

8 years of surveying ion channel screening – has anything changed?

No review of ion channel screening over the past decade can avoid discussing the pivotal role played by automated electrophysiology. Arguably this technology, more than any other, has opened up the field to wider investigation, made ion channels more accessible as drug targets and facilitated the drive towards highest possible data quality. In this review we will hear how automated patch clamping systems are continuing to evolve and are increasingly positioned at the centre of most ion channel discovery activities.

Many advances have been made in ion channel screening over the past decade. From a technology perspective perhaps the greatest of these was the emergence of the planar patch used in automated patch clamping (APC) devices which has opened up the possibility of higher throughput direct electrophysiological assay of ion channel activity. However, the deployment of APC in screening was initially met with some skepticism by electrophysiology purists in the industry¹. APC has evolved considerably in recent years with a diversity of new third generation systems now addressing both voltage and ligand-gated channels at much higher throughputs and at higher seal resistances. In addition, the use of population patch clamp and multi-hole approaches has largely mitigated initial concerns over data consistency and patch success rates. Many of the newer APC systems utilise cutting edge developments in

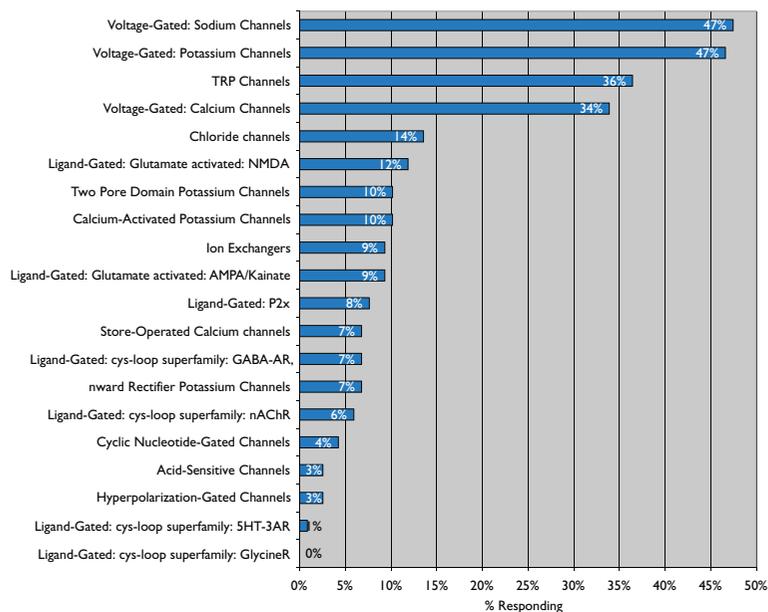
microfluidics. APC systems have also played a pivotal role in enabling the early (non-compliant) investigation of hERG. Increasingly primary and stem cells are being used in ion channel testing and investigated by APC. Although screening technology has been regarded as the key to successful ion channel drug development², the growing realisation that leads discovered against ion channel targets are less likely to make it through to the market than candidates from other disease areas has led to the recent realignment or closure of some Pharma ion channel screening programmes and this is expected to promote greater reliance on biotech collaborations and outsourced testing.

HTStec first got involved in tracking developments of the ion channel screening market in 2004, and it is the only area where annual report updates have been undertaken, thanks largely to the interest and support of the vendor community.

By Dr John Comley

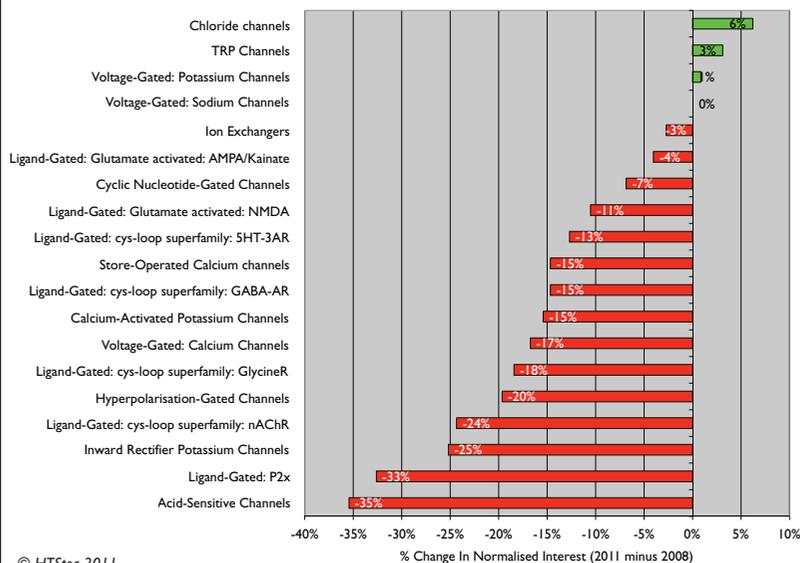
Screening

Figure 1: Ion channels classes of most interest to survey respondents today



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Figure 2: Changing interest in ion channels classes (2011 versus 2008)



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In March 2011, HTStec undertook the latest update of its Ion Channel Screening Trends 2011 report³. The main objectives of this year's study were to better understand respondents' interest and experience of: 1) Ion channels screened, assay technology used and screening metrics generated; 2) the use and purchase of automated patch clamp (APC) systems for ion channel screening; 3) cell requirements for ion channel assays; and 4) the use of outsourced ion channel testing services. In this article we report on the current status of ion channel screening as found in this year's report, and where consistent report data permits show how the landscape has changed over the past eight years. We also review the latest developments in APC devices and some of the options for outsourced testing today.

Ion channel classes of greatest interest

The class of ion channels of greatest interest to survey respondents today was voltage-gated sodium channels (47% interested). This was very closely followed by voltage-gated potassium channels (47% interested); TRP channels (36% interested), and then voltage-gated calcium channels (34% interested). These classes were distantly followed by chloride channels (14% interested). All other classes including all ligand-gated channels had less than 12% interest to respondents (Figure 1).

Figure 2 charts the changing interest in ion channel classes today relative to 2008. This shows that interest had only increased since 2008 with respect to three ion channels classes, ie chloride channels, TRP channels and voltage-gated potassium channels. Interest in voltage-gated sodium channels was unchanged. Interest in all other ion channel classes had decreased since 2008, with the decreases most marked with respect to ligand-gated P2x and acid-sensitive channels (Figure 2).

Ion channel screening activities undertaken in-house

Of the ion channel screening activities undertaken in-house today (2011), the majority of survey respondents were carrying out screening of lead compounds against other specific ion channel targets (59% undertaking), followed by primary screening of ion channels with diversity libraries (53% undertaking) and then safety assessment of lead compounds against specific ion channel liabilities (50% undertaking). The least percentage (36%) of survey respondents were carrying out selectivity profiling against a panel of ion channel targets using membrane potential dyes (Figure 3).

Figure 4 charts the changing focus of ion channel

activities undertaken in-house in 2011, relative to 2008. This shows that in-house screening undertaken had only significantly increased since 2008 with respect to selectivity profiling against a panel of ion channel targets using both membrane potential dyes and other technique(s). In-house screening undertaken had remained essentially unchanged with respect to screening of lead compounds against other specific ion channel targets and the primary screening of ion channels with diversity libraries. All other ion channel activities undertaken in-house have declined, with this being most pronounced for safety assessment of development candidates against specific ion channels liabilities.

Outsourced ion channel testing today

Most survey respondents still prefer not to outsource ion channel testing, but to run it in-house today. In the case of primary screens/HTS and secondary screens the majority of respondents preferred to run these assays in-house (73% and 72% respectively). In the case of safety assessment (non-compliant) assays and compound profiling assays most respondents prefer to run these assays in-house (49% and 44% respectively). Safety assessment (compliant) assays were the only ion channel testing activity where the majority (52%) prefer to outsource (Figure 5).

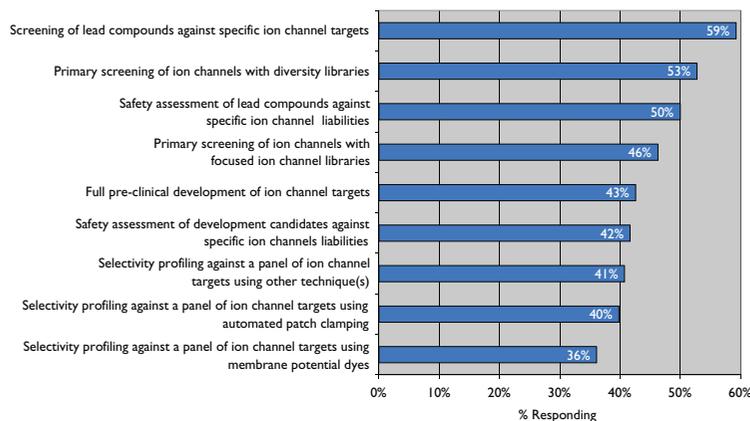
Where hERG testing is initiated

The stage in the drug discovery/development process where the majority (44%) of survey respondents begin hERG liability testing was hits-to-leads (lead optimisation). This was followed by secondary screening (20% deploying); compound profiling (15% deploying); primary screening/HTS (13% deploying); and then safety assessment (non-compliant) (only 9% deploying) (Figure 6).

Main assay technologies used to study ion channel targets

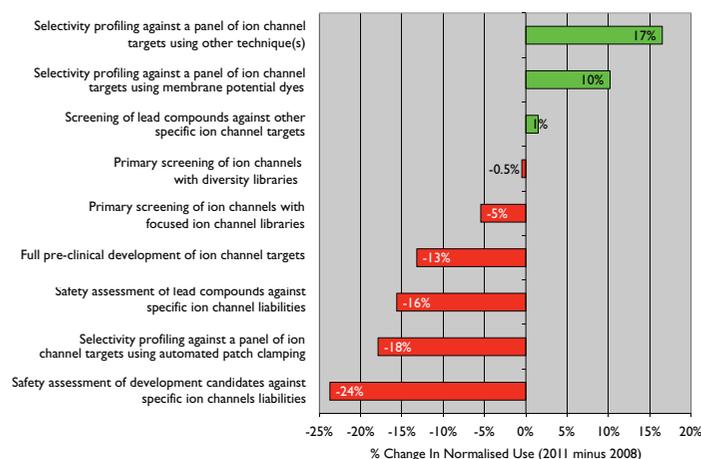
The main assay technologies used by survey respondents in house to study ion channel targets in drug discovery in 2005 and 2011 are presented in Figure 4 and 5 respectively. In 2005 manual patch-clamping was used by most survey respondents in safety assessment (eg hERG compliant assays), therapeutic areas (target identification/validation) and compound profiling. Automated patch-clamping (APC) was used by most survey respondents in early non-compliant hERG liability testing, hits-to-leads (Lead optimisation) and secondary screening. Fluorescence membrane potential assays were used by most survey respondents in: primary screening of focused/targeted libraries;

Figure 3: Ion channel activities undertaken in-house today



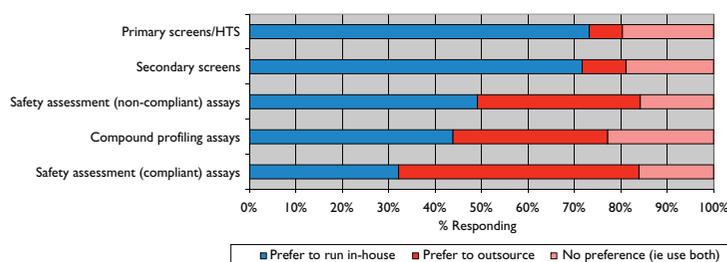
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Figure 4: Changing ion channel activities undertaken in-house (2011 versus 2008)



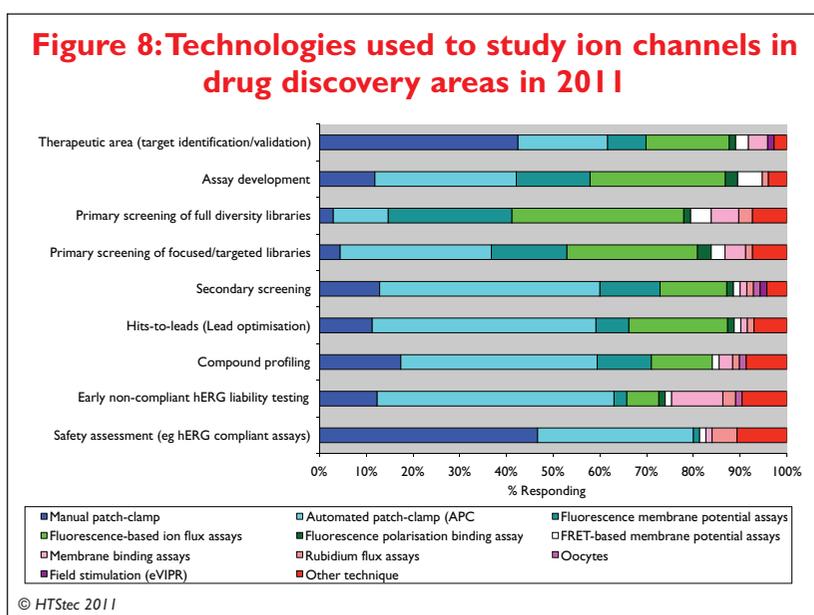
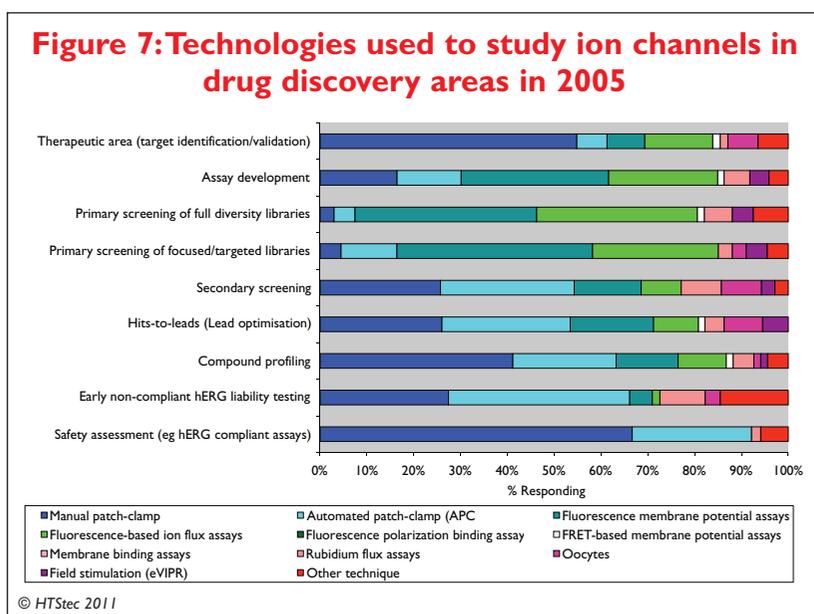
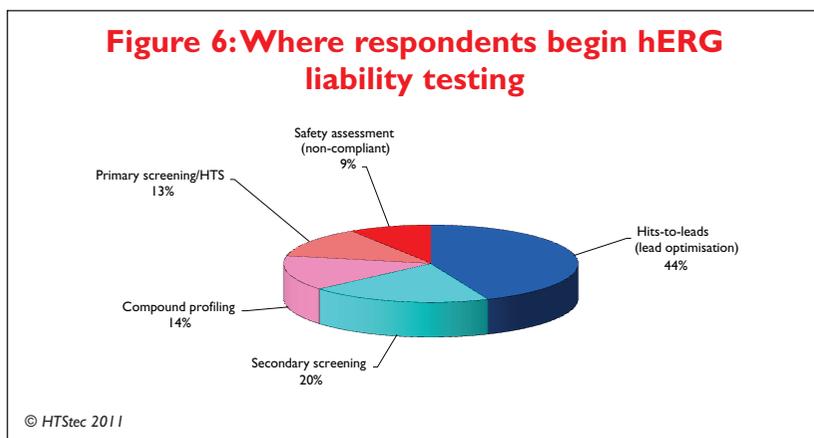
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Figure 5: Ion channel testings respondents prefer to outsource today



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Screening



primary screening of full diversity libraries; and assay development (Figure 7). In 2011 manual patch-clamping was now used by most survey respondents in safety assessment (eg hERG compliant assays) and therapeutic areas (target identification/validation) only. Automated patch-clamping (APC) was now used by most survey respondents in early non-compliant hERG liability testing, compound profiling, hits-to-leads (lead optimisation), secondary screening, primary screening of focused/targeted libraries and assay development. Fluorescence-based ion flux assays were used by most survey respondents in primary screening of full diversity libraries. 2011 also saw the emergence of membrane binding assays, and to a lesser extent fluorescence polarisation binding assays, particularly for early non-compliant hERG liability testing (Figure 8).

Changes in ion channel screening metrics over the past few years

Table 1 reports the primary ion channel screening metrics we have collected since 2004. The absence of a few years reflects the fact we did not collect comparable data in those years. In large pharma labs the median number of ion channel screens was four in 2004 and 2005, peaking at five in 2007 and then declining to three in 2011. This trend is roughly mirrored by the median number of data points per screen which rose from 250,000-500,000 in 2005 to peak at 500,000-1 million in 2007 and 2008, to decline back to 250,000-500,000 in 2009 and 2011. In small/medium pharma and biotech labs the median number of ion channel screens was two in 2004, peaking at three in 2005 and 2007, and then declined back to two in 2008, 2009 and 2011. This trend was again roughly mirrored by the median number of data points per screen which rose from 50,000-100,000 in 2004 to peak at 100,000-250,000 in 2005 and 2007, to decline back to 50,000-100,000 in 2008, and then further declined to 10,000-50,000 in 2009 and 2011.

Will APC systems ever be used in primary screening?

In Figures 7 and 8 we see that primary screening of full diversity libraries for ion channel activity is still mainly done by fluorescence membrane potential assays and fluorescence-based ion flux assays. Which raises the question under what circumstances would survey respondents consider using APC systems for primary screening of 100,000 to 1 million compounds. Respondents' views on this are summarised in Figure 6. The

majority (25% choosing either option) would consider it if the cost per data point equals the current cost of primary screening (FLIPR costs), or if they see that a direct assay will increase compound specific information significantly. A further 17% indicated they would consider it if the target requires a technology with high data quality readout and 16% if the timeline and throughput for such a screen would equal our current primary screening method (eg FLIPR). That left 17% who indicated there were no circumstances where they would consider it (Figure 9).

What represents quality in APC measurements?

What represents best quality in APC measurements to survey respondents is presented in Figure 10. The biggest proportion (35%) of survey respondents thought giga-seal resistances represents best quality in APC measurements. This was very closely followed by stable pharmacological responses and then stable whole-cell conditions (32% responding) and then stable whole-cell conditions (27% responding). Only a small minority (3% or 4%) of respondents thought temperature control or Rs compensation represented best quality in APC measurements (Figure 10).

Use of more relevant cell backgrounds

Survey respondents most want to use primary cells or more relevant cell backgrounds for ion channel testing in hits-to-leads (lead optimisation) (50% wanting); compound profiling (47% wanting), and then safety assessment (non-compliant) (31% wanting). Survey respondents were least interested in using primary cells or more relevant cell backgrounds in primary screening/HTS (only 15% wanting) (Figure 11).

Latest developments in ion channel screening

The following snapshots provide details of how various vendors support work on ion channel screening through provision of new automated patch clamp instruments and testing services.

BioFocus (www.biofocus.com) is a leading provider of drug discovery services. With comprehensive capabilities, from target identification and validation to candidate drug nomination, BioFocus works in partnership with clients to deliver project milestones. BioFocus' ion channel team and its array of core technologies provide breadth and throughput to perform high-throughput screening for voltage- and ligand-gated, multimodal and

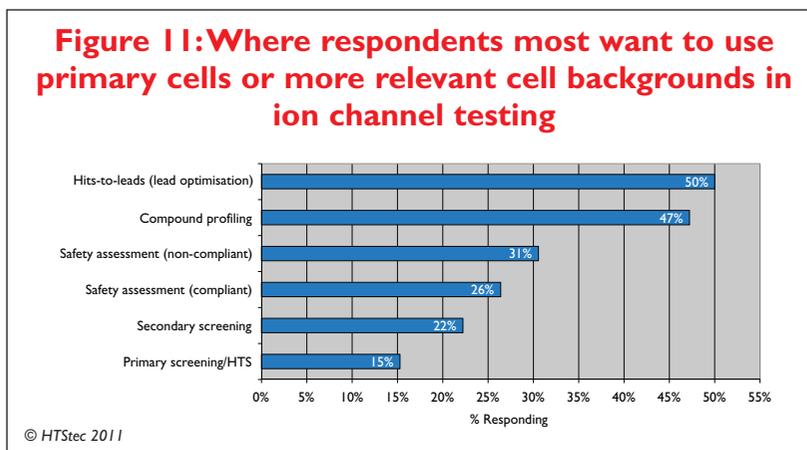
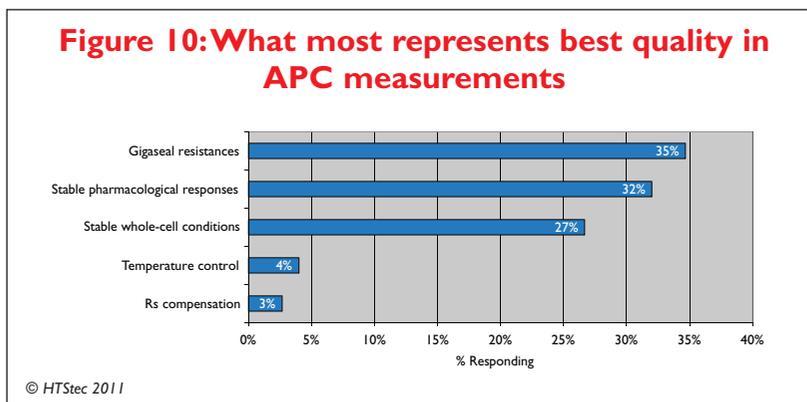
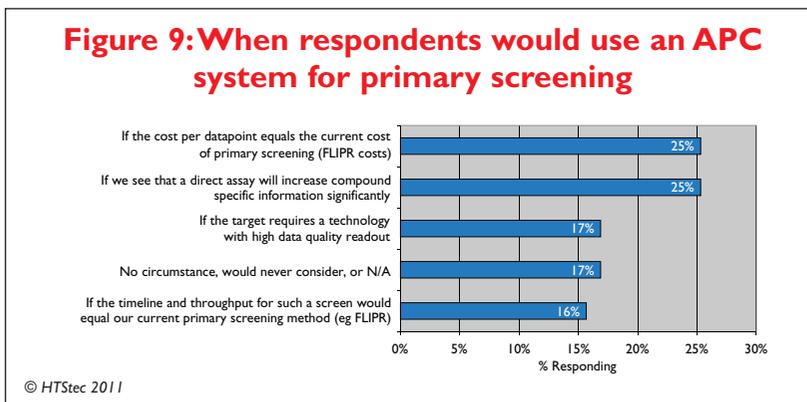


Table 1: Ion channel primary screening metrics over the past eight years

	2004	2005	2007	2008	2009	2011
Large Pharma						
MEDIAN No. Screens/Year	4	4	5	4	4	3
MEDIAN No. Data Points/Screen	250K-500K	250K-500K	500K-1M	500K-1M	250K-500K	250K-500K
Small/Medium Pharma & Biotech						
MEDIAN No. Screens/Year	2	3	3	2	2	2
MEDIAN No. Data Points/Screen	50K-100K	100K-250K	100K-250K	50K-100K	10K-50K	10K-50K

Screening

Figure 12
Celletricon's Dynaflo®HT system is now for driving medicinal chemistry optimisation of ion channel compound activity at BioFocus



'leak' ion channel programmes. Clients can access custom cell line generation – BioFocus has access to Millipore PreciSION™ cell lines and MaxCyte STX bulk transient transfected cell lines. FLIPR and radiometric screening technologies are available as alternative diversity screening technologies for rapid prioritisation of compound activity in follow-up studies. BioFocus' two IonWorks® Quattro™ assay systems combined with custom voltage protocols are designed to: 1) Reveal infor-

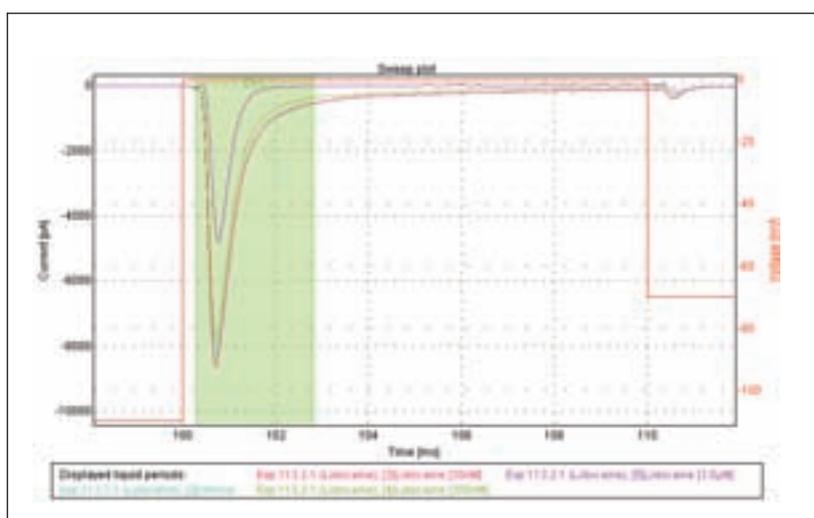


Figure 13: Sweep plot of Nav1.5 currents done using automated patch clamp as part of B'SYS routine testing (IC₅₀ Lidocaine: 1.53 mM; Hill 1.243)

mation regarding compound mechanism of action; and 2) enable automated electrophysiology screens of up to 120,000 compounds. The use of these instruments has led to the delivery of validated hits to its clients for multiple programmes. Dynaflo®HT is a new electrophysiology technology available at BioFocus that provides 96 individual recording channels grouped in clusters of six. The Dynaflo system is built around a consumable containing microfluidic flow channels and provides excellent data suitable for driving medicinal chemistry optimisation of compound activity. PatchXpress® 16 channel giga-seal quality recording and conventional electrophysiology techniques are also used for detailed follow-up/mechanistic studies. Combining the BioFocus diverse collection of 900,000 compounds with the available in-house expertise and APC technologies provides client programmes with a wealth of strategic options for hit finding and hit expansion and provides a rapid route into medicinal chemistry optimisation (Figure 12).

B'SYS (www.bsyst.ch) is a GLP-accredited CRO focusing on functional ion channel screening using automated and manual patch-clamp, fluorescence techniques and assay development including cell line design. Quality control systems apply to all assays in both GLP and non-GLP studies. B'SYS operates a single facility which houses scientists, technical platforms and administration under a single roof, resulting in short ways and fast and qualified responses. Expansion over the past few years has served mainly to satisfy the quality requirement of clients that left academia-related testing labs and to clients that rely on fast turnaround of studies in an auditable CRO. During the past few years B'SYS observed an increasing need of high quality ion channel screenings. For the classical cardiac screening, automated patch clamp systems such as QPatch are available that provide data quality almost comparable with manual patch-clamping. Screening of validated CNS targets requires sophisticated assays: voltage-gated channels often have to be tested on use dependence or in different voltage dependent states. Strict control of high seal resistance and low serial resistance are crucial to record reliable data. Testing ligand-gated channels like GABA, Gluamate or Acetylcholine receptors requires precise, fast and reproducible drug application systems to avoid desensitisation and to provide robust data. Finally, critical data analysis is essential. Following these requirements, B'SYS broadened its library of ion targets, including validated mammalian and

human stem cells which are now increasingly being used. Services at B'SYS include the rapid development of high-expression cell lines and include fluorescence activated cell sorting that represents an ideal method of clone selection (Figure 13).

High throughput screening of voltage-gated ion channels has been performed for close to a decade now and is an established tool in drug discovery. Ligand-gated ion channels have proved to be more difficult to incorporate into a high throughput screening model based on automated electrophysiology. A major limitation so far has been the need to rapidly apply compounds to the cells while continuously measuring the signal. This is essential for being able to resolve rapidly activating and desensitising ion channel currents such as those produced by potential drug targets such as GABA_A and nAChR. Furthermore, the compound application must be performed in such a way that recording duration and success rate is maximised. Using a microfluidic patch clamp technology, the Dynaflow®HT from Celectricon (www.celetricon.com) offers a low-cost and effective method to screen large number of compounds against both ligand- and voltage-gated targets. The microfluidic technology makes it possible to apply and remove compound very rapidly (low ms range) while not exposing the cells to strong forces that would disrupt the cell and terminate the recording. Before applying compound the chip is rinsed of non-trapped cells to avoid compound loss due to binding to non-recordable cells. All cell handling is performed online to proven optimal cell conditions. The Dynaflow HT offers up to eight hours walk away time and provides excellent data quality and assay stability making it optimal for high throughput automated electrophysiology work (Figure 14).

ChanTest (www.chantest.com) is a CRO specialising in ion channels and their role in drug discovery and safety. Improvements in automated electrophysiology have made ion channels more accessible as drug targets. At ChanTest, all major voltage- and ligand-gated ion channels are expressed in cell lines as replication-competent or division-arrested. Cell lines are validated on a large array of manual and automated patch clamp instruments and high throughput automated fluorescence instruments. HTS at between 3,000 data points per day (dps/d) and 10,000 dps/d or greater has been achieved with automated megaohm seal patch clamping or automated fluorescence readouts of membrane potential, calcium ion flux or potassium ion flux. Profiling and QSAR are done



Figure 14: Image showing a cell trapping zone in the microfluidic patch clamp plate utilised in Celectricon's Dynaflow®HT system

on these instruments or with automated gigaohm seal patch clamping at throughputs between 100 and 300 dps/d. State of the art protocols are adapted for each type of instrument keeping in mind the differences in data quality between megaohm and gigaohm patch clamping and the absence of voltage control using fluorescence



Figure 15
Ion channel testing at ChanTest is undertaken using a wide variety of manual and automated patch clamp instruments and high throughput automated fluorescence imagers

Screening

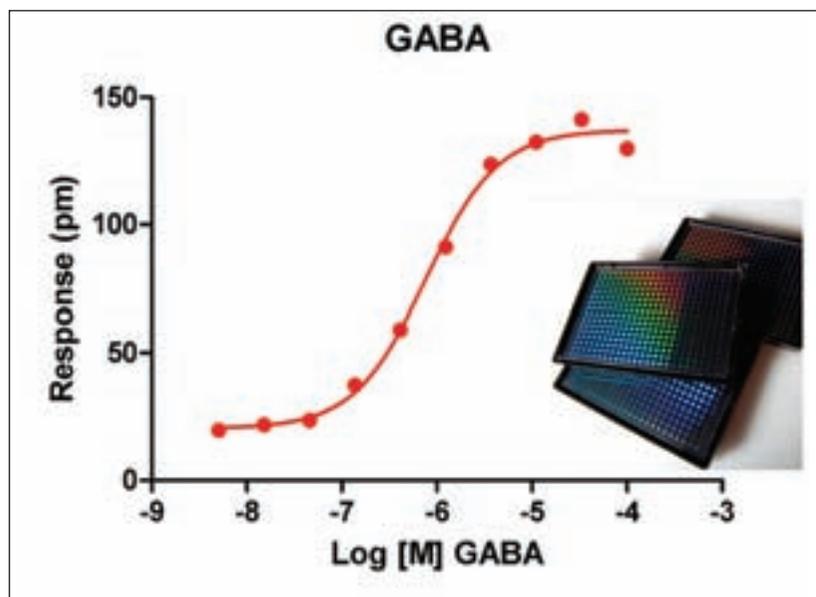


Figure 16: GABA_A channel activity in primary cortical neurons detected by Corning's Epic® Label-free Technology

readouts. The protocols identify conformation-dependence of drug effects on voltage- and ligand-gated ion channels and can discriminate subtle differences in on- and off-target efficacy and safety. The hERG assay was a milestone delineating the importance of off-target effects on ion channels, since then cardiac channel profiling and trafficking have been introduced. More recently, the elec-

trophysiology of stem cell-derived human cardiomyocytes (SCHCMs) and its usefulness in drug discovery and safety has been described. Extension of stem cell electrophysiology to other excitable tissues should greatly benefit the drug discovery effort (Figure 15).

Corning Label-free Epic® Technology (www.corning.com/epic/) monitors the translocation of cellular mass upon the activation of a target protein by using resonant waveguide sensors integrated into the bottom of 384-well microplates. This label-free detection technique is referred to as dynamic mass redistribution (DMR). The DMR signal is a novel quantifiable measurement and highly specific method of investigating GPCR targets. In recent years, the DMR methodology has been applied to ion channel targets with significant success for both recombinant systems (eg TRPV1-CHO, TRPA1-HEK293, P2RX1-CHO) and endogenous models (eg GABA_A channel in primary cortical neurons and CRAC channel in HEK293 cells). Unlike traditional electrophysiological methods which focus on transportation of ions across the cell membrane, Epic ion channel assays focus on the translocation of large cellular molecules associated with ion channel activity. Epic's DMR response profiles could provide additional information about the functionality of ion channels and their activity modulators. For instance, research has demonstrated that DMR ion channel profiles were not only indicative of the specific activation and phosphorylation of ligand-gated ion channel Slack-B, but also provided more insight into the interaction between the ion channel and its regulatory proteins⁴. Epic's ability to detect more than the movements of ions across the cell membrane is helping researchers validate hypotheses about ion channels' link to cell function and diseases. Label-free Epic Technology is available in a high-throughput screening platform from Corning and also Perkin Elmer's Enspire® multi-mode benchtop unit. Corning also offers services including target screening, compound profiling and assay development (Figure 16).

Figure 17
Cytocentrics CytoPatch™
Professional system for ultra-high data quality in automated patch clamping



CytoPatch™ technology from Cytocentrics (www.cytocentrics.com) offers a new dimension in data quality in automated patch clamping. Both instruments, the CytoPatch™ Academic and the CytoPatch™ Professional, meet the demands of a flexible device with highest data quality. Moreover, the CytoPatch™ Professional is fully automated and suited for high throughput with several hours walk away time. The key to the high data quality is

Screening



Figure 18: EMD Millipore's Centre of Excellence for Cell Based Assays

the novel cytocentring chip technology that differs from the present patch clamp automation approaches. With an additional suction channel the cell is positioned on to the patch pipette first, the sealing process starts afterwards. The separation of the two process steps allows keeping the patch pipette clean by positive pressure. Giga-seals can be accomplished easily using CytoPatch™ technology. Seal-forcing chemicals, as Fluoride in the intracellular solution, can be omitted. Another feature contributing to high data quality is the permanent perfusion system. Two sophisticated liquid-handling systems enable a non-stop perfusion of the cell with either buffer or compound for several minutes. In addition, with an ultra-rapid and precisely triggered

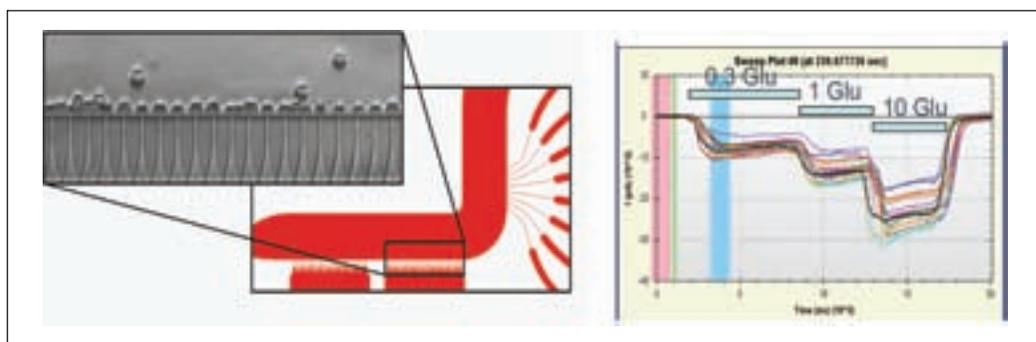
agonist application of <10ms, the proper activation of fast desensitising ligand gated ion channels is possible. A comparative study, demonstrating the high data quality of the CytoPatch™ technology, was recently published in collaboration with Bayer Schering Pharma AG⁵. An additional software package manages the study set-up as well as the automated data analysis and allows a fast access of the raw data for analysis. Furthermore, the CytoPatch™ instrument is GLP compliant and features the possibility to withdraw compound after cell perfusion for HPLC analysis (Figure 17).

EMD Millipore (www.millipore.com/LeadDiscovery) is the Life Science division of Merck KG&A, Germany, supporting research, development and production of biotech and pharmaceutical solutions. Its extensive range of recombinant PrecsION® ion channel cell lines expressing a variety of biologically relevant targets are ideal for both manual and automated patch clamp systems. These purpose-built ion channel cell lines underpin a broad portfolio of high quality and well validated screening and profiling assays, including CardiacProfiler™ and IonChannelProfiler™ services. Its service capabilities combine the range of ion channel cell lines with FLIPR and both manual and automated electrophysiology platforms. Whether profiling several compounds across a range of ion channels or screening thousands of compounds against a single channel, EMD's product and service portfolio can provide the perfect complement to in-house programmes and meet requirements for turnaround, cost, data and quality (Figure 18).

Essen Bioscience (www.essenbioscience.com) is a privately held company specialising in cell-based assays. As inventors of two paradigm shifting ion channel assay technologies, IonWorks and FLIPR (both acquired by Molecular Devices), it has a deep understanding of the biological and technical requirements for constructing relevant and translational ion channel assays. Its Discovery Services contract research division integrates cutting edge molecular biology, gene expression, electrophysiology (automated, manual patch clamp) and fluorescence (eg Ca²⁺, Tl⁺ detection) methods to configure custom reagents, assays and integrated *in vitro* workflows for its clients. Managed and staffed by experienced ion channel drug discoverers, and with laboratories both in the US and UK, this provides a unique ion channel problem solving resource. Essen's specialty is automated electrophysiology which it has been doing since 2002 with the manufacture and release of the first seven commercial



Figure 19: Essen BioScience has two laboratory facilities (Ann Arbor, MI USA and Welwyn Garden City, UK). Our Ann Arbor facility is supported by three IonWorks systems, including the very first instrument (Serial #1) developed by Essen in the left-foreground of the photo

**Figure 20**

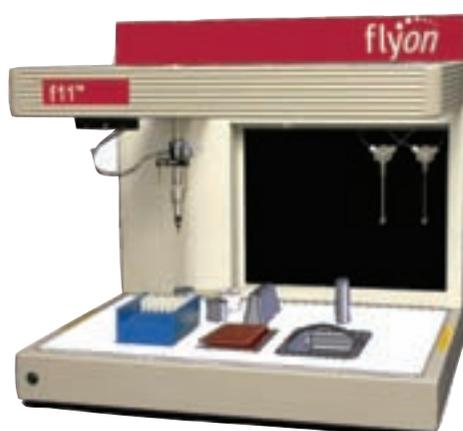
Schematic of the microfluidics for one experimental pattern of Fluxion's IonFlux system. A main channel intersects trapping regions containing intracellular solution that capture cells at the ensemble recording array. All compound channels converge just upstream of the 20-cell recording array. Inset left: Micrograph of an ensemble recording array composed of 20 microfluidic channels occupied by captured cells. Right: A cumulative dose response consisting of four compound applications (three agonist concentration and wash buffer) injected across the entire consumable (64 recording channels, one quadrant shown) to obtain 384 responses to compound additions in a space of 20s

IonWorks systems. With five IonWorks platforms worldwide, it has the capacity to conduct large electrophysiology screens (up to 50K samples) with high quality and fast turnaround for both voltage and ligand-gated targets. In parallel, Essen can support multiple SAR and safety profiling campaigns as well as high throughput mechanistic analyses. Our higher throughput approach couples the precision and relevance of translational electrophysiology protocols with an affordability that permits early and wider scale testing (eg for cardiac safety assays such as hERG and hNaV1.5). Essen has also adopted a multitude of business relationships to meet the needs of its clients ranging from fee-for-service to collaborations involving structured milestone payments (Figure 19).

Fluxion Bioscience's (www.fluxion.com) IonFlux system applies well plate microfluidics technology to ion channel screening. This next generation automated patch clamp instrument is the only screening system to include continuous compound perfusion and continuous recording during the entire protocol. This combination enables superior assay flexibility and key advantages for ligand-gated screens, where throughput is increased by an order of magnitude over other automated systems. The use of interconnected microfluidic channels that are coupled to a pneumatic interface removes the need for liquid handling; the unit operates much like a plate reader and can be used either stand-alone or coupled to standard liquid handlers where more throughput is required. Another unique feature is the available heating module, which will be improved by the upcoming introduction of a chiller plate which can also lower recording temperatures to 4°C. The modular nature of the instrument allows for multiple readers attached to the same liquid handling robot to scale up assay throughput easily. Recent customer data from NMDA and Ach receptors highlights the advantages of simultaneous compound application and efficient washout, achieving very high (>95%)

success rates for these difficult ligand-gated ion channel targets. While the Fluxion's current microfluidic consumable plate records currents from 20-cell ensembles, a single-cell recording plate, called the F1, will be introduced in Q4 2011. The F1 plate includes gigaohm seal resistance capability, and will bolster the effectiveness of the IonFlux for characterising heterogeneous cell populations and other applications requiring single-cell recordings or gigaohm seals. The F1 single-cell recording plate is fully compatible with existing IonFlux systems (Figure 20).

In June 2011 flyion (www.flyion.com) released its f11™ series of automated patch clamp instruments which will succeed all previously offered products. The f11 has been co-developed with a medical devices manufacturer in Switzerland. It is assembled in an ISO 13485 certified production environment as a GLP compliant device. The f11 is based on the patented Flip-the-Tip™ technology which performs the patch clamp experiment inside a regular glass pipette. Instead of pressing a micropipette against a cell membrane and applying suction to form a high resistance seal between the glass and the cell membrane, a cell suspension is infused into the micropipette and suction is applied from the aperture until the cell seals the pipette from the inside.

**Figure 21**
flyion's f11™ – the only commercially available automated patch clamp instrument using glass pipettes

Screening

The electrical resistance is as high as in the conventional manual method. The addition of compound solution is accomplished through a fine quartz capillary inside the micropipette. The autofocusing of solution application on to the cell surface allows extremely rapid solution exchange (<10ms). Small volumes (~1µL) enable experiments with only ~10 cells per measurement for very reliable pharmacological data. Ion channel screening is performed with high and stable success rates using purely physiological solutions without addition of Fluoride. The f11 is available with 1-6 measurement chambers. Temperature control and intracellular perfusion are available options. By moving to an industrial production environment, a single channel f11 is available for less than \$100,000 and the consumable pipettes cost less than \$2. flyion also offers ion channel screening, assay development and cell line development services (Figure 21).

Human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) could prove to be an unlimited source of cardiomyocytes. Stem cell tech-

nology offers great potential for toxicology studies in drug discovery. Cardiomyocytes derived from primate ESCs /iPSCs responded to and predicted side-effects of drugs which have been reported for QT prolongation *in vivo*. The Hamamatsu FDSS/uCELL (www.sales.hamamatsu.com), equipped with the high speed and high sensitivity camera, enables the detection of Ca²⁺ and membrane potential from human ESCs /iPSCs-derived cardiomyocytes in a 96-well plate format. The Ca²⁺ oscillations originate from the sarcoplasmic reticulum and are dependent on the IP₃ and the ryanodine receptor. The Ca²⁺ oscillations activate the Na⁺-Ca²⁺ exchanger, giving rise to subthreshold depolarisations of the membrane potential and/or action potentials. Using 3µM Fluo-8AM, the FDSS/uCELL measured the stable and uniform oscillations of the cardiomyocytes, 100-200µm wide in single clamp, and demonstrated the drastic reduction of oscillations by adding 10µM channel blocker (TTX) with the simultaneous dispensing head of the FDSS/uCELL. Having the correlation with the standard electrophysiology assay, the cell-based assay is

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Automated patch clamp system

Ready for the most complex assays...

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At a price that meets your budget.

The IonFlux System is the only automated patch clamp platform that offers high capacity, continuous perfusion, benchtop format, temperature control, and cost efficiency.

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getting commonly used for the drug safety pharmacology and applying the ESCs /iPSCs is a consequent trend. The newly developed FDSS/uCELL is a compact, simple to use, affordable system, yet equipped with the simultaneous dispensing head, active tip wash and high speed and high sensitivity camera. The Hamamatsu FDSS/uCELL and the assay system are suitable for miniaturisation to save both time and cost from early to late stage drug discovery (Figure 22).

Librede (www.librede.com) is developing a platform for cell-free electrophysiological ion channel screening. Librede's technology measures ion channels in artificial membranes made from solutions derived directly from simply processed source cells, eliminating the need for cell incubation and handling by the end user. Electrophysiological measurements of ion channels are comparable to patch clamp and automated patch clamp but with 10-100 GΩ seal resistance and high yield. In addition to ion channels obtained from common cell membranes (eg HEK, CHO), measurement of ion chan-

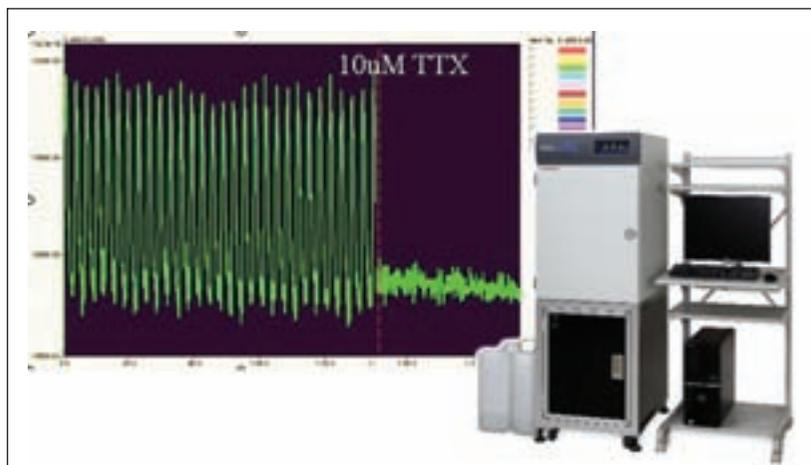


Figure 22: Ca²⁺ oscillations in cardiomyocytes detected by Hamamatsu's FDSS/uCELL

nels from difficult to patch primary cells or sub-cellular organelles is straightforward. Ion channels are measured in inexpensive consumable array plates that allow simultaneous optical and electrical measurement. The array plates (Figure 23) are

New Cell-based Assay Plate Reader, FDSS/μCELL



The **FDSS/μCELL** is a new compact and cost-effective plate reader for fluorescent kinetic cell-based assays.

Features

- 96/384 well dispensing
- All wells dispensed simultaneously
- Fast data acquisition - uses Hamamatsu CCD camera technology
- Whole plate imaging
- Agonist/Antagonist assays with tip washing
- Compact
- Simple to use

Applications

- Intracellular ion measurement (Ca²⁺, fluo-3/-4)
- Membrane potential (FMP)

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Screening

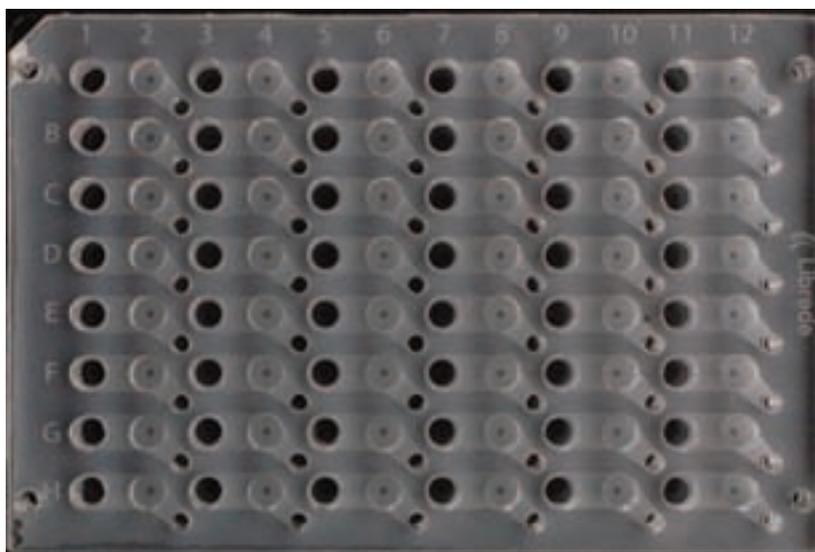


Figure 23: Librede's array plate used for cell-free electrophysiological ion channel screening

compatible with SBS standard 96- and 384-well formats, with 1536-well format in development. The number of measured ion channels can be controlled as desired to obtain single channel currents for detailed biophysical studies or the measurement of thousands of channels, equivalent to whole cell patch clamp for drug screening. Measurement solutions may be easily exchanged during experimentation, reducing screening time and cost while increasing throughput. Librede's platform has been validated with a number of mammalian ion channels including hERG with measured IC_{50}/EC_{50} matching published values (in

review for publication). In addition to ion channel solutions and consumable array plates, Librede will offer a benchtop workstation for scientific studies and an automated parallel instrument for increased throughput.

Molecular Devices (www.moleculardevices.com) offers instrumentation for ion channel research ranging from fully automated high throughput screening systems to amplifiers for traditional low-throughput manual patch clamp setups. IonWorks instruments are typically found in laboratories requiring the screening of large compound libraries. They have historically offered the highest throughput and lowest running costs of commercially available systems. The IonWorks Barracuda system, released in 2010, screens both voltage- and ligand-gated channels and generates a throughput of 1,100 and >6,000 data points per hour for screening and compound profiling, respectively. The IonWorks Quattro system, released in 2005, introduced the Population Patch Clamp (PPC) recording technique to ion channel screening. This breakthrough technology boosted the throughput of IonWorks systems four-fold and allowed for the first time ion channel screening of large libraries up to several hundred thousand compounds. PPC well-to-well data consistency allowed the generation of novel assays which resulted in multiple peer-reviewed publications. The PatchXpress system also offered by Molecular Devices is a 16-channel automated patch clamp system with data quality identically comparable to traditional patch-clamp set-ups. Released in 2003 it has proven to be a workhorse in many of our large pharmaceutical, biotech and CRO customer's labs. We also offer Axon CNS amplifiers that are used on more traditional patch clamp set-ups than any other brand. Overall Molecular Devices has the largest presence of instrumentation for ion channel research ranging from screening and safety laboratories to academic research (Figure 24).

Figure 24
Molecular Devices' IonWorks Barracuda instrument is a high-throughput screening system for the measurement of ligand- or voltage-gated ion channels



Nanon Technologies (www.nanion.de) is a provider of automated patch clamp platforms supporting high quality electrophysiology recordings. Starting as a spin-off from the Center of Nanoscience, LMU in Munich, Germany, Nanion has grown into a profitable company with branches in the USA and China. The Port-a-Patch – “the world's smallest patch clamp rig” – was launched in 2003 and is used in more than 200 research laboratories around the globe. It reduces an entire patch clamp set up to something you can hold in your hand, however still providing high quality, giga-seal recordings. The Patchliner followed in

Screening

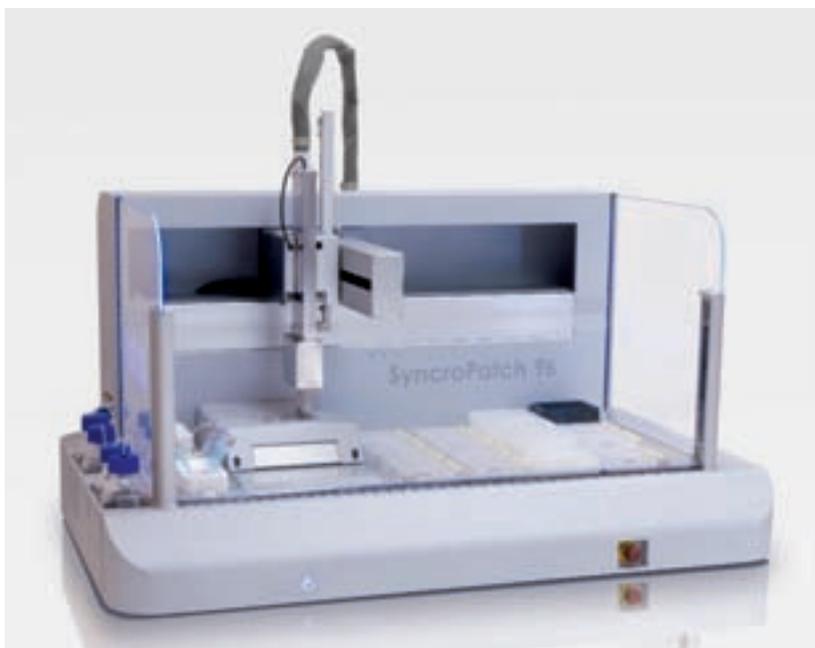


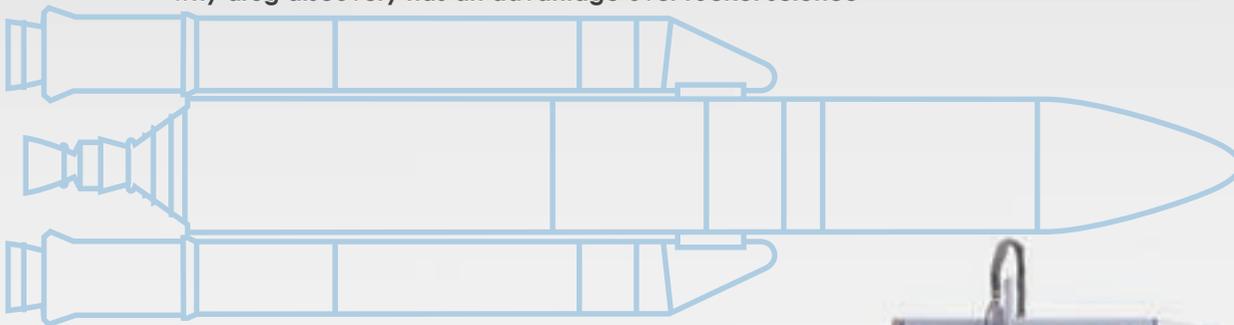
Figure 25: Nanion's SyncroPatch 96 ion channel screening platform records from 96 cells in parallel

2006, offering full automation of patch clamp recordings from eight cells in parallel. Both platforms support versatile functions such as temperature control, current clamp recordings, fast perfusion and automated internal solution exchange. The Patchliner has shown great compatibility with primary cells, as described in high rank journals such as Nature and Nature Protocols^{7,8}. Currently, the Patchliner receives much attention because of the possibilities to run automated current clamp recordings, for example for investigating compound effects on action potentials from cardiac cells. Nanion's screening flagship, the SyncroPatch 96, was launched in the beginning of 2010 and was quickly adopted by the pharmaceutical industry. Like all other Nanion products, the SyncroPatch 96 supports giga-seal recordings, however with a drastically increased throughput: up to 9,000 data points can be collected per day. The SyncroPatch 96 records from 96 cells in parallel and has been validated with both voltage- and advanced ligand-gated ion channels such as nicotinic acetyl choline receptors ($\alpha 7$) and TRPA1. Set-up and running the

Task defines tool

– why drug discovery has an advantage over rocket science

nan|i[on



Rocket science basics:

- How far do you want to get?
- Build a powerful, lightning fast rocket that fits your task
- Discover!

Drug discovery basics:

- How far do you want to get?
- The **SyncroPatch® 96** is made for your mission – just use it
- Discover!



Supercharge your **ion channel drug discovery** project: the **SyncroPatch® 96** is the most powerful, lightning fast automated patch clamp platform you can get on this planet.

experiment is straightforward using a graphical user interface. Data analysis is finished within seconds, also with new cursors and analysis settings, yielding a data output format so flexible that it can be transferred to any existing database (Figure 25).

Sophion Bioscience's (www.sophion.com) entry into the APC market with the QPatch 16 in 2004 gave a major boost to the field of ion channel research. In 2009 Sophion launched the multi-hole technology for the QPatch. The multi-hole technology makes experiments with low-conductance or transiently transfected ion channels more efficient. It provides nearly 100% success rate and hence the highest possible throughput and the lowest possible price per data point. Sophion provides three QPatch models, QPatch 8, QPatch 16 and QPatch HT which mainly differ in their number of channels. The systems are applicable in all phases of drug discovery and on all types of ion channels. Sophion's strategic focus is data quality. Therefore all QPatch systems provide high-quality data based on true giga-seals and glass-coated compound pathways. A novel and patented 100% R-series compensation makes experiments on fast voltage-gated ion channels very accurate and adds to the quality focus. Sophion provides an upgrade pathway from lower to higher throughput systems. A focus area for QPatch has been to avoid the potential bottleneck of data analysis. The QPatch Assay Software provides automated data analysis. The software is very comprehensive and is updated one to two times per year based on input from Sophion's customers. In the very near future Sophion will launch an upgrade package to the QPatch that facilitates experiments based on stem cells and primary cells. This includes current clamp, different patch clamp hole sizes and ability to use fewer cells for the experiments. A 384-channel high throughput automated patch clamp system providing very high-quality data based on true giga-seals is also around the corner (Figure 26).

Discussion

The products and services offered by the vendors reported in this review are detailed in Table 2. The new developments presented in the vendor snapshots can be broadly summarised under the following categories:

Cells: Most service providers cited (BioFocus, B'SYS, ChanTest, Cytocentrics, Essen, EMD-Millipore) have access to all major voltage- and ligand-gated ion channels at high expression in a variety of cell lines or can offer custom cell line development. In addition, several instrument developers



Figure 26: Sophion's QPatch has been installed globally at pharmaceutical companies, CROs and academic research centres contributing to a major boost to the field of ion channel research

also offer cells and cell line development matched to their specific APC technology (Cytocentrics, fly-ion, Molecular Devices, Nanion and Sophion). Few currently offer primary or stem cells (eg human cardiomyocytes); although their usefulness in drug discovery and safety is under investigation by label-free (Corning) and with APC instruments and protocols being modified by system developers (Nanion, Sophion) and service providers to support them (B'SYS, ChanTest). Oscillating cardiomyocytes derived from human ESCs/iPSCs were reported to have been investigated for Ca²⁺ and membrane potential in a 96-well plate fluorescent dispense and read assay format (Hamamatsu).

Megaohm and gigaohm patch clamping: Increasingly service offerings are combining fluorescence imaging with both manual patch clamping and several different APC platforms from multiple vendors. These support both megaohm (population patch clamp or multi-hole devices) and gigaohm patch clamping (single cell recordings), at varying throughputs (BioFocus, B'SYS, ChanTest, EMD-Millipore, Essen). There appears to be an increasing demand for high quality ion channel screening, particularly for detailed follow-up/mechanistic studies. In addition to first-generation APC systems such as the PatchExpress (Molecular Devices), this is now being addressed by new third-generation platforms that offer true giga-seal recordings against single cells at drastically increased throughputs with 96 records

Screening

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Table 2: Ion channel products or services offered by vendors reported

VENDOR/ PROVIDER	ION CHANNEL PRODUCTS OR SERVICES					
	Fluorescent Imaging Instruments	Manual Patch Clamp Instruments	Automated Patch Clamp Instruments	Other Instruments For Ion Channel Testing	Contract Screening Services	Cells and Cell Line Development
BioFocus					✓	✓
B'SYS					✓	✓
Cellectricon			✓			
ChanTest					✓	✓
Corning				✓	✓	
Cytocentrics			✓		✓	✓
EMD Millipore					✓	✓
Essen Biosciences	✓				✓	✓
Fluxion Biosciences			✓			
Flyion			✓		✓	✓
Hamamatsu	✓					
Librede				✓		
Molecular Devices	✓	✓	✓			✓
Nanion Technologies			✓			✓
Sophion Biosciences			✓			✓

read is parallel (Nanion); or in other flexible new platforms supporting ligand-gated testing at gigaohm seal resistance (Cytocentrics, Fluxion, Flyion).

Microfluidics and rapid solution exchange: The inclusion of microfluidic technology into APC devices has enabled continuous compound perfusion and continuous recording during the entire protocol (Fluxion). Microfluidic technology also makes it possible to apply and remove compound very rapidly (low ms range) while not exposing the cells to strong forces that would disrupt the cell and terminate the recording (Cellectricon, Sophion). Other instruments achieve extremely rapid solution exchange (<10ms) by using a permanent perfusion and sophisticated liquid-handling systems enabling ultra-rapid and precisely triggered agonist application (Cytocentrics); or use a fine quartz capillary inside the micropipette to deliver the compound solution directly on to the cell surface (flyion).

Other technologies and approaches: Response profiles focused on the translocation of large cellular molecules associated with ion channel activity monitored by label-free can provide additional information about the functionality of ion channels and their activity modulators (Corning). Cell-free electrophysiological screening of ion channels in mem-

branes derived directly from cells of interest have also been described (Librede). New methods enabling 100% R-series compensation make experiments on fast voltage-gated ion channels more accurate and add to the quality focus (Sophion).

In conclusion, the availability of a rapidly evolving range of APC systems over the past decade and their widespread global adoption by pharmaceutical companies, CROs and academic research centres has revolutionised ion channel screening. Although for the present fluorescence imaging assays is still preferred in primary screening, it seems only a matter of time before APC is fully adopted as an alternative to manual patch clamp for compliant hERG. **DDW**

Dr John Comley is Managing Director of HTStec Limited an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation and assay reagent technologies) to drug discovery and the life sciences. Since its formation eight years ago, HTStec has published more than 70 market reports on enabling technologies and Dr Comley has authored more than 35 review articles in Drug Discovery World. Please contact info@htstec.com for more information about HTStec reports.