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Engineered Ion Channels as Emerging Tools for Chemical Biology

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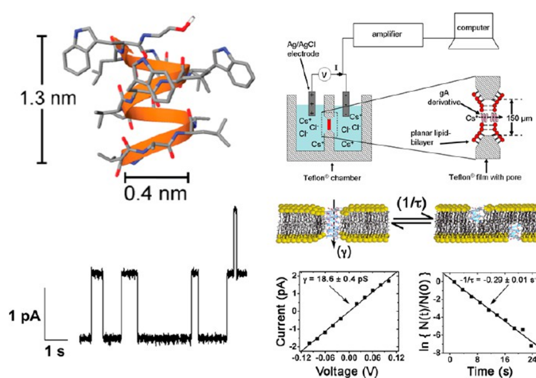
CONSPECTUS

Over the last 25 years, researchers have developed exogenously expressed, genetically engineered, semi-synthetic, and entirely synthetic ion channels. These structures have sufficient fidelity to serve as unique tools that can reveal information about living organisms. One of the most exciting success stories is optogenetics: the use of light-gated channels to trigger action potentials in specific neurons combined with studies of the response from networks of cells or entire live animals.



Despite this breakthrough, the use of molecularly engineered ion channels for studies of biological systems is still in its infancy. Historically, researchers studied ion channels in the context of their own function in single cells or in multicellular signaling and regulation. Only recently have researchers considered ion channels and pore-forming peptides as responsive tools to report on the chemical and physical changes produced by other biochemical processes and reactions. This emerging class of molecular probes has a number of useful characteristics. For instance, these structures can greatly amplify the signal of chemical changes: the binding of one molecule to a ligand-gated ion channel can result in flux of millions of ions across a cell membrane. In addition, gating occurs on sub-microsecond time scales, resulting in fast response times. Moreover, the signal is complementary to existing techniques because the output is ionic current rather than fluorescence or radioactivity. And finally, ion channels are also localized at the membrane of cells where essential processes such as signaling and regulation take place.

This Account highlights examples, mostly from our own work, of uses of ion channels and pore-forming peptides such as gramicidin in chemical biology. We discuss various strategies for preparing synthetically tailored ion channels that range from *de novo* designed synthetic molecules to genetically engineered or simply exogenously expressed or reconstituted wild-type channels. Next we consider aspects of experimental design by comparing various membrane environments or systems that make it possible to quantify the response of ion channels to biochemical processes of interest. We present applications of ion channels to answer questions in chemical biology, and propose potential future developments and applications of these single molecule probes. Finally we discuss the hurdles that impede the routine use of ion channel probes in biochemistry and cell biology laboratories and developments and strategies that could overcome these problems.

Optogenetics has facilitated breakthroughs in neuroscience, and these results give a dramatic idea of what may lie ahead for designed ion channels as a functional class of molecular probes. If researchers can improve molecular engineering to increase ion channel versatility and can overcome the barriers to collaborating across disciplines, we conclude that these structures could have tremendous potential as novel tools for chemical biology studies.

Introduction

Chemical biology emerged, to a large extent, from the need for new tools to study biological systems. Many of these tools encompass methods to isolate or purify biomolecules and biomolecular complexes or to study the function of biological systems in their native environment. The ideal tool for investigating living systems, therefore, can be applied inside and outside of cells, is nontoxic, and

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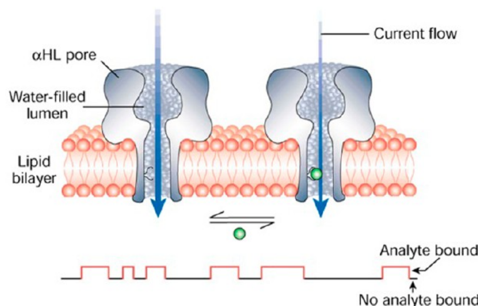

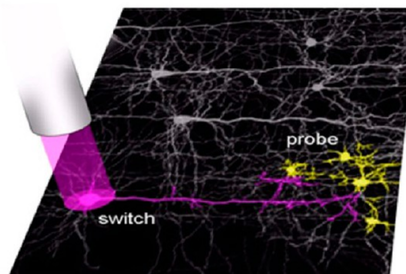

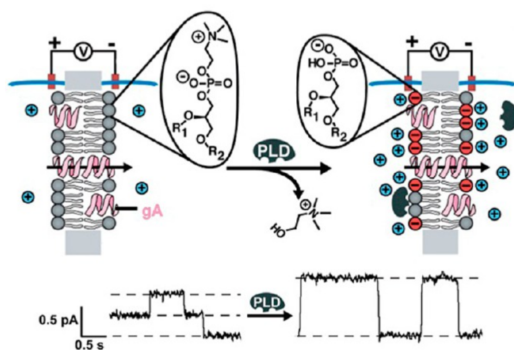

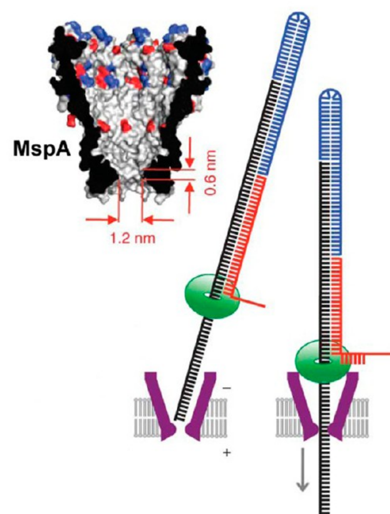

FIGURE 1. Ion channels as enabling tools in chemical biology. Stochastic sensing makes it possible to detect individual biochemical reactions in artificial lipid membranes and possibly in living cells. Light-activated neural firing is the central feature of neuroscience studies by optogenetics. Pore-forming peptides enable monitoring of heterogeneous catalysis on membranes, and genetically engineered protein pores have recently been used for DNA sequencing. The figure illustrating stochastic sensing was reprinted by permission from Macmillan Publishers Ltd: *Nature* (ref 5), Copyright 2001. The figure illustrating light-activated neural firing was reprinted from ref 13 with permission from Elsevier. The cartoon depicting monitoring heterogeneous catalysis was reprinted from ref 4 with permission. Copyright 2009 American Chemical Society. The DNA sequencing illustration was reprinted by permission from Macmillan Publishers Ltd: *Nature Biotechnology* (ref 8), Copyright 2012.

does not perturb the process under study, unless, of course, perturbation is desired to trigger and monitor a response.

Chemical, electrical, fluorescent, radioactive, and spectroscopic signals provide common ways to investigate biological processes. These approaches are well established, and tremendous progress over the last decades has made these tools indispensable for the life sciences. Nonetheless, chemical probes that provide improved target localization, sensitivity, specificity, and ease of use combined with accurate quantification are still needed to increase our understanding of the vast number of biological reactions inside and outside living cells.

Among cellular reactions, processes at biological membranes pose particular challenges due to the heterogeneous nature of the environment.[1] Pore-forming molecules and ion channels are emerging tools to study processes in or around membranes (Figure 1).[2,3] These tools make it possible to follow the dynamic behavior of individual molecules (the pore) and provide information about its specific environment.[4] Stochastic sensing, for instance, has made it possible to gain information about individual biochemical processes and reactions, generating distributions that can represent rare events, which may be lost in an ensemble measurement.[5] Another compelling example is the field of optogenetics, which takes advantage of ion channels to perturb living systems from a point source followed by monitoring the response in a network of cells or throughout

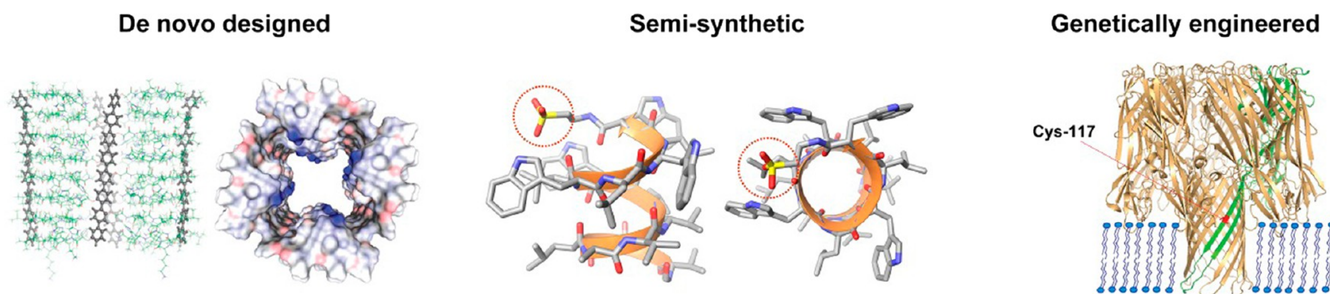

FIGURE 2. Strategies to prepare ion channels as tools for applications in chemical biology. Ion channels can be formed from self-assembly of entirely synthetic molecules, from derivatives of native pore formers, or by genetic engineering of ion channel proteins. The cartoons depicting *de novo* designed and semisynthetic channels are reprinted from refs 14 and 10 with permission. Copyright 2004 and 2007 American Chemical Society. The illustration of a genetically engineered pore was reprinted by permission from Macmillan Publishers Ltd: *Nature Chemistry* (ref 17), Copyright 2010.

an organism.[6] Light-gated ion channels have, for instance, been used for light-activated remote control of neuronal firing.[7] The well-defined conductance of ions through ionic pores makes it possible to study translocation of individual macromolecules with high sensitivity, which has been the driving force for many groups attempting to use ion channels to sequence DNA molecules as they travel through the pore.[8] Additionally, semisynthetic and natural ion channels allow *in situ* monitoring of heterogeneous biochemical processes occurring at the membrane.[4] In comparison to fluorescent probes, which are among the most common tools for chemical biology studies, ion channel probes have the following complementary advantages: large signal amplification,[2] amenability to colored solutions that may contain fluorescence quenchers,[9] capability to interrogate the surface potential on either side of a biomembrane independently,[4,10] potential for switch-like response due to cooperative nature of pore formation by oligomerization of certain pore-forming peptides,[11] potential for miniaturized, low-power, highly parallel analysis,[2,12] and minimized local perturbation of the membrane environment due to small quantities of pore former (e.g., picomolar concentration) required to obtain a measurable signal.[9]

This Account focuses on the use of pores as a tool for chemical biology and addresses two main questions: What does it take to build synthetic ion channels and how can biological systems be investigated with these tools? We provide our perspective, based to a large extent on our own work, and highlight one platform that employs derivatives of a pore-forming peptide to study a number of biological and chemical processes. We discuss challenges in the field, future directions, and hurdles that need to be overcome to get there.

Preparation of Synthetically Tailored Ion Channels

A number of synthetic ion channels have been explored as tools for studying biological processes. These engineered channels can be separated into three classes (Figure 2): (1) *de novo* designed synthetic pore formers, (2) semisynthetic pores derived from natural pore-forming proteins and peptides, and (3) genetically engineered ion channel proteins.

De novo designed molecules that form transmembrane pores include amphiphilic molecules based on macrocyclic scaffolds such as cyclodextrin or calixarenes, as well as peptide lipid hybrid structures, artificial β barrels,[14] steroid-based transporters, or synthetic polymers (reviewed recently by Matile et al.[15]). We reported recently, for instance, that a structurally simple amphiphilic molecule (BTA-EG_4) comprising a small hydrophobic block of aromatic rings covalently attached to a short tetra(ethylene glycol) chain could form cation-selective pores in bilayer membranes at concentrations that were also toxic to mammalian and bacterial cells.[16]

With regard to genetically engineered ion channels, Lu et al. have used site-directed mutagenesis methods to incorporate specific functionality at well-defined locations within the lumen of the channels.[17] This approach made it possible to detect individual reactions or binding events on these sites. Excellent examples of this approach have been reported by Bayley's group using α-hemolysin (αHL) pores as biosensors, gateways into cells, and functional nanodevices.[12,18]

During the past decade, our laboratories have focused on developing semisynthetic ion channels starting with commercially available pore-forming peptides. In particular, gramicidin A (gA) has emerged as a useful starting material for developing pores with tailored functionality.[10] Attractive

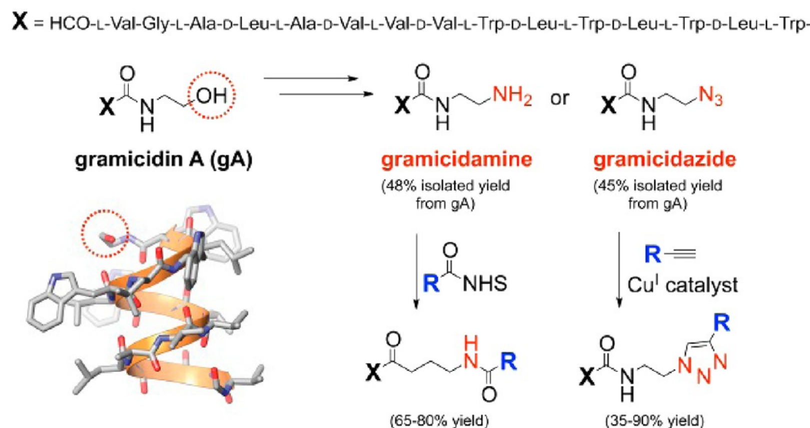

FIGURE 3. Synthesis of chemically reactive gramicidin derivatives (gramicidamine and gramicidazide) enables rapid preparation of pores with tailored functionality.

characteristics for using gA as a probe for chemical biology studies include (1) its availability in gram-scale quantities at low cost, (2) its amenability to straightforward incorporation of chemical groups with predictable channel behavior or function, (3) its capability to incorporate spontaneously into membranes, (4) its capability to form a well-defined, cation-selective pore, which results in a quantized current signal that is straightforward to interpret, and (5) its capability to report small changes to its chemical environment in or near a membrane.[10,19] In comparison with other useful pore-forming molecules or ion channel proteins, the well-defined, transient nature of dimeric gA pores and the resulting lifetime information in the lipid environment offers the opportunity to study fundamental aspects about the membrane (such as membrane thickness and viscosity) that are not readily accessible using permanent pores such as αHL. The unique advantages of permanent pores, such as aHL, MspA, etc., have been discussed in several recent reviews.[2,12,18,20]

While native gA is useful for a number of fundamental studies in membrane biophysics,[21] synthetic derivatives of gA have expanded the capability of this peptide to report changes to the membrane environment in response to a range of alterations of the chemical groups presented at the C-terminus of the peptide.[2,22−24] Since gA dimerizes via its N-terminus to form a pore, the C-termini are exposed at the lipid/water interface.

Two main approaches have been explored for synthesizing C-terminal derivatives of gA: solid-phase peptide synthesis and synthetic derivatization of native gA.[3] While it is possible to perform some simple modifications on the C-terminal alcohol of gA using standard chemical transformations, we found that formal conversion of the primary alcohol in native gA to a primary amine or azide (to produce gramacidamine or gramicidazide) provided new C-terminal chemical moieties that had superior chemical reactivity to the native alcohol present on gA (Figure 3).[25] Both gramicidamine and gramicidazide can be readily prepared on a scale of several hundred milligrams and can be used to generate gA derivatives carrying a diverse set of chemical functionality using standard amidation or copper-catalyzed azide−alkyne cycloaddition (CuAAC or "click") reactions (Figure 3). We used this synthetic methodology to rapidly generate ion-channel-forming probes that we designed to respond to specific stimuli for a variety of chemical biology studies.[25]

Experimental Design

The common element of all pore-based assays for biological applications is the lipid bilayer membrane. These membranes can be composed entirely of synthetic lipid molecules, or they can be cellular membranes of living cells inside a live animal. Figure 4 illustrates six different "membrane systems" that are typically used for pore-based assays.[2]

Liposomes are usually employed for flux assays based on fluorescence or radioactivity detection. In the context of chemical biology, liposomes typically harbor reconstituted transmembrane proteins while pore-forming peptides and proteins are often used to set up a membrane potential or to facilitate transport of ions and other polar molecules across the liposomal membrane.[26]

Supported lipid bilayers are usually used in combination with impedance spectroscopy, and the presence of pore formers can modulate the resistive properties of the bilayers.[27] So far, most applications of this technology have been for detecting binding events on bilayers or on pores embedded in bilayers.[27] Applications for addressing

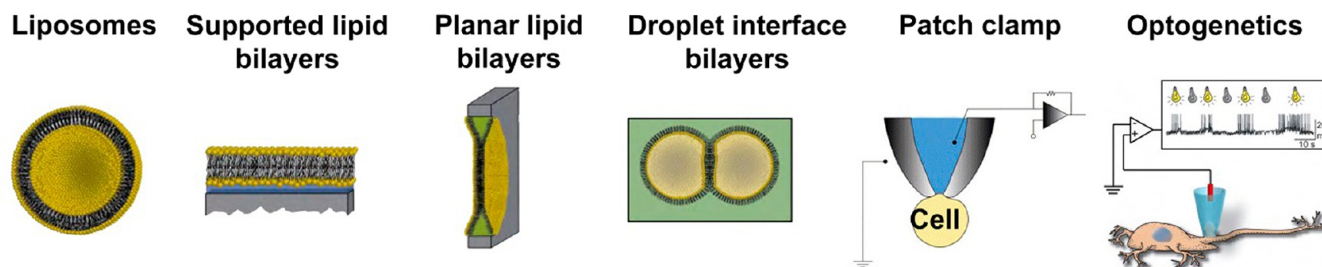

FIGURE 4. Various lipid membrane systems for studying ion channels. Reproduced from several figures within ref 2 with permission from Elsevier.

problems in chemical biology with supported lipid bilayers and pore formers are relatively rare, in part because this approach can currently not resolve ionic currents through individual ion channel proteins or pores.

Planar lipid bilayers (PLBs), also called black lipid membranes or bilayer lipid membranes (BLMs), are established tools for studying the function of ion channels and transport proteins under well-defined experimental conditions.[28] The use of pores in PLBs *as tools* to study other biochemical and biophysical processes is also relatively established in specialized laboratories.[2] Gramicidin pores, for instance, are capable of reporting the viscosity of a membrane, the energy required to bend the membrane, and the local concentration of monovalent cations near the membrane.[21] Reactions that affect these parameters can, therefore, be monitored by electrophysiological recordings of ion flux through gramicidin pores.[4]

Droplet interface bilayers (DIBs) can be used for similar applications as PLBs. Two important differences are that (i) an oil phase surrounds the two water droplets that form a lipid bilayer at their contact zone and (ii) the volume of the water droplets is typically much smaller than the electrolyte volumes of PLB setups. The oil phase needs to be considered when lipophilic molecules are studied, while the small aqueous volumes can be advantageous for processes that require high concentrations of reactants such as protein reconstitution by proteoliposome fusion.[29] These small volumes can, however, also induce limitations with regard to changes in the physicochemical properties of the droplets when ions (and, hence, charges) and molecules enter or leave these volumes.[29]

Patch clamp electrophysiology is the most established approach to study pore formers and ion channels; typically with the intention to better understand the ion channels themselves rather than employing pores as tools for studying other processes. One particularly powerful approach in electrophysiology employs oocytes from *Xenopus laevis* frogs in order to study the function of ion channel proteins that the oocyte expresses in response to injection of the ion channel's mRNA.[30] In this sense, any process that may be reported by an ion channel could, in principle, be studied with patch clamp experiments on these oocytes.

Optogenetics is the most recent experimental approach that uses ion channels as tools for studying biological systems (particularly the nervous system).[6] At the heart of this revolutionary technology is a light-activatable channelrhodopsin protein that can be exogenously expressed in select neurons. Spatially and temporally controlled illumination of these cells with optical fibers, for instance, triggers a depolarization of transfected neurons leading to neuronal firing of action potentials. The response of the neural network can be monitored by either electrophysiological recordings or biosensors that are typically based on fluorescence detection.

Applications of Gramicidin Pores in Chemical Biology

Focused on the example of pores based on gA, this Account highlights applications that use ion channels to study biochemical systems.

Monitoring Chemical and Biochemical Reactions. Single channel recordings of various gramicidin derivatives revealed that the conductance of ions through the pore is dependent on the functional group presented at the entrance for ions into the pore.[10] In particular, two factors appear to dominate the effect of functional groups on the conductance: steric bulk and charge. Based on these observations, it is possible to design ion channels that respond to various external stimuli. For instance, chemical modification of the ionic charge near the entrance of a gramicidin pore is typically accompanied by a measurable change in single channel conductance.[10,31] As expected for a Coulombic phenomenon, this effect is particularly pronounced in solutions with low ionic strength. Figure 5 illustrates this type of charge-based sensing. Here, the negative charge of the sulfonate group presented at the opening of the pore in the reaction product increased the conductance of cations

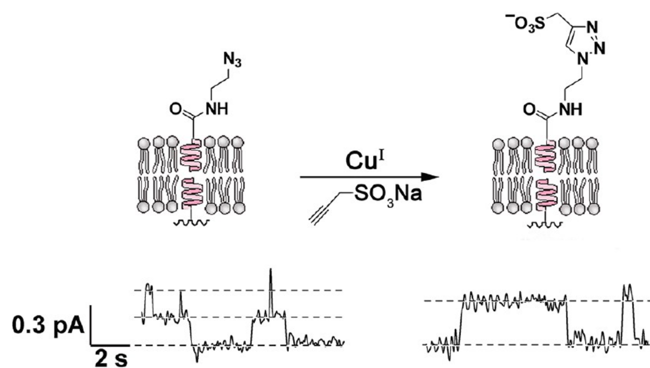

FIGURE 5. *In situ* monitoring of a chemical reaction using ion channel recordings. Reprinted from ref 25 with permission. Copyright 2008 American Chemical Society.

through the channel compared with the starting azide. This approach, therefore, allowed for monitoring of this chemical reaction *in situ* and in close to real time.

In order to apply this ion channel technique to biologically relevant samples, we designed three conceptually different types of experiments that used charge-based sensing to analyze different enzymatic processes. In the first case, we demonstrated that a gramicidin derivative carrying a C-terminal phosphate group (gA-P) could act as a substrate for the enzyme alkaline phosphatase (AP).[9,32] Here, AP added to the aqueous compartment of a bilayer setup reacted with gA-P already present either at the membrane or free in solution and catalyzed the hydrolysis of the phosphate group. Ion channel recordings captured this process because the hydrolyzed gA product (comprising an electrically neutral hydroxyl group) exhibited a measurably smaller single channel conductance than the negatively charged gA-P substrate (Figure 6A). Recording single channel events over the course of this enzymatic reaction made it possible to follow the conversion of substrate to product over time (Figure 6B). We demonstrated that this method required only 600 pM concentrations of the enzyme to follow its activity (Figure 6C) and revealed kinetic parameters such as k_{cat} or K_M for this enzyme–substrate system using a modified kinetic analysis model developed by Morrison.[24]

A second experimental design we developed for charge-based sensing involves the use of charged groups attached to gA to affect partitioning of the ion-channel-forming peptide in the membrane. Native gA is an extremely hydrophobic molecule that partitions strongly into membranes.[33] In order to demonstrate a method that exploits partitioning of gA derivatives to monitor enzymatic activity, we reported a derivative of gramicidin (Figure 7A, gA-LF substrate) carrying a highly positively charged nine amino acid peptide substrate for *Anthrax* lethal factor (LF).[9] We designed this gA derivative to be cleaved by LF near the C-terminus of gA, resulting in removal of the four positively charged amino acid residues on the gA substrate. Before enzymatic cleavage by LF, this gA-LF substrate did not show any ion channel activity. Upon addition of LF, however, we observed a rapid increase in macroscopic channel activity (Figure 7B,C). We attribute this stimuli-responsive behavior to increased partitioning into the membrane after hydrolysis by LF of the gA product, which we presume is much more hydrophobic than the highly positively charged gA-LF substrate. Steric blocking of the pore by the charged amino acid residues prior to reaction with LF may also contribute to the observed stimuli-responsive behavior of the gA-LF substrate.

Finally, we demonstrated a third type of experimental design for charge-based sensing by monitoring the activity of membrane active enzymes (Figure 1). In this case, we used single channel conductance measurements to monitor the activity of phospholipase D (PLD) and phospholipase C (PLC).[4] The substrates for this enzyme are the membrane lipids themselves. In the case of PLD, the enzyme hydrolyzes phosphatidylcholine groups on the membrane, turning the zwitterionic headgroups into negatively charged lipids. As the fraction of negatively charged lipid headgroups increases over time, the local concentration of positively charged counterions near the membrane surface also increases. This increase in local concentration of cations, in turn, increases the observed singe channel conductance of ions through the gramicidin pore. A key feature of this simple experimental design is the capability to study the kinetics of such a heterogeneous catalytic process (i.e., occurring on the membrane surface as opposed to in bulk solution) *in situ* and in close to real time.[60]

Gating Using Protein–Ligand Binding Interactions. We and others have developed methods to gate transmembrane ion flux by incorporating small molecule ligands on synthetic ion channels to turn on or turn off their conductance behavior upon binding to specific proteins in solution.[11,25,34] For instance, we previously reported a derivative of gA carrying a benzene sulfonamide group (gA-sulfonamide) that can bind to the protein carbonic anhydrase (CA). We showed that introduction of gA-sulfonamide to a bilayer resulted in macroscopic currents of ions across the membrane (Figure 8A). Addition of CA to the aqueous compartments of the bilayer setup resulted in nearly complete abolishment of ionic current across the membrane (Figure 8B). Although the mechanistic details of how binding of the ligand on gA to CA results in loss of current activity

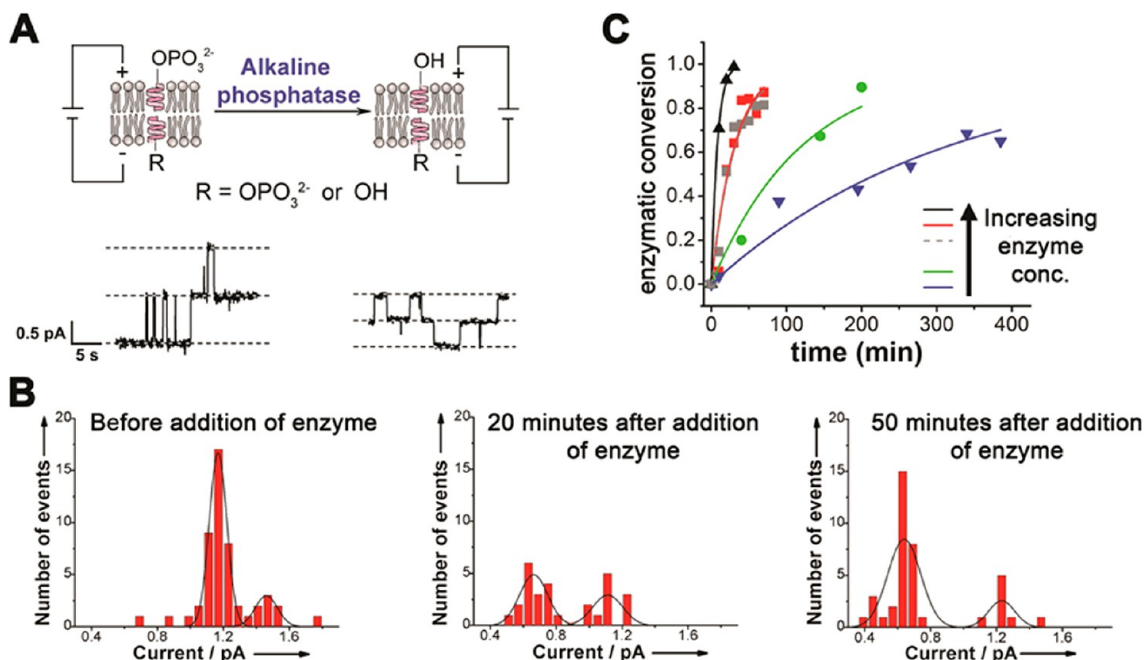

FIGURE 6. Monitoring of alkaline phosphatase (AP) activity using ion channel recordings. (A) The single ion channel conductance of a gramicidin peptide carrying a phosphate group (gA-P) is significantly reduced upon hydrolysis of the phosphate group by AP. (B) The fraction of ion channel events originating from gA-P (with larger current values) and the enzymatically hydrolyzed product gA-OH (with smaller current values) can be monitored over the course of the biochemical reaction. (C) Enzymatic conversion of gA-P over time as a function of AP concentration. Reproduced from several figures within the main text and supporting information of ref 9 with permission. Copyright 2009 American Chemical Society.

remain unclear,[34] addition of free 4-carboxybenzenesulfonamide (to outcompete the gA-sulfonamide from binding to CA) to the aqueous compartment resulted in restoration of macroscopic currents. This simple experiment, thus, demonstrates the capability to reversibly control the flow of ions across a membrane using protein–ligand binding interactions.

Gating Using Light. Genetic engineering has made it possible to use light to gate the conductance of protein channels, which has opened up many exciting opportunities to study complex biological systems *in vitro* and *in vivo*.[7,13,35,36] In an attempt to translate the concept of gating channel conductance with light to a simple and synthetically accessible platform, we have explored the capability to incorporate light-responsive spiropyran molecules on the C-terminus of gA.[37] We demonstrated that we could use UV or visible light to reversibly interconvert the uncharged spiropyran moiety to a positively charged merocyanine form in acidic solutions, making it possible to control the charge at the opening of the pore dynamically. Interestingly, we found that this light-driven reaction of the spiropyran could be used to control both the single channel conductance and the stability (i.e., lifetime) of the pore, depending on the ionic strength of the recording buffer (Figure 9). Thus, controlling the charge of functional groups presented at the entrance of a gA pore (here, by using light) makes it possible to control the total charge transported across the membrane under a variety of electrolyte conditions. A major advantage of such light-gated gramicidin pores is their ease of preparation and the capability to incorporate them into a variety of *in vitro* and *in vivo* systems. In addition to gA, other pore formers and ion channel proteins have been explored in the context of light gating; several of these have found use in neuroscience studies including *in vivo* studies (for recent reviews, refer to Fehrentz et al.[38] and Fenno et al.[39]).

Selected Applications of Non-gramicidin Pores in Chemical Biology

Characterization of Macromolecules. Probably the most common application of ion channels for studying macromolecules focuses on biophysical characterizations of single and double stranded DNA.[40] Several groups have demonstrated the use of protein pores for force spectroscopy measurements.[41] Simmel and co-workers characterized the unzipping and unfolding reactions of various nucleotide hairpin structures.[42] Movileanu and co-workers demonstrated unfolding studies of proteins with α-hemolysin (αHL),[43] while the earliest uses of pores for characterization of macromolecules determined the hydrodynamic

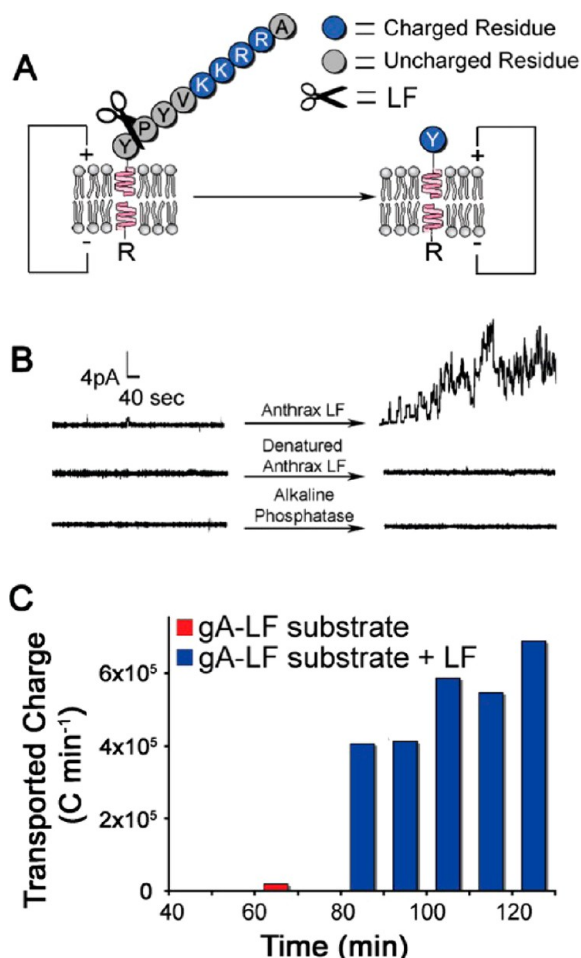

FIGURE 7. Detection of the enzymatic activity of *Anthrax* lethal factor (LF) through ion channel amplification. (A) Cartoon of the cleavage of gA-LF substrate by LF resulted in a net removal of three positive charges. (B) Current versus time traces demonstrating a large increase in transmembrane current upon addition of active LF enzyme to a bilayer containing gA-LF substrate. This large increase in transmembrane current is not observed upon addition of denatured LF or alkaline phosphatase. (C) Time-dependent increase in the flux of charge transported across the bilayer upon addition of LF to a bilayer containing gA-LF substrate. Reprinted from ref 9 with permission. Copyright 2009 American Chemical Society.

radius of synthetic polymers such as poly(ethylene glycol) (PEG).[44]

Nucleotide Sequencing. The promise of the seemingly simple and compelling idea to read out the sequence of nucleobases while single stranded polynucleotide strands are pulled through a protein pore strongly accelerated the use of pores in chemical biology.[45,46] Although it took two decades until the first examples of nanopore-based sequencing were realized, work toward this goal led to engineered ion channel proteins, to fusion proteins that slow the translocation of nucleotides through the pores, and to significant improvements in experimental setups to study ion channels. Several companies emerged from this effort and the first commercial pore-based DNA sequencing devices have been announced.[45] These automated devices are based on parallel PLB recordings from hundreds of pores and it is likely that these devices will find use for applications of pores that go beyond sequencing, including clinical assays, biosensing, and environmental monitoring.

Fundamental Biophysical Studies. Ion channels and pore-forming peptides are attractive tools to probe characteristics of lipid membranes. For instance, the lifetime of gramicidin pores depends on the thickness of the membrane, while their single channel conductance depends on the local concentration of cations and, hence, the surface potential of the lipid membrane.[10,21] Ion channels, in particular light-gated ion channels, also make it possible to manipulate the membrane potential and, therefore, to study the effect of this manipulation in live cells.[6]

Studying Selectivity of Pore-Based Antibiotics for Different Cells. Semisynthetic versions of pore-forming antibiotics make it possible to investigate the importance of charge and other physicochemical properties for targeting microorganisms of interest.[47] Given the dramatic problem of resistance to antibiotics, these studies might help to understand, minimize, or circumvent resistance.

Emerging Applications of Pores in Chemical Biology

Probably the most exciting demonstrated application of ion channels as a tool for chemical biology is optogenetics.[6] The possibility to trigger nerve signals remotely at a specific time and location combined with monitoring downstream signaling and behavior is a breakthrough for neuroscience and neural engineering. Now that this technique is established, one intriguing question is: what bio-orthogonal triggers, other than light, might be used to activate ion channels at specific locations. Could magnetic stimuli or localized thermal or mechanical stimulation be used to open or close engineered ion channel or pore-forming peptides? If these molecules could be targeted to cells or organ regions of interest, then the toolkit for the life sciences would expand significantly. First attempts at making light-switchable gramicidin pores have been reported.[48] Alternatively, ion channel proteins may be genetically engineered to respond to bio-orthogonal stimuli. One of the most dramatic approaches may be to use the recently introduced DNA origami pores combined with engineering stimuli responsive domains in this entirely new class of computer designed, DNA-based ion channels.[49−51]

Many assays in cell biology and biochemistry require transport of membrane-impermeable molecules from the

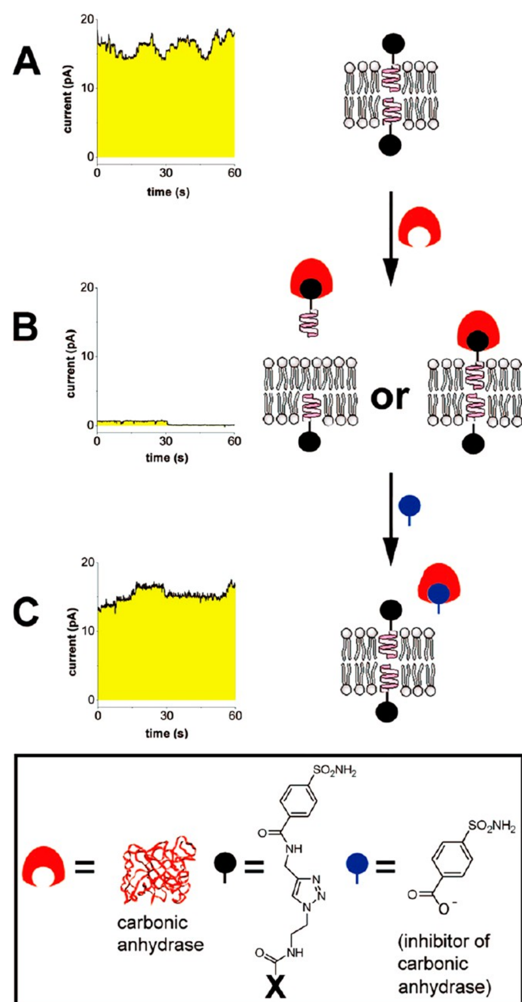

FIGURE 8. Gating of transmembrane ionic current using protein–ligand binding interactions. (A) Cartoon of a gramicidin derivative carrying a benzenesulfonamide group that dimerizes to form open pores across a membrane. The current versus time trace indicates the presence of multiple simultaneous open pores in the membrane. (B) Binding of carbonic anhydrase (CA) to this gA derivative almost completely abolishes transmembrane current. (C) Addition of a competitive inhibitor of CA (4-carboxybenzenesulfonamide) restores ion channel activity. Reprinted from ref 25 with permission. Copyright 2008 American Chemical Society.

extracellular milieu into cells. Typically these molecules are retained in the cells to detect calcium ions or other intracellular species or processes of interest. Although elegant solutions for transmembrane transfer and retention are commercially available for a range of molecular probes, efficient strategies for the general transfer of molecules into cells remain a challenge.[52] If sufficiently big pores could be engineered in such a way that they can be transiently opened at will to allow for diffusion of probes through these pores into the cells, and if these channels would be straightforward to use and cause minimal stress to cells in their closed state, such molecules could be versatile tools for transfer of molecules ranging from drugs and siRNA to aptamers and fluorescent probes.[2]

Finally, recent technological breakthroughs in high-throughput instrumentation for measuring ion channel activity are making it possible to consider entirely new applications of ion channels. For instance, the capability to record from hundreds of individual cells in parallel opens the door to performing measurements on heterogeneous populations of cells, such as primary blood cells. We demonstrated recently that the distribution of ion channel activity of the voltage-gated potassium channel Kv1.3 in human T lymphocytes can be used as an activation marker of these important immune cells.[53,54] Upregulated activity of this channel is implicated in autoimmune diseases such as type I diabetes mellitus, multiple sclerosis, rheumatoid arthritis, and psoriasis.[55] High throughput measurements of ion channel activity in populations of cells may have applications beyond clinical diagnostics and therapeutic monitoring. Ion channels are *functional* markers whose *activity* can now be measured in an automated fashion.[56,57] This technology, therefore, enables the use of ion channels as sensitive reporters of cell function and status. We and others have begun exploring these options.[53,54,57]

Concluding Remarks

While the development and implementation of ionic pores as a tool for chemical biology is in its infancy, barriers to entry into the field slow their widespread use (recently reviewed by Majd et al.).[2] For instance, translation of ion channel probes into complex systems such as living cells poses additional challenges due to the presence of endogenous ion channel proteins. Potential solutions to these challenges include hyperpolarization of cells to a membrane potential that maintains voltage-gated ion channels in their closed state or addition of a mixture of ion channel blockers to inhibit either the intrinsic channels[58] or specifically the engineered ion channel probe.[54] One technical challenge is the availability of ready to use, fail-safe, fully automated instruments in combination with robust *in vitro* and *in vivo* assay platforms for analyzing ion channel activity with high precision across a variety of experimental conditions.[2] A number of bilayer setups and suitable high gain amplifiers are becoming increasingly affordable but the sensitivity of the instrumentation (e.g., to sources of external and internal noise and to the "quality" of the lipid membranes) poses challenges for nonexperts to set up, use, and troubleshoot a system in their own laboratories without outside help. The lack of widely available, robust materials and

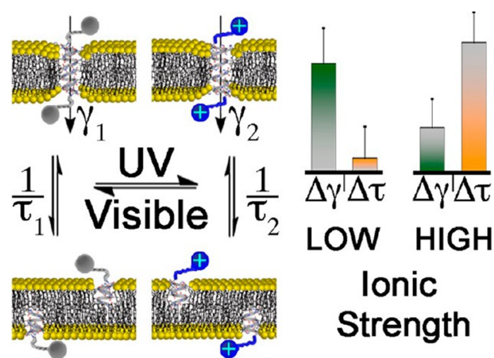

FIGURE 9. Conductance (γ) and stability (as estimated by the lifetime, τ) of pores from dimerization of a photoactive derivative of gA can be controlled by exposure to different wavelengths of light in electrolytes with low and high ionic strengths. Reprinted from ref 37 with permission. Copyright 2011 Wiley.

physical tools that facilitate the formation of stable supported or suspended membranes[59] or cellular patches over long periods of time also imposes limits on what can currently be studied with engineered pores. A more intangible challenge that affects all areas of chemical biology is the breadth of knowledge and expertise required to execute electrophysiological studies such that they provide meaningful results and conclusions. Chemists, biologists, physicists, and engineers historically tend to work on self-imposed "islands" that often prevent interdisciplinary communication and collaborative research. These psychological barriers ultimately limit the speed of development and reach of these tools for revealing new insights into biological systems. Based on our own experience, those interested in entering this exciting field may benefit from seeking out and creating interdisciplinary teams that bring together not only the expertise to execute ion channel experiments or to synthesize tailored pores but also the creative environment that stimulates new, interesting scientific questions.

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