# HTS approaches to voltage-gated ion channel drug discovery

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Voltage-gated ion channels are emerging as a target class of increasing importance to the pharmaceutical industry because of their relevance to a wide variety of diseases in the cardiovascular, CNS and metabolic areas. In the quest to identify novel lead molecules against these targets, drug discovery programmes are increasingly making use of HTS approaches. The authors describe the current technologies available for voltage-gated ion-channel screening, their application to HTS campaigns and the current limitations and emerging technologies within this area.

Voltage-gated ion-channel modulators currently represent a multi-billion pound worldwide market for the treatment of cardiovascular and CNS disorders. Given this success, together with the emerging links between disease and channel dysfunction, and the progress in molecular and functional characterization of the ion-channel families, voltage-gated ion channels are being actively pursued as targets for a wide variety of diseases in the CNS, cardiovascular and metabolic areas. Recent listings of voltage-gated ion-channel compounds nearing or at clinical development reflect an ever growing level of investment in voltage-gated ion-channel R&D[1].

Voltage-gated ion channels play a critical role in shaping the electrical activity of neuronal and muscle cells, and in controlling the secretion of neurotransmitters and hormones through the gating of calcium ion entry. Large families of voltage-gated sodium ($Na^+$), potassium ($K^+$) and calcium ($Ca^{2+}$) ion channels have been defined using electrophysiological, pharmacological and molecular techniques[2,3]; they are named according to their selective permeability for a particular cation with reference to their voltage dependence, kinetic behaviour or molecular identity (Table 1).

Although the structures of $Na^+$, $Ca^{2+}$ and $K^+$ channels are quite different, there are common functional elements represented in each (Figure 1). The channels are all transmembrane proteins with an ion-selective aqueous pore that, when open, extends across the membrane[2]. Channel opening and closing ('gating') is controlled by a voltage-sensitive region of the protein containing charged amino acids that move within the electric field. The movement of these charged groups leads to conformational changes in the structure of the channel resulting in conducting (open/activated) or nonconducting (closed/inactivated) states. These ion-channel states provide unique opportunities for drug discovery, enabling state-dependent molecules to be developed that, for example, only bind to nonconducting (inactivated) channels. The overall effect is to target drugs to tissues exhibiting abnormal electrical activity, while leaving normal channels in active tissues unaffected.

Designing high-throughput screens for voltage-gated ion channels requires a different approach than for drug targets such as 7-transmembrane receptors or ligand-gated ion

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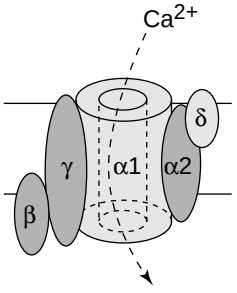
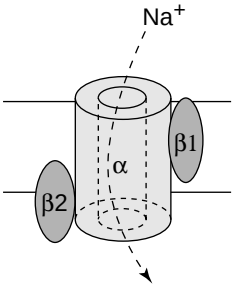
**Table 1. Voltage-gated ion-channel families**

| Subunit structure | Nomenclature and tissue distribution | Molecular identity | Disease targets |
|---|---|---|---|
| **$Ca^{2+}$ channel** | | **Of α1 subunit** | **For $Ca^{2+}$-channel blockers** |
| Subunits: α1, β, γ, α2–δ | P/Q-type (neuronal) | α1A | Angina, arrhythmia, hypertension, atherosclerosis, pain, neuroprotection, migraine, stroke, Alzheimer's, cognitive enhancement and dementia |
|  | N-type (neuronal) | α1B | |
| | L-type (cardiac) | α1C | |
| | L-type (neuronal) | α1D | |
| | R-type (neuronal) | α1E? | |
| | T-type (neuronal) | α1G | |
| | L-type (skeletal) | α1S | |
| | T-type (cardiac) | ? | |
| **$Na^{+}$ channel** | | **Of α subunit** | **For $Na^{+}$-channel blockers/ modulators** |
| Subunits: α, β1, β2 | Brain I | BI | Epilepsy, pain, anaesthesia, neuroprotection, arrhythmia and migraine |
|  | Brain II | BII, BIIA | |
| | Brain III | BIII | |
| | Brain VI | BVI | |
| | Peripheral neuronal 1 | PN1 | |
| | Sensory neurone-specific | SNS (PN3) | |
| | Glial | NaG | |
| | Skeletal muscle 1 | SKM1 | |
| | Heart/skeletal muscle 2 | SKM2 | |
| **$K^{+}$ channel**[a] | **Delayed rectifier (Kv family)** | **Of α subunit** | **For $K^{+}$-channel blockers** |
| Subunits: α (tetramer forms pore), β1, β2, β3 (not shown) | Brain/heart/skeletal muscle | Kv1.1 | Multiple sclerosis, Alzheimer's, arrhythmias, immunosuppression and depression |
| | Brain/heart | Kv1.2 | |
| | Brain/lung | Kv1.3 | |
| | Brain/heart/skeletal muscle | Kv1.4 | |
| | Brain/heart/kidney/skeletal muscle | Kv1.5 | |
| | Brain | Kv1.6 | **For $K^{+}$-channel openers** |
| | Brain | Kv1.7 | Angina, hypertension, asthma and urinary incontinence |
| | Brain/heart/kidney/skeletal muscle | Kv2.1 | |
| | Brain/heart | Kv2.2 | |
| | Brain/lymphocytes/skeletal muscle | Kv3.1 | |
| | Brain | Kv3.2 | |
| | Brain/liver | Kv3.3 | |
| | Brain/skeletal muscle | Kv3.4 | |
| | Brain | Kv4.1 | |
| | Brain/heart/aorta | Kv4.2 | |
| | Brain/lung | Kv5.1 | |
| | Brain | Kv6.1 | |
| | **$Ca^{2+}$-activated $K^{+}$ (BK)** | | |
| | Smooth muscle/brain | hslo | |
| | **Other** | | |
| | Heart | herg | |

[a]Only $K^{+}$ channels with S4 voltage-sensitive domain are presented in this table.

channels, because of the nature of the activation process. As mentioned above, voltage-gated ion channels do not require agonist binding for activation. Hence, there is no physiologically relevant agonist binding site, but rather sites that have been identified experimentally to be important in enabling conformational transitions of channels from conducting to nonconducting states. Such sites may be exploited in drug discovery programmes.

Techniques used in building high-throughput ion-channel assays include functional and radioligand-binding approaches applied to cells (vesicles or membranes) expressing native or cloned channels, or to whole-cell assays. Functional whole-cell assays may use electrophysiological techniques, such as patch clamping (which provides a controlled voltage stimulus and direct electrical readout of ionic current), or may make use of toxins, venoms or compounds that bind to and open channels (see Table 2). In the latter cases the kinetics of ion flux through open channels can be measured using fluorescence, end-point radiotracer or cell viability techniques.

The challenge posed in designing and running HTS campaigns lies in translating these assays into low cost, miniaturized, high-density formats. Typically, screening programmes are designed to assay 50,000–200,000 samples depending on the degree of automation and format (96- or 384-well) of the assay. Smaller campaigns tend to employ directed compound sets, selected around known ion-channel chemistries, whereas larger campaigns often use more diverse or random compound selection. Hence, the ability to perform highly automated, high-density assays increases the chances of discovering and exploiting completely novel chemical leads.

The following sections describe examples of ion-channel assays that have been reported in the literature or explored in-house at Glaxo Wellcome, and highlight technological developments in each area.

## Techniques

### *Radioligand binding*

Radioligand binding studies have revealed that voltage-gated ion channels possess distinct binding sites for different classes of channel activators and modulators[4]. For $Na^+$ channels, five neurotoxin binding sites have been well characterized[5] (Table 3) by either direct measurement of specific binding of labelled neurotoxin to the site or competitive displacement of a labelled neurotoxin by other unlabelled neurotoxins. Additional sites are also implicated for other classes of neurotoxins and compounds including insecticides, local anaesthetics and anticonvulsants[6].

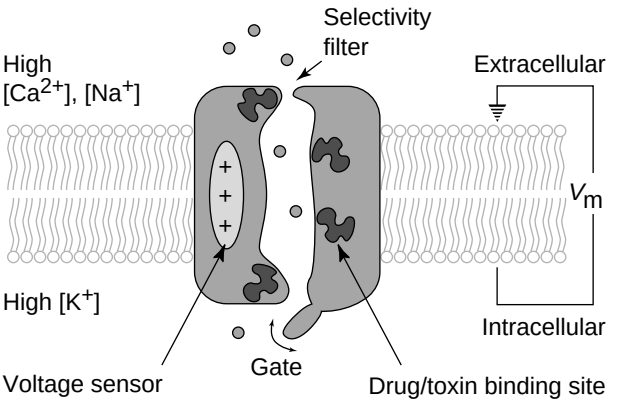



***Figure 1.*** *Schematic representation of a voltage-gated ion channel. All voltage-gated ion channels possess a positively charged region, which acts as a dipole detecting the electric field across the membrane. The pore contains a region that selectively favours passage of a particular ionic species ($Na^+$, $Ca^{2+}$ or $K^+$). A gating mechanism controls the ionic conductance of the channel. Multiple binding sites for toxins and pharmacological agents are found on these channels – only in the case of BK $Ca^{2+}$-activated $K^+$ channels is a binding site known to be physiologically relevant in modulating the channel state.* $V_m$*, membrane potential.*

For some ligands, the affinity for a particular site has been found to vary according to the channel state (open, closed or inactivated), and hence they exhibit voltage or state-dependent binding. For example, with $Na^+$ channels, lamotrigine, lidocaine and phenytoin bind preferentially to the ion channel in the inactivated state[6,7], whereas batrachotoxin and veratridine bind with high affinity to open channels[6]. Interestingly, lidocaine appears to competitively displace batrachotoxin, but the interaction does not take place through competition at site 2; it occurs through allosteric antagonism – by stabilizing the channel in the inactivated state when site 2 exhibits low affinity for batrachotoxin[6]. Such interactions between binding sites, the voltage-dependency of binding and the multiplicity of potential binding sites available on ion channels affecting function, inevitably means that great care is required in designing a meaningful high-throughput assay.

To design a binding assay for a voltage-gated ion channel, an ideal situation would be to identify a high-affinity

**Table 2. Some toxicolocical/pharmacological agents for voltage-gated ion-channel assays**

| Ion channel | Blockers | Activators |
|---|---|---|
| **$Ca^{2+}$ channel** | | |
| N-type | ω-Conotoxin MVIIA peptide toxin from *Conus magnus* and ω-conotoxin GVIA peptide toxin from *Conus geographus* | |
| P/Q-type | ω-Agatoxin-IVA (peptide from *Agelenopsis aperta*) | |
| Q-type | ω-Conotoxin MVIIC (peptide toxin from *C. magnus*) | |
| T-type | Mibefradil (anti-arrhythmic/antihypertensive) | |
| L-type | 1,4-Dihydropyridines e.g. nimodipine (anti-arrhythmic/antihypertensive) | BAYK8644 (pharmacological tool) |
| **$Na^+$ channel** | | |
| | Tetrodotoxin (heterocyclic guanidine toxin from *Tetradon stellatus* and other fish of the Tetraodontiformes order); local anaesthetics e.g. lidocaine; μ-conotoxin GIIIB peptide toxin from *Conus geographus;* anticonvulsants (e.g. lamotrigine, phenytoin); antiarrhythmics (e.g. quinidine, mexiletine) | Batrachotoxin (steroidal alkaloid from *Phyllobates aurotaenia*); aconitine (alkaloid toxin from *Aconitum* sp.); veratridine (alkaloid toxin from *Veratum* sp.); scorpion venom (from *Leiurus quinquestriatus*); ATXII (toxin from *Anthopleura xanthogrammica*); pyrethroids (insecticides) derived from *Chrysanthemum* sp.; brevetoxins (toxin from *Ptychodiscus brevis*) |
| **$K^+$ channel** | | |
| Kv1.1, 1.2, 1.6 | α-Dendrotoxin peptide from *Dendroaspis angusticeps* | |
| Kv1.3 | Charybdotoxin peptide from *Leiurus quinquestriatus hebreaus* | |
| Kv1.3 | Margatoxin from *Centruoides margaritatus* | |
| Kv1, Kv3, Kv4.2 | 4-Aminopyridine (pharmacological tool) | |
| Kv1.1, 1.6, 2.1, Kv3 | Tetraethylammonium (pharmacological tool) | |
| $Ca^{2+}$-activated $K^+$ (BK/hslo) | Paxilline alkaloid toxin from *Penicillium paxilli*<br>Iberiotoxin peptide from *Buthus tamulus* | NS1608 (pharmacological tool) |

**Table 3. Neurotoxin binding sites for $Na^+$ channels**

| Site | Toxin | Effect on $Na^+$ channel |
|---|---|---|
| 1 | Tetrodotoxin<br>Saxitoxin | Block $Na^+$ flux through channel pore |
| 2 | Batrachotoxin<br>Veratridine<br>Aconitine | Stabilize open state |
| 3 | α-Scorpion toxins<br>Type-1 sea -anemone toxins | Delay channel inactivation |
| 4 | ω-Scorpion toxins | Enhance channel activation |
| 5 | Brevetoxins<br>Ciguatoxins | Cause persistent activation |

ligand that binds to the site of interest. But such an approach is not generally possible in HTS programmes, as the binding site linked with the desired modulatory effect is often unknown, unspecified or a high-affinity ligand is not available. Approaches involving noncompetitive displacement of a radioligand through allosteric interactions with other sites may be substituted, although many potentially useful compounds will be missed if there is either no or little allosteric modification. These limitations mean that in essence only very few voltage-gated ion-channel targets are suitable for radioligand binding in HTS and usually other approaches are required.

*Cell-based fluorescence and radiotracer assays*

Ion-channel function may also be monitored through the measurement of changes in intracellular concentration of

permeant ions by using fluorescent-ion indicators or radiolabelled ions. Both approaches lend themselves to HTS in cell-based, 96-well formats. In these assays, ion channels are generally activated using toxins or compounds that promote prolonged channel opening, or if applicable to the channel type, high $K^+$ depolarization or elevation of intracellular $Ca^{2+}$. For example, $Na^+$ channels, which completely inactivate in <10 ms after voltage stimulation, can be induced into an open, ion-conducting conformation over many minutes using toxins such as veratridine or scorpion venom[8,9] (Figure 2). The $Ca^{2+}$ channels exhibit no or a lesser degree of inactivation and hence can be opened by high $K^+$ depolarization as an alternative to using compound activators. Calcium-activated $K^+$ channels can be opened following addition of $Ca^{2+}$ ionophores or as a consequence of $Ca^{2+}$ influx through $Ca^{2+}$ channels.

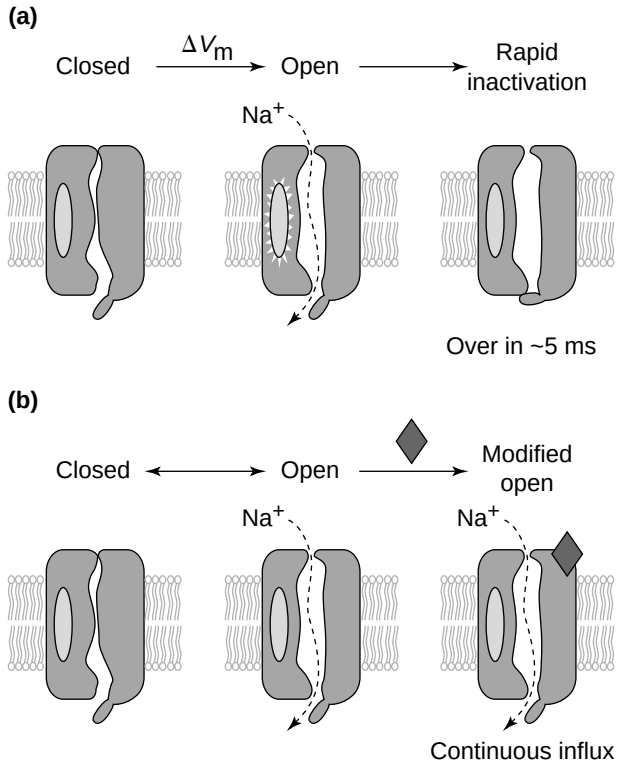



***Figure 2.*** *$Na^+$ channels can exist in multiple ion conducting (open) and nonconducting (closed/inactivated) conformations. (a) $Na^+$ channels open and then rapidly inactivate following voltage stimulation. Transitions between these states occurs in a voltage and time-dependent manner. The time course and voltage dependency of $Na^+$-channel activity can be described by separate activation and inactivation gating processes. Activation takes place upon depolarization of the membrane ($\Delta V_m$) and the channel adopts an open pore conformation allowing $Na^+$ influx. Inactivation processes then change the channel conformation to a nonconducting, non-activatable state. Repolarization returns the channels from inactivated to resting conformations. (b) $Na^+$ channel opening may be prolonged by toxin binding. Toxins such as veratridine and batrachotoxin bind to channels in the open conformation and stabilize the channel in a modified conducting state. This in effect removes or slows down the inactivation process allowing ion flux to continue from minutes to hours.*

*Fluorescence* readout is widely used for $Ca^{2+}$ channels, as influx of $Ca^{2+}$ through open channels causes large transient changes in intracellular $Ca^{2+}$ levels (typically 100–1,000-fold) that can be detected using a range of commercially available fluorescent $Ca^{2+}$ dyes such as Fluo-3 and Calcium green-1 (Molecular Probes, Eugene, OR, USA). In these assays, $Ca^{2+}$ channels can be activated by depolarizing the membrane with an isotonic solution containing a high concentration of $K^+$. The resulting transient movement of intracellular $Ca^{2+}$ can be measured over a duration of 20–60 s.

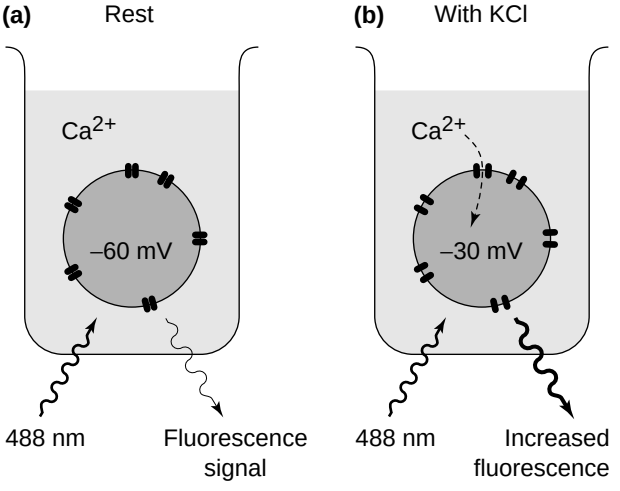



***Figure 3.*** *Schematic representation of a fluorescence-based 96-well $Ca^{2+}$-channel assay. Cells, loaded with the fluorescent $Ca^{2+}$ indicator Fluo-3AM (a), are depolarized by addition of isotonic KCl (b). The depolarization activates the $Ca^{2+}$ channels allowing $Ca^{2+}$ entry and increases the fluorescence signal. Note that the single cell represents a cell monolayer or suspension.*

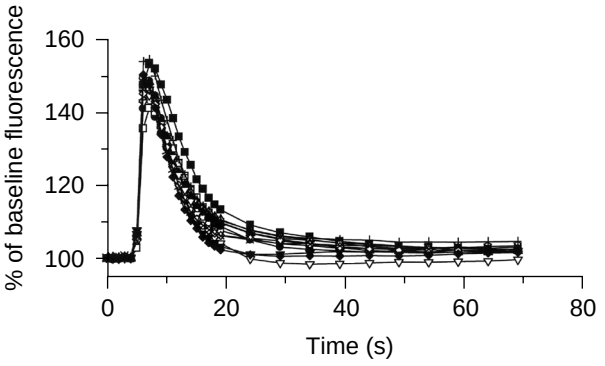



***Figure 4.*** *Fluorescence recording of $Ca^{2+}$ responses in human neuroblastoma cells. Data from assay scheme described in Figure 3 run with adherent cell monolayers in a 96-well plate. Transient rises in fluorescence are recorded in the FLIPR system and show high reproducibility from well to well (time courses in signal from wells A1 to A12 are shown superimposed).*

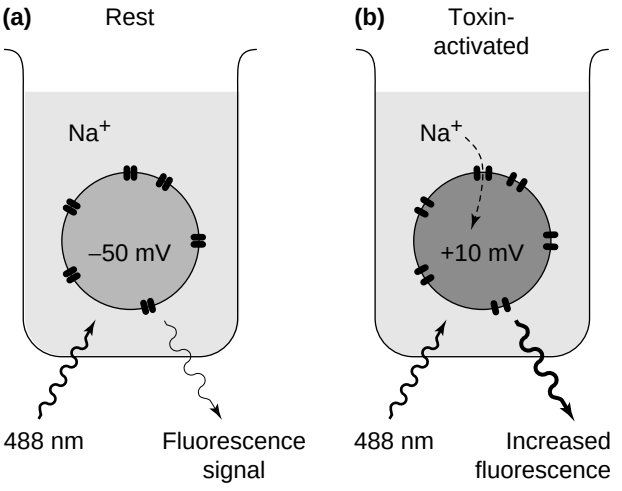



***Figure 5.*** *Use of bis-oxonol membrane potential dyes in $Na^+$-channel assays. Cells expressing $Na^+$ channels are bathed in media containing negatively charged bis-oxonol dye. At rest (a), the cells are hyperpolarized and the negative potential opposes dye entry. When $Na^+$ channels are continuously opened by the addition of an activating toxin (b), influx of positively charged $Na^+$ occurs and depolarizes the cell. A more positively charged cell leads to dye accumulation and hence an increase in fluorescence signal from the cell layer. Note that the single cell represents a cell monolayer.*

The measurement of rapid kinetic changes in fluorescence for HTS purposes has recently become possible with the availability of a charge-coupled device (CCD)-based fluorescence plate reader equipped with integral 96-well pipettors, capable of reading 96 wells simultaneously at rates as fast as one plate per second. A typical $Ca^{2+}$-channel assay performed in-house measuring the transient movement of $Ca^{2+}$ in a fluorescent-imaging plate-reader (FLIPR) system[10] (Molecular Devices, Sunnyvale, CA, USA) is shown in Figure 3 (assay scheme) and Figure 4 (kinetic data). Plate-throughput capacity for each assay is limited by the reading time and the time taken to change pipette tips; for a typical assay measuring the $Ca^{2+}$ response over one minute, each plate is processed approximately every three minutes.

The FLIPR system[10] has optics designed to discriminate fluorescence from the cell monolayer and precise temperature control, enabling the slow membrane-potential-sensitive dye – bis-(1,3-dibutylbarbituric acid)trimethine oxonol [$DiBAC(4)_3$] – to be used in cellular ion-channel assays[11]. $DiBAC(4)_3$ is negatively charged and undergoes potential-dependent distribution between the cell cytoplasm and the extracellular medium. $DiBAC(4)_3$-based $Na^+$-channel FLIPR assays have also been developed in-house with cells expressing $Na^+$ channels, and using scorpion venom or veratridine to promote prolonged channel opening (Figure 5). Toxin activation of $Na^+$ channels causes resting cells to depolarize, which leads to a slow accumulation of dye in the cytoplasm and an increase in fluorescence signal. A similar approach can be used to follow $K^+$-channel activation through hyperpolarization and dye depletion[11,12]. The disadvantages of using the oxonol dyes are that they can only be used for slow membrane-potential changes that occur during the redistribution of dye between the cytoplasm and extracellular medium over a timescale of minutes rather than seconds. Furthermore, several test compounds directly influence the 'fluidity' of the cell membrane, leading to an increase in intracellular dye concentration and a false-positive response.

*Radiotracers* have long been used to follow ion flux through channels in cells and synaptosomal preparations. The radiotracers $^{22}Na^+$ and [$^{14}$C]-guanidinium are commonly used for pharmacological and toxicological analysis of $Na^+$ channels, and in compound development programmes[12,13]. Similarly, $Ca^{2+}$-channel activity has been measured in synaptosomes and cells following KCl depolarization using $^{45}Ca^{2+}$ as the radiotracer[14]. Assays for

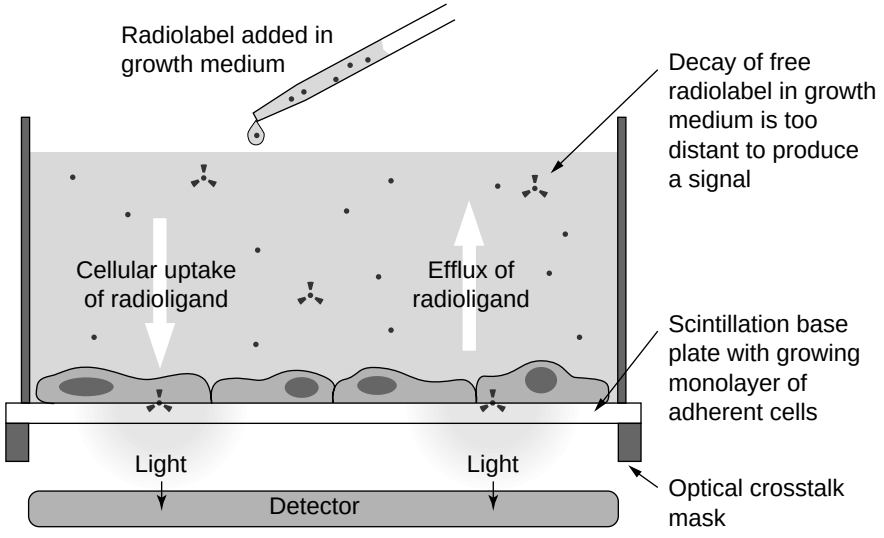



***Figure 6.*** *Principles of Cytostar-T scintillating microplate technology. Cytostar-T plates are standard format tissue culture-treated plates in which the transparent base of each well is a composite of polystyrene and scintillant that permits cultivation and observation of adherent cell monolayers. Radioisotopes that have suitable decay characteristics interact with the scintillant when brought into close proximity with the base of the plate by virtue of the biological process within the cells, thereby resulting in the generation of a light signal.*

$K^+$-channel activity are possible using $^{86}Rb$ as the $K^+$-channel permeant ion. One application in glioma cells follows $Ca^{2+}$-activated $K^+$-channel activity through $^{86}Rb$ influx following addition of the $Ca^{2+}$ ionophore ionomycin[15] – it is a useful technique for screening toxins and crude venoms for $Ca^{2+}$-activated $K^+$-channel activity.

Recently, the development of Cytostar-T scintillating microplates (Amersham International, Little Chalfont, UK) has enabled some radiotracer-flux assays to be performed much more efficiently and at higher throughput than protocols involving liquid scintillant, because the need for separation and washing is reduced[16] (Figure 6). A cell-based $Na^+$-channel assay has been designed in-house to take advantage of these plates. Chinese hampster ovary cells that are stably over-expressing a $Na^+$ channel are cultured within the Cytostar-T plates to form a confluent monolayer. Scorpion venom and veratridine are added to the cells to activate and maintain the $Na^+$ channels in an open state. Cells are loaded with $^{14}C$-labelled guanidinium by addition to the well – this radiolabel is approximately the same physical size as $Na^+$ and passes through the open channel into the cell. After a fixed time period to allow the accumulation of radiolabel within the cytoplasm, the open $Na^+$ channel is blocked with tetrodotoxin. By virtue of the biological process, the accumulated radioactivity within the cell is brought into close proximity with the scintillant incorporated into the base of the plate, causing the generation of light that is measured using a scintillation counter. Test compounds that inhibit the opening of the channel, or block the pore, will inhibit the movement of radiolabel into the cell. With this technique, scintillation fluid is not required and no separation of the incorporated cellular radiolabel from that in the assay buffer is needed, because the confluent monolayer and the cell membrane prevent the non-incorporated radiolabel from generating a light signal. Assays developed in these plates are very suitable for automation and HTS (see below).

### *Cell viability*

Cell viability assays using both yeast and mammalian cell lines have also been developed for ion-channel targets and may have potential for use in HTS to identify novel ligands[17–19]. In these assays, ion-channel activity and the flux of a particular ion is directly related to cell survival. Yeast-based assays employing $K^+$-uptake-defective *Saccharomyces cerevisiae* strains have been widely used for both expression cloning and structure–function analysis of plant inward-rectifier $K^+$ channels[19]. In these assays, expression of functional $K^+$ channels restores $K^+$ uptake and promotes cell survival. A screen based on this approach may prove to be valuable for identifying $K^+$-channel blockers in high-throughput screens.

A semi-automated assay for detecting and quantifying three classes of marine $Na^+$-channel toxins has also been developed using mammalian neuroblastoma cells with a colorimetric cell viability readout[18]. Here, cells are treated with a $Na^+$-channel opener (veratridine) and a $Na^+/K^+$-pump inhibitor (ouabain, which blocks $Na^+$ efflux), to potentiate a lethal intracellular $Na^+$ overload. Cell viability is enhanced by toxins that block the channel and is

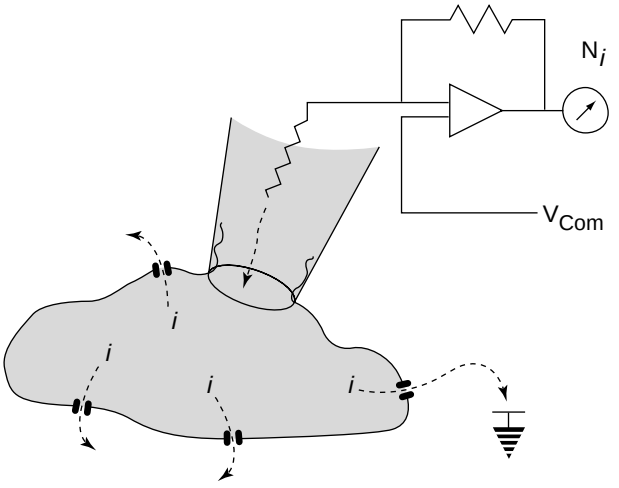



***Figure 7.*** *Patch clamp technique. The whole-cell mode of the patch clamp technique enables the sum of all the individual ion-channel currents to be recorded from the entire cell. Glass pipettes are sealed onto a cell membrane and the membrane patch ruptured to allow electrical access to the whole of the cell membrane. Patch clamping is the most sensitive technique for recording ion-channel activity and allows full voltage control of the cell membrane. Single cells are required for this technique. N denotes the number of channels,* i *the single channel current, hence N*i *is the total current measured through N channels.* $V_{Com}$ *denotes the command voltage applied via the pipette to the interior of the cell.*

reduced by toxins that further promote $Na^+$-channel opening. This screen was proposed as a useful adjunct to reduce the need for *in vivo* toxicity testing of seafood products in mice. The assay protocol has recently been developed further for contract routine testing of paralytic shellfish poisons (MIST bioassay, Jellett Biotek, Dartmouth, NS, Canada)[20].

### *Electrophysiology*

Electrophysiological voltage-clamping techniques encompass the most powerful approach for detailed biophysical analysis of ion-channel function through measurement of ionic current flowing through one or many ion channels. Patch clamping uses a single microelectrode for controlling the membrane voltage whilst measuring the current flow through a single cell or membrane patch[21] (Figure 7). Since the development of this technique, our understanding of ion-channel function and mechanisms of drug action have moved forward rapidly. However, along with other voltage clamp methodologies, patch clamping has not yet evolved into a high-throughput process for compound screening. Perhaps the best solution to date for using patch clamping in a screening programme has been designed around an automatic compound delivery system that uses HPLC autoinjectors coupled with on-line data sampling. NeuroSearch A/S (Glostrup, Denmark) have used this approach in their large-conductance $Ca^{2+}$-dependent $K^+$ (BK)-channel screening programme, enabling many compounds to be studied in excised patches from transfected HEK cells[22]. Further automation of the patch clamping process would further improve throughput capacities.

## HTS program

How would these kinds of technique fit within a typical screening programme? For a high-throughput campaign (200,000 sample), binding assays remain the first choice in terms of cost, automation and throughput rate. This reflects the technical ease of these types of assay and, hence, their ability to be automated. The types of screening sample will vary and will usually include corporate compound collections, natural product extracts and combinatorial libraries presented either discretely or as pools.

Cellular functional assays are used as primary or secondary assays to determine functionality of compounds from a binding screen and also to assess toxicity. As cellular functional screens are typically more labour intensive, at Glaxo Wellcome we screen around 50,000 samples in an equivalent time-frame to a 200,000 sample binding assay. However, these types of assays are information rich and therefore potentially offer greater rewards to the drug hunter. On-going developments in cellular-screening automation and compatibility with 384-, 864- and 1,536-well plates (in terms of cell plating, reagent addition and detection) are expected to increase the throughput of cellular assays to match that of binding assays. At the higher densities (864- and 1,536-well plates), plate washing of adherent cells is more problematic.

Patch clamping remains an important tertiary assay in a hits-to-leads programme, yielding information about voltage, rate- and use-dependence of compound binding. Throughputs here are very low, at best in the order of 10–20 compounds per day, depending on the assay protocol.

## Chemistry

Over the past few years a massive amount of effort has been put into the design of combinatorial chemical

libraries and technology for use in HTS. Although most libraries finding their way into ion-channel screens will undoubtedly be those used in any other target screens (that is, a diverse set of small molecules designed to probe as much molecular space as possible), there has been some interest in designing libraries around small-molecule structural units commonly associated with ion-channel modulation – such as dihydropyridine libraries for $Ca^{2+}$-channel blockade[23,24], dihydrobenzopyran libraries for $K^+$-channel openers[25] and biphenyl-derivative libraries for $K^+$-channel blockade[26]. Other workers have prepared peptide libraries drawing lessons from the multitude of bioactive venom peptides[27,28].

## Automation

Technological advancements are being made in automation and miniaturization of HTS in order to allow larger sample capacities per day at reduced cost. Currently, there are two approaches to HTS automation: the integrated systems and the work stations. The relative merits of these approaches have been described elsewhere[29]. In Glaxo Wellcome, the HTS operation is based around integrated robotic systems that handle assays in 96-well and 384-well plates[30]. The challenges for ion-channel screening using this type of automation are the design of robot-compatible assays and integration of readers like FLIPR into these robotic systems. Once achieved, full automation will enable 24 h continuous operation without requiring shift-work and allowing assays to be performed more efficiently and economically. For example, the $Na^+$-channel assay utilizing Cytostar-T plates, described above, has been automated for screening in-house using the Beckman (Fullerton, CA, USA) Biomek 2000 plus side-loader fully integrated to a Wallac (Turku, Finland) Trilux Microbeta scintillation counter. This approach has significantly increased the throughput of the assay, while reducing the degree of practical work involved.

## Future technologies

A key issue in developing new high-throughput approaches for voltage-gated ion channels is designing equipment and techniques by which rapid and repetitive changes in membrane potential can be induced in a 96-well or higher density formats.

For relatively slow responses, such as intracellular ion accumulation, electrical-field stimulation through extracellular electrodes may be useful for assaying rate- and use-dependent effects of drugs on ion channels. Current technologies that may be applicable to this area include planar microelectrode arrays designed for stimulating and recording from neuronal networks in long-term culture[31]. Such arrays lend themselves to high-density format designs.

For fast membrane-potential responses, recording through extracellular electrodes is possible[32] but yields signals that are very difficult to interpret in terms of ion-channel activity alone. Thus, alternative recording techniques are required that will match intracellular electrical electrodes in terms of potential waveform discrimination and allow analysis of ion-channel activity. It is possible that the development of optical recording systems for use with fast membrane-potential dyes will provide a way forward.

Recent emerging ion-channel technologies have been described in the field of biosensors. Ion channels (gramicidin) incorporated into artificial membranes and linked to antibodies are being used to detect receptor–ligand interactions[33]; here, ionic currents only pass when partners of a dimeric channel align with each other. These ligand–receptor interactions tether the gramicidin molecules, preventing pore formation and thus reducing current flow. Such techniques lend themselves immediately to highly miniaturized formats, given the high resolution and sensitivity of current measurement circuitry. But whether the development of this type of technology is directed towards ion-channel assays *per se* remains to be seen.

## Conclusions

Various technologies and screening approaches are evolving to meet the requirements for voltage-gated ion-channel HTS. Current techniques that can be translated into 96- and 384-well plate format are all associated with a lack of precise voltage control, so that information regarding voltage, use- or rate-dependence remains unknown. This is frustrating given the advantages offered by state- or rate-dependent ion-channel modulators. Patch clamping, although allowing such precise dynamic voltage control of channel state is by no means a technique for HTS. The incorporation of dynamic voltage control into microtitre plate assays therefore remains a key challenge in the ion-channel technology arena.

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## In short…

**DuPont** announced on 19 May an agreement to acquire **Merck & Co**.'s interest in their joint venture – The DuPont Merck Pharmaceutical Co. – which was formed in 1991. The $2.6 billion transaction is expected to be completed in July when DuPont Merck will become an integral part of DuPont and operate under the name **DuPont Pharmaceuticals**. Charles O'Holliday, Jr, President and CEO of DuPont, said that 'this action will enable us to more fully integrate our materials and life science research platforms'. The acquisition is the third major action that DuPont has made recently to strengthen its life sciences portfolio. Kurt M. Landgraf, DuPont Executive Vice President, says that 'the acquisition of Protein Technologies International and the formation of Optimum Quality Grains joint venture with Pioneer Hi-Bred International have made DuPont a leader in agricultural biotechnology. And now we have added the biotechnology potential of a wholly owned pharmaceutical company'.

**Oxford Molecular** aspires to be the world leader in supplying drug discovery research services. As part of this goal, the acquisition of **Chemical Design** and its Chem-X software will provide a broader range of product offerings to scientists in chemoinformatics, molecular design and combinatorial chemistry. For Oxford Molecular's customers it will mean that the right compounds can be brought through the drug discovery process faster and at lower cost. The company will also have the opportunity to integrate the acquired products with selected Oxford Molecular products to provide more-comprehensive software solutions for discovery research scientists.

**Therapeutic Antibodies Inc**. and **G.D. Searle & Co**., the pharmaceutical division of **Monsanto Co**., announced on 21 May the signing of a research collaboration agreement for the identification, development and marketing of a new antibody-based drug. Therapeutic Antibodies will develop, manufacture and register a highly purified polyclonal antibody for a target indication nominated by Searle. It is forecast that Searle will pay U$8 million in R&D payments and product supplies based on achieving certain milestones. The first milestone payment of $1 million was made on signing. While Searle anticipates having the worldwide marketing rights of the product, Therapeutic Antibodies will be responsible for the ongoing supply. There will be shared revenues from the successful commercialization of the product.

**Exelixis Pharmaceuticals** and **Bayer AG** have entered into a collaboration to identify novel screening targets for the development of new crop protection agents. The collaboration will bring together Exelixis' expertise in model system genetics, genomics and bioinformatics and Bayer's experience in the development and commercialization of products for the agricultural market. Exelixis may receive up to $30 million in license fees, research support and milestone payments based on program success, and will receive royalties paid on Bayer's sale of any product arising from the collaboration.

Exelixis will also develop a novel EST database for a pest species of strategic importance to Bayer; assays developed by Exelixis will be used by Bayer to screen against its extensive libraries of chemical compounds, evaluate lead structures *in vivo* and develop and commercialize crop protection products. 'We believe that biology-based approaches to pesticide discovery being pioneered by Exelixis will identify novel targets that will lead to new highly effective crop protection agents', says George Scangos, President and CEO of Exelixis.