An Interview with Min Li, Ph.D.

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Dr. Min Li is Professor of Neuroscience and Director of the Johns Hopkins Ion Channel Center. He was born in China and came to the United States in 1985 for graduate school. He received his Ph.D. in molecular biology and genetics from Johns Hopkins University and carried out his postdoctoral training in neuroscience at the University of California, San Francisco. He worked at Affymax Research Institute on peptide and antibody display technologies after post doctoral research. In 1994, he returned to join the Johns Hopkins faculty. His research has focused on chemical and molecular regulation of membrane excitability, drug regulation of ion transport, and technological development for high-throughput ion channel screening. Work by his laboratory has provided considerable mechanistic insights into subunit assembly and macromolecular organization of ion channels. He is the author of more than 70 research articles and inventor for 12 patents on ion channels.

Dr. Li, what is inherent to ion channels as a class that makes it so challenging to develop assays to study their function?

Most importantly, Ion channels as a class function by allowing passage of inorganic ions, which results in a small electrical current. In contrast, other biological events tend to be slower and are often coupled to an amplification such as diffusible second messages. Ion movement from one side of the membrane to another generates transient current and there is no amplification. This combination of a small, transient electrical signal with little or no amplification represents the biggest challenge. The second major challenge is that in their physiological context many ion channels are gated by a common factor, voltage. Most human cells have several classes of voltage-gated ion channels. In heart cells, for example, there are specific ion channels for sodium, calcium, and potassium, and these are all gated by voltage. Comparatively, imagine if all G-protein–coupled receptors (GPCRs) were gated by one ligand; how could you distinguish one from another?

Nature has created an amazing repertoire of ion channels, many of which are sensitive to voltage. While challenging, they are fascinating protein machines. The way ion channels sense the voltage occurs at different thresholds of membrane potential. These channels open and close with an amazing repertoire of properties. If one imagines that a channel is like a door, then several questions come to mind: how much energy is required to open it; once open how long will it stay open; and when it begins to close how long will it take to shut? Because of the differences in gating and kinetics, you can apply a single voltage that is supposed to open, say, five different channels, but instead you get a beautiful cascade of "door" openings and closings, like a symphony orchestra. The cohort of different channels open and close at different times, creating an ensemble of currents across the cell membrane that carries the signature of a particular cell type in response to a given physiological stimulus, which in this case is membrane potential change.

When you think about developing an assay, you have to consider the target channel of interest and how to analyze it in the appropriate context so you don't end up with an assay that works just fine in the "shooting range," so to speak, but does not work when you go to the "forest" because there is higher background that masks the target of interest. A major reason there is a need for a special focus on ion channel assays is that they are a very important class of targets, but require specialized tools for measuring them and particular expertise for analyzing channel activity. Most importantly, they require a device capable of electronically detecting cellular current known as a patch clamp.

In your laboratory you investigate native ion channel complexes, their organization and regulation, and novel compounds capable of inhibiting or activating potassium channels. In your opinion, does ion channel screening need to be done at a specialized center? What are the advantages/disadvantages of this approach?

My laboratory has been working on ion channels for many years. We are particularly interested in the macro-organization of ion

channel complexes. As a result of our investigations, and of course, as well as research performed in other laboratories, it has become clear that a chemical probe that modulates specific classes of ion channels would be very useful for understanding the role of an ion channel in its native context. We know there are many potential active compounds in nature, such as animal toxins and plant extracts. In addition, there are large libraries of synthetic compounds with diverse chemical structures. Some of the known compounds and toxins, such as tetraethylammonium (TEA) and tetrodotoxin (TTX), are extremely useful tools and play a vital role in our understanding of ion channels. Both were serendipitously discovered many years ago. Indeed, there is no question that it would be ideal to have a systemic approach for isolating compounds such as TEA and TTX; that would be a useful tool. However, because of the unique technological requirements to measure channel activity, ion channels as a class need a dedicated enterprise to focus on target selection and analysis. This focused effort can also take advantage of the technology developed over the past few years for analyzing ion channels in high throughput. Currently, the hardware alone, in terms of its cost and the engineering expertise needed to operate the technology, as well as the informatics, are not within the budget or capabilities of an individual laboratory. Because ion channels are more difficult to study compared with other targets, there is the potential for them to receive less attention. But they are so important that there is a need to focus on this class through diligent efforts and continued investment. This view is also highlighted by the current market size of existing drugs that target ion channels. In the late 1990s, ion channels captured 20%–25% of the total market, and that range probably remains about the same today. Fortunately, or unfortunately, because of the challenges associated with developing small molecules, ion channels have not been developed as prominently as some other drug classes such as GPCRs, kinases, and maybe proteases. If you think about those classes, they share considerable similarities in assay technologies, whereas ion channels are distinct. So there are considerable opportunities if the costs and technologies for studying ion channels are more manageable.

As recently as 5 years ago we did not even have the hardware to run ion channel assays at the throughputs possible for kinases, for example. But now there are several platforms available, most notably Ionworks Quattro and Q-Patch, that are capable of performing high- and medium-throughput ion channel screening using automated patch clamp. Having an ion channel screening center as a dedicated enterprise in an academic setting to capitalize on the rapid pace of technological advances is necessary to support development of this strategically important target class. It is also interesting that during the pilot phase of the molecular library initiative, prior to 2008, there were very few screens that targeted ion channels, but a huge number of compounds and primary screens developed for other target classes. That speaks volumes about the need for a specialized and dedicated expertise for this important but difficult target class.

In terms of any disadvantages, I would say that ion channels, along with a couple of other target classes such as infectious diseases (for which the work has to be done in a specialized environment to prevent the risk of disease spread), still require a lot of nurturing. For ion channels you need to prepare cell lines for the targets of interest, and, in some cases, this presents considerable challenges. We have worked very hard to get the community to participate and provide the channels of interest. Luckily, we have received many cell lines and have benefited from collaborative efforts by colleagues in the ion channel community. A majority of our projects are collaborations with non-Hopkins laboratories. During the pilot phase of MLPCN program until October 2008, there were 10 national centers which generated 79 reports in PubChem for large scale screens (>150,000 compounds). But none of them was for an ion channel target. Since the inception of production phase after October 2008, there are 148 reports for screens of more than 300,000 compounds. Among them, ten are for ion channel targets; nine of them were from Hopkins Ion Channel Center (JHICC).

At the Johns Hopkins Ion Channel Center the hardware now in use allows us to screen electrophysiological properties, namely the current of a given ion channel in a throughput of almost 10,000 compounds per day, depending on the duration of recording. This is particularly exciting when you consider that for a typical receptor or enzyme you measure its activity for maybe a second or a minute, but for ion channels there is a special benefit to being able to measure their activity in a variety of kinetic contexts ranging from milliseconds to minutes. The hardware actually allows us to screen many more compounds per day, but because we can measure the activity over a period of 20 minutes—typically generating 100,000 data points per compound—the throughput seems to be limited, though it is quite adjustable depending on the purpose of the screen.

I should add that electrophysiology represents the earliest high content assay ever developed. In the 1950s people were able to measure a current across the cell surface in a squid giant axon, but they did not know what was producing that current. They were very aware of this current, with its characteristic, reproducible, and temporal behavior over time in response to changes in voltage. They did not realize, however, that they were actually seeing the molecular basis of how nerves send signals from the brain to various nerve terminals as we sense our environment, and that the signals are all triggered by these electric circuits. Ironically, TEA and TTX were the key tool compounds that allowed the research teams of Clay Armstrong and Bertil Hille to propose and ultimately demonstrate that the current was mediated by an ionic conduction pore, or ion channels.

How would you summarize some of the key advances in ion channel assay technology of the past few years?

Three important factors have motivated technology development. First, from the 1950s it became progressively clear that all biological samples, when measured with patch clamp, have a detectable ion current. The second most important advance was the completion of the Human Genome Project (HGP) and the ability to link ion channels to disease states. Without the HGP one could not appreciate how many ion channels and transporters exist. For example, when the sequence of *Escherichia coli* first became available, many people were surprised at the number of transporters relative to other proteins. Later we came to realize that unicellular systems survive by exchanging nutrients and toxic materials with the surrounding en-

vironment through an elaborate system of transporters. The third factor is the validation of the causal involvement of ion channels in genetic diseases and their critical roles in drug development.

Validation of ion channel targets in general has been catalyzed by the well-known harmful activity of the human *Ether-à-go-go* related gene (hERG) potassium channel, which appears to be a target for cardiovascular toxicity. The basic observation is that about 5–15% of compounds in a naïve collection are inhibitory to the hERG channel. So drugs and compounds in development have a high probability to block hERG. This laid the foundation for the need to catalyze the development of high-throughput technology to study ion channels. While we had been able to do this manually, the throughput was not sufficient. So the most important technology development has been the automated patch clamp using the planar electrode-based recording. A typical patch clamp comprises a glass electrode containing a tip that punctures the cell membrane to create a circuit. A planar electrode works differently; it is a flat surface on which a tiny hole is created using a laser or etching technique. One engineers a hole in the well of a microtiter plate and positions the cell to sit directly above the aperture; when you create a seal between the cell and the hole, the area underneath the well will serve as the extracellular compartment. When the membrane is ruptured the intracellular compartment will be connected to the buffer underneath the plate. In this way you can record many different cells and testing components across a microtiter plate.

During the past few years, this technology has been applied to cell lines and has also become applicable to native cells, allowing us to transfer the technology from the "shooting range" to the "forest." There is also emerging label-free technology that measures cell behavior in response to stimuli and can record the opening and closing of ion channels based on a reorganization of the mass distribution or cellular impedance. In this case you do not have to create a seal, you essentially measure a proxy of the cellular signature in response to the channel opening or closing. One could argue that it is difficult to determine what part of the channel activity you are measuring. As I mentioned earlier, with a voltage-gated channel, you have only one type of ligand, which is the voltage change, so you need to be able to distinguish between different effects. It is too early to tell whether the label-free technology can do this. Nevertheless, this could provide an exciting high-throughput proxy readout of channel activity.

What is the structure of the prototypical potassium channel and how does the structure vary from one subtype to the next?

The potassium channel is an exciting class. All ion channels can be compared to a door, with two major components: the pore, which allows the ions in and out, is like the part of the door that opens and closes; and the gating machinery, which is analogous to the hinges of the door in combination with a key that can unlock the door. The pore has two very important functions—to determine ion selectivity and the rate of ionic flow. Potassium channels are one of the most selective channels. A potassium channel might allow thousands of potassium ions to pass without allowing a single sodium ion to pass through. We are talking about two very similar monovalent ions, so you can imagine the exquisite level of ion selectivity.

The channels have a series of binding sites that fit potassium beautifully but fit sodium poorly, allowing them to distinguish one ion from the other. Interestingly, nature has evolved the system allowing the channels to differentiate one physiological ion from another despite their anatomic similarity. Even though the potassium channel has this amazing selectivity over sodium, it will let a rubidium or thallium ion go through; note that they are non-physiological ions absent in animal cells. This has provided opportunities to develop surrogate assays for potassium channel activity; for example, a fluorescent dye for thallium can be used to monitor channel activity based on the fluorescent signal.

For the gating component, several factors affect function. Channels can be gated by voltage (a membrane potential change), by ligand binding (a small physiological metabolite), or by mechano-sensing (the physical configuration or stretching of the channel that allows it to open or close). Also, a certain kind of algae uses light sensing to control the opening and closing of ion channels. The "hinges" convey an important property related to the kinetics of the channel. When a channel opens, even if the "key" remains in the "lock," the door may only open transiently and then become nonconductive because the door is blocked by another intrinsic "blocker." This process is known as inactivation and it is a very important part of its properties. A neuron, for example, might not want too many ions going in and out at one time to limit propagation of the signal. So it may have a very rapid, transient opening and closing event during a prolonged depolarizing period. This is not true closing; it is simply an inactivation event. In the cardiovascular system, for example, each heart beat is a single depolarization event that involves 8 to 11 different channel classes, all opening and closing in a symphonic fashion.

How many distinct potassium channels have been identified in humans and how does the function of a given subtype vary from one tissue to another?

Potassium channels are found in almost all tissues. According to the International Union of Basic and Clinical Pharmacology (IUPHAR) database, at least 78 human genes encode protein subunits that form the pores of potassium channels. Among these, 40 genes encode voltage-gated potassium channels. The expression of different subsets of genes determines the overall potassium conductance in a cell. The channels differ primarily based on how they gate, which can be influenced by multiple factors including sensitivity to voltage, intracellular second messages, post-translational modifications, and association with other regulatory proteins. These channels all select for potassium ions, but their "doors" differ in properties such as unitary conductance, inactivation, and, of course, voltage sensitivity.

What other factors contribute to the diversity of the potassium channel family?

In almost all potassium channels the pore is an oligomer. In other words, more than one subunit is needed to form a functional door. In

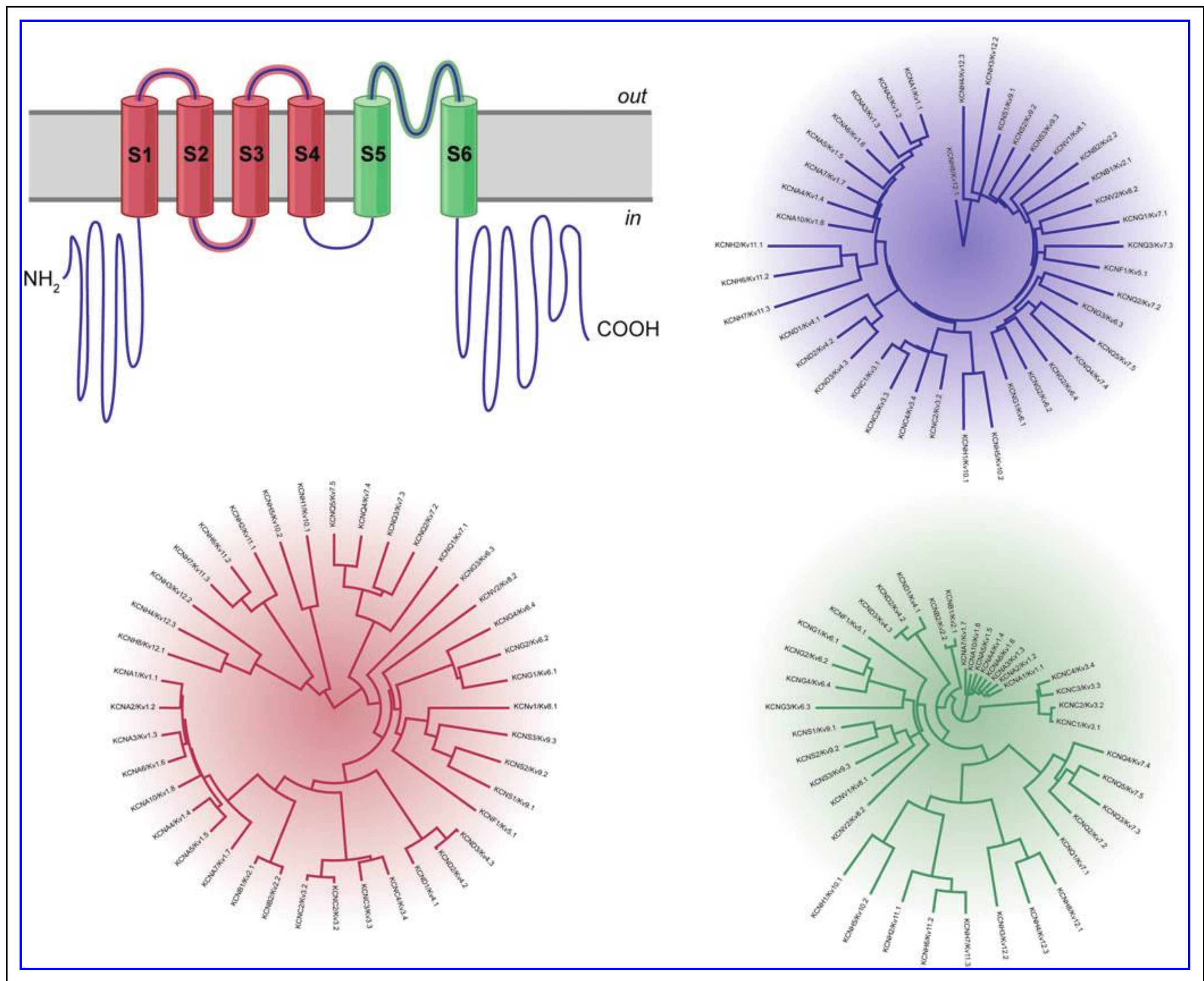

Fig. 1. Schematic topological diagram of a typical voltage-gated potassium channel. The helices that form the voltage sensing domain (VSD) are highlighted in red. The pore helices are highlighted in green. The three circular diagrams display phylogenetic relationships of amino acid sequences corresponding to VSD (red), pore region (green) and full length channel protein (purple).

the late 1980s to early 1990s it was discovered that potassium channels selectively form oligomers through a mix-and-match mechanism. If, for example, a channel has an A and a B subunit, there can be several possible tetramers: A_4, A_3B_1, A_2B_2, A_1B_3, or B_4. Imagine if you could tweak the expression of A and B in a cell; then you could dramatically change the properties of the channel if A and B have different gating phenotypes. For instance, A may be able to inactivate but B cannot. This provides for a tremendous continuum of biophysical properties through regulation of the transcription of A and B. This exciting mix-and-match property is not present for voltage-gated sodium or calcium channels, because their pores are formed by one subunit.

Another factor relates to the pore lining and other components that participate in the gating process, such as the nonpore regulatory subunit through which a protein can regulate pore activity. Post-translational modification of a channel can also affect its properties. Additionally, it is important to point out that because amplification is not part of the channel's properties, the subcellular location of the channel plays a critical role in its function, especially in the nervous system. A typical neuron's axonal terminal has a dramatically different potassium channel organization compared to its dendritic terminal. The dynamic redistribution of channel localization, known as channel trafficking, is an important biological process. Another aspect that contributes to the diversity of potassium channels is based on recent evidence suggesting that noncoding RNAs affect the channels, altering their expression and stability by regulating the half-life of transcripts.

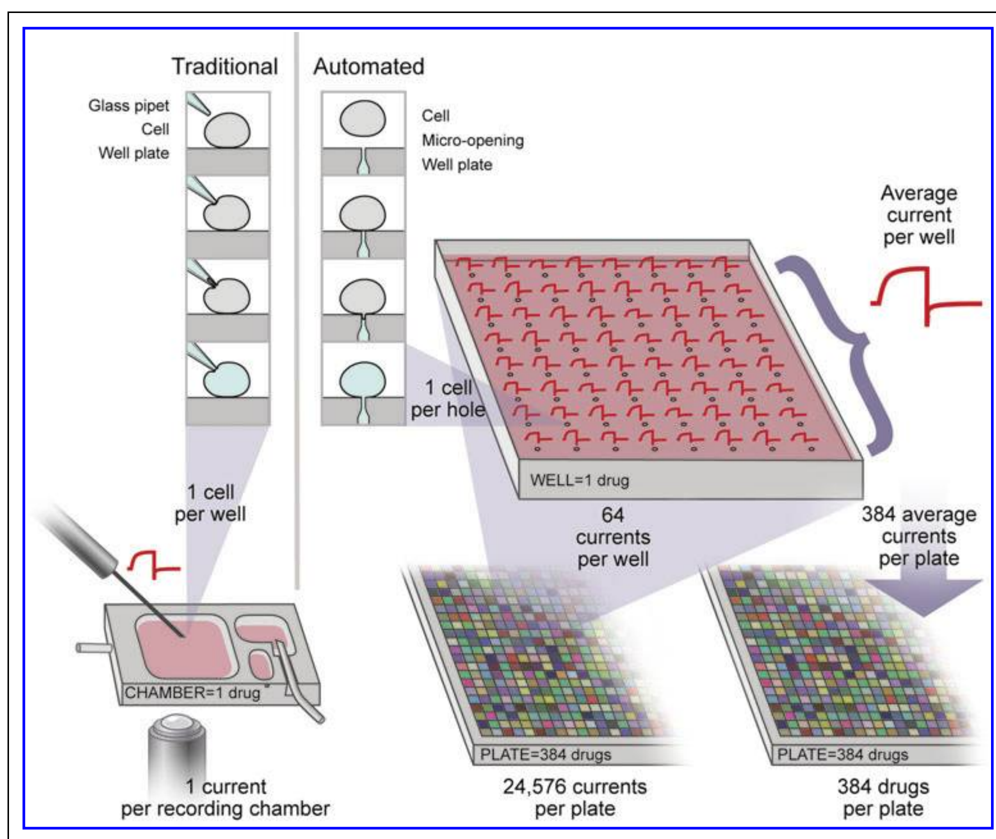

Fig. 2. Schematic diagram comparing conventional manual patch clamp (left) with the automated patch clamp (right).

What are the physiological roles that can be ascribed to potassium channels? In organisms other than humans do ion channels have other functions?

The most commonly known potassium channel activity is electrical excitability, such as its function in the heart and nervous system. Also important is the link between membrane potential change and cellular signaling. This is a key component of potassium channel function in nonexcitable cells such as lymphocytes, cancer cells, and pancreatic cells. Membrane potential is directly coupled to hormone secretion, oxygen sensing, cell cycle control, and a variety of other functions. In breast cancer, for example, it is known that expression of some potassium channels may increase from 40- to 200-fold. It is speculated that the increase of channel expression reduces a cell's sensitivity to oxygen and serum so the cancer cell can divide in a hypoxic environment in the absence of serum.

In terms of nonhuman organisms there are a lot of interesting examples. Perhaps the simplest is the seasonal migration of squid between warm water and cold water. Squid use the same potassium channel and rely on RNA-editing that changes the protein sequence according to the water temperature and allows for proper excitatory activity. Another dramatic example is leaf movement in plants, which correlates with the direction and intensity of sunlight. The important but perhaps not broadly appreciated example is viruses, almost all of which carry ion channels. Many drugs developed against viruses such as the influenza virus are targeted to ion channels. For example, the M2 channel in influenza is a proton channel, and when a virus enters a cell there is a pH-induced conformational change that allows the virus to inject its nucleic acid into the host cell. Without a functional proton channel the virus will not release its nucleic acid. Thus, drugs that block this channel prevent viruses from propagating in the host.

The hERG channel is well-known for its ability to bind to and be inhibited by a wide variety of chemotypes, creating a significant cardiac liability that has resulted in the withdrawal of numerous drugs from the market. How well understood is the molecular basis of this phenomenon, and do all ion channels have similar propensities to bind large classes of drugs? Is hERG special only because of its connection to cardiac arrest?

The activity of hERG is a very exciting and challenging area. The ability of hERG to bind a variety of chemical structures is an important but poorly understood behavior. A protein target should have some sort of chemical specificity, but hERG is almost like a magnet for small molecules; it absorbs a large number of compounds from different classes. Because hERG plays a critical role in cardiac function, this promiscuous behavior becomes a liability in the cardiovascular system. Until

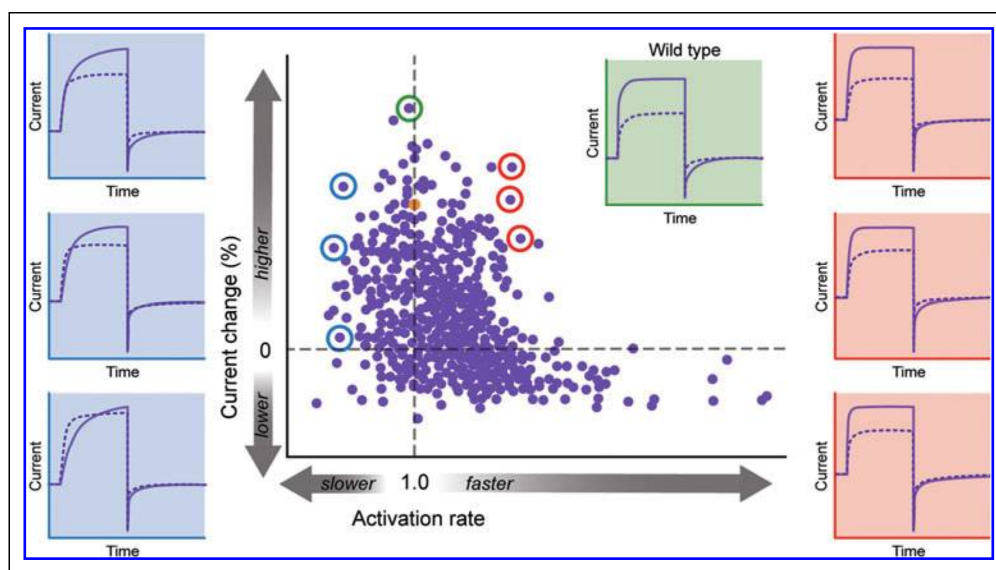

Fig. 3. Schematic diagram of a scatter plot displaying biophysical property changes caused by 1,000 compounds. The representative traces with (solid line) or without (dashed line) compound are corresponding to the circled compounds of matching color.

the structure is resolved and different structures of blocking compounds are carefully examined, it will be difficult to fully understand the mechanism by which hERG can bind to so many different chemical classes.

Regarding other aspects of hERG, there are several particularly exciting examples. Genetic analysis of patients with schizophrenia suggests a role for one of the hERG splice variants, and this has led to an interesting hypothesis that there may be a link between the cardiovascular and nervous systems. In some model organisms, a hERG mutation appears to be associated with cell migration and proliferation as well as an inflammatory response. So hERG's role may go well beyond its cardiovascular significance.

What are the main gaps in our knowledge about potassium channel families and networks that, when better understood, may help advance drug discovery?

The disease link has been clearly established. The main knowledge gap relates to the fact that the combinatorial assembly and complex regulation of the large number of channels limits the resolution of the genetic approach to understanding channel function and, in many cases, the specific defects. If you knock down or knock out a channel you will find that it is responsible for a broad spectrum of biological processes, but to target a channel you need a specific, reversible perturbation of channel function in the appropriate context. In order to understand a channel's role in a given symphony of responses related to a physiological process we need specific pharmacological tools that allow us to probe individual channels. A good parallel is GPCRs because one of the factors that contributes to their significance is the number of agonistic and antagonistic ligands available to study specific targets.

Kinases are rapidly becoming a major drug target class. Can this be exploited in any way to improve our ability to target ion channels as therapeutic targets?

In kinase drug discovery people have learned that you can readily block kinase activity, but you tend to find that the active compounds often attack the ATP binding site. The beauty of this target class is the availability of a large number of active compounds. The drawback is that many of these compounds do not have good selectivity for one kinase over another because the ATP binding domains of different kinases are similar. To overcome this, one needs to target a small molecule to a regulatory site, which triggers kinase activity.

Similarly, with potassium channels, to achieve high isoform specificity you do not want a compound that blocks the selectivity filter of an ionic pore because they all look alike. Instead, you want molecules that recognize, for example, the gating domain—the architecture outside the pore.

GPCRs seemingly experience a resurgence in prominence every decade or so as new pharmacological properties are ascribed to their function and new assay technologies are developed to reveal these properties. In your view, what lies ahead for the field of ion channels and transporters?

This is a very intriguing question. I think Ion channels and transporters are very important target classes for many reasons. The most important reason is that many ion channels are validated targets. The bottleneck is to identify high-quality, active compounds and develop them beyond the preclinical phase of development.

Your laboratory is part of the National Institutes of Health's Molecular Libraries Probe Production Centers Network (MLPCN). Do you see the initiative serving the greater scientific community as you anticipated? Do you think initiatives of this type are going to play increasingly larger roles in translational therapeutics, or give way to some other resource in the near future? What benefits does an initiative like this have to offer individual principal investigators who do not have their own screening and medicinal chemistry capabilities?

MLPCN as a national program is providing a great service to the community. The most important characteristic of the program is that all data are uploaded and shared through a public database, PubChem. There is an ongoing discussion about how to increase the overall value of the program. Over the past few years there has been a major shift in therapeutic R&D activity from industry to an academic environment. This shift, seen in the United States and globally, is an excellent trend for academia and promotes information-sharing. In industry, if a compound does not move forward into drug development it has little value to the company. It is not known what happens to those compounds and knowledge if the research division is reorganized or eliminated. Whereas in an academic environment, whether or not a compound moves forward into the clinic, the knowledge derived from it will be better inherited and publicly shared, whether in the form of publications, progress reports, laboratory notebooks, or training materials for graduate students and post-docs. Knowledge is an important part of scientific and translational advancement, whether or not it comes from a spectacular success or a spectacular failure.

Another important point is that both the achievements and trajectory of the MLPCN, which is in the middle of its third year, are very exciting as a whole. In year one the focus was on whether sufficient targets would be identified, and year two was mainly about compound identification. In year three we are focusing on generating high quality active compounds. We have seen rapid progression and, over the next three to four years, I think we will see more progress. The full potential for MLPCN is unknown, but I am very optimistic.

Individual investigators can benefit in three ways. While it is too expensive for an individual lab to perform a large screen, MLPCN has made it possible by allowing every investigator to use one of our centers essentially for free. Investigators simply have to write a brief application to justify a target of interest to be screened. A whole center may work on an individual investigator's project for months. It is like having an unpaid but highly skilled subcontractor at one's disposal. The second point relates to the chemistry capabilities an investigator might feel are needed to do the follow-up and to characterize hits. Extensive follow-up is beneficial but not always necessary depending on the objective. There are many examples of compounds derived from a primary screen that have reasonable affinity and provide a great deal of mechanistic information about the target. A good example is tetraethylammonium, a small organic cation that blocks potassium channels. This discovery led to the ability to identify the sodium current, which became the most important tool for identifying inactivation behavior in sodium channels, in the squid giant axon, in which one has to block the potassium current to see inactivation of the sodium channel. Finally, the PubChem has considerable contents already, and the contents and utility features will increase dramatically as the program progresses. One can use the database in a variety of ways, ranging from individual compound pharmacology to large-scale data mining.

On a more personal note, how did you become interested in science, and what led to your eventual focus on ion channels?

My parents are university professors in the Department of Botany. I grew up in China during the Cultural Revolution; fortunately, I was too young to understand what was going on. During that time my father was sent to work on a farm. To keep me out of trouble he taught me how to use an outdated microscope to see a variety of plant specimens taken from the pond in the village where we lived. My assignment was to cut a little piece of the plant, put it under the microscope, and draw what I saw. It was amazing to watch the small, colored vesicles moving inside of plant cells, carrying nutrients from the roots to the leaves.

So the short answer to your question is I do not remember precisely when I became interested in science, but I was always comfortable working with a microscope.

In terms of ion channels, it is kind of ironic. I vividly remember one particular experience when I was in graduate school: I failed one of the questions in my Ph.D. qualifying exam, and that was a question about ion channels. That led to a "conditional pass," which was not well-received at Johns Hopkins! After graduate school I was lucky enough to join Lily Jan's lab at the University of California, San Francisco as a postdoctoral fellow, which was a great place for me to shape up my interest, knowledge, and views about ion channels.

—Interview by Vicki Glaser

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