## References

1 Lindon, J.C. *et al.* (1996) Direct coupling of chromatographic separations to NMR spectroscopy. *Prog. NMR Spectros.* 29, 1–49

2 Bayer, E. *et al.* (1979) On-line coupling of high-performance liquid chromatography and nuclear magnetic resonance. *J. Chromatogr.* 186, 497–507

3 Haw, J.F. *et al.* (1981) Continuous flow high field nuclear magnetic resonance detector for liquid chromatographic analysis of fuel samples. *Anal. Chem.* 53, 2327–2332

4 Laude, D.A., Jr. and Wilkins, C.L. (1984) Direct-linked analytical scale high-performance liquid chromatography/nuclear magnetic resonance spectrometry. *Anal. Chem.* 56, 2471–2475

5 Pullen, F.S. *et al.* (1995) Online liquid chromatography-nuclear magnetic resonance- mass spectrometry – a powerful spectroscopic tool for the analysis of mixtures of pharmaceutical interest. *Rapid Comm. Mass Spectrom.* 9, 1003–1006

6 Lindon, J.C. *et al.* (2002) Biomedical and pharmaceutical applications of HPLC-NMR and HPLC-NMR-MS. In *On-line LC-NMR and related Techniques*. (Albert, K., ed.), pp. 45–87, John Wiley & Sons

7 Nicholson, J.K. *et al.* (2001) High-performance liquid chromatography linked to inductively-coupled-plasma mass spectrometry (ICPMS) and orthogonal acceleration time-of-flight mass spectrometry for the simultaneous detection and identification of metabolites of 2-bromo-4-trifluoromethyl-[$^{13}$C]-acetanilide in rat urine. *Anal. Chem.* 73, 1491–1494

8 Wolters, A.M. *et al.* (2002) NMR detection with multiple solenoidal microcoils for continuous-flow capillary electrophoresis. *Anal. Chem.* 74, 5550–5555



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# Ion channels in drug discovery and development


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Approximately 100 attendees and speakers enjoyed the atmosphere and information exchange at the second annual *Ion Channels* conference at the historic Bellevue Hotel (14–15 July 2003, Philadelphia, USA), hosted by the Strategic Research Institute. A good mix of pharmaco scientists, academics and technology reps made up the attendees and presenters.

The conference was co-chaired by Wilhelm Lachnit (Molecular Devices; http://www.moleculardevices.com) and Chris Mathes, who opened with a brief historical overview of the patch-clamp technique. The patch-clamp method has been an enabling technology in the study of ion channels since it was first reported in 1976 [1], being improved, thereafter, by the discovery of gigaseal recordings for low-noise, single-channel recordings [2]. In fact, the patch-clamp is one of the only methods for viewing the biological activity of proteins working in action in living cells. In 1991, Neher and Sakmann received the Nobel Prize in Medicine for inventing the patch-clamp technique. This technique uses glass micropipettes, with tips of 1–2 microns, that form tight seals (>1 gigaohm) against cell membranes. With this technique, single channel or whole cell currents through ion channels can be recorded with specialized amplifiers that enable recordings of miniscule currents (i.e. $10^{-12}$ A; see, for example, http://www.axon.com).

Conventional patch-clamp is slow and tedious, but well suited for PhD-level scientists, working out the detailed biophysical properties of ion channels. Until recently, however, there was no hope that this method could be used for screening ion channels in the drug discovery process. Seven of the talks at the conference highlighted new technologies in automated patch-clamp, several of which use planar patch-clamp electrodes. These planar electrodes are simply flat substrates (usually glass) with a 1–2 micron aperture separating intracellular and extracellular solution. 'On the surface', planar patch-clamp appears to be a modern technology, but it was actually first reported by Russian scientists in 1975 [3].

## The new frontier: automated and chip-based patch-clamp systems

Seven talks presented recent advances in automated patch-clamp. Appropriately, the first technology reported was the AutoPatch® from Xention Discovery (http://www.mvfund.com/xention.htm), presented by John Ford, Director of Biology. The AutoPatch-1 (AP-1) was one of the first automated patch-clamp systems to hit the scene in 1998; at the heart of it is the Interface Patch technology. This clever method involves

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a vertically and upwardly oriented patch pipette that enters a meniscus of extracellular solution, containing cells in suspension. Ford reported that this method is able to achieve gigaseals and whole cell recordings in 80% of cases. The AP-1 is a one-channel patch-clamp system; the AP-2 Sequential Patch-clamping System performs 48 recordings, in sequence, with the AutoPatch® Patch-Plate. There are six AP-2s presently in operation at Xention. The AP-3 provides even higher throughput because of its parallel operation. The AutoPatch® system is not currently commercially available, but instead is used internally at Xention for ion channel drug discovery.

Niels Willumsen (Sophion Bioscience; http://www.sophion.dk/) provided an update on the Qpatch™, 'a new and scalable automated ion channel screening technology'; explaining that Q denotes 'quality', 'quantity' and 'quick'. Approximately three years ago, Sophion emerged from Neurosearch, after developing one of the first automated patch-clamp systems, called the Neuropatch™ (a robotic patch-clamp rig). Later, the name was changed to Apatchi-1™. The recently developed Qpatch™, uses planar patch-clamp electrodes for parallel recording of ion channel currents and the planar electrode substrate is a silicon chip with a surface of $SiO_2$. The QPatch™ includes patch-clamp amplifiers developed by engineers at Sophion. The Qplate™ enables recording from 16 channels in parallel. Data from various cell types (HEK 293, CHO, Sf9, MEL) and channels (Na and K) was reported.

One of the first planar patch-clamp electrodes made from glass for gigaseal recordings came from Nanion Technologies (http://www.nanion.de). Andrea Brueggemann represented Nanion in her presentation entitled, 'Tools for Ion Channel Drug Discovery', discussing three automated patch-clamp systems that are currently in development. The first in this family is the Port-a-Patch one channel system, which is compact enough to be moved around in the lab or between labs, and can be used for whole cell or single channel recordings. The second Nanion family member is the NPC-16s, a 16-channel sequential patch-clamp that should be ready for release at the Biophysical Society conference, 2004. Completing the family is the NPC-16p, which is a 16-channel parallel patch-clamp.

Co-chair, Lachnit presented the IonWorks™ HT system from Molecular Devices, which has been on the market for about a year. The IonWorks™ system is a 48-channel automated, parallel system. Cells are positioned on apertures in the Patch-Plate wells in parallel, using a robotic pipettor (12 channels) and positioned via suction. An electrical seal (~100–200 M) is formed between the cell membrane and the pore periphery. Whole cell access is obtained using the perforated patch technique. Lachnit emphasized that a technician can run the IonWorks™ system but an electrophysiologist might be needed for assay development and data interpretation. Cell lines tested with this system include CHO-K1, HEK-293, CHL, and neuroblastomas, and channel types include Kv1.5, Na (1.5, 1.3), hERG, Ca (L-, N- and R-type).

Jia Xu (AVIVA Bioscience; http://www.avivabio.com) described the SealChip™-16 planar patch-clamp electrodes. Made exclusively for the PatchXpress™ (Axon Instruments), the SealChip™ affords parallel and patch-clamp recordings from 16 cells in parallel. AVIVA's proprietary surface modification of the glass substrate provides a gigaseal and high whole cell success rate. Data from hERG channels and other voltage-gated channels were presented. The PatchXpress™ and SealChip™ electrodes also enable continuous measurements of currents through ligand-gated channels. Xu discussed the importance of tight gigaseals and low access resistance (Ra) for high-quality whole cell recordings; by creating the right balance of aperture size and throughhole depth, AVIVA has designed the SealChip™ to be optimized for both gigaseals and low Ra values.

In the only user presentation of the conference, Francesco Belardetti (NeuroMed Technologies; http://www.neuromedtech.com) reported recent results from ion channel studies using the PatchXpress™. NeuroMed focuses a substantial amount of its drug discovery efforts on calcium channels. For example, N-type Ca channels are specifically targeted because they play a role in presynaptic inhibition, which is important in alleviating pain. Specific N-type targets would be preferable to morphine, which has undesirable side effects in treating pain. NeuroMed also uses patch-clamp early in the pipeline in an intelligent way – by combining available information about drugs that are known to act on ion channels and determining how they perform in a patch-clamp assay. The disadvantage of using this method has been the turtle-pace of patch-clamp, that is, until the recent development of automated patch-clamp systems, such as the PatchXpress™. Biophysical properties of Ca channels recorded with the PatchXpress™ are indistinguishable from conventional patch-clamp recordings. Recent experiments with known blockers have provided $IC_{50}$ values that also matched $IC_{50}$ values from conventional patch experiments.

Finally, Albrecht Lepple-Wienhues (Flyion; http://www.flyion.de) described the Flyscreen® automated patch-clamp system. This unique approach involves typical patch pipettes used in an atypical manner. Patch-clamp electrodes are usually filled with an intracellular solution, then the tips are positioned on top of a stationary cell and a gigaseal is obtained by suction. With the Flyion approach, cells in suspension are pipetted into the patch pipette and pulled down toward the aperture. Stable gigaseals are formed between

the cell membrane and the inside glass walls of the patch pipette. Additional negative pressure from the tip (or alternatively perforants, such as nystatin or amphotericin) ruptures the cell membrane so that whole-cell recordings are made with this 'flip-the-tip' method. Data from CHO-hERG cells, CHO-Kv1.5, and Kv1.3 channels in lympocytes was presented.

## Ion channels as therapeutic targets

The first day of the conference highlighted technologies, whereas the second day focused on ion channels as therapeutic targets. Walter Stühmer (MPI for Experimental Medicine; http://www.mpiem.gwdg.de) started the day with the keynote lecture entitled, 'The EAG Potassium Channel: A New Cancer Target'. EAG stands for 'Ether à go-go', a hyperexcitable *Drosophila* (fruit fly) mutant. When expressed in CHO, HEK or 3T3 cells, EAG1 induces a transformed phenotype. Under conditions that normally prevent cell growth, EAG1 expressing cells exhibit significantly faster growth, growth factor-independent growth, loss of contact inhibition and growth in soft agar. In mice, EAG1 favors tumor progression. Normally, EAG1 is expressed primarily in the brain; however, in many cancer patients, EAG1 is highly expressed in their tumors. Stühmer presented evidence that a blocker of EAG1 (IOT101) also inhibits tumor formation in mice. The problem, however, is that IOT101 also blocks hERG (human EAG-related gene) potassium channels, which is not surprising, given the similarity of these channels. The goal of future research in this area is to develop specific EAG1 blockers that do not block hERG, an important potassium channel that regulates action potential duration in the heart.

George Chandy (University of Irvine; http://www.uci.edu) provided an interesting presentation on Kv1.3 potassium channels as therapeutic targets for autoimmune disorders. Multiple sclerosis (MS), for example, is a chronic demyelination disease of the CNS caused by T cells that recognize and destroy the myelin sheath covering neurons. Chandy and his colleagues discovered that T cells in MS patients have up-regulated Kv1.3 levels. Kv1.3 blockers prevent MS formation in a mouse model.

## Safety assessment for HERG

The grand finale of the two-day conference came with the last three speakers, on the topic of hERG channels. David Rampe (Aventis; http://www.aventis.com) provided an excellent introduction to hERG channels in his presentation entitled, 'Drug-induced QT prolongation'. Rampe pointed out that from 1995–2000, 50% of drugs recalled by the FDA were hERG channel blockers. Some popular examples are Terfenadine (antihistamine), Astemizole (antihistamine) and Cisapride (anti-psychotic). Blocking hERG channels prolongs the action potential duration, leading to long QT syndrome, which, in some individuals, can be fatal. In some cases, compounds block hERG channels without prolonging the QT interval; for example, Verapamil blocks hERG potently, but does not prolong QT, probably due to the fact that this compound also blocks Ca channels.

Arthur Brown (ChanTest; http://www.chantest.com) discussed some of the biophysical properties of hERG channels. He noted that differences in pulse protocols might account for the wide spread in reported $IC_{50}$ values for compounds that are known to block hERG channels. Brown also described a novel assay, called hERG-Lite, which uses a luminometer to measure chemiluminescent signals from cells. The signal comes from a mutant hERG channel with an epitope tag. These mutant hERG channels are also detained in the endoplasmic reticulum (ER) of cells because they are not core glycolsylated. Blockers of hERG channels somehow rescue the channels stuck in the ER, resulting in incorporation in the plasma membrane. Brown asserted that this assay should be able to test ~10 000 compounds per day.

Craig January (University of Wisconsin; http://www.wisc.edu) closed the conference with an interesting talk entitled, 'Clinical and Basic Science of Long QT syndrome'. January's lab has created a HEK-293 cell line, expressing hERG channels that are used in laboratories around the world. In his presentation, he made a distinction between congenital and acquired long QT syndrome: he described a woman with congenital long QT who lived to be 102. She was advised to avoid medications that tend to prolong QT. Congenital LQT has been found to be a more complex polygenic disease of both K and Na channels. Frequently, it involves the failure of a mutated hERG channel protein to traffic normally to the cell surface. Acquired long QT occurs in patients without any previous history of syncope or long QT-related problems and coincides with taking a medication that prolongs QT [i.e. the antihistamine Hismanal (astemizole)]. Acquired LQT is a monogenic disease, caused almost entirely by hERG channel block. Long QT syndrome is the most common of the inherited arrhythmia syndromes and has ~1/5 the incidence of cystic fibrosis.

## References

1 Neher, E. and Sakmann, B. (1976) Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature* 260, 799–802
2 Hamill, O.P., *et al.* (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* 391, 85–100
3 Kostyuk, P.G. *et al.* (1975) Effect of internal fluoride and phosphate on membrane currents during intracellular dialysis of nerve cells. *Nature* 257, 691–693