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Review

High throughput screening technologies for ion channels

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Ion channels are involved in a variety of fundamental physiological processes, and their malfunction causes numerous human diseases. Therefore, ion channels represent a class of attractive drug targets and a class of important off-targets for *in vitro* pharmacological profiling. In the past decades, the rapid progress in developing functional assays and instrumentation has enabled high throughput screening (HTS) campaigns on an expanding list of channel types. Chronologically, HTS methods for ion channels include the ligand binding assay, flux-based assay, fluorescence-based assay, and automated electrophysiological assay. In this review we summarize the current HTS technologies for different ion channel classes and their applications.

Keywords: ion channels; high throughput screening; ligand binding assay; flux-based assay; fluorescence-based assay; automated electrophysiological assay; drug discovery

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Introduction

Ion channels are a very important membrane protein family involved in a variety of fundamental physiological processes. Their malfunction causes a variety of human diseases. Therefore, ion channels represent a class of attractive drug targets[1-4] and a class of important off-targets for *in vitro* pharmacological profiling[5]. Traditionally, patch clamp electrophysiology is the gold standard for ion channel studies. However, the method is labor-intensive with a low throughput and requires highly trained staff to perform the experiments. Ion channels are difficult targets to be investigated using high throughput approaches, which hinders the use of ion channels compared with other targets. Recently, the rapid progress in developing functional assays and instrumentation has enabled high throughput screening (HTS) campaigns on an expanding list of channel types. Consequently, HTS was designed to identify active compounds for ion channel targets, which are of great interest to academic and industrial researchers. The methodologies for studying ion channels can be divided into non-electrophysiological and electrophysiological methods. This review will summarize the current technologies and commonly used screening methods for different ion channel classes.

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High throughput screening technologies

In the past, HTS methods for ion channels have been extensively developed and applied to most ion channels. In chronological order, the approaches include: the ligand binding assay, flux-based assay, fluorescence-based assay and automated electrophysiological assay.

Ligand binding assays

Ligand binding assays have been widely used to screen for ion channel modulators. However, these assays are not considered as functional assays because they detect the binding affinity of a compound to an ion channel rather than the ability of altering channel function. Ligand binding assays require a previous knowledge of the target binding sites and of the formation of a radio-labeled ligand which is specific to those binding sites. Activity of the test compound is indicated by the displacement of the labeled ligand. Consequently, conventional instrumentation may be used, in which throughput represents its major strength. Because the method only discovers compounds that influence radioligand binding, it misses allosteric modulators of ion channels[6-9]. Binding assays identify affinity data but do not identify the functional change of ion channels. For example, an agonist cannot be distinguished from an antagonist in a binding assay. Secondary assays are necessary to determine if the compound is an agonist, antagonist or neither. Furthermore, the scope of binding assay is limited by the availability and affinity of radio-labeled ligands[7-9].

The sensitivity of a binding assay is often determined by the affinity of a known labeled ligand. A high-affinity ligand may not allow the detection of weak binders. However, the use of a low-affinity ligand can lead to an increased detection of non-specific binding. When the ligand affinity is within a certain range (*eg*, from nano- to micro-molar concentration), the IC_{50} values obtained from binding assays have a reasonable correlation[10] with those obtained from patch clamping. Thus far, the assay format has been rarely used for general screening but is still good for identifying modulators specific to some given ligands.

Flux-based assays

Ion flux assay has been successfully applied to directly access a functional change of ion channel activity. Radioactive isotopes have been used to trace the cellular influx or efflux of specific ions, such as $^{22}Na^+$, $^{45}Ca^{2+}$ and $^{86}Rb^+$, for the studies of Na^+, Ca^{2+} and K^+ channels, respectively. A commonly used assay format is the $^{86}Rb^+$ efflux for K^+ channels or non-selective cation channels. In this format, the cells that express the ion channel of interest are incubated with a buffer that contains $^{86}Rb^+$ for several hours before they are washed and stimulated with an agonist to allow for $^{86}Rb^+$ efflux. Then the cells and supernatant are collected for radioactive counting[11, 12]. However, radioactive-efflux assays suffer from the inconvenience and cost associated with the handling of radioactive materials. Additionally, it is necessary to use different radioisotopes for channels that are selective for different ions. Therefore, a non-radioactive Rb^+ efflux assay was developed that uses atomic absorption spectroscopy to detect rubidium[13]. The flux assay is a format preferred by many screening laboratories because it measures ionic flux that better correlates with the activity[14-16]. This assay technology is widely applied in the pharmaceutical industry for both drug discovery and hERG-related drug-safety screening to identify potential QT liabilities that might cause lethal arrhythmias[6, 17]. However, these assays have the disadvantages of low temporal resolution (typically from seconds to minutes), uncontrolled membrane potential, less information content compared with voltage-clamping and lower throughput compared with fluorescence-based assays. Furthermore, this assay generates a very weak signal for some ion channels, which requires a high level of channel expression to achieve an acceptable signal-to-noise ratio.

Fluorescence-based assays

Fluorescence-based methods do not directly measure ionic current. Rather, they measure either the membrane-potential-dependent or ion-concentration-dependent changes of fluorescence signals as a result of ionic flux. Because fluorescence-based methods produce a robust and homogeneous cell population measurement, these assays are similar to those for other protein classes. Therefore, more instrument choices and expertise are available. Consequently, these assays are relatively easy to implement and to optimize to achieve a higher throughput.

Voltage-sensitive dye assays

Fluorescent voltage-sensitive dyes measure voltage changes across the cellular membrane using either the potential-dependent accumulation and redistribution[18] or the fluorescence resonance energy transfer (FRET) mechanism[19]. Oxonol derivatives, such as bis-(1,3-dibutylbarbituric acid) trimethine oxonol [$DiBAC_4$ (3)], are lipophilic and negatively-charged dyes that display an increased quantum yield in an aqueous environment upon binding to hydrophobic intracellular molecules instead of weak fluorescence. Because the change in fluorescence occurs minutes after the change in membrane potential, these dyes are best suited to detect a steady state instead of kinetic changes of membrane potential. These dyes cannot detect second and sub-second resolution changes of membrane potential. In contrast, the FMP dye, another anionic membrane potential dye from the FLIPR Membrane Potential Assay Kit (Molecular Devices, Sunnyvale, CA, USA), provides a faster response time (in tens of seconds) than $DiBAC_4$ (3)[20] and is good for detecting kinetic signal changes.

The lipophilic character of $DiBAC_4$ (3) and the FMP dye is associated with a lack of membrane selectivity, which indicates that these voltage-sensitive dyes can respond to a membrane potential change from both the plasma membrane and the endo-membrane. For FRET-based assays, the negatively charged, membrane-soluble oxonol dyes (bis-(1,3-dialkylthiobarbituric acid) trimethine oxonol [$DiSBAC_n$ (3)]), are used as voltage-sensing FRET acceptors. The FRET donors are coumarin-tagged phospholipids (CC2-DMPE) that are integrated into the outer leaflet of the membrane when loaded into the cells. An increase or decrease of FRET in response to membrane hyperpolarization or depolarization produces fast, ratiometric changes. The ratiometric nature of the assay helps to eliminate many artifacts associated with DiBAC assays. Unlike DiBAC assays, the use of phospholipid-anchored FRET donor restricts the location of FRET in the plasma membrane, which ensures that the measurement of potential changes occur at the cell membrane rather than in other subcellular compartments, such as the mitochondria. In addition, FRET-based voltage sensors produce sub-second temporal resolution, which allows for kinetic reading. Because the mobile oxonol molecules are charged, a dye-concentration-dependent dye-current may interfere with the change of membrane potential caused by the ionic current through the ion channels, especially when the current is less than a few hundred pico-amperes. One way to reduce this interference is to use less dye, which may cause a decrease in the signal-to-noise ratio if the dye concentration is below a certain limit.

Ion-specific fluorescent probes

The assays that measure intracellular ionic concentrations are widely used in research and pharmaceutical screenings for ion channels. Different ion-specific synthesized probes have been developed, such as calcium indicator dyes (Fura-2, Fluo-3, Fluo-4[21, 22]), potassium indicator dyes (FluxOR[23, 24] and PBFI[25]) and sodium indicator SBFI[26]. In addition to the

synthesized ion indicator dyes, a genetically-coded fluorescent protein can be used as an indicator of ion channels. Specifically, mutations of YFP (H148Q and I152L) further increase the YFP-halide sensitivity, which is suitable for chloride-involved channel assay[27–29]. These indicators are usually used in conjunction with a FLIPR-type kinetic fluorescent reader to achieve high throughput, low-noise detection of both absolute levels and changes of cytosolic corresponding ionic concentrations.

Overall, ion channel assays that use ion indicator dyes are largely limited to the availability of high performance ion-specific indicators. To date, calcium indicators are most extensively utilized and provide a robust performance that can be used for HTS of calcium channels or non-selective cation channels[30–33].

Automated electrophysiological assays

Patch-clamp has been widely considered as the gold standard to directly record ion channel activity. This technology provides high quality and physiologically relevant data of ion channel function at the single cell or single channel (within a small patch of membrane) level. For pharmacological testing of compounds, it provides a standard for measuring the potency of compound–channel interactions. Although the conventional patch-clamp offers a direct, information-rich and real-time method to study the channel function, it has very low throughput and a labor-intensive nature, which requires highly skilled and trained personnels. Over the last decade, the development of an automated planar patch clamp has been a breakthrough. Many automated electrophysiology platforms have been developed and are commercially available. They either provide a giga-ohm seal quality of data comparable to a manual electrophysiology recording, or they rely on the use of a perforated patch clamp technique with higher throughput that does not compromise data quality and pharmacology.

The IonWorks platform was the first commercially available automated electrophysiological screening platform to gain widespread utility and validation in the field. It is now available in its second generation, the IonWorks Quattro™, and a more recent generation, the IonWorks Barracuda™[34–36]. The system operates in a 384-well format in two modes, single-hole mode and population patch clamp mode (PPC). In single-hole mode, one cell per well is recorded in a 384-well plate. In PPC mode, the instrument reports average currents from recordings of up to 64 cells per well. The data recorded using single-hole mode achieved a lower success rate with a considerable well-to-well variability. However, it is the preferred mode for clonal screening and selection during cell line development[37]. PPC mode provides an improved data consistency and success rate in the measurement of ionic currents[34]. A common limitation has been observed for lipophilic compounds with right-shifted potencies due to the use of plastic materials for the plates[36, 38]. However, with good assay optimization, an acceptable correlation between compound potencies derived from IonWorks assays and manual patch clamp electrophysiology can be achieved[36, 39]. The system can not only be utilized to voltage-gated channels but also for fast-desensitizing ligand-gated channels[38, 40]. In addition, ion channel-targeted drug-discovery efforts on a number of diverse ion channel subtypes have benefited from the availability of the IonWorks platform with robust assay properties[35, 40-46]. Thus, the major limitation for Ionworks systems is to establish giga-ohm seals.

To gain a giga-ohm level of seals, several planar array-based automated electrophysiology systems have been developed to incorporate the precision and accuracy of manual electrophysiology recording. The platforms provide a giga-ohm seal with compromised low throughput. However, the platforms have gained prominence and acceptance in the pharmaceutical industry. Some of these platforms are PatchXpress and IonFlux (Molecular Devices, LLC, Sunnyvale, CA, USA), QPatch HT/HTX (Sophion, Copenhagen)[47, 48], and Nanion's Patchliner and SynchroPatch (Nanion Technologies GmbH, Munich)[49]. The rapid solution exchange time (50–100 ms) and perfusion capability of these systems have made them amenable for ligand-gated ion channel investigations. Furthermore, Nanion's Patchliner offers a primary cell recording capability with both the voltage clamp and current clamp recording modes. Recently, automated patch clamp instruments with a much higher throughput and giga-seal have been launched that bridge the gap between the high-throughput and high quality of ion channel assays, including Sophion's Qube (384-well) and Nanion's SyncroPatch 384PE and 768PE.

High-throughput electrophysiology has many theoretical advantages and holds much promise. The continued evolution of existing and new platforms for automated ion channel screening will keep up with the demand both for ion channel safety profiling and for ion channel-targeted drug discovery.

Prevalent HTS methods for the specific ion channel families

Before choosing the ideal screening method(s), it is important to determine what to look for when comparing technologies and their applications. Eight parameters commonly considered include sensitivity, specificity, throughput, temporal resolution, robustness, flexibility, cost, and physiological relevance. Among all the assay formats of ion channels, undoubtedly, the automated patch clamp assay is the best choice that provides a good quality of data and allows a higher throughput. Currently, an automated electrophysiology assay remains expensive. Thus, not every laboratory can afford it. Therefore, as a compromise, a combination of fluorescence-based screening technologies and an automated patch clamp has become the most commonly used method for ion channel-targeted drug discovery. With cost reduction and technology improvement, automated electrophysiology will become the dominant assay format for most ion channel subtypes. For different ion channel subclasses, the high-throughput screening methods differ due to ion selectivity, channel activation kinetics and a consideration of whether a ligand is needed. For a selected ion channel target, the flowchart of the high-throughput screening is summarized in Figure 1. It is divided into 3 stages: a

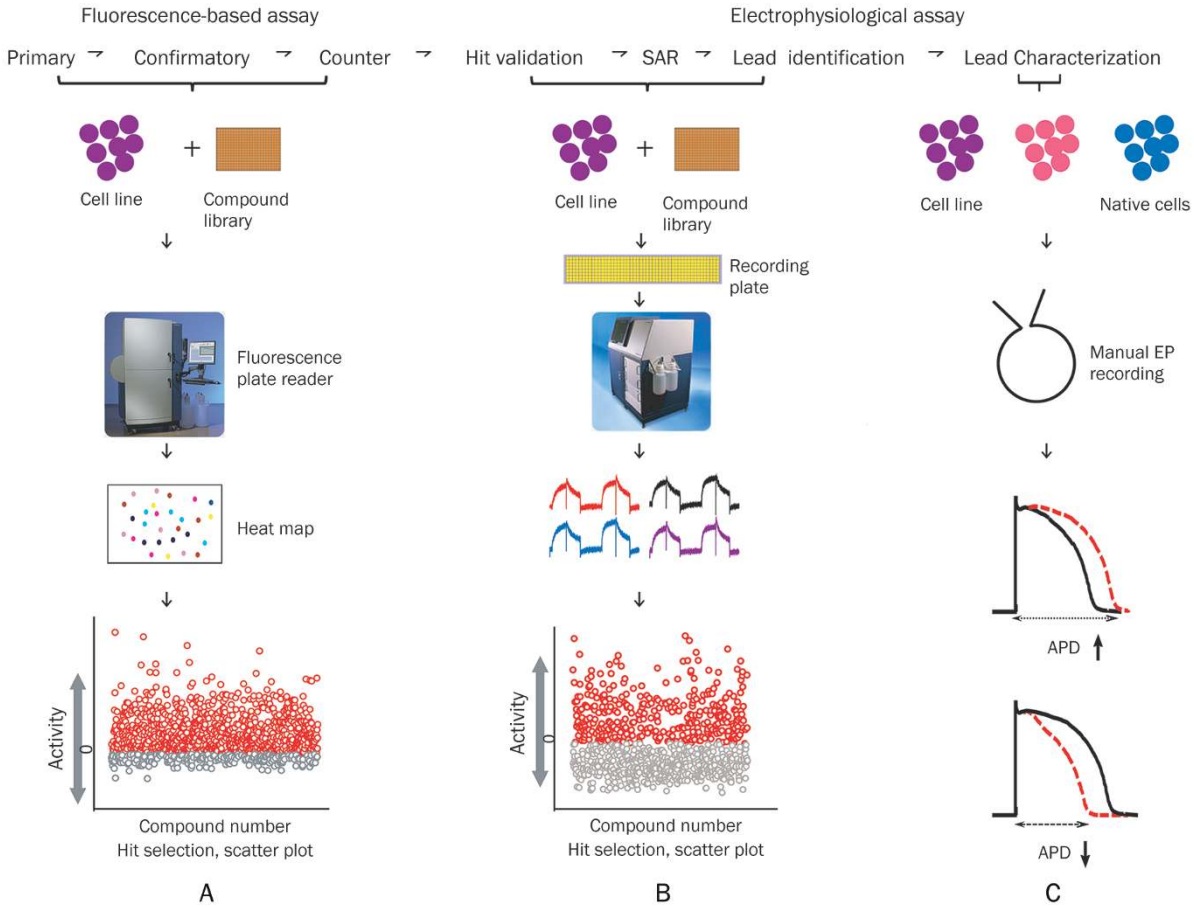

Figure 1. Pipeline of high-throughput screening targeting ion channels. (A) For a large compound library, the compounds are first screened using the fluorescence-based flux assay on an ion channel-expressed stable cell line, and the identified hits are confirmed on the same cells and are counter-screened on the parental cells to exclude non-specific hits. (B) Second, active hits are tested using an automated patch clamp for validation and, the active hits are pharmacologically evaluated for structure-activity relationship (SAR) until lead compounds are identified. (C) Finally, a manual patch clamp is used to characterize biophysical properties on stable cell lines and native cells.

fluorescence-based assay for primary screening, an automated patch clamp validation for secondary screening and a manual patch characterization for tertiary screening. Because there are many ion channel families and subclasses available, the most commonly used screening methods will be discussed based on ion selectivity or permeability. The diagrams for the representative screening methods are displayed in Figure 2.

Potassium-selective channels

Potassium-selective channels are the largest and most diverse group among the ion channel families. The classes of channels include voltage-gated (K_v), inward-rectifying (K_{IR}), two-pore (K_{2P}) and Ca^{2+}-activated (K_{Ca}) potassium channels. Multiple assay formats have been applied to this large family, including the ligand binding assay, $^{86}Rb^+$ flux assay, voltage-sensitive dye-assay and Tl^+ flux assay. Among them, the Tl^+ flux assay is most often used to identify potassium channel modulators[24]. In this assay, Tl^+ is used as a surrogate ion for K^+, and its influx into cells is initially measured using a thallium-sensitive fluorescent dye benzothiazole (BTC)[24, 50], which is a Ca^{2+} indicator with a low Ca^{2+}-binding affinity (K_d=7 μmol/L). Using a commercially available thallium assay kit (FluxOR™, Life Technologies), the method has been successfully developed for a number of potassium channels. The Tl^+ flux assay has been extensively used in potassium channel screenings, such as K_v[24, 51-56], K_{IR}[57-59], K_{2P}[60-62] and K_{Ca}[63, 64] channels. It should be noted that a variety of off-target pathways (*eg*, Na^+/K^+ ATPase) from the native HEK-293 or CHO-K1 (these two parental cell types are most commonly used) cells could interfere with the Tl^+ influx, which will cause a higher false-positive or false-negative hit rate. Therefore, a counter screening against parental cells is necessary to eliminate the false hits interacting with parental cells. In addition, the assay should be handled with great care due to toxicity of thallium.

Ca^{2+}-involved ion channels

An intracellular calcium ion (Ca^{2+}) is a universal second messenger that controls both physiological and pathological processes. Among the mentioned ion indicators, calcium indicators are most commonly used[65] because they alter their fluorescence

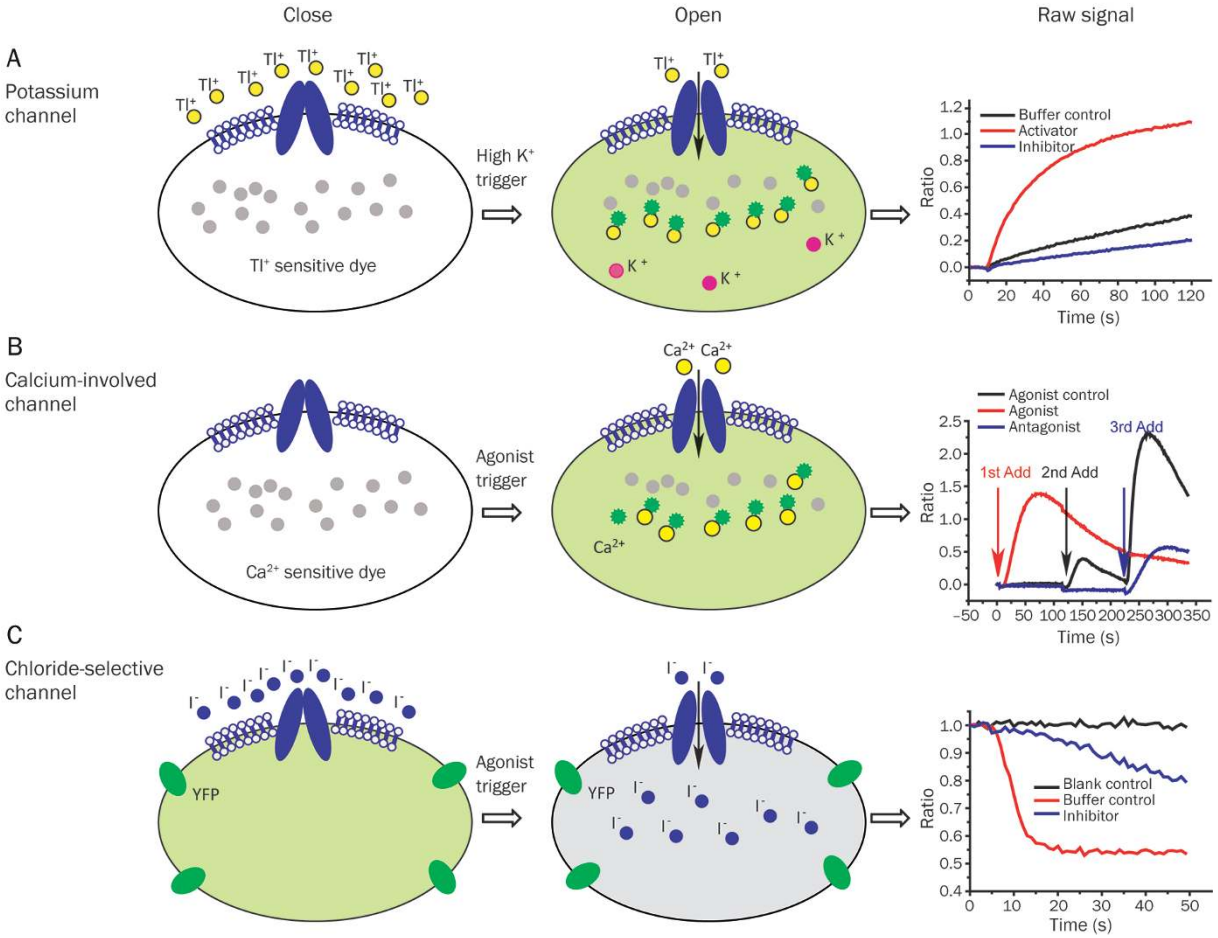

Figure 2. Diagram of high-throughput screening methods. (A) Diagram of the thallium-flux assay for potassium channels, closed state, open state and the raw signal. For a potassium channel, the Tl^+-flux assay is the most commonly used assay format. To trigger the channel to open, typically a high-K^+ solution is used to depolarize the membrane potential. Then, thallium passes into the cells through open potassium channels. Upon the cytosolic thallium binding to the dye, the fluorescence signal is kinetically increased. The raw traces were acquired from the primary screening of KCNQ2 potassium channels. The black, red and blue raw traces represent the effect of a buffer control, an activator and an inhibitor, respectively. (B) Diagram of calcium-flux assay for calcium-involved channels. Fluo-4 is the most commonly used calcium indicator for calcium-involved channels. To trigger the related channel to open, a high-Ca^{2+} or a channel agonist is used to open the channel or to increase cytosol Ca^{2+}. The raw data traces were acquired from the primary screening of the TRPC4 channel. To screen for multiple classes of modulators, three additions were applied for the same well. The first addition is for the screened compounds without DAMGO (a highly selective peptide agonist for the μ opioid receptor) to screen for agonist. The second addition is an EC_{20} concentration of DAMGO for allosteric modulators. The third addition is an EC_{80} concentration of DAMGO for antagonists. Black traces represent compounds without any effect to the three additions. Red traces represent compounds with an agonist effect. Blue traces represent compounds with an antagonist effect. (C) Diagram of chloride-selective channels. Mutated YFP (H148Q and I152L) was co-expressed with chloride-selective channels. When the channel opens, iodide enters the cells via chloride channels, binds to YFP and quenches its fluorescence. The raw data were acquired from the primary screening of a calcium-activated chloride channel (TMEM16A). Ionomycin was used as an agonist to activate the channel. Red trace represents the ionomycin-activated channels. Blue trace represents the effect of an inhibitor.

emission upon calcium binding. Currently, over one hundred chemically synthesized and genetically encoded indicators are available[66]. Chemical indicators that are most commonly utilized include Fura-2, Indo-1, Fluo-3 and Fluo-4, which are the derivatives of a Ca^{2+} selective chelator BAPTA[30, 67].

Depending on the application, calcium dyes are available in a range of affinities to calcium ions[68], excitation and emission spectra, and chemical forms (membrane permeable or not). They show different temporal resolution (from milliseconds, such as Fluo-3 and Fluo-4, to tens of seconds, such as Fura-2), and different degrees of accuracy for each range of calcium concentrations. For example, Indo-1 is preferable to Fluo-3 for measuring large and relatively slow intracellular calcium transients that are associated with cellular contraction. In contrast, Fluo-3 is preferred for measuring small, fast transients that are associated with calcium "sparks"[69]. Therefore, Fluo-3 (including its derivative Fluo-4) is more suitable for detecting Ca^{2+}-involved ion channel signals. For the high-throughput screening of Ca^{2+}-involved ion channels, commercial kits, such as the Fluo-4 calcium assay kits (Life Technologies), are avail-

able. They are extensively applied in Ca^{2+}-involved ion channel assays, such as voltage-gated calcium channels[33, 70], TRP channels[71-75], and NMDA receptors[76, 77].

Furthermore, calcium channels exist among closed, open and inactivated states. To distinguish the state-dependent inhibitors, usually an inward rectifier potassium channel gene (*eg*, Kir2.3) is co-expressed with calcium channels. Thus, the membrane potential can be adjusted by altering the external K^+ concentrations. This approach has successfully been used to identify state-dependent inhibitors and to characterize the molecular selectivity, even offering some advantages over electrophysiology[33, 41]. The strategy can also be applied to sodium channels. Calcium dye-based ion channel assays suffer from interference from other cellular processes that produce changes in the intracellular calcium concentration. The counter screening for the same assay has to be run against parental cells to remove the nonspecific compounds.

Voltage-gated sodium channels

Voltage-gated sodium channels are important targets for treating excitable diseases, such as epilepsy and neuropathic pain. It is known that voltage-gated sodium channels exist in closed, open and inactivated states. Additionally, for the sodium channels expressed in HEK-293 cells, the membrane potential is at a more depolarized level. Taking sodium channel subtype Nav1.7 expressed in HEK-293 cells as an example, the averaged membrane potential was measured to be approximately –24 mV, an averaged value from a panel of approximately 50 cells (data not published). Thus, this will drive most channels into inactivated states instead of their real "resting states". Therefore, a known inhibitor of Nav inactivation, veratridine[78], is commonly used to drive the channels open, which produces a more robust signal.

Ideally, ion-selective indicators are perfect to assay the specific ion channels. However, the available Na^+ sensitive dyes (*eg*, SBFI[26], CoroNa dyes[79] and Asante Natrium Green-2[80]) are not well-suited for high-throughput screening due to low sensitivity and a poor signal-to-background ratio. The fluorescence-based membrane potential dye assay (*eg*, DiBAC and FRET dyes) is often used for sodium channel screenings. For the assay itself, the signal change is primarily affected by the membrane potential. Therefore, any event that changes the membrane potential modulates the signals. Thus, the dye-based method may yield a relative high false-positive and/or false-negative rate when compared with the electrophysiology methods. Recently, a thallium flux (Tl^+ flux)-based method[81], a valuable technique for potassium channels, has been successfully developed as a functional assay for Nav1.7 sodium channels. Tl^+ flux methods produce dramatically larger signals, which are superior to the state-of-the-art Na^+-sensitive dyes and are amenable for HTS of sodium channels. The application of the ligands (*eg*, veratridine) may interact with test compounds and thus increase the false-positive or false-negative rates. In addition, a newly developed calcium flux assay (SoCal assay)[82] has been used as a readout of functional change of Nav channels. In this assay, Nav channels were genetically engineered to produce persistent Nav currents with impaired fast inactivation and enhanced calcium permeability. Thus, the calcium reporters, including both the chemical dyes and genetically encoded sensors, would be the alternative indicators of Nav channels. Although the SoCal assay demonstrates a good estimation of activity for the vast majority of tested compounds, it is still recommended that the hits should be further validated using electrophysiological method in wild-type Nav channels. Due to the limitation in controlling the state of sodium channels compared with electrophysiology, care should be taken when interpreting the inhibitor data from the fluorescence-based approach.

Chloride-selective channels

For chloride channels, the yellow fluorescent protein (YFP) quenching assay has been developed[27]. Initially, YFP-H148Q had a higher selectivity for iodide (I^-) than chloride (Cl^-) due to the halide binding properties of YFP[27]. However, H148Q required substantial concentrations of iodide to produce an acceptable signal, which would manifest cell toxicity. A random mutation approach, YFP-I152L, has a significantly increased sensitivity to iodide[27, 29]. Thus, YFP (H148Q and I152L) combined with iodide was widely used for the chloride channel screening. Upon chloride channel activation, I152L enters the cells, binds to YFP and quenches its fluorescence. Agonist-dependent quench of YFP fluorescence can then be measured with a fluorescence reader and used to determine channel activation, inhibition and modulation. The YFP assay is a noninvasive technique that measures fast responses. The assay has been widely developed for chloride selective channels and receptors, including CFTR[83, 84], calcium-activated Cl^- channels (CaCC) (TMEM16A)[85-88], Glycine receptors[89] and GABA receptors[89, 90]. Additionally, the voltage-sensitive dye method can be used to assay the chloride channels but is rarely used for this class of channels.

Because membrane potential cannot be controlled as electrophysiological assays in the abovementioned fluorescence-based assays, the rationale is to improve the signal-to-noise ratio during the assay development of the HTS. In general, there are two approaches to improve the signal-to-noise ratio: increase the signal or decrease the noise. The signal can be increased by creating conditions under which more channels are opened. This can be achieved by applying high K^+ to induce membrane depolarization or by applying ligands to drive the channels into more activated states. The noise can be effectively decreased using non-physiological surrogate ions, such as thallium for K^+ and iodide for Cl^-. These surrogate ions are almost non-existent within cells and their noise level is therefore very low. Even a tiny amount intracellular change of these surrogate ions can be easily detected.

Conclusion and perspective

Overall, progress and improvements in ion channel HTS technologies have sped up ion channel drug discovery. Detection of ion flux signals can be achieved using fluorescence indicator dyes and a fluorescence plate reader, such as the Fluorometric

Imaging Plate Reader (FLIPR^{Tetra}, Molecular Devices) or FDSS (Hamamatsu Photonics). These assays have relatively low temporal resolution and information content but enable robust and low cost. Electrophysiological methods have the most direct approach to measure ion channel activity and additionally allow flexibility in assay optimization for each channel type. The combination of non-electrophysiological and electrophysiological HTS methods provides an integrated and cost-effective approach for ion channel drug discovery and ensures high quality of data to be generated.

The development of ion channel screening technologies has met most needs for drug discovery. Significant instrumentation development efforts continue to improve the capabilities of automated electrophysiological instruments, which are being used for more ion channel classes and cell types. Emerging trends focus on the exploration of reagents and the development of strategies that may be applied to the screening process of ion channels, including the highly expressed ion channel stable cell lines, sensitive and specific indicators and optimized screening strategies. Expression systems, including off-the-shelf reagents, enable new opportunities for the existing instrumentation. Recently, a number of new products have emerged for the cell line development, including the MaxCyte STX electroporation instrument[91], BacMam system[51] and Jump-In™ Cell Engineering Platform[92]. These products are expected to enhance the stable cell line development process and expedite the screening for drug discovery, bioproduction, and cell-based therapy. Because a constitutive high-level expression of ion channels may cause cell toxicity for some ion channel classes and lead to a drop of the expression level after some passages, the alternative is to use an inducible system that can decrease the toxicity and guarantee long-lasting gene expression. It has been suggested that inducible expression systems should be routinely used because the preparation of a stable cell line is a time-consuming process.

Drug discovery is a slow and complicated process. Identification of the active ingredient is the first and critical step for almost all drug targets. All of the screening-related work is geared towards the first step. Researchers may be interested in searching for multiple classes of chemo-types of ion channel modulators, such as orthosteric modulators, allosteric modulators, or competitive antagonists. Therefore, a flexible design of strategies is encouraged to meet researchers' needs. This flexibility dramatically enhances the possibility of success. Additionally, data analysis and management are also important and critical aspects of the HTS process, especially when analyzing a large amount of data across different assays and targets.

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