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HTS Revisited

Ion channel screening – automated patch clamp on the rise

Cecilia Farre, Michael George, Andrea Brüggemann, Niels Fertig*

Nanion Technologies GmbH, Erzgiessereistr. 4, 80335 Munich, Germany

Ion channel proteins are of major importance for the human physiology and thus highly attractive molecular drug targets. Large-scale ion channel screening of wanted and unwanted drug effects is required, but has been limited by the lack of adequate screening technology, because available methods put a trade-off between high-throughput and high-information content.

The advent of automated patch clamp platforms has revolutionized ion channel screening, enabling investigations from a more functional perspective at a much higher throughput. The current status of automated patch clamp platforms, their strengths and drawbacks as well as future developments are reviewed.

Section Editors:
Phil Gribbon – European ScreeningPort, Hamburg, Germany
Kelvin Lam – Harvard University, Cambridge, MA, USA

Introduction

Ion channels reside virtually in all the cell membranes in mammals, insects and fungi, and are essential for life, serving as key components in inter- and intracellular communication [1]. Because of their essentiality for life, ion channel malfunction has adverse effects on the physiology [2]. As transmembrane proteins, ion channels are accessible for small molecules treatment and are the so-called druggable targets. The pharmacological modulation of ion channel function enables the subtle correction of their dysfunction, being involved in many diseases and disorders particularly in the nervous, gastrointestinal and cardiovascular system. Another aspect of ion channel drug development is the safety liability issues of the developed drug candidates (e.g. acquired QT interval prolongation), which is why the FDA and other regulatory entities require safety testing, for example on hERG for all new chemical entities [3].

The patch clamp technique is the state-of-the-art technology for the study of ion channels, but patch clamping is a laborious process requiring a skilled and highly trained scientist. Owing to the ultra-low-throughput, conventional patch clamping is frequently applied only in the later stages of drug discovery and development. Several screening techniques with high-throughput capabilities are available for drug screening efforts, but those are limited in sensitivity and thereby at risk for false negatives and missing out on a potential blockbuster drug. The development of automated patch clamp technology has the potential to provide detailed information on structure–activity relationship of the compound at much higher throughput earlier in the drug discovery process. New developments within this area hold a promise for increasing the throughput capabilities even more, and lowering the price per data point, which are the two main requirements heard from 'the hit discovery community' in the pharmaceutical industry.

Conventional patch clamp

Ion channel activity is most accurately measured using the patch clamp method, developed in the late 1970s (Nobel Prize 1991, Bert Sakmann and Erwin Neher) [4,5]. In principle, the method utilizes a glass microelectrode that is pressed against the surface of a single cell. A tight interaction is

*Corresponding author: N. Fertig (info@nanion.de)
URL: http://www.nanion.de/

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formed between the cell membrane and the rim of the glass microelectrode, with an electrical resistance greater than a gigaohm, frequently referred to as 'giga-seal' [6]. The electrical contact obtained with the cellular membrane allows for the control of the membrane voltage, and thus the possibility to directly measure the movement of charge, that is current across the membrane. In this way, the investigation of ion channels and their effectors is possible with regard to current/voltage-characteristics, ion selectivity, temperature sensitivity, kinetics and single channel recordings, meaning that very information-rich data can be obtained. Achieving a giga-seal is one of the factors for high quality, low noise recordings with accurate voltage clamp and allows for resolving small currents, even at single channel level.

Although being considered the gold standard for investigations of ion channels and their effectors, patch clamp suffers from being very labor intense, and the fact that it takes years to learn. The data throughput is ultra low, and does not allow for using patch clamp as screening tool in drug development and optimization. Typical data throughput of conventional patch clamping is approximately five to ten data points per day.

Ion channel screening technologies with high-throughput capabilities

Ion channel activity can also be monitored using indirect methods, which offer a throughput compatible with HTS. Current workhorses for primary drug screening using cellular assays are mostly based on fluorescence, affinity binding or radioactive flux assays. Most of these methods have low temporal resolution (in the range of seconds to minutes, as compared to submilliseconds for patch clamp), often require extensive assay development and are known for their high false-negative and false-positive rates. Some of them are not applicable to all ion channel types because the readout is coupled to a specific ion species, for example, calcium-sensitive dyes, or rubidium efflux assays, which also require the use radioactive compounds. Ion channels activated by a change in transmembrane voltage sometimes offer a challenge to investigate, owing to the lack of voltage control of the cellular membranes. On the positive side, these methods allow for the screening of 100,000s of compounds in limited time frames. Table 1 summarizes the strengths and drawbacks of the most commonly used HTS techniques. Detailed analysis and discussion of high-throughput ion channel screening methods can be found in several recent reviews [7–9].

Using the indirect screening techniques, listed in Table 1, a 'Yes/No' type of answer generates to the question of compound activity, and they are limited in sensitivity and information content, with the risk of false negatives. Therefore, a follow-up, secondary screening with more information dense methods like patch clamp are required for further characterization of the 'hits' from the primary screening. Ideally, electrophysiological measurements should be performed as early as possible in the screening cascade, to obtain better information about the drug candidates, and also possible safety liability issues. We will hence focus here on the automated patch clamp technologies which recently have become available and quickly found its way into the drug discovery process.

Automated patch clamp

Most automated patch clamp platforms on the market place today, utilize planar chips as seal forming substrates either of

Table 1. Comparison of ion channel assay methods using cell based sensors

Method	Information content	Throughput	Costs	Sensitivity	Temporal resolution	Comments
Automated patch clamp	High	Low to medium	High	High	Submillisecond	High data density with increased throughput
Manual patch clamp	Very high	Ultra-low	Very high	High	Submillisecond	Gold standard
Automated oocyte TEVC[a]	High	Low	High	High	Milliseconds	Nonmammalian system, requires RNA preparation
Radioactive flux assays	Medium	Medium to high	Low to medium	Medium	Seconds to minutes	Requires synthesis of radiolabeled probe
Redistribution of voltage-sensitive dyes	Medium	Medium	Medium	Medium	Minutes	Requires high expression, prone to artefacts, long assay development
Ca^{2+}-dyes	Medium	Medium to high	Low	Medium to high	Several seconds to minutes	Limited to Ca^{2+} (or Ca^{2+}-coupled) channels
FRET-based voltage sensors	Medium	Medium	Low	Medium	Several seconds to minutes	Voltage changes need many open changes

Throughput data points per day: low < 1000, medium = 1000–10,000, high > 10,000; sensitivity means the relative number of channels per cell needed for good signal-to-noise ratio. Modified from Gonzalez [25].

[a] Two electrode voltage clamp (TEVC).

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glass, silicon nitride or plastic. The patch clamp substrate contains a micron-sized aperture, where the cell is captured [10]. A few of these are capable of giga-seal recordings and allow for sophisticated liquid handling.

Instead of approaching the cell with a micropipette as with conventional patch clamping, in automated patch clamping, the cell approaches the patch clamp substrate owing to computerized feedback controlled application of suction. The automated patch clamp robots execute preprogrammed protocols to fill the patch clamp substrate with electrolyte solutions, add cells in suspension, seal onto a cell, break through to the whole-cell configuration and perform voltage-protocols in the absence and presence of drugs while recording the ionic currents. The benefit is obvious that the manual handling has been removed from the process, making it more straightforward to run these machines also by nonelectrophysiologists and also increasing reproducibility of data obtained. Because the technology is scalable to array formats, recordings from many individual cells can be done in parallel.

Apart from using cells in suspension and the approach of choosing cells randomly, the actual measurement is analogous to that of conventional patch clamp. For a detailed discussion we refer to a historical perspective of the development of the planar patch clamp technique [11].

Available automated patch clamp systems

Several automated platforms are commercially available, the first one on the market being the Ionworks HT and later Ionworks Quattro (Molecular Devices Corporation, now MDS Analytical Technologies, http://www.mdsinc.com/) followed by the PatchXpress (MDS, formerly Axon Instruments Inc.), and Nanion's Port-a-Patch (Nanion Technologies GmbH, http://www.nanion.de/). Other automated patch clamp devices are Sophion's QPatch-16 and -48 (Sophion Biosciences A/S, http://www.sophion.dk/), Flyion's Flyscreen (Flyion GmbH, http://www.flyion.com/) and Nanion's Patchliner platform.

The true giga-seal patch clamp recording platforms are the PatchXpress, Port-a-Patch, Patchliner, Flyscreen and the QPatch. In Table 2 the different instruments are compared and their features and capabilities are listed.

Ionworks

Although not achieving giga-seals, the Ionworks platform [12], offers the highest throughput by recording from 48 out of 384 chambers simultaneously. The instrument is claimed to achieve a throughput of about 10,000 data points per day, making it the only option to get somewhere in the range of the throughput demands of drug screening. It uses a perforated plastic foil as planar patch clamp substrate in a 384-well plate format.

Table 2. Features and performance of the available automated patch clamp instrumentation

Instrument	Patchliner	Port-a-Patch	QPatch	PatchXpress	Ionworks HT	Flyscreen 8500
Company	Nanion	Nanion	Sophion	MDS	MDS	Flyion
Substrate supplier	Nanion	Nanion	Sophion	Aviva	MDS	Flyion
Recording configuration	Whole cell, perforated patch	Whole cell, perforated patch, bilayer	Whole cell	Whole cell	Perforated patch (loose patch clamp)	Whole cell, perforated patch
Electrode type	Planar, glass	Planar, glass	Planar, silicon	Planar, glass	Planar, plastic	Pipette, glass
Recording chambers	16	1	16, 48	16	384	3–6
Amplifier channels	4–8	1	16, 48	16	48	3–6
Data points per day	250–500	50	250–3500	300	3000	100–500
Recording during application	Yes	Yes	Yes	Yes	No	Yes
Compound wash out	Yes	Yes	Yes	Yes	No	Yes
Unlimited cpd/wash additions	Yes	Yes	No	Yes	No	No
Seal resistance	>1 GΩ	>1 GΩ	>1 GΩ	>1 GΩ	50–1000 MΩ	>1 GΩ
Rs and Cslow compensation	Yes	Yes	Yes	Yes	No	Yes
Internal perfusion	Yes	Yes	No	No	No	No
Temperature control	Yes	Yes	No	No	No	Yes
Current clamp	Yes	Yes	No	No	No	Yes
User intervention during experiment	Yes	Yes	No	No	No	No
Consumable pricing	Moderate	Moderate	High	High	Moderate	Moderate

Success rates for stable whole-cell recordings are in the range of 50–90% for all instruments according to provider websites, but experiences by individual users do vary. Perfusion time constants are claimed to be around 50 ms for all systems.

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In 2006, the Ionworks Quattro was launched, introducing the population patch clamp, where the recording substrate contains multiple apertures for capturing and recording from a multitude of cells [13]. The recorded currents are averaged from up to 64 cells in one recording well, and are hence larger which eliminates at the same time for cell-to-cell differences within the population. A major drawback of the Ionworks is that it does not support giga-seal recordings; rather the seal resistance is on the order of 100 MΩ [14]. The low seal resistance confers low data quality, incomparable to conventional patch clamping, due to lower signal-to-noise ratio. Furthermore, a pore-forming agent, not suction is used to permeabilize the cell membrane. The use of pore formers such as amphotericin for obtaining electrical contact with the cell membrane, results in a drastically higher access resistance compared to conventional patch clamping, which in turn entails a poor voltage control over the membrane possibly altering the pharmacology of the ion channel and drugs under investigation. Another drawback with the system is the discontinuous recording during compound administration. This is because the recording electrodes have to be removed to make compound addition, meaning that the initial response of the compound cannot be measured.

This limits the use of the Ionworks to measuring cell lines with high current density, that is, many ion channels expressed in the membrane, and excludes direct measurements of ligand-gated ion channels, where the current response is instantaneous on drug addition. Also, many ligand-gated ion channels generally exhibit a phenomenon called desensitization where the current response diminishes despite the constant presence of the drug. This makes the Ionworks suboptimal for investigations of ligand-gated ion channels owing to the discontinuous recording mode.

Still, if the user considers the limitations and designs the experiment accordingly, the Ionworks is widely used and accepted by screeners thanks to its throughput capabilities, which so far is unmet by any other platform. The Ionworks reports a practically achievable data throughput of about 3000 data points per day.

PatchXpress

The PatchXpress, developed by Axon Instruments (now MDS) was the first automated patch clamp device on the market supporting true giga-seal recordings [15]. It uses SealChips, provided by Aviva Biosciences (http://www.avivabio.com/), for the formation of giga-seals on a perforated glass substrate in a linear 16-well format. The SealChips utilizes a surface coating to promote seal formation. Chips are delivered in solutions and need to be prepared before actual use, which according to users takes several minutes.

The PatchXpress allows for continuous recordings during ligand applications, it uses a capillary for the fast addition of compound to the recording well allowing the studies of both ligand-gated and voltage-gated ion channels. It uses in-house developed software for operation and data analysis, with versatile programming features. The stated data throughput for the machine is 250–300 data points per day.

QPatch

The QPatch-16 platform from Sophion Biosciences [16], was launched in 2004, and also allows true giga-seal recordings. The QPatch has received quite good market acceptance because of high-quality recordings and the increased throughput. The QPlates, used for the recordings are made from silicon structures with plastic channels for solution administration. Channels as well as silicon chips are coated with silicon oxide to make the surface glass like and also to reduce potential compound adsorption problems.

On compound addition solutions are collected in a waste reservoir of limited volume. This can be a restriction in dose–response analysis of compounds affecting ligand-gated ion channels, where many additions often are required for a full dose–response curve.

Protocols are preprogrammed using in-house developed software, which also includes data analysis. Compounds affecting both ligand- and voltage-gated ion channels can be screened with true giga-seals and acceptable success rates. The liquid handling of the QPatch is quite sophisticated, allowing fast applications of solutions, which is important when investigating ligand-gated ion channels.

To increase the throughput possibilities further, Sophion recently launched the QPatch-HT, allowing for the parallel recordings of 48 cells at a time. It is a linear scale up of the 16-amplifier system and the stated throughput of the machine is 250–1200 data points for the 16- and 750–3500 data points per day for the HT-system.

Flyscreen

The Flyscreen is offered as a 3- or 6-channel system and claims to have a throughput of 100–500 data points per day, by parallel, asynchronous recordings. Recordings are performed in the so-called 'Flip-tips' or 'Chip-tips', which basically are patch clamp glass pipettes where the cells are patch clamped on the inside rim of the pipette tip, rather than on the outside rim of the glass pipette as with conventional patch clamp [17]. Although being technically very elegant, the technique suffers from limited perfusion capabilities because of the dimensions of the tip, and the limited access of the perfusion capillary. Flyion introduced modified patch pipettes where the tip of the patch pipette was expanded (Chip-tips), to allow for more controlled administration of solutions.

When using the Flip-tip/Chip-tip technology, the cell is wedged in the taper of the patch clamp pipette with a risk that parts of the cell membrane will never be exposed to the surrounding solution, only the glass wall of the pipette. That

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may cause variability in the whole-cell response. The Flyscreen is also available with temperature control.

Port-a-Patch and Patchliner

Nanion introduced the Port-a-Patch in 2003, which is a miniaturized patch clamp set-up with bench top format. It basically is meant to replace a conventional rig and analyzes a single cell at a time. In 2006, the Patchliner was launched, which is a fully automated patch clamp workstation supporting true giga-seal recordings from up to eight cells simultaneously. Both platforms use borosilicate glass chips as recording substrates, where patch clamp measurements are possible in the whole-cell, cell-attached and perforated-patch recording configurations. Solution exchange of the external as well as internal recording solution is straightforward with the Port-a-Patch and the Patchliner allowing modulation of the ion channels from both sides of the membrane [18].

The NPC-chips are produced with various chip-resistances ranging from 0.5 MΩ to 10 MΩ, and can also be customized to fit specific applications, for example, for primary cells [19] or automated lipid bilayer recordings [20]. The NPC-chips require no special treatment and can be stored in their vacuumized packages at room temperature for 24 months without change in performance. On the NPC-1 chips for the Port-a-Patch, solutions are simply pipetted on the chip where they stay by surface tension, and a laminar flow perfusion chamber is available. For the NPC-16 chips, each of the 16 recording chambers contains 2 microfluidic channels, one for the administration of solutions to the external side, and the other for the administration of internal solutions.

Three NPC-16 chips can be mounted on the chip loading area which means that up to 48 experiments can be preprogrammed and executed without any user interaction being required. The Patchliner not only operates in stand-alone mode, but also allows for on-the-fly modification of the protocol at any time during the course of experiments, which make the platform flexible in operation. Both platforms support experiments at physiological temperatures as well as allow for internal solution exchange.

The Port-a-Patch has an approximate date throughput of 50 data points per day, whereas the Patchliner is capable of 500 data points per day.

Other platforms for increased throughput

Cellectricon (http://www.cellectricon.se/) offers a multichannel perfusion system called Dynaflow, which can be combined with a conventional patch clamp setup. The system uses microfluidic channels made from silicone elastomere to enable rapid perfusion and increased throughput in conventional patch clamp analysis [21].

Besides the currently commercially available automated patch clamp systems, there are upcoming platforms from companies like Cytocentrics (http://www.cytocentrics.com/) using a silicon chip based system [22] and from Fluxion Biosciences (http://www.fluxion-bio.com/) employing the so-called lateral patch clamp principle. Fluxion uses PDMS-based microfluidic chips, where cells are trapped at junctions of small, lateral channels with a main channel, thereby opening the pace to highly integrated arrays of patch clamp sites [23].

General requirements for automated patch clamp screening

Parallelization of patch clamp recordings implies higher throughput in the characterization of compounds and ion channels. The electrophysiologist can generate and manage much more data in less time for screening and safety profiling, which is likely to cut the time to market in the development of drugs. At the same time automated patch clamp platforms are restricted to using cell-lines for the expression of ion channels where the quality of the cell suspension is crucial for acceptable success rates. A cell is caught randomly from a cell suspension, which means that the expression must be homogenous within the suspension and most cells must be healthy. For most systems on the market it is therefore not feasible to use primary or transiently transfected cells with low expression rates because of the blind approach of capturing cells.

Primary cells can also be problematic for automated patch clamp platforms, because the cell preparation often contains different cell types and that the suspensions are not 'clean' enough. There are, however, examples where the Ionworks and Patchliner have been used for primary cells and more publications in this area are anticipated in the future [19,24].

Conclusion

Automated electrophysiology has made a significant impact on drug discovery so far, with a surprising rapid implementation of what only five years ago were scientific proofs of concepts. As a result, a portfolio of different established instruments is available to the user today. Currently, automated patch clamp technology is mainly employed in safety pharmacology (hERG screening), secondary screening efforts and lead optimization, where it certainly is a decisive factor for the increase in efficiency. It shortens cycle times to get results, for example on the safety profile of a compound and it also allows for better qualification of hits, which over all decreases the drop out rate in late phase drug development and shortens the time to market for new drugs.

Still, automated patch clamp has not found its way into routine use for primary drug screening. Commercially available systems today only offer low to medium throughput of several 100–1000s of data points per day yet (although a quantum leap as compared to manual patch clamp) and costs per data point are considered high for (primary) screening standards (see http://www.htstec.com). The ambition of

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both, providers and users, is to use high-quality screening methods, such as automated patch clamping, earlier and earlier in the drug discovery process. However, for this dream to come true, users argue that consumable pricing has to decrease to make screens of tens of thousands of compounds affordable. Costs will certainly drop over time, but one needs to consider that using these more sophisticated methods results in a more qualified hit list of drug candidates and it probably can cut time to market for new developed drugs.

In any case, automated patch clamp technology is further being scaled up and instrument providers will offer higher throughput devices with lower cost per data point in the near to midterm future. Sophion already offers a system for recording from 48 cells at a time, with up to 3500 data points per day the highest throughput machine with giga-seal recording available today. Still, screening costs for this device are considered high.

For 2009, two new patch clamp platforms are announced to be launched: one by Cellectricon and the other by Nanion. Both new platforms will record from 96 cells at a time, thereby getting closer to the throughput required by screeners. We are not aware yet of detailed technical capabilities/specifications of Cellectricon's new machine. Nanion's new SyncroPatch-96 platform will be using a 96-well-plate patch clamp substrate (glass) enabling full dose–response measurements by the addition of multiple drug concentrations to every single of the 96 cells. So with these and surely other advancements, new developments and new instruments, there certainly is more to come in the following years with regard to increasing the throughput, and lowering the price per data point in patch clamp based ion channel screening technology.

Currently there is a clear paradigm shift when thinking in terms of how patch clamp recordings can and should be carried out in the drug discovery process. It is the authors' firm belief that automated patch clamp will eventually become the standard tool for ion channel screening in safety pharmacology and the development of ion channel modulating drugs.

References

1 Hille, B., ed. (2001) *Ion Channels of Excitable Membranes*, Sinauer Associates Inc.
2 Ashcroft, F.M., ed. (2000) *Ion Channels and Disease*, Academic Press
3 Redfern, W.S. *et al.* (2003) Relationship between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de point for a broad range of drugs: evidence for provisional safety margin in drug development. *Cardiovasc. Res.* 58, 32–45
4 Neher, E. and Sakmann, B. (1976) Single channel currents recorded from membrane of denervated frog muscle fibers. *Nature* 260, 799–802
5 Sakmann, B. and Neher, E., eds) (1995) *Single Channel Recordings*, Plenum Press
6 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
7 Molokanova, E. and Savchenko, A. (2008) Bright future of optical sensors for ion channel drug discovery. *Drug Discov. Today* 13, 14–22
8 Treherne, J.M. (2006) Exploiting high-throughput ion channel screening technologies in integrated drug discovery. *Curr. Pharm. Des.* 12, 397–406
9 Terstappen, G.C. (2004) Non-radioactive rubidium ion efflux assay and its applications in drug discovery and development. *Assay Drug Dev. Technol.* 2, 553–559
10 Fertig, N. *et al.* (2002) Whole cell patch clamp recording performed on a planar glass chip. *Biophys. J.* 82, 3056–3062
11 Behrends, J.C. and Fertig, N. (2007) Planar patch clamping. In *Patch Clamp Analysis: Advanced Techniques* (Walz, W., ed.), pp. 414–433, Humana Press
12 Schroeder, K. *et al.* (2003) IonWorksHT: a new high-throughput electrophysiology measurement platform. *J. Biomol. Screen.* 8, 50–64
13 Finkel, A. *et al.* (2006) Population patch clamp improves data consistency and success rates in the measurement of ionic currents. *J. Biomol. Screen.* 11, 488–496
14 Lizhen, Y. *et al.* (2005) ShK, a pharmacological tool for studying Kv3.2 channels. *Mol. Pharm. Fast Forward* 10.1124/mol.105.011064 http://molpharm.aspetjournals.org
15 Tao, H. *et al.* (2004) Automated tight seal electrophysiology for assessing the potential hERG liability of pharmaceutical compounds. *Assay Drug Dev. Technol.* 2, 497–506
16 Mathes, C. (2006) Qpatch: the past present and future of automated patch clamping. *Expert Opin. Ther. Targets* 10, 319–327
17 Lepple-Wienhues, A. (2003) Flip the tip: an automated, high quality, cost-effective patch clamp screen. *Receptors Channels* 9, 13–17
18 Farre, C. *et al.* (2007) Automated ion channel screening: patch clamping made easy. *Expert Opin. Ther. Targets* 11, 557–565
19 Xu, S.Z. *et al.* (2008) TRPC channel activation by extracellular thioredoxin. *Nature* 451, 69–72
20 Kreir, M. *et al.* (2008) Rapid screening of membrane protein activity: electrophysiological analysis of OmpF reconstituted in proteoliposomes. *Lab-on-a-Chip* 8, 587–595
21 Sinclair, J. *et al.* (2002) A cell-based bar code reader for high-throughput screening of ion channel–ligand interactions. *Anal. Chem.* 74, 6133–6138
22 Stett, A. (2003) A novel technique enabling automated cell-by-cell patch clamping with the CYTOPATCH™ chip. *Receptors Channels* 9, 59–66
23 Ionescu-Zanetti, C. *et al.* (2005) Mammalian electrophysiology on a microfluidic platform. *Proc. Natl. Acad. Sci. U. S. A.* 102, 9112–9117
24 Estes, D.J. *et al.* (2008) High-throughput profiling of ion channel activity in primary human lymphocytes. *Anal. Chem.* 80, 3728–3735
25 Gonzalez, *et al.* (1999) Cell-based assays and instrumentation for screening ion channel targets. *Drug Discov. Today* 4, 431–439