Screening Services & Assay Development

Solutions for Cardiac Safety & Drug Discovery

Preclinical cardiac safety screening

- Cardiac ion channels
- in vivo models

Screening of therapeutic ion channels targets

- Cell line validation
- Estimation of Ions in Biological Samples

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What are ion channels? What is their therapeutic importance?

Ion channels as therapeutic targets:

Ion channels are cell membrane embedded proteins that act as highly specific filters, allowing only desired ions, such as Na^+, K^+, Ca^{2+} or Cl^-, to pass through the cell membrane. These channels provide a hydrophilic pathway (pore) across the hydrophobic interior of the membrane. They are able to discriminate between size and charge of the different ions, conducting only the ion of interest through the membrane. The opening and closing of ion channels may depend on changes in membrane voltage, changes in membrane mechanical stretch, or binding of ligand molecules to the channel.

Mutations in genes coding for ion channels are associated with a number of "channelopathies", including a large group of cardiac diseases. As such, ion channels represent one of today's most promising and exciting classes of therapeutic targets.

Ion channels and transporters in cardiac disease:

In addition to mutational effects, unintentional activity of cardiac ion channels have become an important concern in the field of safety pharmacology. In human cardiac myocytes, action potential events are mediated by a number of ion channels and transporters (Fig. 1)[2].

Patients with Long QT Syndrome (LQTS) have mutations in some of these ion channels, causing a decreased rate of depolarization or repolarization that leads to prolonged action potential duration. Unintentional drug activity on these ion channels has shown similar effects. This has led to a number of compounds to be withdrawn from the market or removed from clinical studies.

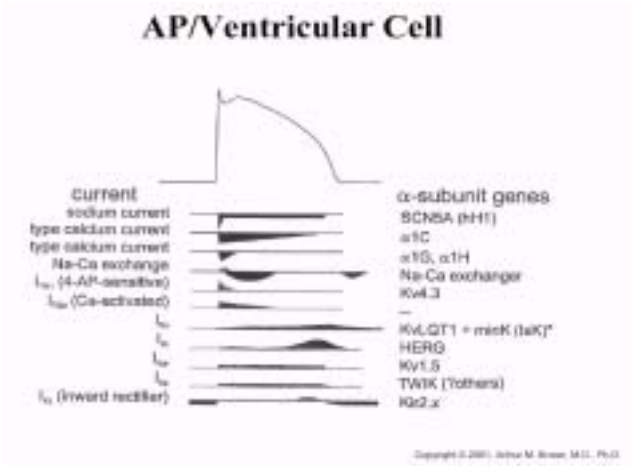

Fig 1. Ion channels involved in the action potential of ventricular cells

References:
1. Akar et al.: Ion channels as novel therapeutic targets in heart failure. Ann Med. 2005;37(1):44-54.
2. Brown et al.: Ion channels in drug safety testing and drug discovery. 2005, PPT.

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Aurora Biomed's screening technology

For ion channel screening, Aurora Biomed employs flux assay technology in which non-radioactive ions are used as tracers for specific ion channels. Tracer ions are loaded either into the cells or into the extracellular solution. The cells are then bathed in a solution containing the compound(s) of interest to determine its effect on channel activity. The amount of tracer ion present in the intra- and extracellular portions of the cells is measured using Aurora Biomed's sensitive, robust, and high-throughput proprietary atomic absorbance-based Ion Channel Reader (ICR) series.

The following tracers are used for different ion channels and transporters:

- Potassium ion (K^+) channels: Rubidium (Rb^+) for K^+ ion[1].
- Sodium ion (Na^+) channels: Lithium (Li^+) for Na^+ ion[2].
- Chloride ion (Cl^-) channels: A known concentration of silver (Ag^+) is used to precipitate Cl^- from the samples as AgCl, and then free Ag^+ is measured[3].
- Calcium ion (Ca^{2+}) channels: Ca^{2+} or Strontium (Sr^{2+}) for Ca^{2+} ion[4].
- Na^+,K^+-ATPase: Rb^+ for K^+ ion[5].
- K^+,Cl^- co-transporter: Ag^+ precipitation for Cl^- ion[6].

Advantages of flux screens in drug discovery?

1. Flux assays are functional, robust, and widely used by various pharmaceutical companies.
2. ICR measurements of the ions are very sensitive with extremely low percentage CVs among the replicates.
3. Assay volumes are less than 100 µL, minimizing the amount of compound used.
4. The screens are carried out in the presence of 1% DMSO, minimizing compound solubility issues.
5. Z' values of the screens are always higher than 0.8 indicating high robustness and low variability.
6. With a large window of detection presented by the screens, test compounds emerge as either clear blockers/non-blockers or activators/nonactivators[7].
7. Well-established compounds of known activity against specific ion channel targets are used as positive controls.
8. Drug potencies determined by flux screens are highly correlated with those of electrophysiology. Calculated drug rank orders between the two methods are identical.
9. Flux assays involve about $1.38x10^5$ cells per well and thus provide multicellular response compared to other techniques measuring only a few ion channels.

References:
1. Terstappen: Nonradioactive rubidium ion efflux assay and its applications in drug discovery and development. Assay Drug Dev Technol 2004; 2(5):253-7.
2. Gill et al.: HTS cell-based flux assays for sodium channels. (Poster: Biophysical Society Meeting 2003).
3. Gill et al.: Development and validation of HTS flux assay for endogenously expressed chloride channels in a CHO-K1 cell line. Assay Drug Dev Technol 2006; 4(1):65-71.
4. Gill et al.: Development of HTS flux assay for Ca^{2+} activated chloride channels (Poster: SBS Conference, 2004).
5. Gill et al.: Development of a HTS assay for Na^+,K^+-ATPase using nonradioactive rubidium ion uptake. Assay Drug Dev Technol 2004; 2(5):535-42.
6. Gill: Flux assays for screening compounds against ion channels and transporters. Ion Channel Retreat, Vancouver, Canada, 2004.
7. Wang et al.: Validation of an atomic absorption rubidium ion efflux assay for KCNQ/M channels using the Ion Channel Reader 8000. Assay Drug Dev Technol 2004; 2(5):525-34.

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Importance of hERG in cardiac safety screening

1. Many drugs withdrawn from the market induce acquired Long QT Syndrome (LQTS) by predominately modulating the *human ether-a-go-go* (hERG) K^+ channel.
2. Between 25-40% of all lead compounds show some binding to hERG[1], thus increasing the need for hERG screening in early phases of drug discovery.
3. Regulatory agencies now require pharmaceutical companies to screen novel chemical entities (NCEs) for hERG liability prior to commencing clinical studies.
4. Detection and elimination of possible hERG inhibitors in the early stages of drug development represents substantial decrease in drug development time and resources.

Aurora's hERG Flux Screen

1. Aurora Biomed offers the cold Rb^+ flux assay in a high throughput format to determine hERG liability of NCEs[2].
2. In the presence of test compounds, Rb^+ concentration is measured in both the extracellular and intracellular samples. The effect of the NCE is calculated from the % efflux of Rb^+.
3. Astemizole and terfenadine are used as positive controls in this screen. Curve fits and SEM values are shown (Fig 1).
4. IC_{50} determinations of a group of known compounds are shown in Table 1.
5. The potency rank order of specific inhibitors of hERG determined with Rb^+ flux assay matches that of patch clamp[3,4] (Fig 2).
6. The correlation coefficient of 0.88 has been observed between Rb^+ efflux and manual patch clamp is[3], whereas that between automated and manual patch clamp is only 0.53[5]. Similar to the automated patch clamp, the flux IC_{50} values are a few fold higher than patch clamp. Moreover, flux assay averages the response from a population of cells. This suggests that flux assay is a reliable technology for hERG screening.

Drug	IC_{50} (μM)
Pimozide	0.018
Dofetilide	0.021
Astemizole	0.044
E-4031	0.1
Terfenadine	0.94
Domperidone	2.8
Verapamil	3.5
Thioridazine	3.7
Fluoxetine	4.1
Risperidone	8.6
Diltiazem	62
Disopyramide	120
Aspirin	No block
Chloramphenicol	No block

Table 1. Relative potencies of hERG blockers as determined by the Rb^+ Flux Assay.

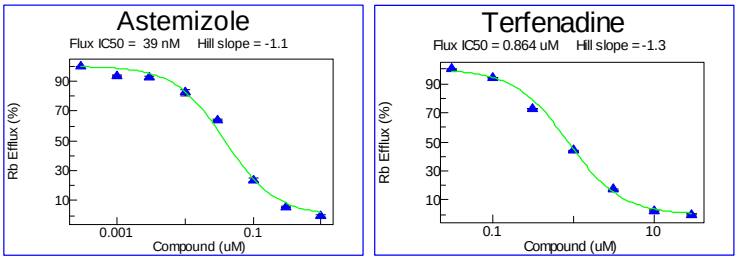

Fig 1. Curve fits and IC_{50} of astemizole and terfenadine with flux assay

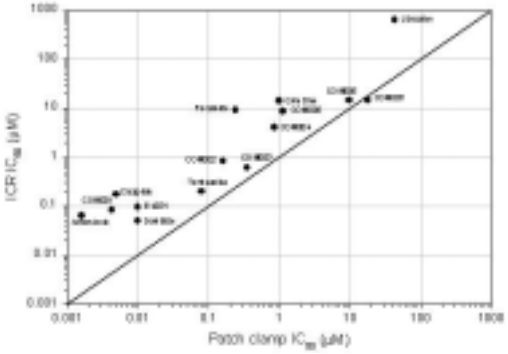

Figure 2. Comparison of potency estimates for hERG block between patch clamp and ICR.

References:

1. Razvi: HERG-technology and market analysis. Report # 9195 DMD Publications, 2005: pp14.
2. Terstappen: Nonradioactive rubidium ion efflux assay and its applications in drug discovery and development. Assay Drug Dev Technol 2004; 2(5):253-57.
3. Gill et al.: Flux assays in high throughput screening of ion channels in drug discovery. Assay Drug Dev Technol 2003; 1(5):709-17.
4. Murphy et al.: Evaluation of functional and binding assays in cells expressing either recombinant or endogenous hERG channel. J Pharmacol Toxicol Methods 2005; in press
5. Guthrie et al.: A place for high-throughput electrophysiology in cardiac safety: Screening hERG cell lines and novel compounds with the IonWorks HT system. J Biomol Screen 2005; 10(8):832-40.

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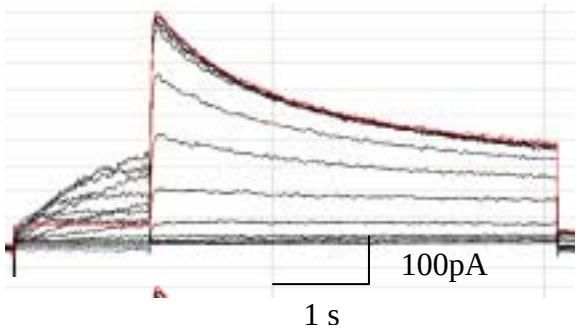
Whole cell patch clamp recordings for hERG

Combining preclinical data from various assays can assist in better decision making on safety margins for moving novel chemical entities (NCEs) forward. In this view, Aurora offers whole cell patch clamp as a secondary screen for hERG liability. In addition, patch clamp is considered a gold standard amongst ion channel screening technologies and can be employed on its own.

Protocol used for hERG whole cell patch clamp screening

1. For I-V plots, cells are held at -80 mV, stepped to a depolarizing voltage, and then held at -50 mV for 3 s (Fig. 1). The current at the end of the depolarizing step is used in the I-V plot.

2. For determining IC_{50} values of compounds, a test pulse is applied until the peak tail current is stabilized (Fig. 2). IC_{50} curves of two commonly known hERG blockers, astemizole and terfenadine, are shown in (Fig 3).



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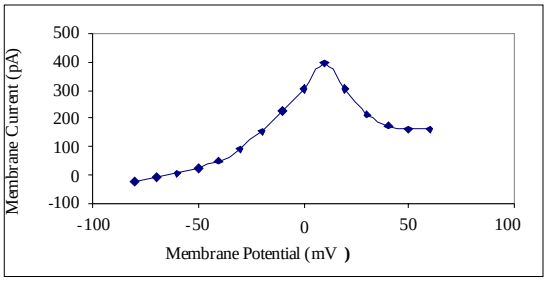

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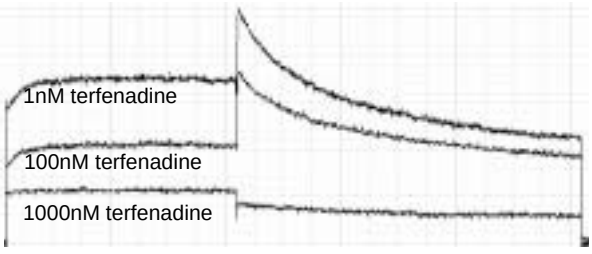
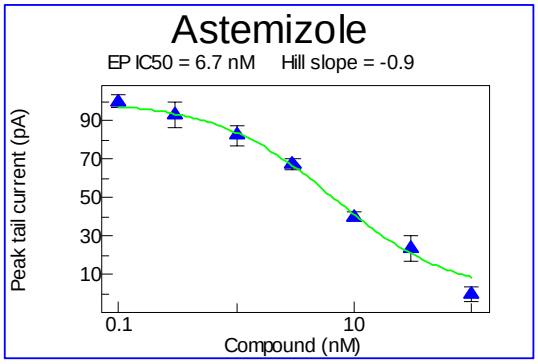


1c

Fig 1a,b,c. I-V plots of hERG currents using CHO-hERG cell line



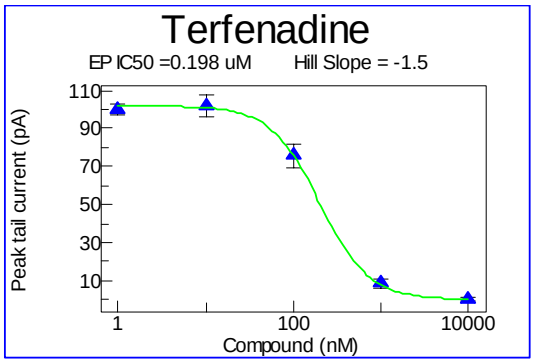


Fig 2. Typical IC_{50} curves obtained using patch-clamp electrophysiology

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Importance of Nav1.5 in cardiac safety screening

1. Mutations in the cardiac sodium ion channel (Nav1.5) gene, SCN5A, have been found to contribute to a type of long-QT syndrome (LQT3)[1]. Furthermore, abnormal heart rhythms due to this mutation are more likely to be fatal than those due to types LQT1 (KVLQT1) or LQT2 (hERG)[2].
2. As such, any non-specific binding of drugs to Nav1.5 can alter normal cardiac rhythm [2,3].

Aurora's Nav1.5 Flux Screen

1. Aurora Biomed offers its patented cold Li^+ flux assay in a high throughput format to investigate Nav1.5 channel activity, both as a stand alone service and as part of a panel of cardiac safety screens.
2. In the presence of test compounds, Li^+ influx through Na^+ channels is measured using the ICR.
3. Using the Li^+ flux assay, the potencies of two known Nav1.5 blockers, TTC, and Amitriptyline have been presented (Fig. 1).

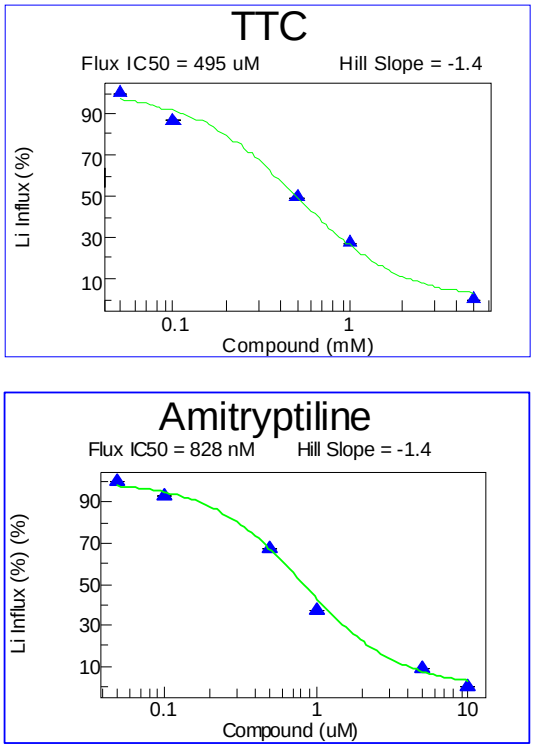

Fig 1. Typical IC_{50} curves obtained for tetracaine (TTC), and amitryptiline using Li^+ flux assay with the ICR.

References:
1. Viskin et al.: Long QT syndrome and torsade de points. Lancet 1999; 354:1625-33.
2. Schwartz et al., Genotype-phenotype correlation in the Long-QT Syndrome. Circulation 2001; 103:89-115.
3. Kuryshev et al., Interactions of the 5-Hydroxy tryptamine 3 antagonists class of antimetic drugs with human cardiac ion channels. J Pharmacol Exp Therapeut 2000; 295:614-20.

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Whole cell patch clamp recordings for Nav1.5

1. Aurora Biomed offers patch clamp whole cell recordings for screening of Nav1.5 channels.

2. The cell line transfected with SCN5a is robust cell line and the potencies of two known Nav1.5 blockers, TTC and procainamide have been determined using patch clamp (Fig 2).

Protocol used for Nav1.5 whole cell patch clamp screening

1. Electrophysiology experiments are conducted using standard patch clamp techniques. The bath solution contains (in mM) 0.90 $CaCl^2$, 2.67 KCl, 1.47 $KHPO^4$, 0.50 $MgCl^2$, 138 NaCl, and 8.10 Na^2HPO^4. The pipette solution contains (in mM) 140 KCl, 1 $MgCl^2$, 1 EGTA, and 20 HEPES.

2. For I-V plots, cells are held at -70mV, stepped to -120mV for 20ms, and then stepped to a depolarizing voltage for 20ms to record the peak current (Fig 1).

3. Dose response curves of two known Nav1.5 blockers, TTC and procainamide, are shown.

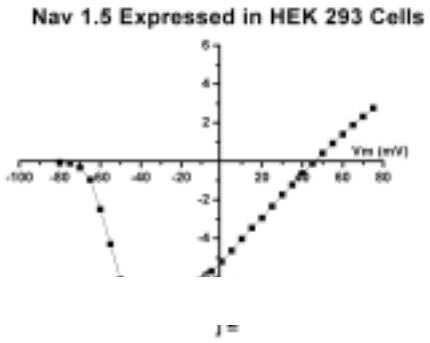
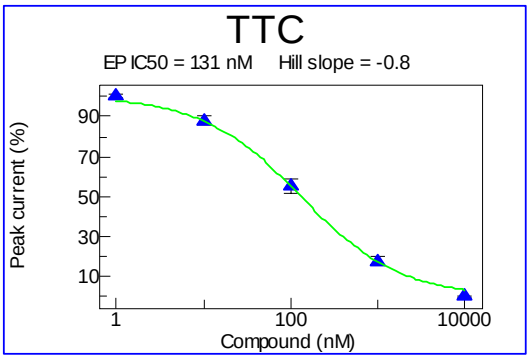


Fig 1. I-V plot of Nav1.5 currents



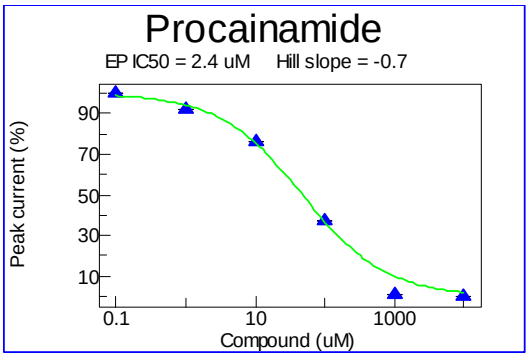


Fig 2. Typical IC_{50} curves obtained using patch-clamp electrophysiology

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Importance of stretch activated ion channels involved in cardiac safety screening?

1. Stretch-activated channels (SAC) are involved in volume regulation in cardiac cells; it has been shown that mechanical stress leads to mechanoelectric feedback in the cells

2. Stretching intact hearts or excised muscles can raise beat rate, change action potential configuration, and induce arrhythmias. In this case, SACs are considered to be the origin of mechanoelectric transduction[1].

3. Pharmacological blockade of SACs has been reported to alter the cell volume of isolated rabbit ventricular myocytes during hypo-osmotic stress. The cationic SAC blocker gadolinium (Gd^{3+}) reduces the amount of swelling in hypo-osmotic solutions by 24% and induces a regulatory cell volume decrease[2].

SAC flux assay for high throughput screening

1. The high throughput SAC flux screen uses Rb^+ flux assay technology where cold Rb^+ is used as a tracer for K^+ and employs the ICR.
2. In the presence of test compounds, Rb^+ concentration is measured in both the extracellular and intracellular samples. The effect of the test compound is calculated from the % efflux of Rb^+.
3. The assay generates a Z' > 0.8 and an approximately seven-fold window of detection (Fig 1)
4. Gadolinium, a widely used blocker of SAC, is used as a positive control; a curve fit with SEM values are shown (Fig 2).

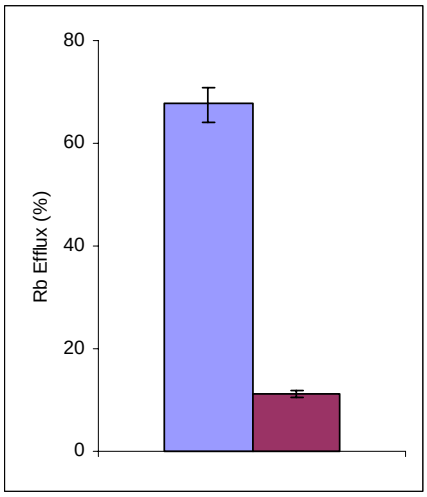

Fig 1. Window of detection: activated efflux (blue bar) and basal efflux (red bar).

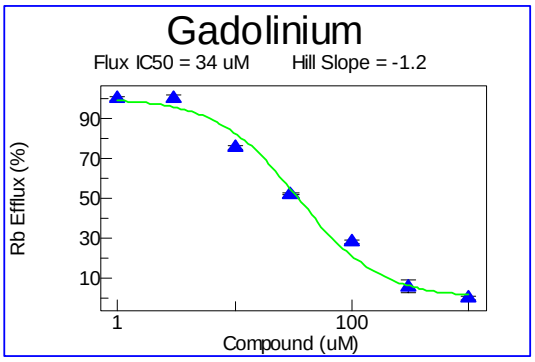

Fig 2. Curve fit and IC_{50} of gadolinium

References:
1. Zeng et al.: Stretch-activated whole cell currents in adult rat cardiac myocytes Am J Physiol Heart Cir Physiol 2000; 278:H548-57
2. Suleymanian et al.: Stretch-activated channel blockers modulate cell volume in cardiac ventricular myocytes. J Mol Cell Cardiol 1995; 27(1):721-728.

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Na+,K+-ATPase is an important therapeutic target

1. Human Na^+,K^+-ATPase, also known as the Na^+,K^+-pump, is responsible for maintaining membrane potential of the cells by creating ionic gradient of Na^+, and K^+ across the membrane. In addition to pumping ions, the Na/K-ATPase serves as a receptor that not only regulates the function of protein kinases, but also acts as a scaffold for other cell surface proteins[1]. Studies in isolated muscle preparations have demonstrated an important role for Na^+,K^+-ATPase in muscular fatigue[2].

2. For playing an important role in the pathogenesis of many cardiovascular diseases, this pump serves as a selective target for cardioglycosides such as digoxin and digitoxin which are used to treat heart disease and related conditions, and are two of the leading prescribed therapeutics worldwide [1-3]. This pump, therefore, serves as an important target for development of novel therapeutic agents.

Na+,K+-ATPase Flux Assay for HTS

1. A high-throughput screen for Na^+,K^+-ATPase uses Rb^+ as a tracer for K^+ to measure the functional activity and modulation of this transporter[4].
2. Na^+,K^+-ATPase endogenous to CHO or HEK cell lines is used for this screen.
3. This assay generates a Z' > 0.7 and an approximately 4-fold window of detection (Fig 1)

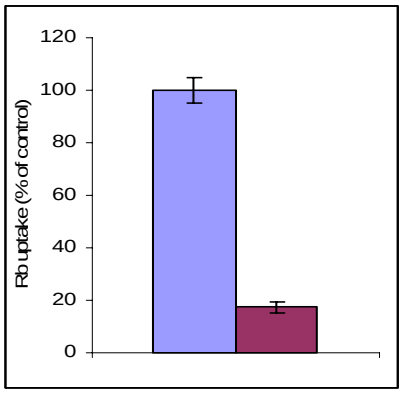

Fig 1. Window of detection in Na^+,K^+-ATPase screen. Activity and the inhibition by ouabain shown as blue bar and red bar, respectively.

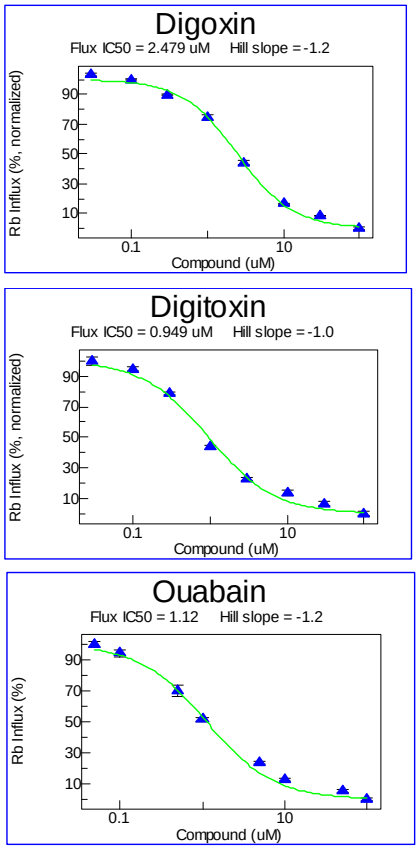

Fig 2. Curve fit and IC_{50} of digoxin, digitoxin, and ouabain

References:
1. Xie and Xie: The Na/K-ATPase-mediated signal transduction as a target for new drug development. Front Biosci. 2005; 10:3100-09
2. Rose et al.: Understanding the sodium pump and its relevance to disease. Clin Chem 1994; 40(9):1674-1685.
3. Perlin: Ion pumps as targets for therapeutic intervention: Old and new paradigms. Electron J Biotechnol1998, 1:1-5
4. Gill et al. : Development of an HTS assay for Na^+, K^+-ATPase using nonradioactive rubidium ion uptake. Assay Drug Dev Technol; 2004; 2(5):535-42.

Importance of Voltage-dependent calcium channels

1. These channels link membrane potential changes of excitable cells to various important intracellular processes[1].
2. This role makes Ca^{2+} channels attractive targets to develop novel pharmacological agents for a broad variety of diseases.
3. Currently, Ca^{2+} channel-blockers are most widely used to treat cardiovascular ailments largely via block of just one type of Ca^{2+} channel, the L-type.
4. Aurora Biomed offers patch clamping technique for screening the activity of compounds against Ca^{2+} channels including L-type (Figure 1).

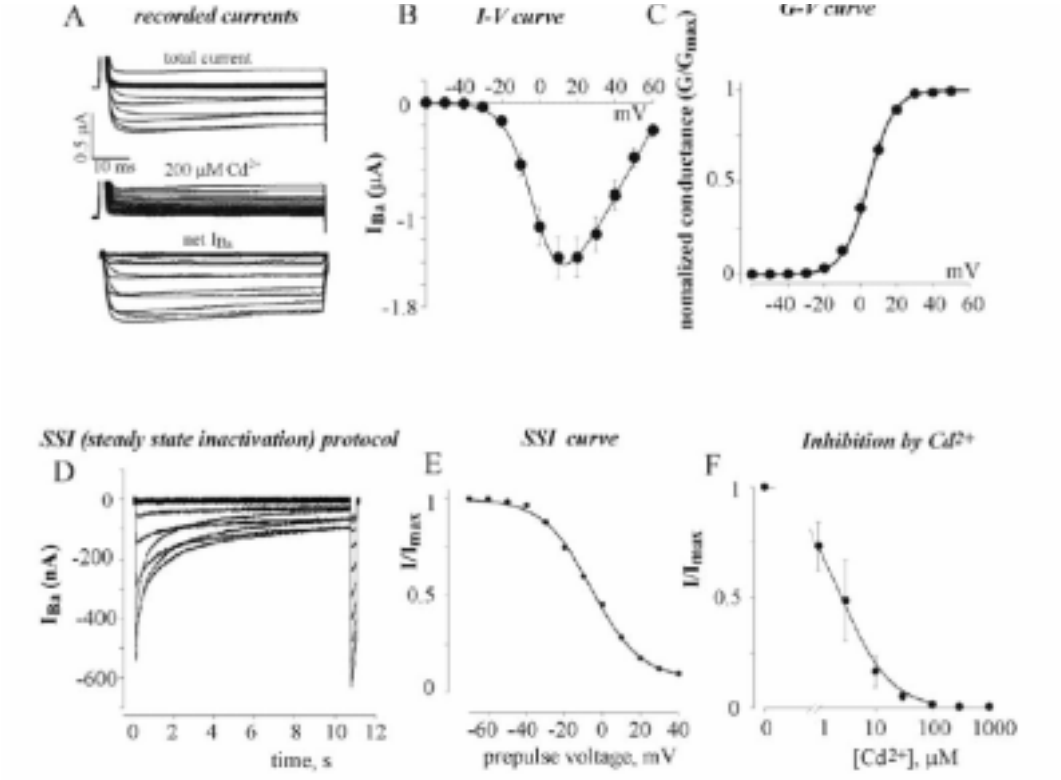

Figure 1. Electrophysiological characterization of cardiac L-type Ca^{2+} channels of α1C/α2δ/β2 subunit composition. **A**: The currents evoked by square pulses from a holding potential of -80 mV to 70 to +60 mV in the normal Ba^{2+} solution and then containing 200 μM $CdCl_2$. The net Ba^{2+} currer was obtained by digital subtraction of the second records from the first one. **B, C**: The current voltage (I-V) and conductance-voltage (G-V) curve averaged from four oocytes of one donor. **D, E** steady-state inactivation (SSI) protocol in a representative oocyte. 10-s long prepulses from -70 t +40 mV, in 10 mV steps, preceded a 200 ms test pulse to +20 mV. Holding potential was -80 mV Net Ba^{2+} currents were obtained by subtracting currents recorded in the presence of 200 μM Cd^{2+}. **F** Dose-response curve of Cd^{2+} inhibition (n=3 oocytes). The curve shows best fit to Michaelis-Mente equation with Ki of 2.6 μM. IBa was recorded at +20 mV, in 40 mM Ba^{2+}.

References:

1. Dolphin AC: A short history of voltage-gated calcium channels. *British Journal of Pharmacology* (2006) **147,** S56–S62.

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Advantages of in vivo cardiac safety screening

1. Primary hERG screening has limited usefulness compared with *in vivo* safety screening for predictive value to clinical testing[1]
2. Adopting *in vivo* QT measurement studies at an early stage in the discovery process would yield important information on compound QT prolongation liability in humans

Animal model and protocol used for *in vivo* screen

1. Aurora Biomed offers non-GLP QT measurement studies in sling-trained, conscious, one to two year-old beagle dogs.
2. Compound doses are administered intravenously, while heart rate and direct ECG (four electrodes) signals are monitored at specific times throughout the testing procedures.
3. QTc values are determined using the Van der Water's correction factor.[2]

How is our model validated?

Using our paradigm, known torsadogenic agents lead to an increase in QT interval (Fig.1). In addition, comparing our data for astemizole with that of a previously published report (Yamomoto et al., 2001)[3] show quite similar effects (Fig 2).

We also compared our drug rank order with those determined by *in vitro* manual patch clamp and Rb^+ efflux assay, as well as published dog QTc data (Table 1). Both *in vitro* techniques and dog QT data resulted in similar rank orders, which create a high degree of confidence in our protocol for *in vivo* dog QT measurements.

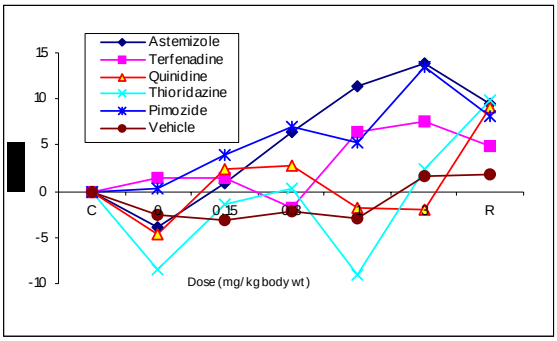

Fig.1. The effects on QT interval of five known hERG modulators.

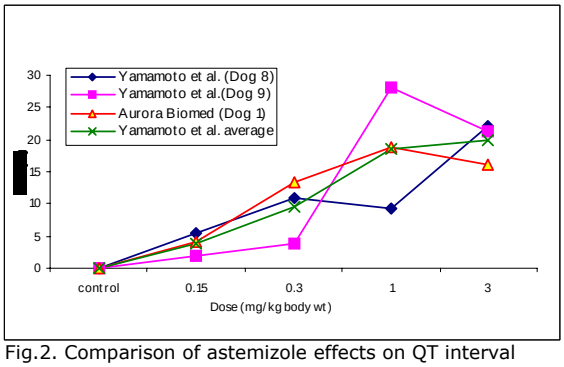

Fig.2. Comparison of astemizole effects on QT interval between Aurora Biomed and Yamamoto et al. (2001).

Compound	EP IC50 (uM)	Flux IC50 (uM)	Aurora *in vivo* QTc (%)	JPI *in vivo* data QTc (%)[2]	Rank
Pimozide	0.018	0.019	13.5	20.6	strong
Astemizole	0.001-.04	0.037	13.8	15.8	strong
Terfenadine	0.079	0.345	7.6	13.4	med
Thiorizadine	1-1.2	2.237	2.4	19.6	weak
Quinidine	0.4-1.4	7.4	2.8	10.2	weak

Table 1. Rank-order comparisons between *in vitro* hERG screening, *in vivo* QT measurement study and published *in vivo* data.

References:
1. Redfern et al. : Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc Res 2003; 58:32-45.
2. Spencer et al. Toxicol. Sci.1998; 45: 274-58.
3. Toyishima et al.: Safety Pharmacology Society Meeting Poster.

Kv1.3 is an important therapeutic target

1. The Kv1.3 channel is involved in regulating membrane potential, cell proliferation and cytokine secretion by T-lymphocytes along with the IK Ca^{2+} channel.
2. Kv1.3 is also expressed by microglia involved in inflammation in CNS disorders, and is an important regulator of peripheral insulin sensitivity and glucose metabolism[1].
3. It is widely recognized as a potential therapeutic target for immunotherapy[2].

Kv1.3 Flux Assay for HTS

1. This assay offers a non-radioactive high throughput format to facilitate the screening of molecules that modulate Kv1.3 activity.
2. The screen incorporates Rb^+ as a tracer for K^+ which is analyzed using the ICR. The assay provides a Z' > 0.8 with more than a 5-fold window of detection (Fig 1).
3. Three known Kv1.3 blockers, agitoxin, margatoxin, and fluoxetine are used as controls and resulted IC_{50} values of 0.6 nM, 2 nM, and 13 uM, respectively (Fig 2).
4. This screen makes a clear distinction between non-peptide compounds like tamoxifen, nifedipine and fluoxetine that inhibit the Kv1.3 channel, and compounds like astemizole and pimozide that do not block Kv1.3 but are potent hERG channel blockers.

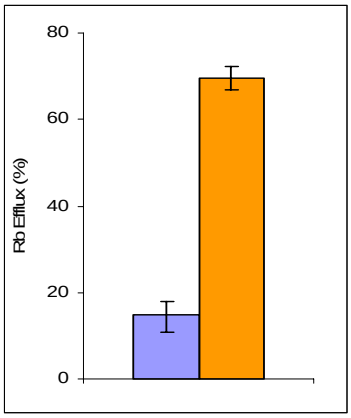

Fig 1. Window of detection in Kv1.3 screen showing basal efflux (blue bar) and activated efflux (orange bar).

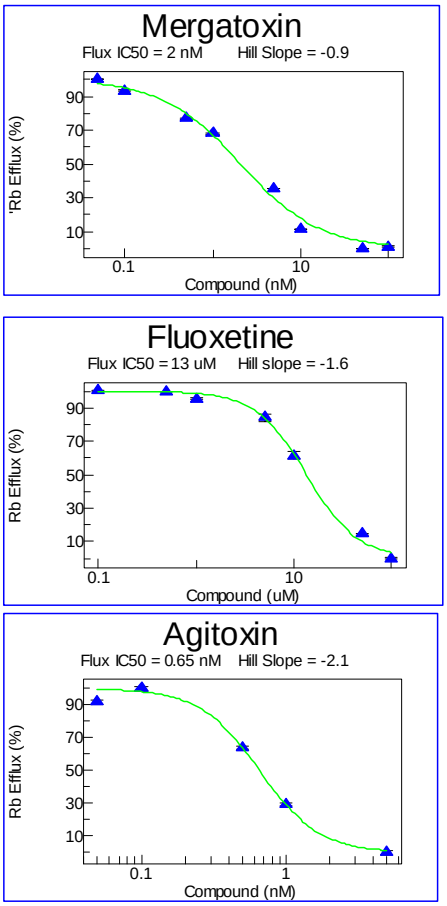

Fig 2. Curve fit and IC_{50} of agitoxin, mergatoxin, and fluoxetine.

References:
1. Fordyce et al.: Microglia Kv1.3 channels contribute to their ability to kill neurons. J Neurosci 2005; 25(31):7139-49.
2. Chandy et al.: Potassium channels in T lymphocytes: toxins to therapeutic immunosuppressants. Toxicon 2001; 39:1269-76.

Importance of Calcium channels N-type:

1. Voltage-activated Ca^{2+} channels play a major role in many physiological processes including release of neurotransmitters and the regulation of neuronal excitability.
2. Dysfunction of Ca^{2+} channels is implicated in numerous CNS disorders ranging from epilepsy to chronic pain.
3. Considerable efforts have focused on the identification of Ca channels modulators for screening and medicinal chemistry. The naturally occurring conotoxins have activity at various Ca^{2+} channels including the N-, P/Q-, R-, and T- type channels and have served as platforms for the development of therapeutic agents[1]. Aurora Biomed has expanded patch clamp screenings for these channels (Figure 1 and 2).

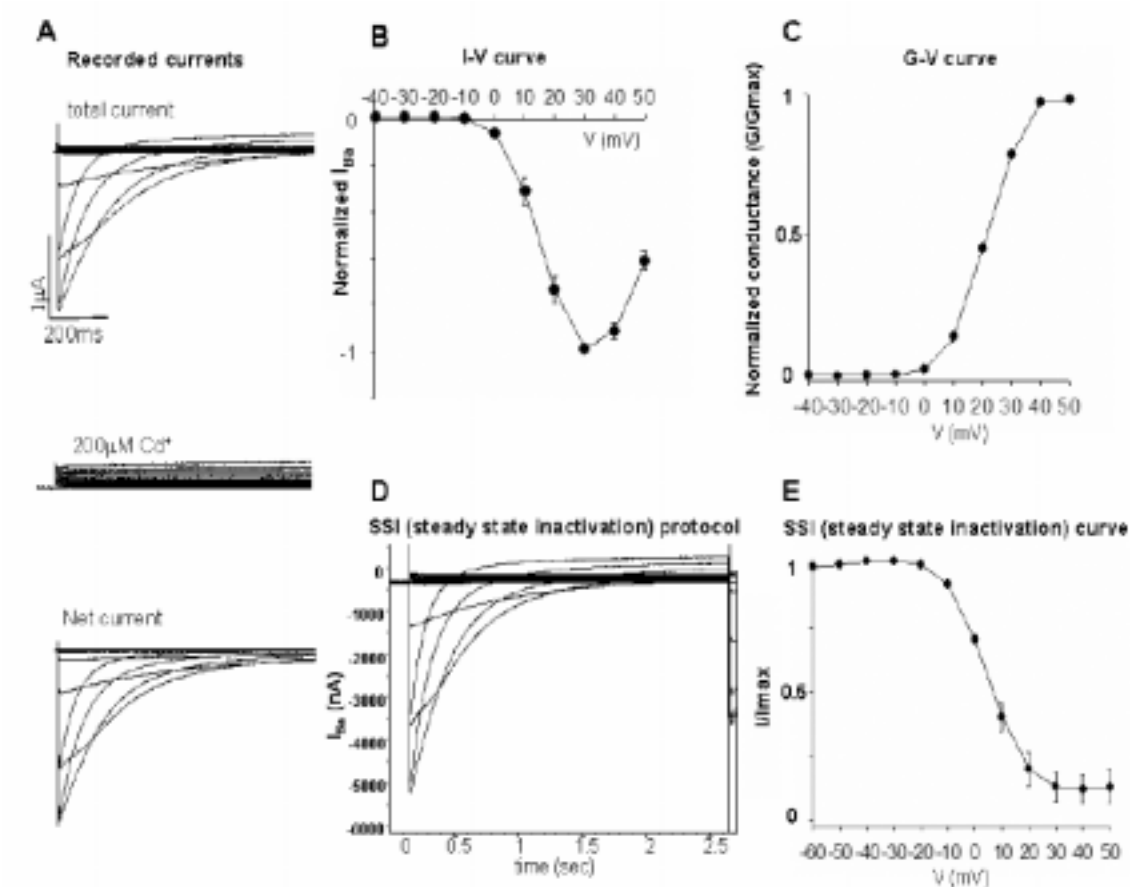

Figure 1. Electrophysiological characterization of neuronal N-type Ca^{2+} channels of α1B/α2δ subunits, expressed in *Xenopus* oocytes. **A**: Currents evoked by square pulses from a holding potential of -80 mV to +50 mV first performed in the normal Ba^{2+} solution (upper panel) and then in the presence of 200 μM $CdCl_2$ in the same solution (middle panel) where net Ba^{2+} current was obtained by digital subtraction of the second records from the first one. **B, C**: The current-voltage (I-V) and conductance-voltage (G-V) curve (n=4 oocytes. **D, E**: Steady-state inactivation (SSI) protocol in a representative oocyte. 2.5-s long prepulses from -60 to +50 mV, in 10 mV steps, preceded a 200 ms test pulse to +20 mV. Holding potential was -80 mV. Total Ba^{2+} currents are shown.

References:

1 Pringle et al.: Selective N-Type Calcium Channel Antagonist Omega Conotoxin MVIIA Is Neuroprotective Against Hypoxic Neurodegeneration in Organotypic Hippocampal-Slice Cultures. *Stroke.* 1996;27:2124-2130.)

Importance of inhibition of N-type Ca^{2+} channels by G-proteins

1. The inhibition of N-type channels by G proteins figures critically in presynaptic inhibition in understanding the molecular mechanisms.
2. In the past several years, there has been considerable progress in understanding the molecular basis of G-protein inhibition[1].

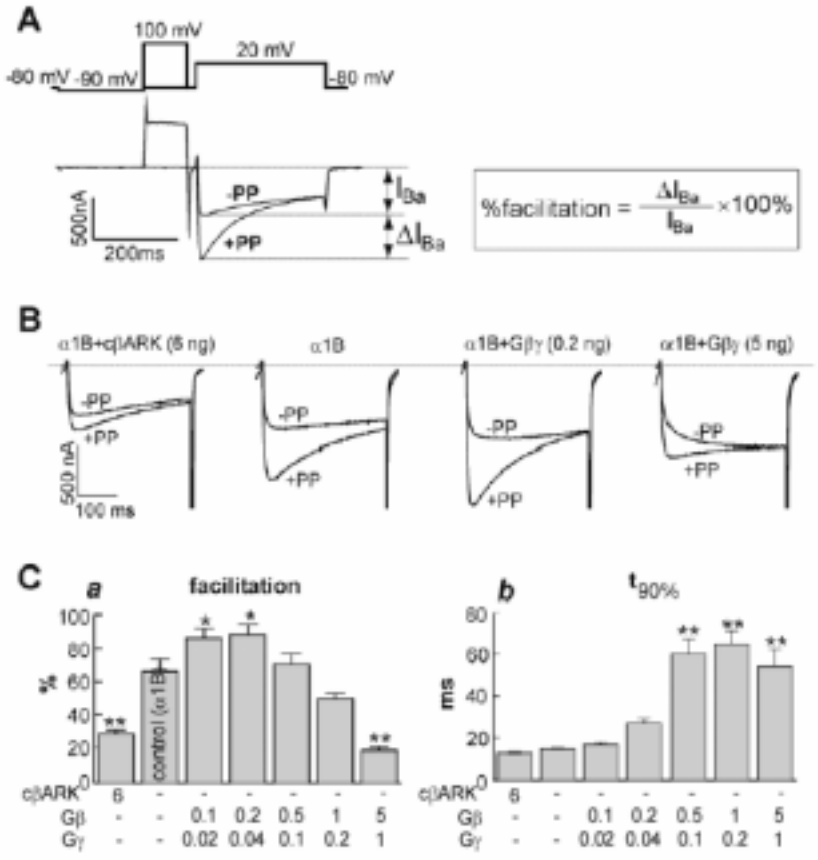

Figure 2. Study of modulation of neuronal N-type channels by G proteins: Effects of co-expressed cβARK and Gβγ on N-type channels in *Xenopus* oocytes. **A**, IBa (*lower trace*) measured by the double pulse protocol (*upper trace*). % facilitation was calculated as ΔIBa/IBa.100%. **B**, effects of coexpressed cβARK and Gβγ on facilitation of α1B. Amounts of coexpressed RNAs are indicated (ng/oocyte; RNAs of Gβ and Gγ were always injected at 5:1 ratio). **C**, summary of effects of cβARK and Gβγ on facilitation (***a***) and t90% (***b***). Each bar represents a group of oocytes injected with the indicated RNAs; n=10 in each group. *,

References:
1 Pringle et al.: Selective N-Type Calcium Channel Antagonist Omega Conotoxin MVIIA Is Neuroprotective Against Hypoxic Neurodegeneration in Organotypic Hippocampal-Slice Cultures. *Stroke.* 1996;27:2124-2130.)

Cl^- channels are therapeutically important targets

1. Chloride (Cl^-) channels play an important role in the regulation of electrical excitability in muscles and neurons. These channels also influence fluid transport, and pH regulation in epithelial tissues and intracellular organelles[1].
2. Patch clamp techniques which represent the gold standard for determining ion channel activity suffer from low throughput[2].
3. As such, the drug discovery industry has turned to using radiotracer flux methods with either ^{36}Cl or ^{125}I, or fluorescence methods to determine ion channel function[3].
4. There are many concerns related to the use of radioactivity in large quantities or fluorescence based indicators[4]. To overcome this issue, Aurora employs a non radioactive flux technique.

Cl^- Flux Assay for HTS

1. This screen employs a cell-based flux assay coupled to Ag^+ precipitation to measure Cl^- levels[5].
2. Intra- and extracellular Cl^- is titrated against Ag^+ as AgCl. Free Ag^+ is then measured using the ICR. A highly linear correlation (r^2 = 0.996) was observed between the ideal and calculated Cl^- indicating that this method is reliable (Fig 1).
3. This screen is very sensitive with the linear working range of the ICR between 0.02-10 mg/L.
4. The screen shows high robustness with a Z > 0.8 and free from interference by various ions present in assay reagents (Fig 2).
5. Potencies of NPPB (5-nitro-2-(3-phenylpropyl amino)-benzoate) a blocker of CFTR, derived by Aurora Biomed's non-radioactive method (Fig 3) are comparable to those derived from radioactive flux assays[6].

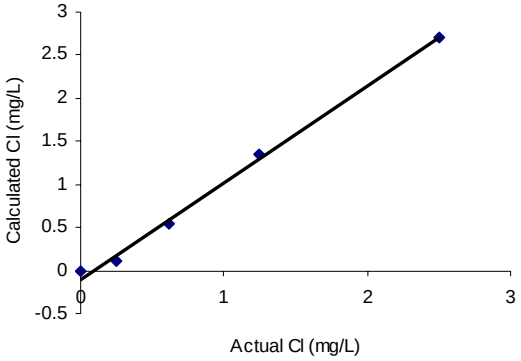

Fig 1: Correlation of theoretical and calculated Cl^- concentrations. The free Ag^+ precipitation measurements show a linear relationship against actual concentrations of Cl^- with r^2=0.996.

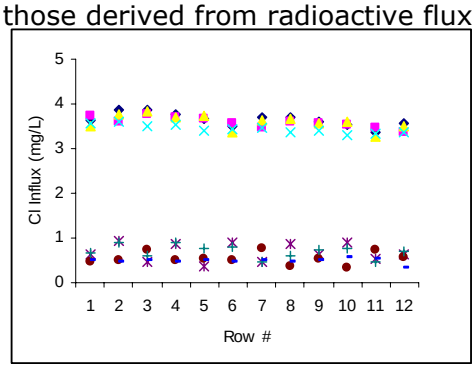

Fig 2: Window of detection: activated (upper rows, n=48) and basal influx (lower rows, n=48) signals.

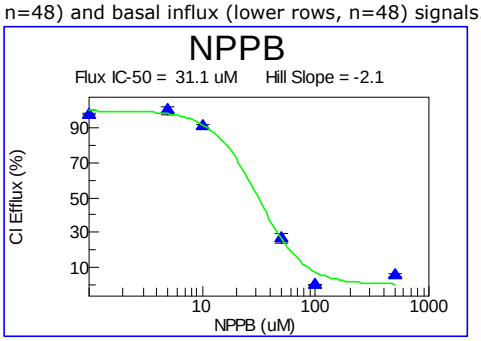

Fig 3: Curve fits and IC_{50} of a CFTR blocker NPPB.

References:
1. Rogers: Airway mucus hypersecretion inasthma: An undervalued pathology? Curr Opin Pharmacol 2004; 4(3):241-50.
2. Andersson et al.: Measurement of chloride efflux from nasal epithelial cells using the fluorescent indicator MQAE. Authors: The European Working Group on CFTR Expression 2001; 3.
3. Yamazaki et al.: Inhibitory effects of glybenclamide on CFTR swelling, activated and calcium activated chloride channel in mammalian cardiac myocytes. Circ Res 1997; 81:101-9.
4. Mohr et al.: Investigation of potential-sensitive fluorescent dyes for applicationin nitrate sensitivepolymer membranes. Fresenius J Anal Chem 1997; 357:284-91.
5. Gill et al.: Development andvalidation of HTS flux assay for endogenously expressed chloride channels in a CHO-K1 cell line. Assay Drug Dev Technol 2006, 4(1):65-71.

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Assay Development

Aurora Biomed has a highly trained molecular biology team with solid understanding of ion channels and flux assays.

We enjoy a challenge and feel confident in providing solutions to further your ion channel screening needs

Cell Line Validation

We can accept cell lines to develop assay for your HTS needs in the ion channel drug discovery field as part of our services. We also carry validation of cell lines for ion channel targets included in this brochure.

Detection and Estimation of Ions in Biological Samples

Aurora Biomed provides services for sensitive and reliable estimation of ions like Na^+, K^+, and Cl^- in any sample using the ICR.