

The Ninth Annual Ion Channel Retreat, Vancouver, Canada, June 27–29, 2011

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ABSTRACT

Nine years ago Aurora Biomed Inc. (Vancouver, Canada) committed to gathering the brightest minds and the most innovative research companies at one conference. The Ion Channel Retreat provides a podium for scientific discourse spanning a wide range of ion channel disciplines. This conference has consistently provided a venue for people to share knowledge, exchange ideas, and establish partnerships. This conference continues to expand and grow each year, demonstrating the value of such a conference. Attendees at the 2011 Ion Channel retreat presented ion channel research from 12 different countries, representing research groups located on 5 of the 7 continents. Aurora Biomed's 2011 Retreat covered a variety of topics including Ion Channels as Disease Targets, Ion Channels as Pain Targets, TRP-channels, Ion Channel Screening Technologies, Cardiac Function and Pharmacology, Cardiac Safety and Toxicology, and Structure and Function of Ion Channels.

INTRODUCTION

Over the past year corporate and academic research laboratories have been reshuffled as organizations increased efficiency and reduced operational costs. Researchers have moved to new laboratories and groups have fine-tuned their research focus as organizations continue to streamline their approach in the face of global economic uncertainty. Mergers and acquisitions have allowed organizations to strengthen their market position, providing a strong foundation for future growth. The global representation of ion channel researchers at this year's Retreat proved that research is continuing and new technologies continue to be launched. Additionally, as well-established drugs continue to go off-patent, research continuing to fuel the pipeline is a necessity. The continued success of the Ion Channel Retreat proves that investors still believe in the value of research and discovery in ion channels.

Ion channels have been associated with a wide range of afflictions and pathophysiological conditions affecting numerous organisms. To address these issues, Aurora Biomed has relied on the time, expertise, advice, suggestions, and affiliations of the Scientific Advisory board to create a globally inclusive program with internationally recognized ion channel research scientists. Together with the Scientific Advisory Board, Aurora Biomed assembled a diverse program covering a wide range of ion channel research topics to be discussed at the 9th Annual Ion Channel Retreat. The 2011 Scientific Advisory Board was composed of Dr. Greg Kaczorowski (Founder, Kanalis Consulting LLC), Dr. Joe McGivern (Director of Research, Amgen), Dr. Robert Petroski (Project Manager, Dart Neuroscience), and Dr. Catherine Smith-Maxwell (Senior Research Scientist, Gilead Sciences). Aurora's annual Retreat welcomed scientists from a variety of research laboratories representing the full spectrum of nonprofit, academic, biotechnology, and pharmaceutical industries. The program presented at the 2011 Ion Channel Retreat showcased both the importance of new drug discovery research, as well as the importance of producing new screening technologies, while maintaining or improving safety and efficacy of target compounds.

The purpose of this meeting review is to provide a general overview of the conference proceedings. Due to the necessity for confidentiality agreements for ongoing research projects, the discussion summaries presented in this review may lack significant detail. Please contact the speakers directly for further detail about their research findings.

ION CHANNELS AS DISEASE TARGETS

An increasing number of human diseases have been linked to defects in various ion channels leading to the development of channelopathies. Due to the critical role that ion channels play in virtually all tissue types and organs, they are also involved in a number of pathophysiological conditions. Observing the importance of ion channels in drug discovery and drug pharmacology, Aurora Biomed has continued to focus strongly on ion channel research associated with pain and disease targets while organizing the annual Retreat. These topics were discussed in detail over the course of the first day of the meeting. The first session, on Ion Channels as Disease

ABBREVIATIONS: AF, atrial fibrillation; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate; CB, cannabinoid; CF, cystic fibrosis; CHO, Chinese hamster ovary; CRAC, calcium-release activated calcium; EAD, early after depolarization; ENaC, epithelial sodium channel; FGF14, fibroblast growth factor 14; FLIPR, fluorometric imaging plate reader; GlyRs, glycine ion channel receptors; hERG, human Ether-à-go-go; HHC, hereditary hemochromatosis; HTS, high-throughput screening; iPS, inducible pluripotent stem; LCA, luciferase complementation assay; LQTS, long QT syndrome; NMR, nuclear magnetic resonance; NSAIDS, nonsteroidal anti-inflammatory drugs; SCID, severe combined immunodeficiency; ssNMR, solid-state nuclear magnetic resonance; THC, Δ^9 -tetrahydrocannabinol; TRP, transient receptor potential; TRPV1, transient receptor potential cation channel subfamily V member 1.

Targets, centered on recent work by researchers seeking solutions for diseases such as memory loss, arrhythmias, autoimmune/inflammatory disease, and cystic fibrosis.

In the opening session, Dr. Robert Petroski, project manager from Dart Neurosciences, introduced several important advances made by his company in the search of memory-enhancing drugs. He described the research work carried by Dr. Tim Tully, CSO of Dart Neurosciences, in which mutant fruit fly and rodents were used to show that the CREB gene plays an important role in regulating the conversion of short-term memory to long-term memory. This finding suggested that small molecule modulators of the CREB pathway might enhance memory and therefore would be useful in treating memory-related disorders such as Alzheimer disease. By using an RNAi-mediated gene knockdown approach, Dart Neuroscience has been interrogating various proteins involved in the signaling cascade that mediates memory formation to find candidate genes that can be used as targets for memory-enhancing drugs.

This presentation was followed by Dr. Robert Kass (Columbia University) who focused the session more narrowly on channelopathies. Such diseases are caused by inherited mutations in genes coding for ion channel or channel-associated proteins. By studying known channelopathies such as long QT syndrome (LQTS), fundamental mechanisms underlying human cardiac electrophysiology have been elucidated. Congenital LQTS is an important cause of sudden cardiac death. LQTS has been shown to arise from a variety of mutations in different genes—each is classed as a separate type. Type 1 LQTS (LQT1) is the most common. It has previously been reported that LQT1 results from loss-of-function mutations to the gene encoding the I_{Ks} channel and its subunits (KCNQ1/KCNE1). Similarly, gain-of-function mutations of genes encoding the $Na_{V1.5}$ sodium channel resulted in type 3 LQTS. On the basis of these findings, Dr. Kass investigated the cellular electrophysiology of patient-derived inducible pluripotent stem (iPS) cells. Dr. Kass concluded that his approach presented opportunities to screen drugs for effective disease management of cases in which multiple genes were thought to be involved in the disease phenotype. Additionally he proposed that this technique appeared to offer an affordable option as researchers continue to move towards personalized medicine solutions.

Continuing the theme of channelopathies, the topic of hereditary hemochromatosis (HHC) as a common genetic disorder was discussed by the next speaker, Dr. Clint Young (Xenon Pharmaceuticals). HHC is caused by a defect in the gene responsible for regulating iron metabolism, leading to over-absorption and build-up of iron in vital organs, joints, and tissues. Based on previously published work that found that HHCs are characterized by an up-regulation of DMT1 in the intestine and hyper-absorption of dietary iron, Dr Young's group thought that an effective inhibitor of DMT1 could also be a potential therapeutic for HHC. Dr. Young's research team adopted a fluorescence quench assay to find suitable a DMT1 inhibitor. After screening more than 175,000 compounds, they identified the compound XEN601, a small molecule inhibitor of hDMT1 that potently blocked DMT1. Using voltage clamp measurement on Chinese hamster ovary (CHO) cells expressing hDMT1, they confirmed that XEN601 acted as

an inhibitor. XEN601 is one of several promising agents under development by Xenon Pharmaceuticals for the treatment of iron hyper-absorption disorders such as HHC and thalassemia intermedia.

Dr. Catherine Howsham (Novartis Institutes of Biomedical Research) centered her talk on cystic fibrosis (CF). This disease is caused by mutation in the CF transmembrane conductance regulator, which normally inhibits the epithelial sodium channel (ENaC), thus producing hyperactive sodium channels. Previously, pyrazinoyl-acylguanidines such as amiloride represented the only class of potent ENaC blockers that could enhance mucociliary clearance in CF. In order to identify novel ENaC blockers, Dr. Howsham used 2 approaches that had been previously developed by Novartis. First, a high-throughput screening (HTS) assay was used to identify a non-pyrazinoyl-acylguanidine based hit. Based upon the pharmacological profile, a series of close analogues were synthesized and identified with reasonable *in vitro* potency and modest *in vivo* efficacy. Secondly, a literature class of ENaC blockers was used as a starting point for optimization for inhaled delivery using a bioisostere approach. A rapid expansion of the structure-activity relationship based on potency similar to that exhibited with amiloride by specialized chemical scaffolds afforded analogues with excellent *in vitro* and *in vivo* ENaC blocking potency. It is hoped that these approaches will yield novel ENaC blockers that are inhalable and have improved duration of action so as to further enhance CF therapy.

Rounding out the first session, Dr. Kenneth Stauderman (Calci-Medica) highlighted how mutations in STIM1 and Orai1 proteins, both of which are central to the activity of calcium-release activated calcium (CRAC) channels, led to a form of severe combined immunodeficiency (SCID). By inhibiting the CRAC channel pharmacologically, it should be possible to suppress the adaptive immune response of T cells and thereby treat autoimmune diseases such as psoriasis. Dr. Stauderman presented the discovery made by his company of a novel small molecule (compound B) that selectively inhibits CRAC channels. It was determined that compound B displayed more than 100-fold selectivity for CRAC channels over L-type Ca^{2+} and human Ether-à-go-go (hERG) encoded channels. Further testing revealed that compound B effectively blocked interleukin-2 release from Jurkat T cells and human $CD4^+$ T cells, and blocked degranulation in the RBL-2H3 mast cell line. *In vivo* testing also confirmed reduction of arthritis in rat models. Taken together, this evidence suggested that compound B and other CRAC channel inhibitors might serve as useful therapeutics in the treatment of autoimmune disorders.

ION CHANNELS AS PAIN TARGETS

Continuing the day's theme of ion channels as important targets for the treatment of physiological disorders, the next session highlighted research on several targets suggested to be potentially useful for the treatment of pain. Pain caused by the constant activation of ion channels is an area of increased interest throughout the pharmaceutical and scientific communities. Researchers are constantly looking for new drugs to soothe pain. However, the mechanisms of these pathways are unclear, and issues such as specificity and side effects of treatment compounds require further investigation.

The presentations in this session reflected the challenges scientists face and the experimental approaches they follow to maximize the analgesic effect while ensuring the safety of the drugs and treatments.

Opening this session, Dr. Li Zhang (National Institute on Alcohol Abuse and Alcoholism) discussed how cannabinoid (CB) potentiation of glycine receptors contributes to cannabis-induced analgesia. Δ^9 -tetrahydrocannabinol (THC) and other CBs have long been established as providers of analgesic and therapeutic effects. Glycine ion channel receptors (GlyRs) in particular were chosen as a target for their study since previous evidence suggested that THC and other CBs increased the activity of glycine receptors independent of the CB₁/CB₂ receptor mechanism. Several preliminary tests demonstrated that CBs enhanced the function of GlyRs. To determine more about the mechanisms and behavioral implications of the CB-GlyR interaction, mutagenesis and nuclear magnetic resonance (NMR) analyses were used to identify a key serine residue critical for activation of THC-induced potentiation of IGly. GlyR knock-out mice lacking this protein did not experience CB-induced analgesia, while mice that only lacked CB₁ and CB₂ receptors experienced analgesia. In conclusion, CB potentiation of GlyRs was linked to cannabis-induced analgesia and therapeutic effects.

Next, the founder of Kanalis Consulting, Dr. Greg Kaczorowski switched the focus to Cav_{2.2} channels as targets for chronic pain. N-type Cav_{2.2} calcium channels in particular are found to be present and up-regulated in many pain pathways, identifying these channels as important therapeutic targets for pain treatment. Previous studies had established that Cav_{2.2} null mice are unaffected by chronic inflammatory and neuropathic pain. A peptidyl Cav_{2.2} blocker, Prialt[®], has also been effectively used to manage chronic pain in clinic settings. Based on this evidence, Dr. Kaczorowski's group used an HTS assay to identify small molecule candidates with the potential to offer enhanced therapeutic effects over previously identified peptide channel blockers. State-dependent selectivity assays using cell lines containing Cav_{2.2} channel complexes were screened using both fluorescence-based techniques and electrophysiological assays. Dr. Kaczorowski concluded that several small molecule inhibitor classes had been identified. Future assays will continue to test the efficacy of these compounds for treating pain in rodents.

Closing this session, Dr. Torben Neelands (Abbott Neuroscience) discussed the idea that in addition to the ongoing importance of identifying new analgesic agents for pain management, it is important to identify novel mechanisms involved in pain transmission, especially given the limitations of products currently available for pain treatment. Transient receptor potential cation channel subfamily V member 1 (TRPV1) in particular has garnered attention recently because it has been shown in the literature to mediate the effects of capsaicin—the active ingredient found in chili peppers. TRPV1 has been shown to be activated by other factors, such as heat and pH. TRPV1 null mice have exhibited decreased pain sensitivity. Preliminary research presented a challenge because potential TRPV1 antagonists had so far been found to elicit an increase in core body temperature in both animal models and humans. Dr. Neelands' group

characterized A-1165442, a TRPV1 antagonist that appeared more promising than existing antagonists since it did not appear to affect core body temperature and was shown to effectively treat osteoarthritis in rats. Moving forward, further testing will need to be conducted prior to this compound entering a clinical testing phase.

TRANSIENT RECEPTOR PROTEIN CHANNELS AS PAIN AND DISEASE TARGETS

As described by Dr. Neelands during the pain session, transient receptor protein (TRP) channels are a family of conserved ligand-gated ion channels that contribute to the detection of physical stimuli. Six TRP channels (TRPV1 [discussed by Dr. Neelands], TRPV2, TRPV3, TRPV4, TRPM8, and TRPA1) have all been detected in pain-sensing neurons (nociceptors), where they act as transducers for chemical, mechanical, and thermal stimuli. The final presentations of the day focused on new discoveries and challenges associated specifically with TRP channels, and how these channels might be potential targets for both analgesic compounds and other pathophysiological conditions.

Opening the discussion, the President and CEO of Algomedix Inc., Dr. Jeffrey Herz, presented TRPA1, a localized nociceptor associated with both acute and neuroinflammatory pain responses. TRPA1 had been validated previously as a pain target for small molecule antagonists. Pro-algesic activators of TRPA1 tested *in vivo* caused, while TRPA1 null mice showed significant decreases in pain sensitivity. Previous research had shown that several compounds are capable of activating this target irreversibly, but only a select few antagonists had been found to act as reversible ligands. Dr. Herz and his team focused particularly on fenamate nonsteroidal anti-inflammatory drugs (NSAIDs). They demonstrated that the activation of TRPA1 in conjunction with the allosteric binding of other compounds provided some insight into structural changes of the TRPA1 channel and provided a means of exploring other reversible ligand interactions with TRPA1 channels. Dr. Herz indicated that Algomedix is interested in the potential use of these findings for structure-guided drug design, and that reversible antagonists may be potential analgesic drug targets.

Continuing with this topic, Dr. Jose L. Mercado (Washington University) presented data on TRPV2 involvement in chemical and thermal pain transduction. Though there are structural differences between TRPV2 and TRPV1 in both their N and C terminal regions, both channels exhibit similar mechanisms of Ca²⁺ desensitization. Dr. Mercado therefore hypothesized that the mechanism was likely also conserved. To elucidate the mechanism of TRPV2 Ca²⁺ desensitization, F-11 cells were transfected with TRPV2 and subjected to prolonged exposure to the TRPV2 agonist 2-APB. Dr. Mercado observed an almost complete desensitization of TRPV2 in the presence of extracellular Ca²⁺ as compared with almost no desensitization in the absence of Ca²⁺. Furthermore, CaM was determined not to play a major role in this process. On the other hand, PIP2 was found to modulate TRPV2 currents in that Ca²⁺-induced desensitization promoted the hydrolysis of PIP2, thus resulting in a significant decrease in overall PIP2 levels. In conclusion, Ca²⁺-dependent

degradation of PIP2 represents the major mechanism involved in Ca^{2+} -dependent desensitization of TRPV2 and TRPV1. This raises the question of whether this mechanism of regulation is conserved among TRP channels and indicates a potential focus for therapeutic research.

Next, Dr. Jack Stewart (Soricimed Biopharma Inc.) presented the recent findings of his group's TRPV6 diagnostic assays in which binding peptides were used to detect and quantify TRPV6-rich ovarian and prostate tumors in conjunction with different imaging technologies. This group has previously demonstrated the ability to treat human tumors in animal models by employing their novel soricidin-derived peptide compound as an antagonist of TRPV6. In their more recent study presented here, Dr. Stewart's group focused on the use of fluorescent and magnetic resonance imaging techniques to detect TRPV6 "signatures." TRPV6 was known to be overexpressed in epithelial-type cancers, so they hypothesized that standard immuno-histochemical detection methods would enable detection of TRPV6 in cancerous epithelial tumors. Using blood samples to detect microvesicular structures shed by cancer cells with a nucleotide-based measurement of cell-free microvesicular TRPV6 mRNA, Dr. Stewart concluded that their finding of elevated levels of TRPV6 mRNA in cancer patients relative to levels seen in healthy individuals might provide a convenient and valuable early diagnostic marker. Their group will continue to use molecular tests to establish if real-time polymerase chain reaction testing might be validated as a mechanism for the early detection of epithelial cancer.

Finishing this session, Dr. Stuart E. Dryer (University of Houston) switched the discussion slightly away from TRP channels, focusing instead on the role of NMDA receptors and NR1 subunits in podocytes as potential targets for glomerulodegenerative diseases. Known to be expressed in the podocytes of the kidney glomerulus, NMDA receptors are required for many forms of neuronal plasticity, but their overstimulation has also been shown to result in toxicity and cell death. In addition to defining several characteristics of these receptors, such as their permeability and sensitivity to various metal ions and amino acids, Dr. Dryer found that exposure of podocytes to NMDA in particular, elicited numerous secondary responses in mouse podocytes. NMDA caused changes not only in the function and production of podocytes but also resulted in a significant increase in the expression of TRPC6 channels and TRPC3. NMDA NR1 receptors were shown to be up-regulated in both acquired and hereditary glomerulodegenerative diseases, including in mouse models of diabetes mellitus. Dr. Dryer concluded that NMDA receptors promote podocyte toxicity that may influence the progression of glomerular diseases.

ION CHANNEL SCREENING TECHNOLOGIES

As researchers make progress gathering knowledge and finding new fields of ion channel research, reliable and reproducible screening technologies are required to handle the growing work load and reduce human errors. Technology companies and research institutes are consistently being challenged to develop state of the art instrumentation, reagents, cell lines, and diagnostic assays to support

and accelerate this research field. The following group of presentations, taking place over the course of the second day of the conference, provided an update of the technologies that have been launched recently along with the challenges and strategies researchers and companies have tackled and overcome over the course of the past year.

Dr. Stephen Smith (Fluxion Biosciences) introduced IonFlux, an automated, high throughput instrument designed for ion channel drug discovery and research. The IonFlux uses microfluidics technology to perform automated patch clamp assays for screening ion channel targets. This compact system features continuous compound perfusion, fast dilution capabilities, and assay flexibility; performs complicated and time-sensitive protocols; and allows precise control of experimental conditions. Dr. Smith showed validation data from a wide range of channels. A glutamate dose-response curve was quickly acquired for the NMDA receptor, while desensitization recovery was tested using HEK cells expressing the human nicotinic acetylcholine receptor, as well as HEK cells expressing sodium channel $\text{Na}_{\text{V}1.8}$. The advantages of the system's temperature control were demonstrated in an experiment with several known hERG blockers. Dr. Smith showed that IC_{50} values fell within published literature ranges at physiological temperatures for most compounds, including terfenadine and propranolol and that these hERG blockers exhibited temperature-dependent pharmacology at physiological temperatures. Results obtained with the Ionflux instrument were similar to those observed by manual conventional patch clamp experiments, as well as for other automated electrophysiology systems. Therefore, Dr. Smith concluded that the IonFlux system was well suited for high-throughput applications and should be expected to provide high experimental reproducibility.

The industry's need for high-throughout ion channel screening technology was discussed by the next speaker, Dr. Adam Hendricson (Bristol-Myers Squibb). Technological advances should allow researchers to carry out more work with fewer resources. Dr. Hendricson described his work with the SyncroPatch 96 instrument (Nanon Technologies), a robotic system with a diversified assay portfolio, advertised as being capable to screen hundreds of compounds per day and compatible with both fast and slow voltage- and ligand-gated channels. The SyncroPatch 96 featured a 96-channel continuous voltage clamp that allowed multiple channels to be patched in a single run, performed continuous recording during compound application, and had plate stacking and transfer capabilities for walk away operation. Rapid solution exchange enabled the addition of multiple compounds to cells. Dr. Hendricson analyzed HEK293 cells expressing hERG and $\text{Na}_{\text{V}1.5}$ channels and determined that SyncroPatch could indeed be used to analyze both voltage- and ligand-gated channels and that internal solution exchange was feasible using this instrument. In conclusion, Dr. Hendricson contended that the SyncroPatch was a flexible platform that provided his team with the ideal fidelity of a patch clamp system at a manageable cost.

Dr. Ali Yehia (Sophion) continued the session by offering a detailed overview of Sophion's proprietary QPatch analysis software.

Dr. Yehia stated that automated patch clamp technologies have simplified acquisition of large amounts of data; however, researchers still struggle to analyze these data in a timely manner, thereby creating a bottleneck in drug-screening programs. Q-patch software fully automated the analysis of data generated by experiments performed using QPatch (Sophion Bioscience Inc.) automated patch clamp systems. QPatch software automatically filters and rejects data, thereby speeding up data analysis process. The software also detects experiment reproducibility and enables users to build assays automatically to recollect data when an instrument fails to acquire data for a certain compound during a run. Dr. Yehia demonstrated how a data analysis report is created and is automatically e-mailed to the user or added to the database of choice once the run is complete. Built-in analyses included IC_{50} and EC_{50} determination, IV plots, and a Boltzmann fit equation along with graphical illustrations and tables. Dr. Yehia asserted that their software is user friendly and compatible with all QPatch instruments.

Next, Dr. Andrew Baxter (Evotec) described his work with $P2X_3$ and $P2X_{2/3}$ receptors. These receptors are members of the $P2X$ purinergic receptor family of ATP-gated cation channels and have an important role in peripheral pain signaling and bladder function in humans, making them promising therapeutic targets. Development of $P2X$ antagonists has been challenging due to their low potency and bioavailability and poor selectivity. With the goal of developing potent selective antagonists to $P2X_3$, Dr. Baxter combined fluorometric imaging plate reader (FLIPR) screening, automated patch clamp (Sophion's QPatch system in HTX format), and manual patch clamp experiments on DR6 and ND6 neurons, along with the administration of candidate compounds directly to rats. His group identified a series of drug-like $P2X_3$ antagonists that selectively blocked whole cell currents mediated by both $P2X_3$ and $P2X_{2/3}$ receptors and calcium influx mediated by $P2X_3$ and $P2X_{2/3}$. Dr. Baxter suggested that these compounds may be good potential drug candidates but further research is required to further test for different selectivity profiles and peripheral action sites.

Dr. Andreas Nolting (AstraZeneca R&D, Södertälje) introduced the Dynaflow HT instrument (Celletricon). This fully automated patch clamp system was equipped with advanced microfluidic chip perfusion technology. The instrument featured on-board cell preparation for experiments, walk-away operation, temperature control, and fast fluid exchange for addition of multiple compounds and washes. During the run, cell statistics could be observed in real time—if an error was detected, then the run could be stopped, thereby saving expensive consumables in the process. This instrument was also advertised as being useful for screening both fast ligand- and voltage-gated ion channels and was capable of performing a variety of complex application protocols. Dr. Nolting evaluated the reliability of the data produced by DynaFlow HT, by characterizing modulators of the GlyR Cl^- ion channel. Dr. Nolting reported that both agonist and antagonist values appeared to have consistent results even over multiple runs. Pharmacology data corresponded well with reference data from manual patch clamp experiments in the literature and was in good agreement with EC_{50} reference data pro-

duced using a QPatch platform. Dr. Nolting advised that his research demonstrated that the DynaFlow HT was indeed a reliable, cost-effective tool capable of screening thousands of wells per day.

Next, Dr. Fernanda Laezza (University of Texas Medical Branch) discussed her work in screening for drug candidates targeting voltage-gated Na^+ channel 1.6 ($Na_{V1.6}$). Na_V channels are ubiquitously expressed in all excitable cells and many pathological conditions in humans are associated with their dysfunction, including epilepsy and neuropathic pain. These channels are important targets for pharmacotherapeutic development; however, no selective drugs against specific Na_V channel isoforms have been developed yet. Her group had focused on screening for molecules that specifically disrupted the macromolecular complex formed by $Na_{V1.6}$ and fibroblast growth factor 14 (FGF14), an intracellular protein that controls gating properties and subcellular localization of native Na_V channels protein-protein interactions with the channel C-terminal tail. Medium throughput screening of a 400-member kinase inhibitor library was performed, using the split luciferase complementation assay (LCA), to search for kinases that could disrupt or modulate the FGF14- $Na_{V1.6}$ channel complex. Twenty-eight compounds were identified as initial hits, and 8 were confirmed by secondary screenings. From these results, Dr. Laezza summarized that LCA was a powerful assay for studying protein-protein complexes in live cells, identification of important mechanisms underlying ion channel activity regulation, and lead development and/or optimization in ion channel drug screening.

In the first part of his presentation, Dr. Sikander Gill (Aurora Biomed) presented an overview of HTS of ion channels and co-transporters as targets for therapeutic development. This was followed by the introduction to Aurora Biomed's family of ion channel screening products including their atomic absorption spectroscopy-based ion channel readers (ICR8000, ICR12000) and the principle of tracer flux technology in which Rb^+ and Li^+ ions are used to analyze K^+ and Na^+ channels, respectively. Rb^+ was used as a tracer to determine the activity of transporters exchanging K^+ ions with Na^+ or Cl^- and Na^+/K^+ ATPase. Embryonic stem cell-derived cardiomyocytes endogenously expressing Na^+/K^+ ATPase were also shown to be functional for HTS of compounds against this target. In addition, a Li^+ tracer was used to study the pharmacology of acid-sensing ion channels that were activated by changes in physiological pH. This Rb^+ tracer was found compatible with HTS of TRPC3 in which a panel of positive inhibitors including flufenamic acid, gadolinium, and ruthenium red were reported to inhibit the TRPC3 channels with IC_{50} rank order matching patch clamp values reported in the literature. Dr. Gill closed his presentation with a short discussion on automation of HTS assay using VERSA automated liquid handling workstations (Aurora Biomed) integrated with an ICR8000. Automated screening results were compared with manually performed assays, and it was shown that IC_{50} values from a panel of standard drugs including terfenadine, E-4031, and astemizole were comparable with manually performed assays.

Continuing this session, Dr. Yuri Kuryshv (ChanTest Corporation) introduced his group's work with a novel IonWorks Barracuda system

(Molecular Devices, LLC). The IonWorks Barracuda features second generation Auto-Patch technology. According to the manufacturer, this revised technology improves data consistency by decreasing the effect of biological variability found when measuring currents in single cells and circumvents common issues associated with conventional patch clamp assays, such as the lack of precision, temporal resolution and voltage control. The IonWorks Barracuda has been advertised as being compatible with both voltage- and ligand-gated ion channels and capable of achieving a throughput of over 10,000 data points per day. Dr. Kuryshev analyzed a number of targets and showed data profiles for several reference compounds tested against target channels including the GABA- $A_{\alpha 4\beta 3\gamma 2}$ receptor complex, HEK-hERG cell lines, and CHO- $Na_{V1.7}$ cell lines. Dr. Kuryshev showed that the instrument permitted multiple additions of various concentrations of compounds. Various classes of inhibitors were used in experiments with $Na_{V1.2}$ and $Na_{V1.7}$ channels, in which the IonWorks Barracuda was shown to detect state and use-dependent blockers with good resolution and reproducibility. Additionally, Dr. Kuryshev showed that very uniform response curves were obtained from cells expressing fast ionotropic serotonin receptor 5-HT_{3A}. From the data presented, Dr. Kuryshev determined that this second generation IonWorks Barracuda improves HTS ion channel assays and extends the range of potential targets.

Next, Dr. Ralf Kettenhofen (Senior Scientist, Axiogenesis) discussed the *in vitro* production of pure cardiomyocytes from genetically engineered embryonic stem cells using puromycin-based selection. In this approach, selected cells remained frozen until required during the screening process and retained their autonomous contractile phenotype for at least 3 weeks after thawing when cultured in a monolayer. Several different automated screening technologies were described during this presentation. Dr. Kettenhofen's team had characterized calcium channels of these stem cells using the L-type Ca^{2+} channel blocker nifedipine, with the assistance of a FLIPR Tetra high-throughput cellular screening system (Molecular Devices, LLC). Dose-response profiling of calcium channel agonists and the effects of hERG trafficking inhibitors such as quinine and cisapride were assessed using a Real-Time Cell Analyzer Cardio Instrument (Roche Applied Science). Tests were also performed using isoproterenol, the muscarinic agonist carbachol, and the Na^+ channel blocker lidocaine. Finally, cardiac Na^+/K^+ ATPases were assessed using a Rb^+ flux assay on an ICR8000. Dr. Kettenhofen showed that each of these 3 paradigms indicated that pure cardiomyocytes derived from iPS cells did indeed offer the possibility of conducting predictive high content screening for a variety of cardiac targets in high-throughput assay systems.

Next, Dr. Stephen Hess (EMD Millipore) described his work on the development of profiling assays for KCNQ (K_{V7}) channels, a family of voltage-gated channels that underlie a number of important K^+ currents throughout the body. Retigabine and flupirtine have previously been shown to increase native K_{V7} -like currents in vascular smooth muscle cells, thereby decreasing vascular resistance. Dr. Hess theorized that KCNQ channels might be potential therapeutic targets for high blood pressure and cerebral vasospasms. In order to develop

a selection of reliable high-throughput profiling assays for K_{V7} channels, Dr. Hess first validated a number of cell lines including CHO- $K_{V7.1/minK}$, CHO- $K_{V7.2/7.3}$, CHO- $K_{V7.3/7.5}$, HEK- $K_{V7.4}$, and HEK- $K_{V7.4/7.5}$. Manual patch clamp protocols as well as automated patch clamp experiments using a PatchXpress instrument (Molecular Devices, LLC) were used to assess the effects of retigabine and celecoxib on these cell lines. Retigabine showed current increase for all channels and was found to have a larger potentiation than celecoxib in both $K_{V7.4}$ and $K_{V7.5}$ channels. Having established reliable assays for K_{V7} activators, Dr. Hess's group will continue to work towards the identification of selective K_{V7} agonists and modulators.

Then, Dr. Steven Petrou (University of Melbourne) talked about the dynamic clamp method. Dynamic clamp (also referred to as conductance clamp) is a relatively novel electrophysiology technique that uses a real-time interface between one or more living cells, together with a computer or other analog device to simulate dynamic processes such as membrane or synaptic currents in living cells via computational models. Dynamic clamp allows researchers to create virtual models for diseases in real time and provides a novel mechanism for assessing treatment strategies. This technique enables researchers to test the membrane potential of a "virtual cell." Dr. Petrou showed data from studies he had performed using HEK293 cells expressing the $Na_{V1.4}$ channel. Dynamic clamp can be used to explore the state dependence of drug interactions faster than standard voltage clamp techniques. Dr. Petrou's group studied numerous risk models for epilepsy. Channels of interest were identified and risk models for epilepsy and drug responsiveness were then explored dynamically. Dr. Petrou showed how this novel technique may be used to explore risk assessments for drug and disease responsiveness, thereby improving safety and hit recognition.

Dr. Jacob Schmidt (UCLA Department of Bioengineering) closed the screening technologies session with a discussion on artificial lipid bilayers. Artificial bilayers had previously shown promise as a novel ion channel screening technology but were found to suffer from a number of serious limitations. Artificial lipid bilayers must be manually formed and tend to be quite fragile, with a short half-life. Dr. Schmidt's earlier research had yielded findings of a novel formulation that could be frozen and stored for later use or even shipped to another location before self-assembly was completed. This formulation could be stored for more than 1 month with no apparent negative effects. Upon thawing, the lipid bilayer would resume the self-assembly process. These bilayers were used to successfully reconstitute several physiologically relevant channels. Dr. Schmidt showed that TRPM8, TRPV1, and hERG channels were successfully reconstituted and performed as expected when subjected to various electrophysiology experiments. Based on these results, Dr. Schmidt stated that these artificial structures provide an alternative mechanism for isolating ion channels and also can be utilized as a platform for cell-free ion channel screening or general protein reconstitution. Additional work with robotic liquid handling platforms suggested that it would be possible to produce reliable HTS platforms using these artificial bilayers. Dr. Schmidt felt that this technology had

potential for use in automated ion channel screening and could be especially useful for pharmaceutical research.

CARDIAC FUNCTION AND PHARMACOLOGY

Cardiovascular disease is one of the leading causes of death in the United States and has consistently been a focus for pharmaceutical companies and other research organizations. Aurora Biomed has recognized the importance of this field since the inception of the Ion Channel Retreat in 2003. The final day's discussions focused on the function and pharmacology of cardiac cells. Cardiac function is controlled by the highly regulated flow of ions across the cell membrane during the cardiac action potentials. Cardiac arrhythmias result from abnormalities in the structure and/or functional ability of a variety of ion channels. These variations result in altered responses to environmental stimuli, resulting in various physiological problems. By understanding the underlying molecular mechanisms of both normal and abnormal cardiac function, researchers may be able to develop safer and more effective anti-arrhythmic therapies.

Dr. David Madge (Xention Limited) focused on atrial fibrillation (AF) to start the session. AF is one of the most common forms of cardiac arrhythmia in North America and has been shown to be correlated with age (occurrence in 1 of 25 people over 60 years old and 1 of 10 in people over 80 years old). Current pharmacological treatments for AF involve a cocktail of ion channel blockers with limited channel selectivity. Recent studies have identified the I_{Kur} (carried by the $K_{V1.5}$ channel) and I_{KACH} (carried by the $Kir_{3.1/3.4}$ hetero-multimer) rectifier currents as promising targets due to their predominant expression in human atrial cells. Dr. Madge used HTS methods and lead optimization strategies to search for potent blockers selective for cardiac cells. This study identified XEN-D0101 and XEN-D0103 as potent selective $K_{V1.5}$ inhibitors and XEN-R0702 as a selective I_{KACH} inhibitor. These drugs prevented inducibility of AF in an *in vivo* atrial tachy-paced dog model and also appeared to be selective *in vitro* in human tissues. The use of selective and well-characterized I_{Kur} and I_{KACH} inhibitors appeared to greatly improve the AF treatment. Clinical trials are being implemented to further test these compounds for efficacy and safety.

Next, Dr. Catherine Smith-Maxwell (Gilead Sciences) discussed the role of cardiac Na^+ channels in ischemic heart disease, describing pathophysiological changes in the heart that can lead to arrhythmia and sudden death. Of the many biochemical changes that occur in the ischemic myocardium, several have been shown to cause incomplete inactivation of cardiac Na^+ channels, leading to a persistent or late Na^+ current (I_{Na}). This pathological increase in late I_{Na} can cause increases in intracellular Na^+ , Ca^{2+} overload, and increases in CaMKII activity that can lead to further increases in late I_{Na} . The increases in late I_{Na} and intracellular Ca^{2+} can lead to arrhythmias, mechanical dysfunction, and increased ischemia, thus leading scientists at Gilead to believe that late I_{Na} is a good target for treatment of ischemic heart disease. The ion channel screening group at Gilead used automated patch clamps (PatchXpress 700A, Molecular Devices and QPatch 16X, Sophion Bioscience Inc.) to select for compounds that block late I_{Na} , and not peak I_{Na} or other cardiac ion channels such

as the hERG K^+ channel. A selective late I_{Na} blocker was identified from the screen with relatively little block of the other major cardiac ion channels. This highly selective late I_{Na} blocker was tested in multiple cellular, isolated heart, and *in vivo* models of ischemia and arrhythmia and was shown to have higher potency and efficacy than the less selective ranolazine (Ranexa), a successful anti-anginal drug with demonstrated clinical efficacy.

Continuing with the theme of cardiac fibrillation, Dr. Guy Salama (Cardiovascular Institute, University of Pittsburgh) centered his talk on the onset of early after depolarizations (EADs). EADs have been shown to potentially progress to ventricular fibrillation. Ventricular fibrillation is seen more commonly in adult women due to their higher expression of the Ca^{2+} channel ($Ca_{V1.2\alpha}$) and L-type Ca^{2+} current ($I_{Ca,L}$) at the base of the epicardium. Dr. Salama's group assessed the effect of sex-steroid hormones on Ca^{2+} channel activation involved in EADs using isolated rabbit ventricular myocytes as a model. He demonstrated that estrogen, but not progesterone, increased the expression of $Ca_{V1.2\alpha}$ and $I_{Ca,L}$. Further tests determined that the estrogen receptor ER α mediated the increase of $I_{Ca,L}$ in ventricular myocytes, while blocking the Ca^{2+} -channels reduced the onset of EADs, further highlighting the importance of sex steroids in the regulation of cardiac ion channels and the risk of arrhythmias. Accelerated activation and/or inactivation kinetics of the Ca^{2+} channel was protective against EADs. Therefore, it is important to consider the effect of sex steroids on membrane excitability in cardiac cells.

Dr. Weinian Shou (Riley Heart Research Center, Indiana University School of Medicine) wrapped up the cardiac structure and function session by discussing the effects of FKBP12 on cardiomyocytes. FKBP12 is a *cis-trans* peptidyl-prolyl isomerase expressed in cardiomyocytes that is known to bind to the immunosuppressant FK506, as well as to rapamycin. Since previous works had provided controversial indications that this isomerase might also be a RyR2 channel regulator, his group attempted to deduce the role of FKBP12 in cardiac arrhythmias. Dr. Shou initially generated FKBP12 and FKBP12.6 transgenic mice. He showed that FKBP12 transgenic mice could not rescue FKBP12-deficient mice, and that FKBP12 transgenic mice had a very high mortality rate within the first few weeks of age. Through patch clamp experiments using whole cells isolated from FKBP12-overexpressing and FKBP12 knock-out mice, Dr. Shou showed that arrhythmias only appeared in FKBP12 cardiomyocytes. Based on the results of patch clamp comparisons between transgenic and nontransgenic mice, Dr. Shou suggested that FKBP12 played a key role in regulating the peak density of the tetrodotoxin-resistant, voltage-gated I_{Na} current in ventricular cardiomyocytes. FKBP12 appeared to slow down the recovery of I_{Na} from inactivation inducing depolarization and increased the occurrence of late I_{Na} . Dr. Shou concluded that FKBP12 directly regulated $Na_{V1.5}$ expression, stability, and trafficking via its isomerase activity and appeared to also indirectly modulate $Na_{V1.5}$ via other channel regulators. Since voltage-gated Na^+ channels have been classified as important to a number of serious diseases, it will be important to further study the roles of FKBP12 in cardiac arrhythmias.

CARDIAC SAFETY AND TOXICOLOGY

Pharmaceutical companies have been targeting various cardiovascular disease and other diseases for a number of years. Unfortunately many of these compounds have been shown to have significant and potentially dangerous side effects related to their effects on the heart. Moreover, cardiac safety concerns are a leading cause for the recall of marketed drugs and abandonment of drug development programs as public safety is paramount. There is an ever increasing need to assess the toxicities and adverse effects of these drugs. New drug development programs must develop strategies for minimizing and/or managing the risks of toxicity so as to avoid the removal of these drugs from the marketplace. The remainder of the presentations in the cardiac session summarized studies and strategies conceived in order to avoid the undesired side effects of cardiovascular drugs.

Dr. Arthur “Buzz” Brown (ChanTest Corporation) opened the session by putting the spotlight back on AF. Blocking hERG channels has been the treatment of choice for AF, but all of the marketed drugs potentially may also associate with different, nontarget channels. Using ChanTest’s “Cardiac Channel Panel” (which included the Ca^{2+} channel, pacemaker current, and a number of K^+ and Na^+ channels) in combination with an automated patch clamp (IonWorks Barracuda) instrument, Dr. Brown screened a series of drugs commonly used for the treatment of arrhythmias and hypertension. His results indicated that drugs like verapamil or vanoxerine blocked more than one type of channel at the same time, but appeared to be safe for clinical use because they do not induce a repolarization current. Other drugs, such as alfuzosin, inhibited hERG while simultaneously inducing Na^+ currents, thereby increasing the risk of unexpected side effects. From these results, Dr. Brown concluded that profiling candidate drugs against a wide panel of channels is an important strategy to eliminate false positives and negatives. This strategy will be useful for improving risk assessments for selected drugs.

Continuing on the topic of hERG inhibition, Dr. Sabina Kupersmidt (Vanderbilt University School of Medicine) discussed the importance of the I_{Kr} current (hERG-encoded K^+ current) for cardiac repolarization. Inhibition of hERG by pharmaceutical drugs can result in LQTS and sudden cardiac death. This has resulted in the removal or restriction of several drugs, such as terfenadine and astemizole from the market and has highlighted the importance of evaluating all new drugs for QT prolongation indications. Preliminary studies suggested that the membrane protein KCR1 was the regulatory component of non-inactivating K^+ channels, but the mechanism by which KCR1 influenced hERG-drug interactions was unknown. Dr. Kupersmidt hypothesized that a molecule with similar properties to KCR1 might also prevent the reduction of I_{Kr} in response to the inhibitor dofetilide. A high-throughput thallium redistribution FluxOR™ assay (Invitrogen) was used to find such a compound, identified as VU0405601. This compound decreased the sensitivity of hERG to dofetilide inhibition by 40%, enhanced hERG currents (4-fold in peak, 2-fold in tail) in a concentration-dependent manner, and did not affect either I_{Na} or I_{Kur} , indicating its specificity for I_{Kr} *in vitro*.

Further testing on isolated rabbit models corroborated these findings since pretreatment with VU0505601 reduced the incidence of dofetilide-facilitated arrhythmias. Therefore, VU0405601 is an important compound that has the potential to decrease the risk of arrhythmias in response to hERG inhibitors.

Dr. Blake Anson (Cellular Dynamics International) closed the session with a discussion concerning the advantages and utility of iCell® Cardiomyocytes (Cellular Dynamics). Cellular Dynamics developed and purified these cells from human iPS cell-derived cardiomyocytes. Through transcriptome analysis, protein expression imaging, metabolic oxygen consumption characterization, single-cell patch clamp, and several automated patch clamp experiments, Dr. Anson demonstrated that iCell Cardiomyocytes featured important advantages for cardiac cell studies. These cells featured stable gene expression, suitable protein expression, and localization properties and were applicable to a range of ion channel monitoring platforms including QPatch, Patchliner (Nanion Technologies), FLIPR Tetra, IonOptix (IonOptix, MA), and the xCELLigence RTCA system (ACEA Biosciences Inc.). In summary, iCell Cardiomyocytes are an important system with wide applicability for electrophysiological and biochemical assays and have vast potential in basic biology and preclinical development research. These cardiomyocytes will be an important resource for researchers interested in safety pharmacology and disease modeling. Additionally these cells represented an excellent human-based test system for *in vitro* screening.

STRUCTURE AND FUNCTION OF ION CHANNELS

Ion channel activity can be modified by a number of factors. Conformational differences between closed and open states are controlled by a number of external factors, including modifying enzymes that may themselves be controlled by modulators and effectors. Ion channel subunits are also modified by glycosylation. Resulting changes in the 3-dimensional structure of these compounds are in most cases not well documented because it is hard to confirm the real *in vivo* structure of most proteins. The final segment of the Ion Channel Retreat summarized 3 novel studies that provided state of the art knowledge about key structural features related to either channel activity or their interaction with ions and other compounds.

Opening this session, Dr. Geoffrey T. Swanson (Northwestern University Feinberg School of Medicine) centered his presentation on the glutamate-gated ion channels, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptors. These receptors are known to be affected by plant lectins, but no lectins with analogous effects have as yet been identified in animals. Dr. Swanson identified several members of the galectin family that increased steady-state currents and slowed kinetics of GluA4 AMPA and GluK2 kainate receptors, while reducing peak current amplitudes of GluA1 AMPA and GluK1 kainate receptors. His study showed that galectins isolated from Conger eels (congerin I and II) are more efficacious in these modulations than the human galectin, galectin-1. Dr Swanson suggested that these differences are linked to the carbohydrate content and structural complexity of these receptors. Structural

analysis of glutamate gated ion channels showed that their activity levels change significantly depending on the dimer structure of the associated galectins. In summary, his study identified galectins as potential endogenous modulators of AMPA and kainate receptor function and hypothesized that galectins may play a potential role in excitatory neurotransmission.

Next, Dr. Heike Wulff (University of California, Davis) discussed $K_{V1.3}$, a voltage-gated potassium channel that has been suggested as a target for memory T cell-mediated autoimmune disease. To further understand the inhibiting mechanism of the immunosuppressant PAP-1 (a high-affinity $K_{V1.3}$ blocker) Dr. Wulff's group collaborated with the molecular modeling group of Dr. Boris Zhorov and combined Monte Carlo minimizations and site-directed mutagenesis to map the binding locus. Tests on the resulting structural model suggested that two PAP-1 molecules coordinate a K^+ ion in the $K_{V1.3}$ pore with their carbonyl groups and anchor themselves by extending their lipophilic side chain into the interface between helices S5 and S6. This appeared to explain PAP-1's high selectivity for K_V channels and voltage-dependent pore blocking. Using a similar strategy, Dr. Wulff and her collaborators at NeuroSearch A/S also characterized the action site of the negative gating modulator NS8593 on the small-conductance Ca^{2+} -activated K^+ channels $K_{Ca2.1-2.3}$. The group created chimeras between the NS8593-sensitive $K_{Ca2.3}$ and the NS8593-insensitive $K_{Ca3.1}$ channel. Tests with these chimeras suggested that the binding site for NS8593 was actually in the pore of $K_{Ca2.3}$, rather than the C-terminus as originally hypothesized. Further mutational studies demonstrated that the residues S507 and A532 mediated the effect on $K_{Ca2.3}$ and conferred sensitivity to NS8593, and suggested that pharmacological modulation of $K_{Ca2/3}$ channels was possible at multiple sites.

Ending this session, Dr. David D. Busath (Brigham Young University) focused his talk on the structure of the M2 proton-selective ion channel, which has been shown to be localized in the viral envelope of the influenza A virus. The M2 protein enables acidification of the influenza A lumen upon endosomal internalization, making it critical to the viral infectivity cycle. This has made M2 a popular drug target; however, mutations in this channel have since resulted in resistance to traditional antiviral drugs such as amantadine and rimantadine. Dr. Busath attempted to characterize the structure of the M2 proton channel, as well as key domains that interacted with amantadine. Using a liposome assay block, he determined that the amantadine binding site is located inside the wild-type channel. Furthermore, his results showed that the M2 conductance domain (residues 22–62) contains drug-resistant mutation epitopes (residues 22–46) and that a mutation in this epitope (S31N) conferred a weaker block of amantadine in oocyte washout assays. Structures were analyzed using solid-state NMR (ssNMR) analysis of the conductance domain to determine their structural properties and deduce potential function of structures and locate binding sites. Dr. Busath also used docking software to assess a library of similar, amantadine-like compounds that had previously been reported as having known antiviral activity. Top scorers were selected for further liposome and oocyte testing. From the results of the analysis, Dr. Busath concluded

that ideal antiviral molecules must be 113–437 Da (with optimal at 205 Da) and prolate-shaped to bind to M2 and inhibit even more strongly than amantadine. The liposome assay was shown to be a good functional HTS test that should be applicable to any ion channel. Additionally, ssNMR was shown to be a very accurate mechanism for determining the structural properties of the conductance domain, eliminating problems associated with x-ray crystallography.

CLOSING REMARKS

Wrapping up the 2011 Ion Channel Retreat, Dr. Dong Liang (CEO, Aurora Biomed Inc.) expressed his gratitude to each of the participants. Dr. Liang highlighted the importance of collaborations between scientists harbored by institutions in both academia and industry, who covered topics ranging all the way from basic science to pharmaceutical discovery, and from research and development to business. Dr. Liang stressed that the Retreat succeeds due to overall variety and quality of the participants.

CONCLUSION

The 2011 Ion Channel Retreat showcased researchers from both academia and industry who presented various novel approaches for targeting ion channels hypothesized to have therapeutic potential. Specific classes of ion channels were repeatedly mentioned throughout the conference highlighting their importance across the field of ion channel research. Several researchers discussed a number of members of the TRP super-family, while other talks frequently mentioned either Na^+ or Ca^+ channels.

Innovative screening technologies, such as the dynamic clamp method and artificial bilayers were introduced to the delegates for the first time, while updated models of familiar HTS instruments were described in detail. These technologies, methodologies, and platforms continue to revolutionize the marketplace. Automation continues to lower costs and improve efficacy for researchers who are required to increase their throughput and testing, while simultaneously being provided with fewer resources to work with.

Aurora Biomed's Ion Channel Retreat is a yearly conference that commenced in 2003 and has consistently provided an environment for researchers and industry leaders to share knowledge, exchange ideas, and establish partnerships. Aurora Biomed would like to thank each of the sponsors and attendees for their continued support. This conference has steadily grown each year, and we would like to invite submissions for speaking and poster presentations at the 10th Annual Ion Channel Retreat to be held in the summer of 2012 (dates to be announced).

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