen and Why Now?


FierceBiotech
THE BIOTECH INDUSTRY'S DAILY MONITOR
NEWS TOPICS ANALYSIS FEATU
Four years after buyout, Pfizer spins Icagen ion channel platform back out
July 2, 2015 | By John Carroll
FierceBiotech
THE BIOTECH INDUSTRY'S DAILY MONITOR


EXCLUSIVE: XRpro's Revamped Icagen, Inc. (ICGN) May See Hiring, CEO Tells BioSpace (DHX)
Like Share Share g+1
7/2/2015 9:51:47 AM
BioSpace
Life Sciences News + Jobs

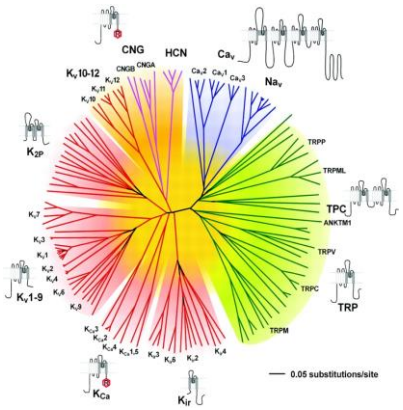

INDUSTRY NEWS › HEALTH CARE
Icagen, acquired by Pfizer in 2011, turns next chapter
Jul 2, 2015, 9:30am EDT Updated Jul 2, 2015, 9:51am EDT
TRIANGLE BUSINESS JOURNAL


Xconomy Xperience EXOME National Regions Channels Events
XRpro Resurrects Icagen With Buy of Some Pfizer Ion Channel Assets

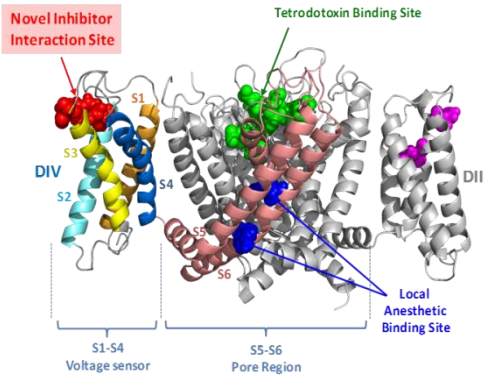
- **Unique Experience & Depth of Expertise**
  - 20+ years of ion channel drug discovery experience
  - Clinical candidates identified independently, with pharma collaborators and as Pfizer
- **Technical Know-How and Capabilities**
  - Very wide array of assay technologies including proprietary X-Ray fluorescence platform (XRpro®)
  - Extensive Cell Line and Reagent Inventory
- **Growing Industry Need for Access to External Expert Technology**

Ion Channel Core Platform
Know-How and Capabilities

Ion Channel Genome

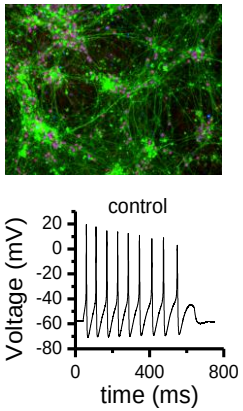


Broad Technological Capabilities



Custom Cell Lines to Identify 1st/Best in Class Molecules Compounds

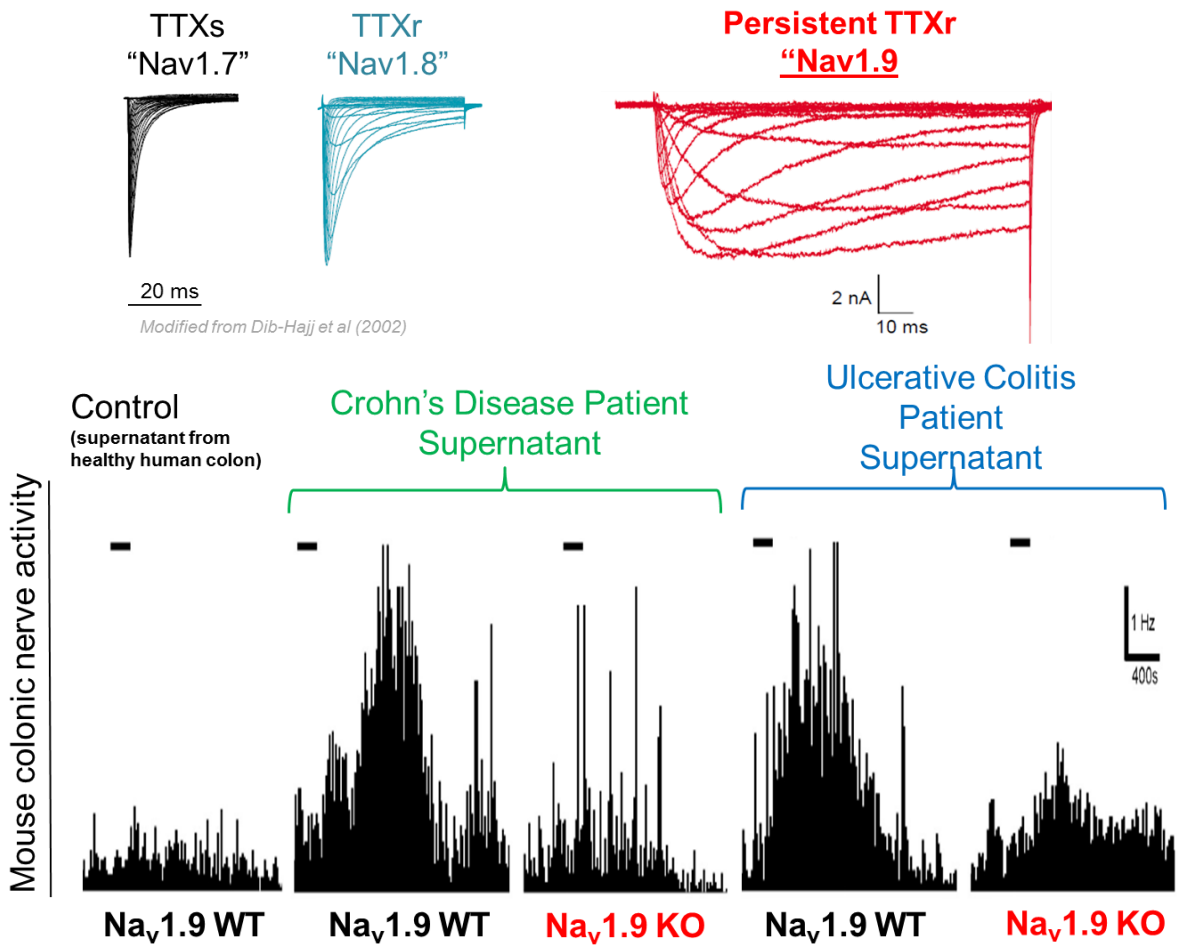
iPSC-Based Electrophysiology



The strongest ion channel drug discovery platform in the industry

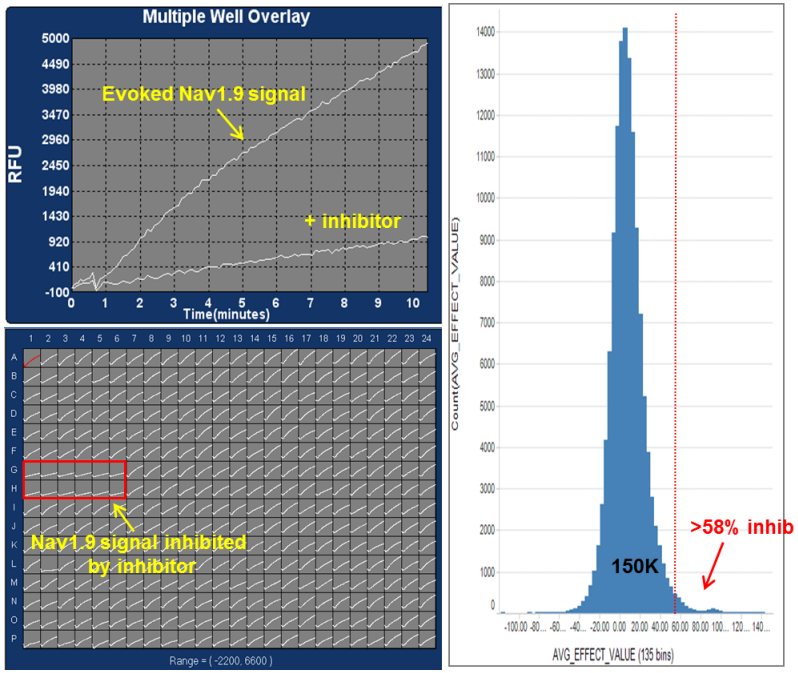
- **applied technology to support small molecule and biologics drug discovery**
- **reagents and tools leading to unique sub-type selective ion channel compounds**
- **stem cell approaches to drive precision medicine**
- **structural biology support to drive SAR (e.g. bespoke channels with modified binding sites)**

$Na_V1.9$
Genetically Validated Target in Pain

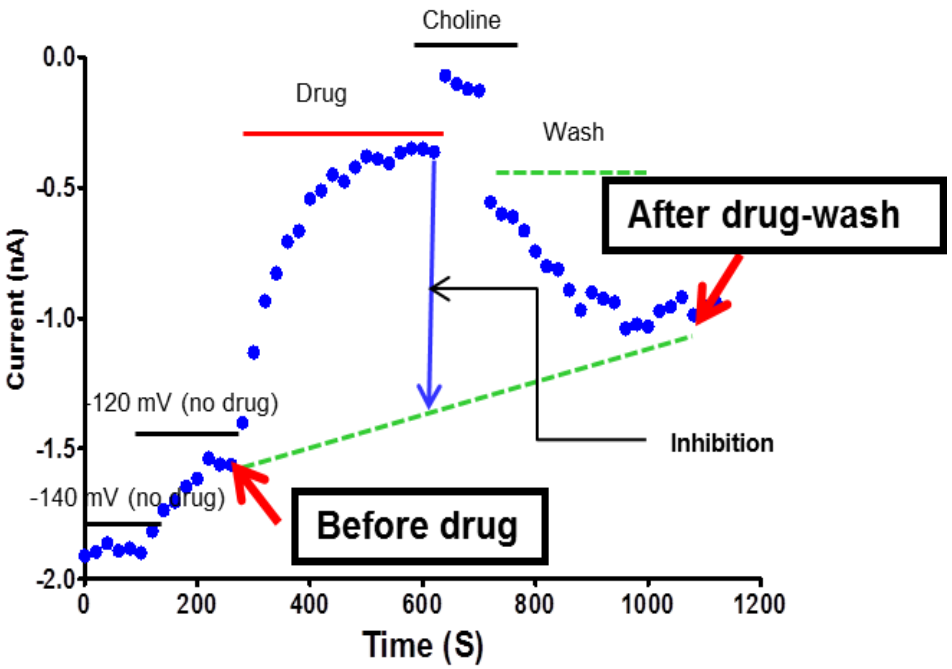


$Na_V1.9$
1st in Class Assay Platform

384-well Nav1.9 HTS Assay



Nav1.9 HT Electrophysiology Assay



Example of Depth in Ion Channels

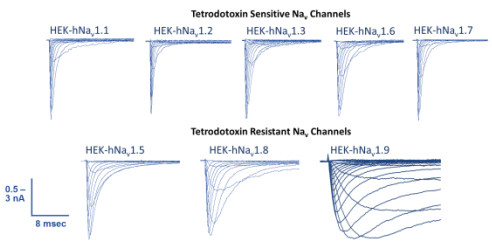
Enabling Platform for Sodium Channel Drug Discovery

Abstract

Voltage gated sodium (Na_v) channels are important drug development targets for a wide variety of therapeutic including pain, epilepsy and cardiac rhythm disorders. For example, in the pain therapeutic area, there is considerable interest in $Na_v1.7$ and more recently $Na_v1.9$ because human gain and/or loss of function mutations of these channels are associated with hypersensitivity or complete loss of sensitivity to pain.

Icagen brings more than two decades of experience in ion channel drug discovery research and development, with a record of successfully moving compounds from discovery into clinical development across a variety of therapeutic areas, both alone and in partnership with leading pharmaceutical developers. Icagen has successfully prosecuted programs for identification and development of modulators for both $Na_v1.7$ and the historically challenging $Na_v1.9$ sodium channel. Utilizing a broad portfolio of recombinant cell reagents and assay platforms, Icagen is able to run high throughput screening of >500K compound libraries, electrophysiological evaluation of Na_v channel subtype selectivity, species ortholog activity along with mechanism and site of action assessment utilizing channel mutation and detailed biophysical and pharmacological analysis.

Comprehensive Portfolio of Na_v Channel Cell Lines

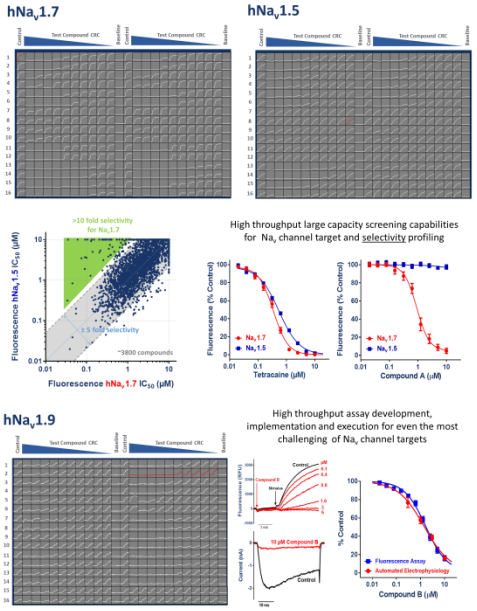


	$Na_v1.1$	$Na_v1.2$	$Na_v1.3$	$Na_v1.4$	$Na_v1.5$	$Na_v1.6$	$Na_v1.7$	$Na_v1.8$	$Na_v1.9$
Human	■	■	■	■	■	■	■	■	■
NH Primate	■		■			■	■		
Dog	■						■		
Rat	■	■	■	■		■	■	■	■
Mouse	■		■			■	■	■	■

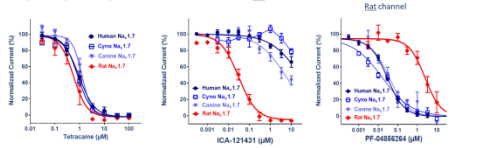
Comprehensive Na_v Channel Assay Platforms

Validated Assays	$Na_v1.1$	$Na_v1.2$	$Na_v1.3$	$Na_v1.4$	$Na_v1.5$	$Na_v1.6$	$Na_v1.7$	$Na_v1.8$	$Na_v1.9$
Manual Patch Clamp	■	■	■	■	■	■	■	■	■
Automated Patch Clamp	■	■	■	■	■	■	■	■	■
Fluorescence Flux HTS Assay					■	■	■		■
Isotope Flux HTS assay			■				■	■	

384-Well HTS for Na_v Channel Modulators

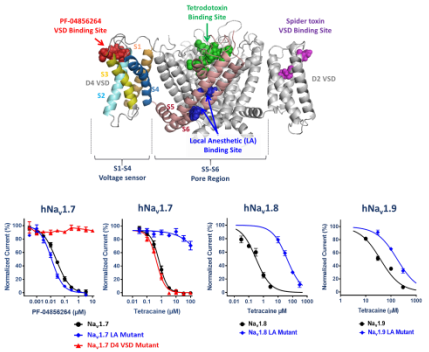


Activity Versus Na_v Channel Species Orthologs



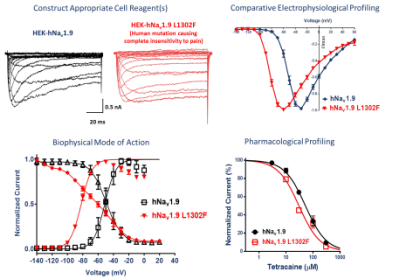
Non-human species are employed throughout the preclinical drug development process to assess on and off target mediated efficacy and/or toxicity. Icagen provides the ability to confirm activity, potency and selectivity for target Na_v channel species orthologs commonly used in such evaluations

Where Does My Compound Bind?



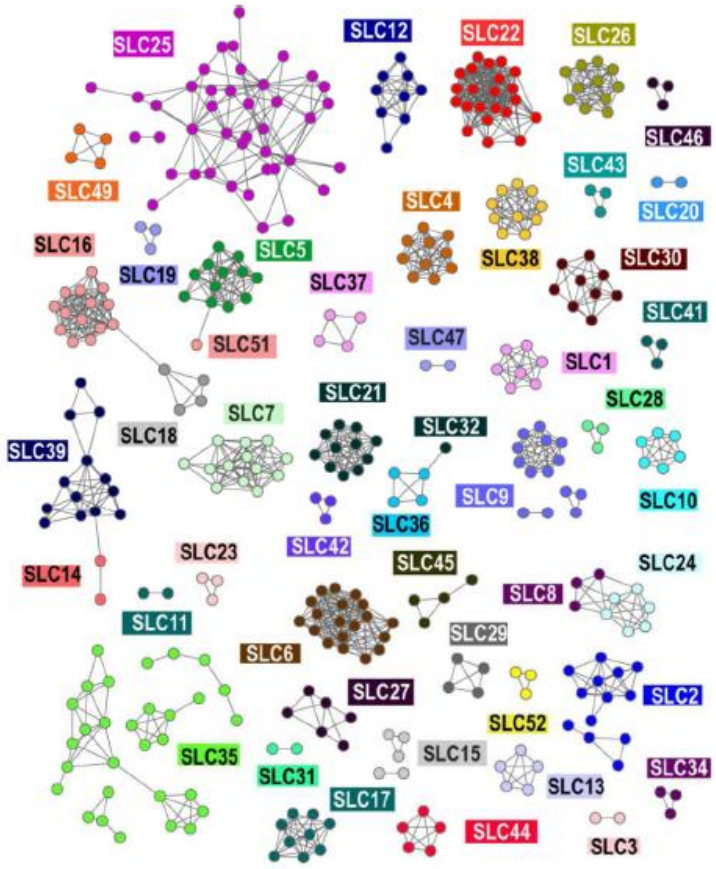
Cell line	$Na_v1.1$	$Na_v1.2$	$Na_v1.3$	$Na_v1.4$	$Na_v1.5$	$Na_v1.6$	$Na_v1.7$	$Na_v1.8$	$Na_v1.9$
Local Anesthetic Binding Site Mutant					■		■	■	■
D4 VSD Inhibitor Binding Site Mutant			■				■		

Detailed Biophysical and Pharmacological Analysis

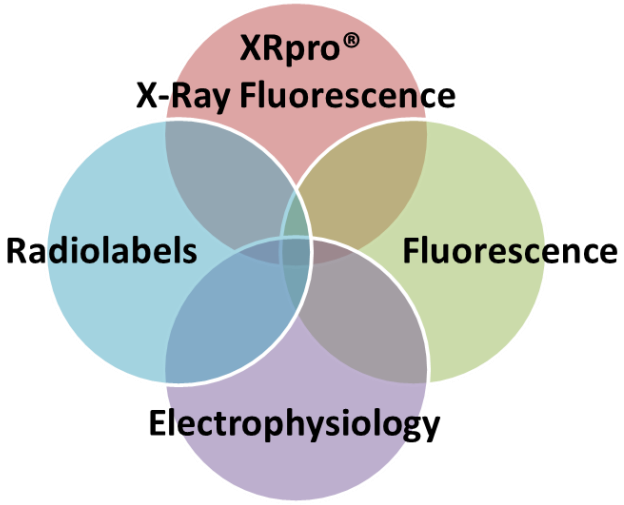


Developing a Transporter Discovery Platform
Leveraging Experience and Tools

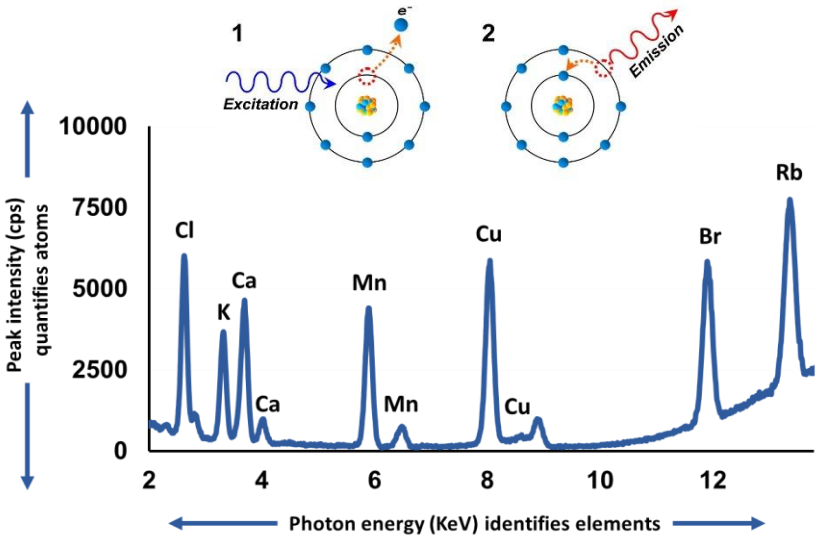
SLC Family of Transporters

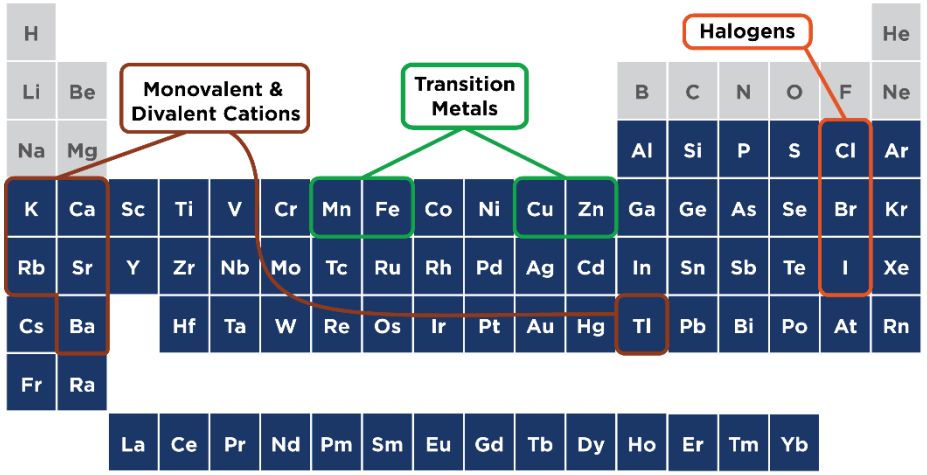


- **SLCs a large emerging family of therapeutic targets**
  - *Lin et al Nat Rev Drug Disc 26-June-2015*
- **Appropriate assay platforms remain a bottleneck**
- **Icagen's array of technologies facilitates drug discovery in this space**



XRpro® Technology
Fluorescence of Atoms

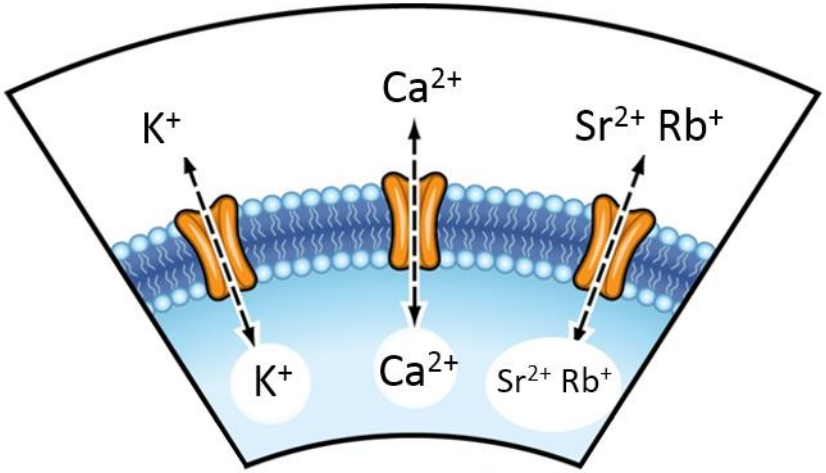




- Direct measurements of elements shown in **blue**
- Standard cell biology, with protocols similar to ^{86}Rb flux assays
- No dyes, fluorophores, or radiolabels
- Biochemically important elements and tracer elements (*e.g.*, K and Rb)
- Measurements in complex and optically opaque matrices, including serum, high DMSO, etc.

XRpro® Ion Flux Measurements

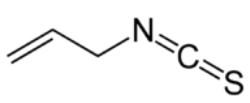
Case Study: TRPA1 Analysis



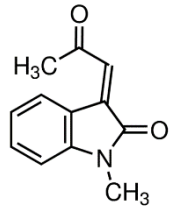
TRPA1

- Drug target for pain
- Nonspecific K^+ / Ca^{2+} channel
- Measure monovalent efflux with Rb^+
- Measure divalent influx with Sr^{2+}
- Assays in buffer or **100% serum**

Agonists

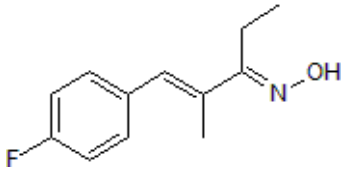


AITC

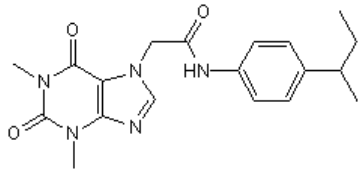


Supercinnamaldehyde

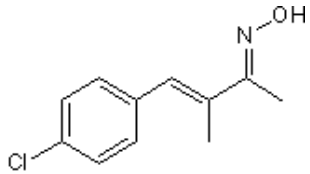
Antagonists



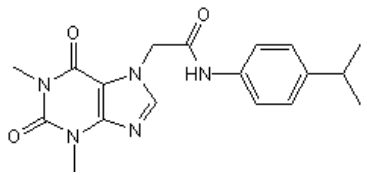
A 967079



TCS 5861528



AP 18



HC 030031

XRpro® Ion Flux Measurements

Case Study: TRPA1 Analysis

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Goals

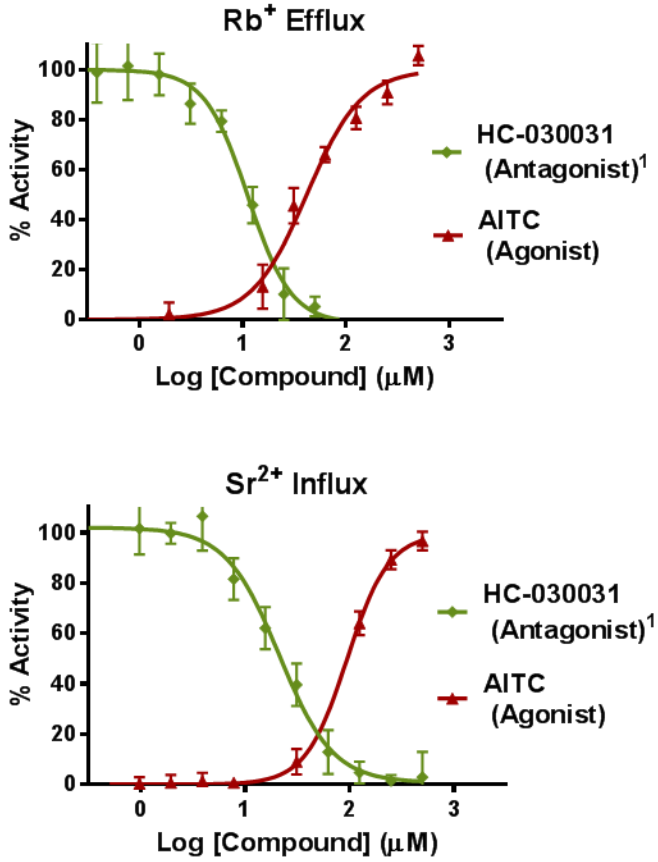
- Monovalent ion (Rb^+) efflux
- Divalent ion (Sr^{2+}) influx

Results

	Rb^+ Efflux	Sr^{2+} Influx	Expected
	EC_{50} (µM)	EC_{50} (µM)	EC_{50} (µM)
AITC	9.8 ± 0.5		3 to 300
Supercinnamaldehyde	10.5 ± 0.3		0.8
TCS 5861528†	12 ± 1	12 ± 1	14
HC 030031†	11 ± 1	22 ± 1	5
A 967079†	0.06 ± 0.03	0.051 ± 0.006	0.07
AP 18‡	0.15 ± 0.01	0.69 ± 0.04	3

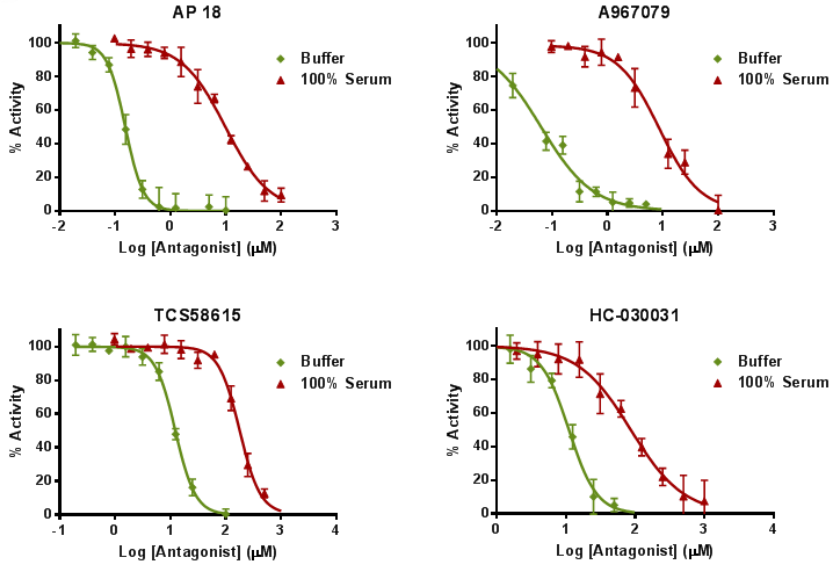
† With 200 µM AITC

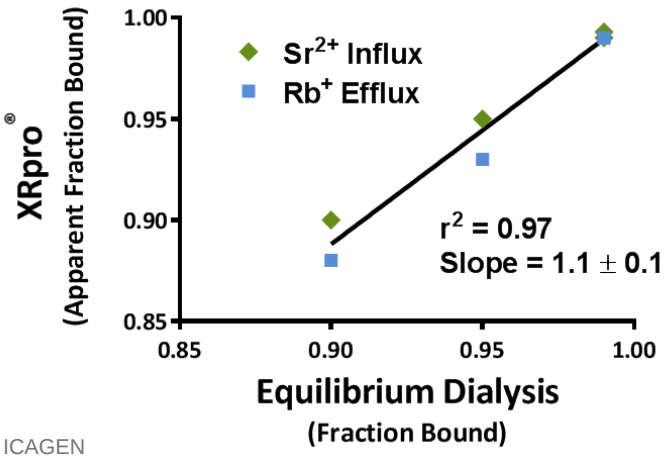
‡ With 100 µM supercinnamaldehyde



XRpro® Ion Flux Measurements

Case Study: TRPA1 Analysis in 100% Serum





Experiment

- Sr^{2+} influx
- Analysis in buffer and 100% human serum

Results

	Without Serum	**100% Serum**
	EC_{50} (µM)	EC_{50} (µM)
AITC	9.8 ± 0.5	41 ± 6
Supercinnamaldehyde	10.5 ± 0.3	47 ± 1
TCS 5861528†	12 ± 1	180 ± 10
HC 030031†	11 ± 1	90 ± 10
A 967079†	0.06 ± 0.03	8 ± 2
AP 18‡	0.15 ± 0.01	10 ± 1

† With 200 µM AITC
‡ With 100 µM supercinnamaldehyde

Conclusions

- Measurements in 100% human serum
- Functional serum shift measurements

XRpro® SLC Transporters
Nonelectrogenic Transporters

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CCC Transporters (SLC12)

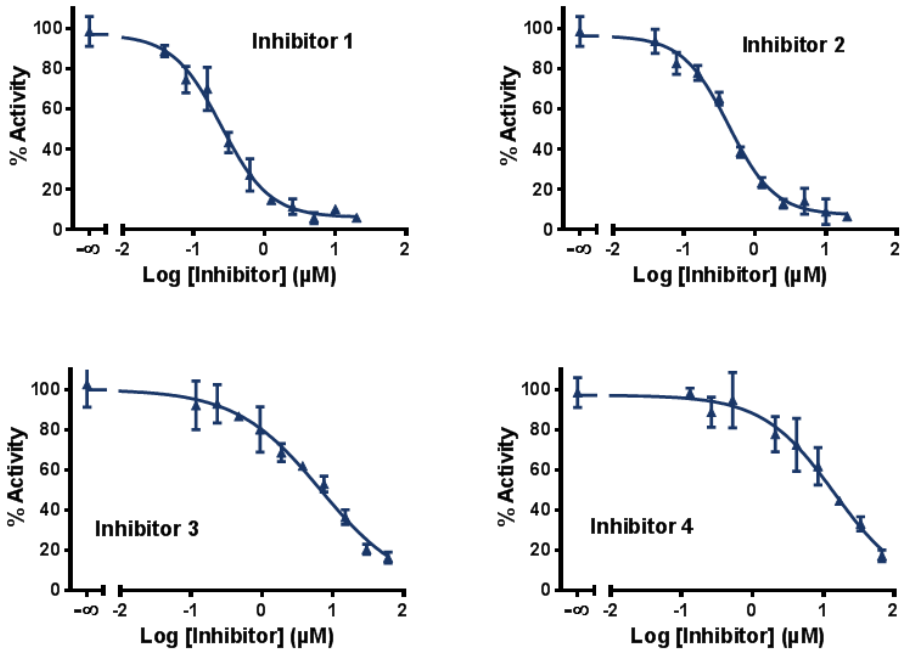
- **Non-electrogenic** symporters
- Family includes neurological targets regulating intracellular [Cl^-]

Goals

- Measure activity with Rb^+ tracer
- Optimize existing assay for XRpro®
- Match blinded validation

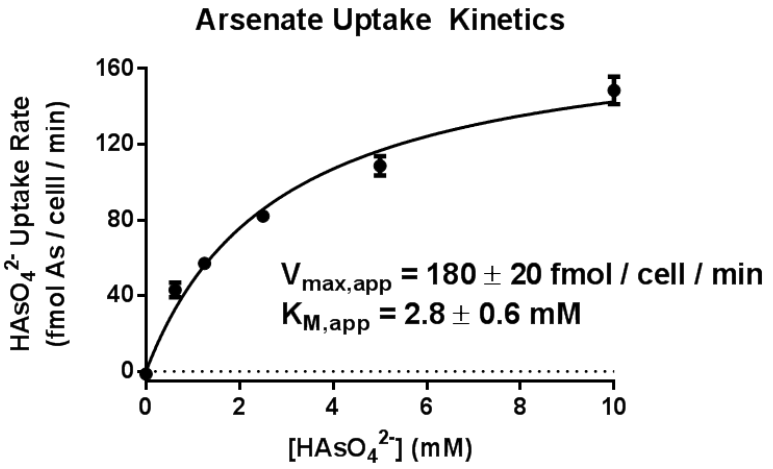
Results

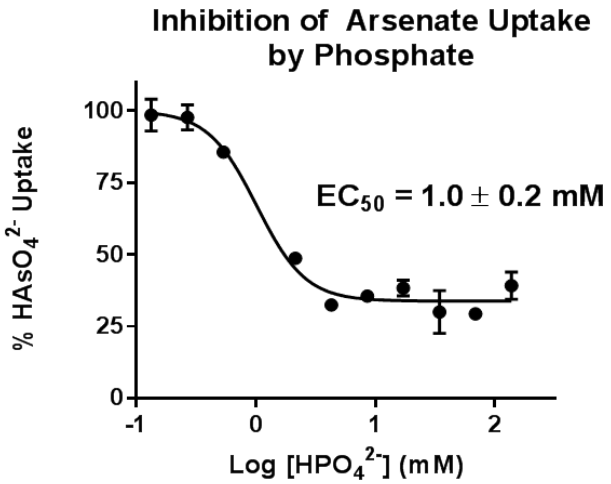
- Z' > 0.7
- Improved assay, reduced costs
  - Removed 3 of 4 wash steps
  - Moved from 96- to 384-well format
- XRpro® matched previous values



	XRpro® EC_{50} (µM)	Client Value EC_{50} (µM)
Inhibitor 1	0.5 ± 0.2	0.5
Inhibitor 2	0.3 ± 0.1	1.1
Inhibitor 3	7 ± 1	9
Inhibitor 4	17 ± 1	18

XRpro® SLC Transporters
Phosphate Transporter Kinetics





Na^+-P_i Transporters (SLC20, SLC34)

- Sodium / phosphate symporter
- Primary transport pathway for arsenate ($HAsO_4^{2-}$) uptake.

Goals

- Measure endogenous transporters
- Establish $HAsO_4^{2-}$ as a tracer for P_i

Conclusions

- ✓ V_{max} and K_M determinations for $HAsO4^{2-}$
- ✓ $HAsO_4^{2-}$ and P_i are competitive
  - Consistent with shared uptake pathway
  - $HAsO_4^{2-}$ is a functional surrogate for HPO_4^{2-}

[1] Maciaszczyk-Dziubinska *et al.*, 2012, *Int. J. Mol. Sci.* **13** (3527-3548)

SLC Transporters

Zn^{2+}: An Emerging Target

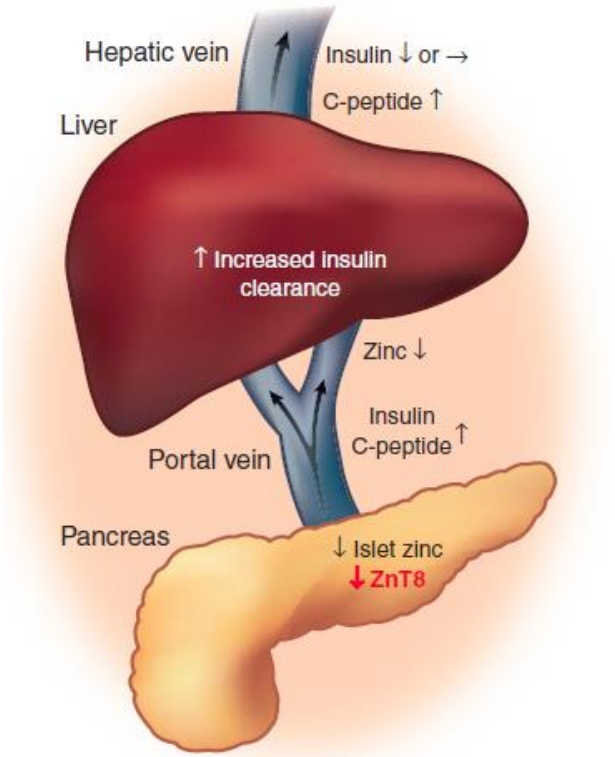
LETTERS

nature genetics

Loss-of-function mutations in *SLC30A8* protect against type 2 diabetes

Jason Flannick[1–3], Gudmar Thorleifsson[4], Nicola L Beer[1,5], Suzanne B R Jacobs[1], Niels Grarup[6], Noël P Burtt[1], Anubha Mahajan[7], Christian Fuchsberger[8], Gil Atzmon[9,10], Rafn Benediktsson[11], John Blangero[12], Don W Bowden[13–16], Ivan Brandslund[17,18], Julia Brosnan[19], Frank Burslem[20], John Chambers[21–23], Yoon Shin Cho[24], Cramer Christensen[25], Desirée A Douglas[26], Ravindranath Duggirala[12], Zachary Dymek[1], Yossi Farjoun[1], Timothy Fennell[1], Pierre Fontanillas[1], Tom Forsén[27,28], Stacey Gabriel[1], Benjamin Glaser[29,30], Daniel F Gudbjartsson[4], Craig Hanis[31], Torben Hansen[6,32], Astradur B Hreidarsson[11], Kristian Hveem[33], Erik Ingelsson[7,34], Bo Isomaa[35,36], Stefan Johansson[37–39], Torben Jørgensen[40–42], Marit Eika Jørgensen[43], Sekar Kathiresan[1,44–46], Augustine Kong[4], Jaspal Kooner[22,23,47], Jasmina Kravic[48], Markku Laakso[49], Jong-Young Lee[50], Lars Lind[51], Cecilia M Lindgren[1,7], Allan Linneberg[40,41,52], Gisli Masson[4], Thomas Meitinger[53], Karen L Mohlke[54], Anders Molven[37,55,56], Andrew P Morris[7,57], Shobha Potluri[58], Rainer Rauramaa[59,60], Rasmus Ribel-Madsen[6], Ann-Marie Richard[19], Tim Rolph[19], Veikko Salomaa[61], Ayellet V Segrè[1,2], Hanna Skärstrand[26], Valgerdur Steinthorsdottir[4], Heather M Stringham[8], Patrick Sulem[4], E Shyong Tai[62–64], Yik Ying Teo[62,65–68], Tanya Teslovich[8], Unnur Thorsteinsdottir[4,69], Jeff K Trimmer[19], Tiinamaija Tuomi[27,35], Jaakko Tuomilehto[70–72], Fariba Vaziri-Sani[26], Benjamin F Voight[1,73,74], James G Wilson[75], Michael Boehnke[8], Mark I McCarthy[5,7,76], Pål R Njølstad[1,37,77], Oluf Pedersen[6], Go-T2D Consortium[78], T2D-GENES Consortium[78], Leif Groop[48,79], David R Cox[58], Kari Stefansson[4,69] & David Altshuler[1–3,44,45,80,81]

Flannick et al 2014 Nature Genetics

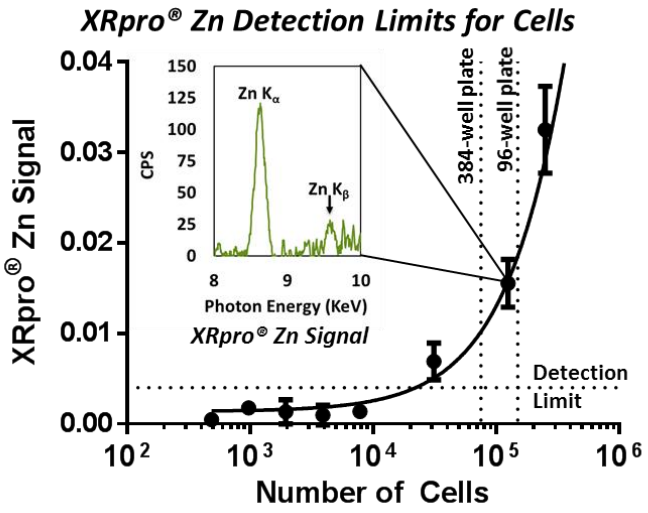


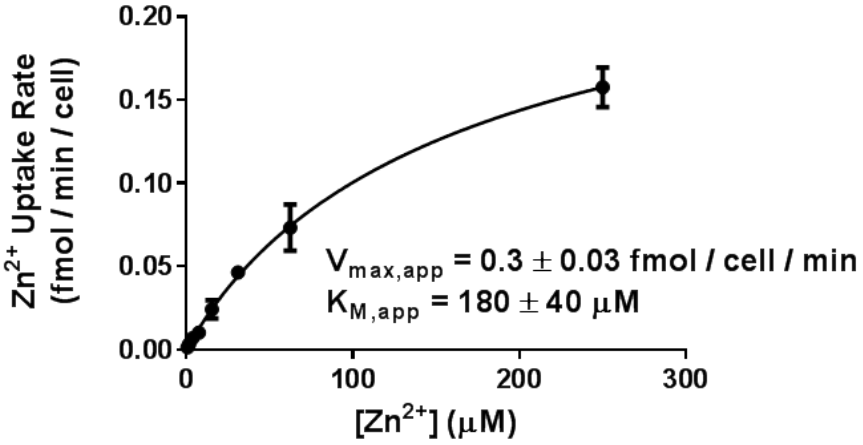
Pearson 2014 Nature Genetics

- Zn^{2+} is a critical, highly regulated trace metal.
- Loss of function variants of SLC30A8 reduce T2D risk by 65%

XRpro® SLC Transporters

Zn^{2+} Transporters





Zinc Transporters (SLC30, SLC39)

- Essential biological trace metal with highly regulated intracellular concentrations
- Central role in insulin packaging and release

Results

- Direct measurement of Zn^{2+} content for cells grown in 96- or 384-well plates
- Time and concentration dependent Zn^{2+} uptake

Conclusions

✓ Measurement of endogenous Zn^{2+} transporters shows an apparent K_M in the μM range

Enabling Ion Channel & SLC Drug Discovery

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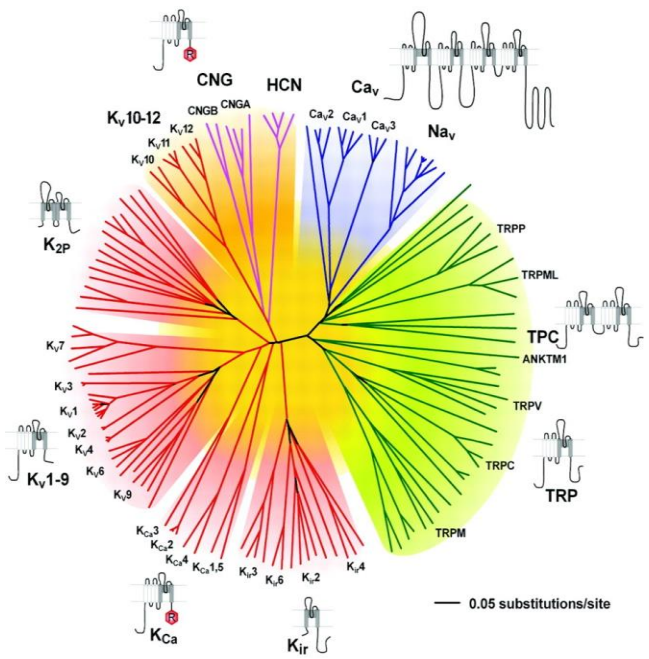
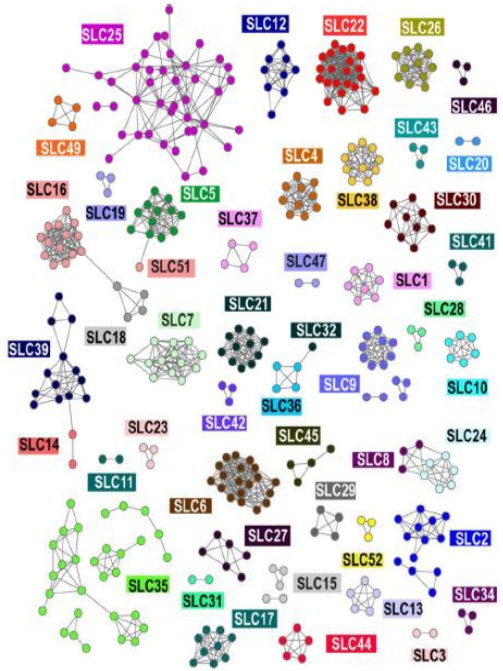
Ion Channel Gene Family



SLC Family of Transporters



Assays (x-ray, fluorescence, radio tracer)
Electrophysiology (PX, IWQ, Q-Patch, Patchliner, Manual)
Assay Development & Custom Cell Line Generation
Drug Discovery Partner (HTS, SAR Development, Lead Optimization)

ICAGEN™

ADVANCING ION CHANNEL & TRANSPORTER DISCOVERY

Douglas Krafte, CSO
Nathan Zahler, Product Manager

Aurora's 13th Annual Ion Channel Retreat
July 7-9, 2015
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