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Patch clamping by numbers

Claire Wood, Christine Williams and Gareth J. Waldron

Many ion channels are recognized as amenable targets for a range of disease states and conditions. However, the process of discovering drugs is highly influenced by the chemical doability, the biological confidence in rationale of the approach and the 'screenability'. To date, the absence of informative high throughput technologies for ion channel screening has resulted in ion channels remaining a largely unexplored class of drug targets. This, however, is about to change – a large increase in the number of data points per day should be achieved by the introduction of automated 'high throughput' patch clamp machines.

Claire Wood
Gareth J. Waldron*
Ion Channel Pharmacology Group
Pfizer Global Research and Development
Sandwich, UK, CT13 9NJ
Christine Williams
Gareth J. Waldron
Hit Discovery Group
Pfizer Global Research and Development
Sandwich, UK, CT13 9NJ
*e-mail: gareth_waldron@sandwich.pfizer.com

▼ In 1998, in an article in *Drug Discovery Today*, Jane Denyer and colleagues from GlaxoWellcome reviewed the technologies then available for screening ion channels as drug targets [1]. In the five years since, the human genome has been sequenced, compound libraries have burgeoned, HTS groups have moved from screening in 96-well plates to 1536 and at least eight drugs have been withdrawn from sale because of ion channel interactions. And yet, the techniques described by Denyer *et al.* [1] have remained largely the same. Indeed, the mainstay of ion channel research, the patch-clamp technique, has not undergone a major revolution since it was described in 1981 [2]. The advent of the next wave of ion channel technologies has been long awaited and anticipated [3–5] until finally, last year, the first commercial parallel patch machine rolled off the assembly line.

Rationale for screening against ion channels

Ion channels are membrane-bound proteins that allow the passage of ions across otherwise impermeable cell membranes. Their most obvious role is to underlie action potentials of neurones and cardiac muscle but they have other wide ranging functions in, for example, cell volume control, growth and differentiation of cells and neurotransmitter release. They can be structurally categorised into two major families – 'ligand-gated' and 'P-loop' (voltage-gated) channels [6,7]; (see http://www.pasteur.fr/recherche/banques/LGIC/LGIC.html and http://www.iuphar-db.org/iuphar-ic/). These superfamilies consist of ~61 and 123 human pore-forming subunits, respectively, with both homomers and heteromers forming different functional units. This, coupled with the fact that accessory subunits and splice variants are common, implies that the potential for ion channel diversity is huge. Despite this, only a few ion channel targets are represented in the current ion channel pharmaceutical market. In 2000, the top 100 best selling drugs acted on 42 different molecular targets, however only three of these 42 targets were ion channels (L-type Ca^{2+} channels, e.g. amlodipine for hypertension; $GABA_A$ receptor modulators, e.g. benzodiazepines as anticonvulsants and anaesthetics; sulphonylureas for diabetes), albeit with sales in the order of US$7 billion [8]. One of the reasons for the paucity of successful ion channel targets is that, although their activity can be modulated by small molecule therapeutics (indeed they are the third most frequent biochemical class of targets for marketed small molecules behind enzymes and G-protein coupled receptors [9]), it is technically challenging to screen large numbers of compounds against them in a meaningful manner. In an environment where the number of druggable targets is much fewer than imagined just a few years ago, Pharma needs to maximize the potential of these precedented targets and efficiently exploit new targets, in order to reverse the year on year decline in new drug applications [10].

In addition to being drug targets, the profile of ion channels in the pharmaceutical arena has been increased over recent years because of the discovery of the h-*erg* channel [11] and its underlying role in Torsade de Pointes. h-*erg* is a K^+ channel that contributes to the repolarization of the cardiac action potential and is the target of certain drugs for

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atrial fibrillation (e.g. dofetilide). However, some prescription drugs have also been shown to interact with h-*erg* to prolong the QT interval of the cardiac action potential, cause Torsade de Pointes and sudden death. Although there is no conclusive evidence that block of h-*erg* inevitably leads to Torsade de Pointes, there is a strong correlation. Thus, a change in the length of the cardiac action potential is now a worry for regulatory bodies, and thus the preclinical testing of candidate molecules against cardiac ion channels, and especially h-*erg*, is now an important part of safety screening [12].

Current methodology for screening for ion channels

The current method of choice for obtaining high-quality data of the functional effects of compounds at ion channels is the patch clamp technique [2,13], (see Figure 1). However, patch clamping is a technically challenging method with a throughput that crucially limits its applicability to drug discovery and safety testing. Many companies are therefore willing to compromise on data quality to increase throughput by using methodologies such as radioligand binding, fluorescence and ion flux measurements [3,5,14]. Although high in throughput, these techniques might not provide appropriate pharmacological values for many classes of ion channels and thus they lack the sensitivity of the patch clamp technique. For example, compounds acting on expressed h-*erg* channels (measured using change in membrane potential) might appear to be 10–1000-fold less potent [15] compared with the same compounds assayed by measuring ionic current (using voltage clamp techniques; see Box 1 for definitions of terms) as readout. This could represent a crucial discrepancy for prediction of the therapeutic index of a compound.

In some respects the availability of the patch clamp technique in recent years has been a double-edged sword. Because of its powerful temporal nature, resolution and sensitivity (millisecond measurements of functional activity with exquisite precision are routinely made) more is known about the differing functional microstates that ion channel proteins can exist in than any other class of protein. Thus, although all proteins exhibit secondary structure motions on this timescale, only with ion channels has it been measured on a routine basis (although some fluorescent techniques for GPCRs are approaching these standards; Ref. [16]). Knowledge of the functional effects of these microstates (grossly categorised as 'open', 'closed' and 'inactivated') has helped characterize ion channel modulating compounds according to the sub-state to which they preferentially bind. This sub-state binding can account for the molecular and tissue selectivity observed for some compounds. Thus, the bar has been set higher in many respects for ion channel drug discovery programmes with respect to other targets. For these reasons the development of a high-throughput patch clamp technology has become crucial to the successful exploitation of ion channels as drug targets.

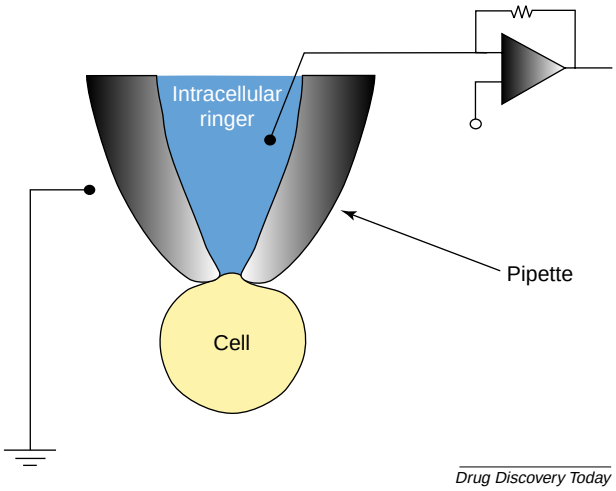

Figure 1. Patch clamp technique. Patch clamp electrophysiology is extensively used to investigate drug effects on the biophysical properties of ion channels. A glass micropipette with a tip diameter of ~1 μm is moved onto the cell to form a high-resistance seal with the cell. The micropipette is manually controlled, on an inverted microscope, by a three-axis micromanipulator to contact (usually) a single cell. Suction is applied and a high-resistance seal ('gigaohm' = $10^9\Omega$) is obtained between the pipette and cell. The membrane underlying the pipette aperture is then broken to enable the amplifier to control the voltage across the whole of the cell membrane. The nature and cleanliness of the glass used to fabricate the pipettes, and its geometry when pulled in heated filament, can have profound effects on the success rates of sealing. Different cell types often require different qualities of pipette, especially when isolated enzymatically from native tissue. Figure courtesy of Sophion Biosciences (http://www.sophion.dk), and reproduced, with permission, from Ref. [26].

New and emerging technologies

New automated approaches to ion channel screening are based on the voltage clamp technique rather than the fluorescence-based technologies that have been responsible for the increases in throughput for these and other classes of target in recent years. Voltage clamp techniques represent the 'Gold Standard' in this area, providing both real time and mechanistic information on ion channels. Using either two-electrode or whole cell voltage clamp, the technologies aim to increase throughput and automate the study of drug effects on ion channels.

An alternative to patch clamp for studying ion channels is the use of two-electrode voltage clamp (TEVC). This requires the use of two micropipettes to pierce the membrane

Box 1. Electrophysiological glossary

Voltage clamp: Form of patch clamp where the cellular transmembrane potential is maintained by the amplifier at a set point and the current required to do so is measured. This current is analogous to the current flowing through the ion channels of the cell.

Whole-cell voltage clamp: Form of patch clamp where electrical access is gained to the whole of the cell (usually by disrupting the membrane underneath the pipette by suction), allowing the electrical activity of the whole cell to be measured.

Cell-attached (single-channel) voltage clamp: Form of patch clamp when the voltage of only a small part of the cell is controlled allowing current flowing through individual ion channels to be distinguished.

Seal resistance: The resistance (in Ohms) of the junction between the pipette (or substrate) and the cell. For high-fidelity recordings to be made, the seal resistance needs to be greater than 1GΩ so that the current 'escaping' between the pipette and cell does not dominate and overwhelm the cellular electrical activity.

Series resistance: The resistance observed when the pipette and cell are in series. Poor compensation can lead to errors between intended and actual voltage that is applied to the cell.

Capacitance compensation: Amplifier-controlled compensation for the capacitance spikes observed when voltage steps are applied to a cell. Poor capacitance compensation can lead to the lack of fidelity when measuring fast (millisecond) channels, e.g. Na^+ channels.

of single cells and hence is unfeasible for small, mammalian cells. *Xenopus* oocytes, however, are sufficiently large (~1 mm diameter) to be used for this technique and they present a relatively null background for the expression of ion channels. There are several marketed automated machines that pursue TEVC in a serial (e.g. Robocyte, MultiChannel Systems; http://www.multichannelsystems.com) or parallel manner (e.g. OpusXpress, Axon Instruments; http://www.axon.com/cs_OpusXpress.html) and several companies using these technologies within partnership arrangements [e.g. Scion Pharmaceuticals (http://www.scionpharma.com), Neurion Pharmaceuticals (http://www.neurionpharma.com)]. In addition, several pharma companies have designed in-house versions [17]. There remains, however, a general reluctance to use oocytes as representative systems for drug discovery because of their non-mammalian nature, the requirement for RNA or cDNA injection into each oocyte and the possibility that lipophilic molecules concentrate in the oocytes leading to differing IC_{50} values between TEVC and patched mammalian cells.

Early attempts to automate whole-cell mammalian patch clamping included the Apatchi 1 (Sophion Biosciences; http://www.sophion.dk). This system attempted to replace the human in a conventional patch clamp rig with computer-based image analysis and robotically controlled micromanipulators. However, this was a serial, not parallel, system and did not provide substantial improvement in throughput over conventional patch. With the need to increase patch clamp throughput acknowledged in the late 1990s, many companies and academics decided to revive the idea that the interface between an electrode and a cell need not be achieved by a glass pipette – an approach that had first been attempted in the 1970s [18]. One of the prerequisites for high fidelity recording is a high resistance ('giga-Ohm') seal between the pipette and cell (although *vide infra*). For this reason, much academic research has been applied into the composition and treatment of the glass from which the pipettes were made – many scientists thought that no other substance could replicate a glass pipette in this manner. However, it would be demonstrated that a planar substrate could be substituted in its place [19]. Thus, by bringing the cell to the substrate to form the seal rather than bringing the pipette to the cell, Planar Electrode Patch could be established. In this way, the physical process of patch clamp is 'de-skilled' and some of the time-consuming components of this assay are removed. Fred Sigworth's group at Yale began to fabricate PDMS ('Sylgard') substrates to replace the pipette and successfully recorded single ion channel current from *Xenopus* oocytes in cell-attached mode [20]. This technology was eventually shared with Axon Instruments. It is comparatively easy to obtain a good cell-attached seal in patch clamp using oocytes, but most electrophysiology is performed in mammalian cells in whole-cell mode. The paradigm shift from cell-attached mode in oocytes to whole-cell mammalian planar patch clamp was first published by Niels Fertig and colleagues at Nanion [21]. These new technologies are designed to overcome many of the hurdles associated with traditional whole-cell voltage clamp by dramatically reducing time and expertise required to perform the assay. Companies competing in this area include Flyion, Axon Instruments, Cytocentrics, Sophion, Nanion and Molecular Devices. Their approaches to higher throughput electrophysiology incorporate automated seal formation, voltage clamp, whole cell recordings, drug perfusion and data analysis and are briefly discussed below.

Planar patch machines

Supportive ion channel data are now available for several systems. Three of these machines, PatchXpress™ 7000A (Axon Instruments; http://www.axon.com), Flyscreen® 8500

(Flyion GmbH; http://www.flyion.com) and IonWorks HT (Molecular Devices; http://www.moleculardevices.com) are commercially available. Others, such as QPatch (Sophion Bioscience A/S), Cytopatch (Cytocentrics; http://wwwcytocentrics.com) and the NPC series (Nanion; http://www.nanion.com) are in β testing mode. Below is a brief description of the major players in the market and the reader is directed to the publications quoted for further details. A comparative table of the technologies has recently been published [22].

Ionworks HT – Molecular Devices

The Ionworks HT system uses a 48-channel amplifier to clamp eight differing regions of the 384-well patch plate at different points in time. The system aspirates from standard 96- and 384-well microplates with a 12-channel dispensing arm. This machine is aimed at the HTS end of the market and has the highest known throughput of all the current versions of machines.

Rather than using suction to break into the cells, this machine uses an antibiotic, amphotericin B, to permeabilise the membrane. This method of obtaining electrical access to a cell (referred to as 'perforated patch') helps to retain intracellular co-factors required for the modulation of some channels. However, as series resistance is not measured, or compensated for, in this machine, there exists the possibility that changes in access resistance over time can lead to variations in current amplitude and create errors in quantification of drug block. The observed median seal resistance is 100 MΩ, which in comparison with seals achieved with conventional whole-cell recordings of >1 GΩ, is low [23,24]. The HT system voltage clamps and records using a 48-format headstage which physically moves out of the way during compound addition, leaving the cells unclamped for parts of the experiment. As voltage clamp is not continuous, the conformation of the ion channels is unclear. This in turn might make the evaluation of state-dependent block problematic and the evaluation of quickly desensitising ligand-gated ion channels impossible. Nevertheless, despite these deviations from classical patch clamp, the machine has been able to reproduce appropriate pharmacological response for several channels and thus has challenged some of the dogma associated with patch clamp [23,24].

PatchXpress 7000A – Axon Instruments

The PatchXpress 7000A from Axon records whole-cell current from 16 individual chambers using planar electrode chips designed and manufactured by Aviva Bioscience (http://www.avivabio.com). The system has a medium throughput, which Axon estimates at ~2000 data points per day.

Whole-cell voltage clamp is achieved by negative pressure that is individually adjusted for each chamber to achieve GΩ seals. These tight seals permit low noise, stable and long-lasting whole cell recordings [25]. The system has incorporated continuous voltage control and measurement/compensation of series resistance to produce low noise, high-quality recordings comparable to that of conventional voltage clamp techniques. As a consequence, errors in the quantification of drug block are reduced. Importantly, PatchXpress software and pipetting robotics work together to apply test compounds only to cells that meet the criteria for a successful recording. In addition, there are no limitations to the number of protocols that can be run or limits to the number of drug additions performed. As a result, concentration response curves can be generated from a single cell to produce IC_{50} values for test compounds of interest. The PatchXpress 7000A fluidics allow automatic washout of test compounds with buffer, permitting the addition of a positive control to each cell. The system has clear advantages for testing of ion channels for cardiac safety by enabling quality control (QC) parameters to be monitored for each cell and the continuous recording of current data.

QPatch – Sophion

Sophion Biosciences has 16- and 48-well machines (the plate format is demonstrated in Figure 2) in development and estimates that these planar patch machines can achieve up to 1250 and 3500 data points per day, respectively. Appropriate pharmacology for voltage-gated K^+ channels, including h-*erg*, has been demonstrated [26]. Sophion recognizes that it is not first to market and thus is attempting to manufacture a fully integrated system that will be 21 CFR Part 11 compliant – a feature that is retrospectively being added to the first machines released.

Cytopatch – Cytocentrics

The Cytopatch approach is not a pure planar approach as the patching substrate is not flat but consists of an inverted quartz glass pipette protruding from a silicon dioxide aperture (see http://www.cytocentrics.com/technology/chip.html). The Cytopatch has a separate microfluidic channel to apply suction, thus attracting the cell to the aperture. In this manner there will be less cross-contamination between intracellular and extracellular fluids (which usually have differing composition). The Cytopatch is also one of the few machines to continually perfuse compound over the patch cell rather than exist in a static bath configuration [27].

NPC series – Nanion

Nanion uses borosilicate glass in its chips that, because of its capacitance qualities, gives superior quality recordings.

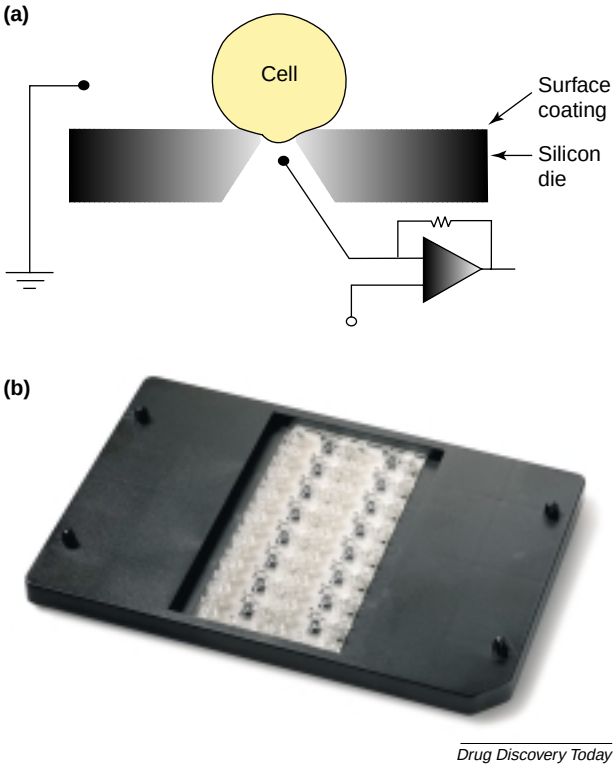

Figure 2. Planar patch clamp. **(a)** A hole is etched or micromachined into a silicon-based substrate. Suction brings one of the cells close to the hole and the gigaohm seal is made. This removes the need for an operator skilled in 3D manipulation to bring patch pipette in contact with a cell. The crucial ingredient is the characteristics of the substrate, which tends to be silica based. In many of the machines, suction and/or an electrical field attracts the cell to the aperture and most of the planar patch technologies are variations on a theme. For example, Cytocentrics has preferred to have the suction mechanism physically separate from the intracellular solution, thus miminizing contamination with extracellular fluid. **(b)** A patch plate from Sophion. Figure courtesy of Sophion Bioscience (http://www.sophion.dk).

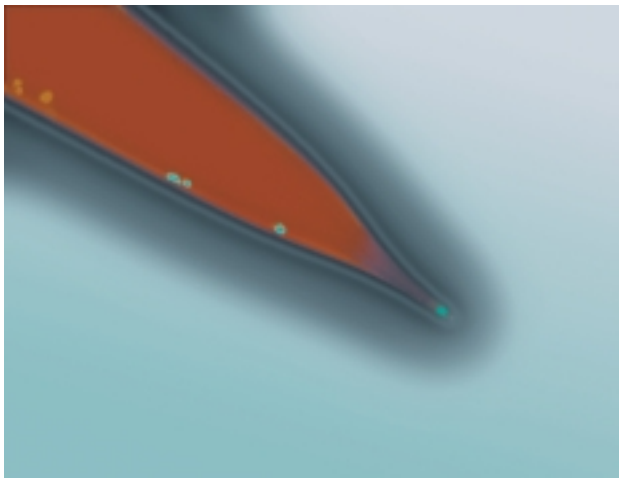

Figure 3. Visualization of GFP-expressing cells injected into the inside of one of Flyion's pipettes. Despite hundreds of cells being added to the pipette, only a single cell (lower right) is recorded from – the remainder adhere to the sides of the pipette. A fine needle dispenses compound in close proximity to the sealed cell. Figure courtesy of Flyion (http://www.flyion.com).

It has also demonstrated fast perfusion (<50 ms exchange time) and single channel recordings. [21,28,29]. A single-well version, the NPC-1 Port-a-Patch, will be available as a bench-top machine as well as a 16-well version.

Non-planar mechanisms of increasing throughput

Flyion has taken an innovative approach to de-skilling patch clamp. Flyion maintains the use of conventional glass pipettes, but injects the cells internally into the pipette – another example of this field challenging the dogma associated with patch clamp. One cell is sucked to the bottom and forms a high-resistance seal inside the pipette (Figure 3). The geometry of the cell and pipette makes for high-resistance seals, and the same pipette glass as used in conventional patch can be used. Compounds can be perfused into the pipette to replicate compound addition to the extracellular face of the membrane without displacement of the cell [30]. The Flyscreen 8500 is designed to be scalable with machines consisting of 1–6 recording positions available.

Formerly part of Cenes, Xention (http://www.xention.com) has produced a mechanism for 'blind' patch clamping. By inverting a conventional patch pipette and lowering a capillary tube containing a cell suspension on to it, this highly innovative technique relies on spontaneous formation of seals with cells positioned towards the bottom of the droplet. Because of an increase in cell density at the bottom of the droplet the chance of spontaneous seal formation with a patch pipette was increased and has thus been termed 'Interface patch clamping'. In its first incarnation the AP-1 showed no improvement in throughput over conventional patch, although it is possible that one operator could work two or three machines simultaneously. Further improvements in throughput of this technique have been proposed by the introduction of 48 pipettes in parallel.

Cellectricon (http://www.cellectricon.com) has developed a high-speed laminar flow microfluidic system that allows multiple applications to a conventionally patched single cell. Although this will be of use for quickly de-sensitising channels, many ion channels develop compound block over tens of seconds to minutes which limits the applicability of this technology.

How can these machines be best employed?

For these machines to be of the most use they need to replicate the pharmacology obtained in manual patch clamp. Several papers have reported positive correlations [23,24,26,29], although full evaluations are still ongoing and differences could occur. For example, most conventional patch data are obtained under flow conditions – the compound is continuously perfused over the cell. Many of the automated machines operate under no-flow conditions, the consequences of which have not been fully explored as yet. It should also be noted that the optimal cell culture and preparation for a given cell type will probably be different between automated and manual patch, which could potentially lead to differing pharmacology. In the worst case, a particular cell line might prove not to be amenable to automated patch.

Because this technology is a measurement of a single cell, as opposed to an average or sum of many thousands of cells in plate-based screening, one might have expected that the assay robustness would have been compromised, but because of the extremely high resolution of the patch clamp technique, and the fact that these are temporal recordings (thus each cell acts as its own internal control) Z′ values greater than 0.5 have been reported [22]. In this respect, automated patch clamp might be used for single point testing. There are drawbacks, however, that occur as a result of testing a single cell as opposed to averaging the signal from thousands of cells in conventional plate-based assays. For example, a seal in a particular well is not guaranteed and might fail during the application of compound as they are temporary in nature. Thus, the technique has less than a 100% success rate and scientists will need to alter their expectations compared with other screening technologies – 384 screened wells will not lead to 384 data points. The smarter machines monitor the state of the cell and the user can define parameters in the software that will allow the machine to QC wells – if criteria are not met then compound is not wasted by being added to a null well. The Molecular Devices Ionworks circumvents this by introducing redundancy into the system by applying each compound to four different wells. If the average success rate for forming appropriate seals is 75%, then the probability of obtaining at least two valid data points is in excess of 98% in these circumstances.

This reduction in ‘success rate’ also impacts on the cost. Many of the comparisons published so far have concentrated on cost per well, and not cost per usable data point. A comparison between the machines for real life throughput is absent from the literature at the present time – the numbers quoted are from the manufacturers, are probably best case and will be highly dependent on how the purchasers use the machines. Potential costs can be calculated – two examples are derived here from the systems that most is known about. Axon’s chips are list price of US$170 for 16 wells (although on a sliding scale with lower costs for higher volume usage). A success rate of 7/16 (44%) is extremely possible, leading to costs per data point of ~US$25. Molecular Devices’ chips are US$150 for 384 wells with fourfold redundancy, i.e. 96 compounds. With a similar success rate in an individual well, and given the desire to achieve at least two data points out of four for each compound, 60% of the 96 compounds would produce data leading to a per data-point cost of ~US$3. However, most of the machines support multiple dosing to a single well, which decreases the cost per data point.

Using conventional patch clamp, a single trained electrophysiologist is able to produce 0–10 points of data per day because of the constraints of cell viability, the sequential patch of cells and the temporal nature of many of the protocols. Thus, the introduction of automated planar patch could increase the rate of data acquisition 100-fold. These numbers of thousands of data points per day are not in the realm of HTS in which companies are screening at 100,000+ compounds per day. With fiscal restraints and assay technologies allowing conventional plate-based screening costs to drop to <10 cents well^{-1}, the probability of a company screening hundreds of thousands of compounds at a financial cost of US$3–25 per data point is minimal. These medium-throughput machines could, however, be used to support a Medicinal Chemistry program, targeted subset screening or as a higher throughput secondary or tertiary assay to some effect. Pharmaceutical companies would like to be able to consider screening ion channel targets as routine and with no more constraints than for any other protein family. With increases in the reliability (in terms of successful patches per attempt), major decreases in cost by large-scale manufacture and an increase in parallelization, patch clamp HTS would then be feasible [31]. At the other end of the scale, several companies are developing single-well versions of the larger parallel machines. These are stand-alone machines that often sit on a bench top, negating the need for a custom-built patch clamp rig which takes up space, requires expensive microscopes, anti-vibration tables and significant ongoing attention from their operators. These machines would facilitate the patch clamp study in areas in which throughput is not an issue, or where the cost of the consumables for the parallel machines is prohibitive. For the parallel machines a decision will need to be made by the companies using them. The full capacity of the machine could be used to generate much more data, it could increase the *n*-numbers on the preclinical compounds, or many more compounds

could be tested, although this comes at a high financial cost with the chips costing ~US$10 per point. Alternatively, managers might choose to maintain the current level of data acquisition and redeploy scientists towards other projects. Either way, it is a nice choice to be able to make.

Although in the literature planar patch techniques have been applied to many cell types, it is unclear as yet whether a single substrate will be able to fulfil the needs of a discovery department with diverse targets expressed in differing cell lines. Having a machine that is flexible and robust enough to facilitate seal formation on a variety of mammalian cells to include different clonal cell lines, primary cultures and isolated native cells such as cardiomyocytes might prove to be problematic, although Sophion has published data from several different cell types including CHO, HEK, MEL and Sf9 [26].

h-*erg* channel testing is not going to go away and *in vitro* testing for QT prolongation is becoming more common in the early stages of drug discovery. Once a reliable substrate for the planar patch is found for the particular type of cells a company has expressed its h-*erg* channels in, then this assay technology will revolutionize cardiac safety testing by allowing an increase in the throughput by two orders of magnitude. The increased throughput might allow companies to extend the number of channels that are tested in preclinical safety, such as the cardiac Na^+ channel, extend the number of compounds that are tested for cardiac safety, and/or develop greater understanding of the interplay between the complement of ion channels that contribute to the action potential to understand more of the pathophysiology of Long QT syndrome.

Because of the rate of acquisition of data during patch clamp (usually measured in kHz and lasting seconds, if not minutes) huge amounts of data are collected. This issue is not unique to electrophysiology and 'high content' screening using image analysis faces the same issue of storage capacity and data reduction. This is a factor that the manufacturers of the current machines are aware of and have partly addressed. Further streamlining of the analysis of this data in terms of automated measurement of user-defined parameters (onset or decay time constants are commonly altered by drugs) would be beneficial.

Conclusion and challenges ahead

In the short term, this new technology has the potential to do for ion channel screening what the microtitre plate did for *in vitro* screening. It can address crucial bottlenecks, particularly in secondary and safety assays. This extra capacity comes at a financial cost and the still modest throughput will severely restrict, if not prevent, the application of automated patch to full file HTS. The continued development of this, or alternative technologies for ion channel screening, remains highly attractive for HTS applications. There is no doubt, however, that in medium-low throughput applications, the full capacity of the machine is a welcome technological advance that significantly increases the feasibility and cost-effectiveness of programmes for the discovery of ion channel modulators.

While this article was in press, Molecular Devices announced that it has entered into a definitive agreement to acquire Axon Instruments

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References

1 Denyer, J. *et al.* (1998) HTS approaches to voltage-gated ion channel drug discovery. *Drug Discov. Today* 3, 323–332
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 Mattheakis, L.C. and Savchenko, A. (2001) Assay technologies for screening ion channel targets. *Curr. Opin. Drug Discov. Devel.* 4, 124–134
4 Worley, J. and Li, M. (2001) Location is key – recent progress in single-cell-based high-throughput assays. *Drug Discov. Today* 6, 454
5 Xu, J. *et al.* (2001) Ion-channel assay technologies: quo vadis? *Drug Discov. Today* 6, 1278–1287
6 Le Novère, N. and Changeux, J-P. (2001) LGICdb: the ligand-gated ion channel database. *Nucleic Acids Res.* 29, 294–295
7 Catterall, W.A. *et al.* (2003) International union of pharmacology: approaches to the nomenclature of voltage-gated ion channels. *Pharmacol. Rev.* 55, 573–574
8 Zambrowicz, B.P. and Sands, A.T. (2003) Knockouts model the 100 best-selling drugs – will they model the next 100? *Nat. Rev. Drug Discov.* 2, 38–51
9 Hopkins, A.L. and Groom, C.R. (2002) The druggable genome. *Nat. Rev. Drug Discov.* 1, 727–730
10 Frantz, S. and Smith, A. (2003) New drug approvals for 2002. *Nat. Rev. Drug Discov.* 2, 95–96
11 Curran, M.E. *et al.* (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* 80, 795–803
12 Fermini, B. and Fossa, A.A. (2003) The impact of drug-induced QT interval prolongation on drug discovery and development. *Nat. Rev. Drug Discov.* 2, 439–447
13 Neher, E. and Sakmann, B. (1976) Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature* 260, 799–802
14 Bennett, P.B. and Guthrie, H.R. (2003) Trends in ion channel drug discovery: advances in screening technologies. *Trends Biotechnol.* 21, 563–569
15 Tang, W. *et al.* (2001) Development and evaluation of high throughput functional assay methods for HERG potassium channel. *J. Biomol. Screening* 6, 325–331
16 Vilardaga, J.P. *et al.* (2003) Measurement of the millisecond activation switch of G protein-coupled receptors in living cells. *Nat. Biotechnol.* 21, 807–812
17 Shieh, C-C. *et al.* (2003) Automated parallel oocyte electrophysiology test station (POETs™): a screening platform for identification of ligand-gated ion channel modulators. *Assay Drug Devel. Technol.* 1, 655–663
18 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

19 Schmidt, C. *et al.* (2000) A chip-based biosensor for the functional analysis of single ion channels. *Angew. Chem., Int. Ed. Engl.* 39, 3137–3140
20 Klemic, K.G. *et al.* (2002) Micromolded PDMS planar electrode allows patch clamp electrical recordings from cells. *Biosens. Bioelectron.* 17, 597–604
21 Fertig, N. *et al.* (2002) Whole cell patch clamp recording performed on a planar glass chip. *Biophys. J.* 82, 3056–3062
22 Wang, X. and Li, M. (2003) Automated electrophysiology: high throughput of art. *Assay Drug Devel. Technol.* 1, 695–708
23 Kiss, L. *et al.* (2003) High throughput ion-channel pharmacology: planar-array based voltage clamp. *Assay Drug Devel. Technol.* 1, 127–135
24 Schroeder, K. *et al.* (2003) Ionworks HT: a new high-throughput electrophysiology measurement platform. *J. Biomol. Screening* 8, 50–64
25 Xu, J. *et al.* (2003) A benchmark study with SealChip™ planar patch-clamp technology. *Assay Drug Devel. Technol.* 1, 675–684
26 Kutchinsky, J. *et al.* (2003) Characterization of potassium channel modulators with QPatch™ automated patch-clamp technology: system characteristics and performance. *Assay Drug Devel. Technol.* 1, 685–693
27 Stett, A. *et al.* (2003) Cytocentering: a novel technique enabling automated cell-by-cell patch clamping with the Cytopatch™ chip. *Recept. Channels* 9, 59–66
28 Fertig, N. *et al.* (2002) Activity of single ion channel proteins detected with a planar microstructure. *Appl. Phys. Lett.* 81, 4865–4867
29 Brüggemann, A. *et al.* (2003) High quality ion channel analysis on a chip with the NPC Technology. *Assay Drug Devel. Technol.* 5, 665–673
30 Lepple-Wienhues, A. *et al.* (2003) Flip the tip: an automated, high quality, cost-effective patch clamp screen. *Recept. Channels* 9, 13–17
31 Comley, J. (2003) Patchers v. screeners. *Drug Discov. World* Fall, 47–57