Synthetic Ion Channels

Sakai, Naomi; Matile, Stefan

How to cite

SAKAI, Naomi, MATILE, Stefan. Synthetic Ion Channels. In: Langmuir, 2013, vol. 29, n° 29, p. 9031–9040. doi: 10.1021/la400716c

This publication URL: https://archive-ouverte.unige.ch/unige:28962
Publication DOI: 10.1021/la400716c

Synthetic Ion Channels

Naomi Sakai and Stefan Matile

Department of Organic Chemistry, University of Geneva, Geneva, Switzerland,

www.unige.ch/sciences/chiorg/matile/, stefan.matile@unige.ch

The objective of this historical review is to recall the development of the field of synthetic ion channels over the past three decades. The most inspiring and influential breakthroughs with regard to structure and function are brought together to give the general reader an easily accessible understanding of the field. Pioneering work in the 80s is followed by the golden age in the 90s with structures emphasizing crown ethers, calixarenes and peptide mimetics. Follows the emergence of questions concerning specific functions such as ion selectivity, voltage gating, ligand gating and blockage, and with π-stacks, metal-organic scaffolds and DNA origami a new wave of innovative structures. The perspectives outline promising directions and major challenges waiting to be addressed.

Introduction

Synthetic ion channels have been around for three decades as one of these topics everyone knows what it is but no one can tell what it really means. Of course they should somehow resemble biological ion channels in form and function, that is span a lipid bilayer membrane, mediate rapid flux of ions

without moving themselves, and cause the stochastic appearance of single-channel currents (Figure 1).[1,2] And they should have a meaning, that is respond to physical or chemical stimulation.

This definition is not as innocent as it looks. The existence of transmembrane architectures can be difficult to prove. If beautiful transmembrane architectures have been created and their activity to transport ions across membranes has been confirmed, but the methods used to do so, for various reasons, do not include the measurement of single-channel currents, do they disqualify as synthetic ion channels? On the other hand, "on/off" currents of single active compounds have been reported for many systems that do not look at all like ion channels, in part very small molecules without obvious tendency to self-assemble into ordered transmembrane architectures. Do they qualify as synthetic ion channels? Finally, synthetic ion channels often contain structural motifs from nature, peptides, DNA, steroids, and so on. How much "nature" is tolerated to qualify as synthetic ion channel?

There is no clear answer to these questions. A meaningful understanding of the topic thus requires a certain amount of common sense that is best acquired by looking at leading examples from the history of the field. This overview over quite exactly three decades of research is provided in the following. Some brilliant highlights were obvious to include. The selection of others was not always easy in the light of above questions. And many wonderful examples could not be considered because of stringent limitation of space and references. Sincere apologies go to all colleagues whose efforts could not be properly appreciated for only this reason.

At the Beginning

The first synthetic ion channel is commonly attributed to the group of the late Iwao Tabushi at Kyoto University. It has been published three decades ago in *Tetrahedron Letters*.[3] The proposed active structure **1** is a dimer of amphiphilic β-cyclodextrins (Figure 2). The hydrophilic macrocycle was envisioned to locate at the surface of the membrane and serve as entrance into the ion channel. The hydrophobic alkyl tails should provide a hydrophobic channel across one leaflet of the membrane.

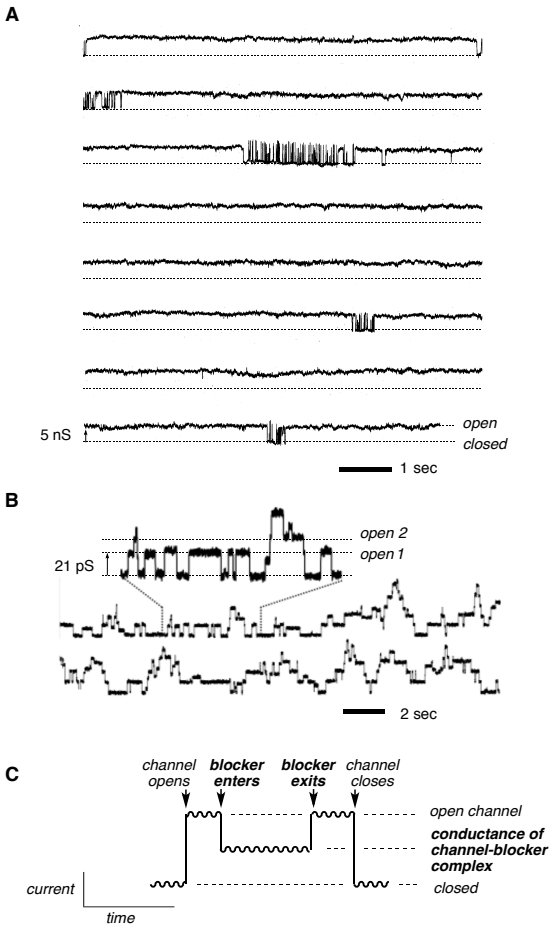

Figure 1. The characteristic behavior of large (A) and small (B) synthetic ion channels in planar bilayer conductance experiments, and schematic drawing of the holy grail in the field, the detection of molecular recognition on the single-molecule level (C). Lipid bilayer as such are insulators because the electrolytes cannot pass across the hydrophobic barrier, ion carriers cause a gradual increase in the macroscopic current and only ion channels transport fast enough to show single-channel currents appearing and disappearing in a stochastic manner. The upper trace shows currents for one active structure with large conductance and long lifetime (A, reproduced with permission from reference 1. Copyright 2004 American Chemical Society). The lower trace shows small currents for at least two active structures with different conductance and shorter lifetime (B, reproduced with permission from reference 2. Copyright 2006 American Chemical Society).

The Tabushi paper appeared at a time when Ben de Kruijff in Utrecht already formulated the barrel-stave model to explain insights on ion channels formed by the antifungal polyketide natural product amphotericin B. Five years earlier, ion-channel formation by the synthetic LSLG peptide **2** has been reported.[4] The tetrad repeat was expected to roll up into a hollow helix. Contrary to the β-helix with all residues at the outer surface, helix **2** has only the hydrophobic leucine (L) residues at the exterior, whereas the hydrophilic serines (S) point toward the center of the channel. Instead of the D-amino acids in standard β-helices, the achiral glycine (G) is used in **2** to reorient the β-sheets formed by the L-tripeptide LSL. The orientation of the amide carbonyls alternates as in β-sheets. The helix thus should not contain a macrodipole and form unimolecular channels that are insensitive to voltage (see below).

The formation of ion channels by peptide **2** is documented with single-channel currents. One might wonder why this channel is usually not considered as the first synthetic ion channel. Perhaps because it's a peptide composed of standard amino acids only? Although the Tabushi paper clearly formulates the objective to synthesize ion channels from scratch, their formation is supported by methods other than single-channel measurements. For systematic efforts to obtain single-channel currents from a transport system that has really nothing to do with biological building blocks, one has to wait for a paper from Kobuke and Sokabe in 1992 on ion pair **3**.[5] But then, on first view, ion pair **3** doesn't really resemble an ion channel, although it has been proposed to self-assemble into transmembrane multicomponent architectures with the more hydrophilic ether tails being surrounded by the hydrophobic alkyl tails. Taken together, the structures **1**-**3**, all candidates to pass as the first synthetic ion channel, illustrate perfectly well the difficulties to clearly define the topic: All contain some of the expected characteristics, but none is perfect (i.e., **1** has no single-channel data, **2** is composed of biological building blocks only, and **3** doesn't really look like a well-defined transmembrane architecture). And the really pertinen t questions concerning specific responses to chemical or physical stimulation came up only much later.

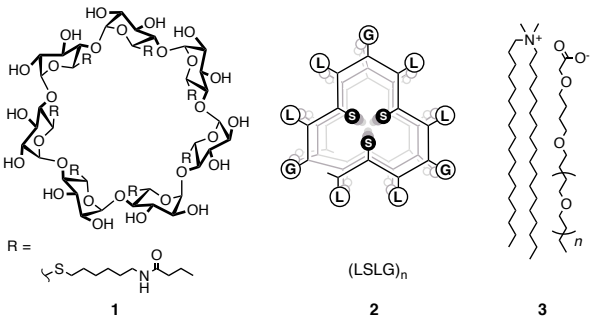

Figure 2. The first synthetic ion channels, with a schematic axial view of the β-helix/β-sheet hybrid formed by peptide **2** (backbone in solid lines, side chains with single-letter abbreviations).

Crown Ethers and Other Macrocycles

Shortly after the Tabushi paper, reports on synthetic ion channels appeared from the groups of Nolte,[6] Lehn and Fuhrhop, the last including a pioneering yet somewhat peculiar example for blockage, that is the reversible inactivation of a synthetic transport system by molecular recognition. The Nolte system introduces crown ethers in the context of helical isocyanate polymers **4** (Figure 3). The crown ethers are placed along the polymer backbone to generate cation-transporting pathways. Significant activities were found, but efforts were discontinued rather soon.

Crown ethers remain the dominant motif in early synthetic ion channels. The covalent ion channel **5**, a pioneering masterpiece of rational design, has been published by the group of Tom Fyles, in Victoria, Canada, in 1989.[7] A central crown is coupled to three amphiphilic macrocycles on each side to produce a hydrophilic channel across the bilayer and hydrophilic carbohydrate anchors at the end to stabilize an extended conformation. Excellent activities were reported. Later on, the Fyles group removed the central crown and focused on membrane spanning, facially amphiphilic macrocycles, including one of the first examples for voltage gating (see below). More recently, the group moved on to even smaller fragments, simple hydrophobic esters, which are being screened for the generation of single-channel currents, some of them being remarkably active.

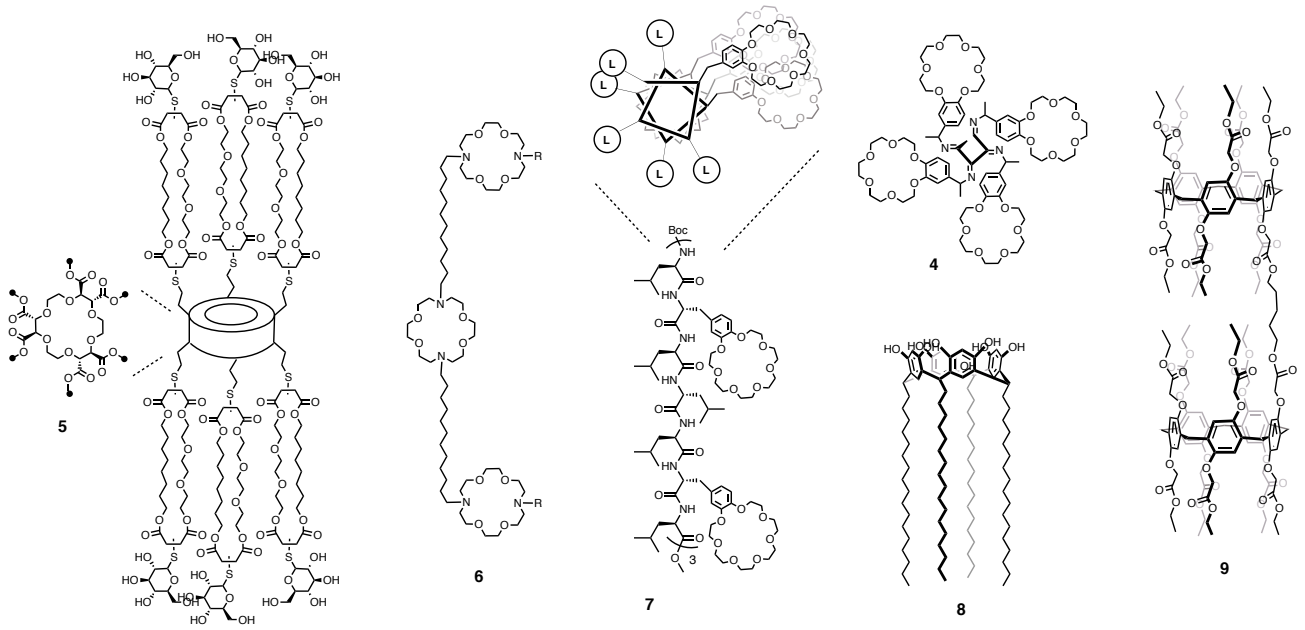

Figure 3. Synthetic ion channels made from macrocycles, including early and classical variations of crown-ether based systems as well as a milestone example for resorcinarenes and a recent example for pillararenes.

The year later, in 1990, George Gokel, another giant in the field, reported for the first time on oligocrown ion channels **6**.[8] By now, this is one of the most charismatic early synthetic ion channel, perfect in its simplicity and complete with regard to all functional and structural requirements, including single-channel currents. Monomeric crown ethers bind cations according to their size, and can function as carriers to shuttle cations across bulk and bilayer membranes. To convert these monomeric ion carriers into ion channels that span a bilayer and stay immobile while the cations are hopping from one binding site to the other, three crowns are simply linked together. These trimers have been studied comprehensively. This includes modifications of crown size, length of the alkyl chains, variation of functional groups and the termini R, as well as functional studies by NMR, with ion selective electrodes, and in fluorogenic vesicles, planar bilayer conductance experiments and cells.

A classical architecture from the group of Normand Voyer at Laval University in Quebec explores the alignment of crown ethers aligned along an α-helical scaffold.[9] Separated by three hydrophobic leucine residues, the folding of the peptide **7** into an α-helix places all crowns on one side of the hydrophobic, transmembrane scaffold to yield a channel for cations to hop from crown to crown. Since the first appearance in 1995, this system has been carefully characterized with regard to length and nature of the helix, nature, number and size of the crowns, as well as possible applications in medicine and materials sciences.

Several macrocycles other than crown ethers and cyclodextrins have been explored in the context of synthetic ion channels. Cyclic peptides and supramolecular macrocycles, G quartets etc, will be mentioned later on, other examples include calixarenes, resorcinarenes, pyrogalloarenes, cucubiturils, porphyrins, calixpyrroles or pillararenes. The resorcin[4]arene channel **8** from the group of Yoshiaki Kobuke has been an early milestone in 1995 because it is the first report on ion selectivity in single-channel measurements.[10] In these measurements, salt gradients are applied to generate currents that flow in the absence of voltage. The voltage needed to stop these currents from flowing gives the reversal potential, which is inserted into the Goldman-Hodgkin-Katz equation to yield permeability ratios. Today, this method is routine in the field, but the reliable determination of ion selectivity topologies is possible in fluorogenic vesicles as well. In channel **8**, the hydrophilic resorcinarene was designed as entry gates to the channel at the membrane surface, the hydrophobic tails to span one leaflet. Potassium selectivity was expected to originate from cation-π interactions within the macrocycle. The smaller sodium is less preferred because binding is too loose, the larger rubidium blocks the channel. The implication that the cations pass through the macrocycle has generated a healthy controversy in the field that lasted for many years.

One of the most recent key examples on macrocycles is the pillar[5]arene **9** from the group of Jun-Li Hou in Shanghai.[11] This is an extremely interesting architecture because in the crystal structure, the internal channel is filled with a single file of water. In single-channel experiments and in fluorogenic vesicles, high selectivity for the transport of protons is observed, presumably by a

hop-turn mechanism along this molecular water wire. In a follow-up study, water transport is explored in more detail, contributing to a current shift of attention in several groups toward water channels. This is attractive with regard to biological water channels on the one hand and possible applications toward water purification on the other. However, the detection of selective water transport in synthetic systems remains challenging. Moreover, lipid bilayer membranes as such are already water permeable, a process that is best known from rapid vesicle swelling and shrinking in response to osmotic stress.

Larger Architectures

The thickness of standard lipid bilayers is about 3-4 nm, transmembrane architectures are thus necessarily large. One of the most significant limitations in the field is the poor development of synthetic methods to create such large architectures. Whereas we are very good at synthesizing small molecules of almost any structure, our performance is much less convincing when asked to build larger systems. One way to bypass this limitation is to use strategies from nature. With biological ion channels being mainly helix bundles, this structural motif has been envisioned early on for the de-novo design of larger ion channels.

One of the most impressive examples from this approach are the "minimalist" ion channels from the group of DeGrado and Lear, then in Philadelphia (Figure 4).[12] Only two amino acids, a hydrophobic leucine and a hydrophilic serine, are used. In the heptad repeat for two turns of an α-helix, the two amino acids are arranged in a sequence that places all hydrophilic serines on one side and all hydrophobic leucines on the other side of helix **10**. In bilayer membranes, these facial amphiphiles self-assemble into helix bundles **11** with a hydrophobic surface to interact with the lipid tails in the hydrophobic core of the membrane, and a hydrophilic ion channel within the bundle. With only two amino acids, ion channel **11** recreates the protein tertiary structure that is present in most biological ion channels.

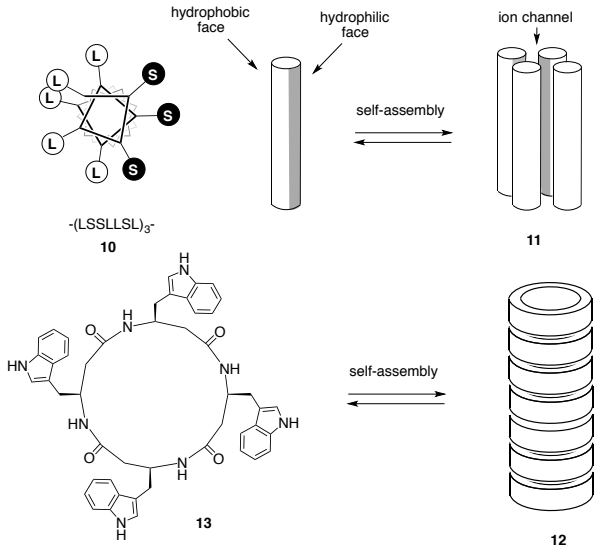

Figure 4. Ion channels formed by α-helix bundles and peptide nanotubes, with a schematic axial view of the α-helix formed by peptide **10** (backbone in solid lines, side chains with single-letter abbreviations).

In water, amphiphilic helices self-assemble into helix bundles in an antiparallel manner to benefit from attractive dipole-dipole interactions of their macrodipoles. The resulting tertiary structure does not have a macrodipole because the macrodipoles of the individual helices are cancelled out in antiparallel bundles. In polarized lipid bilayer membranes, however, self-assembly occurs in a parallel manner and gives bundles with large macrodipoles for powerful dipole-potential interactions. As a result, ion channels formed by helix bundles are usually voltage sensitive. This term stands for a non-linear, exponential change in activity with increasing membrane potentials, characterized by the gating charge z_g (see below). In a milestone publication in 1997, DeGrado and Lear modulated the macrodipole of ion channel **11** with positive and negative charges attached to the N-terminus.[12] A positive charge at the N-terminus increases the macrodipole of helix **10**. As a result, an increase in voltage sensitivity is observed. Formal removal of the macrodipole of helix **10** with a negative charge at the N-terminus gives nearly ohmic channels with linear voltage dependence.

Helix bundles like **11** are not always considered as synthetic ion channels because they are built exclusively from the same amino acids that are present in biological ion channels. A similar

peptide-based approach has been developed with peptide nanotubes **12**. In the first report from the group of Reza Ghadiri at Scripps on this topic, cyclic peptides composed of α-amino acids with alternating stereochemistry have been used.[13] The sequence is as in the well known β-helical ion channel gramicidin, and the assembly of the macrocycles into hollow nanotube thus mimics the folding into a β-helix, with all hydrophobic amino-acid residues at the outer surface to interact with the bilayer and a hollow channel inside without any functional groups, leaving free lone pairs from backbone carbonyls to interact with cations.

Peptide nanotubes have been studied comprehensively and continue to attract interest in several groups, particularly the one of Juan Granja in Santiago de Compostela. For example, larger macrocycles have been shown to give larger channels, exchange of the macrocycles at the membrane surface can modulate ion channel characteristics, stereochemical defects have been used to introduce serine residues at the inner channel surface (as in β-helix **2**, Figure 1), and charged macrocycles provide access to antibiotic activity. In the most innovative example with reduced biomimicry, cyclic β-peptides **13** composed of β-amino acids are used to build peptide nanotubes. This approach is interesting because in β-sheets formed by β-peptides, the amide carbonyls are uniformly oriented. In principle, this should lead to peptide nanotubes with a strong macrodipole that, like helix bundle **11**, should afford voltage-dependent ion channels.

In biology, the transport of ions is usually achieved with helix bundles, whereas the second pure protein tertiary structure, that is the β-barrel, is in charge of transporting molecules. Contrary to the straightforward synthetic access to helix bundles, the synthesis of β-barrels remains difficult until today, mainly because of folding problems and questions how to stabilize large internal space. Our group, on the move from Georgetown University to Geneva, has introduced early on a solution for this problem. In this approach, non-planar *p*-oligophenyls serve as artificial 3D turns to roll planar β-sheets into cylindrical structures (Figure 5).[14] Short peptides attached along the 3D turn interdigitate with peptides from the adjacent 3D turn to form antiparallel β-sheets. In this architecture, the length of the barrel is controlled to match the thickness of the bilayer membrane, and both external

and internal barrel surfaces can be functionalized in a straightforward and rational manner. The transmembrane orientation of the fluorescent *p*-oligophenyl turns could be easily confirmed by depth quenching, the stoichiometry of the supramolecular barrels, sometimes hexamers (**14**), more often tetramers (**15**), is deduced from Hill analysis.

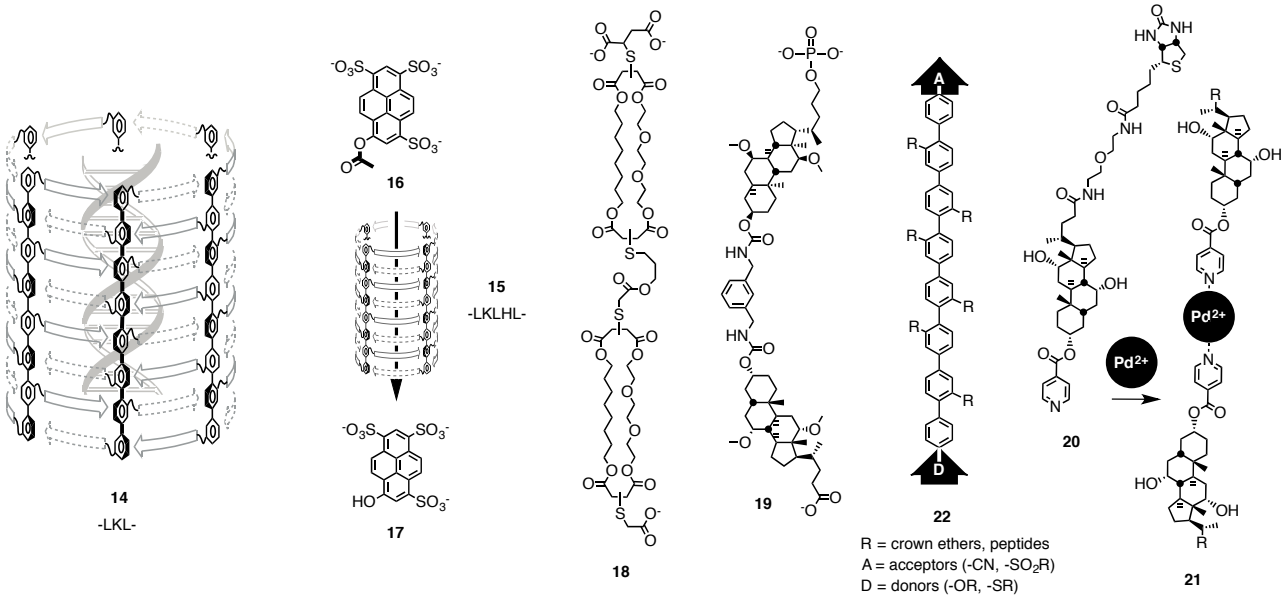

Figure 5. Synthetic ion channels made for voltage gating, ligand gating, blockage and catalysis, including artificial β-barrels blocked by an internal DNA duplex (hexamer **14**) or catalyzing an ester hydrolysis (tetramer **15**). α-Sheets are shown as arrows, sequences with single-letter abbreviations.

The advantages of the artificial β-barrel architecture have been explored to the fullest. Structural variations focus on modification of peptide length and sequence, including artificial amino acids. Push-pull rods are used to build voltage-gated ion channels (see below, Figure 5). The unique possibility to place functional groups at the inner barrel surface along the ion-conducting pathway provides access to synthetic pores that open (ligand gating) and close (blockage) in response to chemical stimulation, with blockage by DNA duplexes in inclusion complex **14** as first example (Figure 5).[14] The possibility of molecular recognition by synthetic ion channels naturally leads to applications in sensing and catalysis, the latter exemplified with catalytic pore **15** that can transform ester **16** into product **17**.[1] Some of these developments will be discussed in the next two chapters.

The search for synthetic methods to create large transmembrane architectures continues until today. Milestones will be described later on with the introduction of π-stack architectures[15] and metal-organic channels and pores.[16] Other examples include the self-assembly of dendronized dipeptides into nanopores reported from the Percec group or branched, linear and cyclic oligocholates from the groups of Regen and Zhao. The most recent breakthrough in this direction is the application of DNA origami to the construction of giant pores (below).[17]

Voltage Gating

Around the turn of the millennium, the hot topic in the field was voltage-sensitive ion channels. They are essential for nerve signal transduction and could be expected to have interesting applications in the materials sciences. The big question was how to do this. Insights from helix bundles **11** suggested that the parallel self-assembly of asymmetric linear oligomers into transmembrane bundles would be the strategy of choice (Figure 4). The first of these asymmetric scaffolds has been reported by the Fyles group in 1998 (Figure 5).[18] It is built with a fragment of the early ion channel **5** from the same group (Figure 3). The central crown ether is removed and two amphiphilic macrocycles are linked together to give the linear scaffold **18** with a hydrophilic and a hydrophobic face. These facial amphiphiles, a bit like facially amphiphilic α-helices **10**, are then expected to self-assemble into transmembrane bundles with a central channel for cations. The asymmetry needed for voltage gating is introduced with one negative charge at one terminus and two negative charges at the other. Despite the small asymmetry the observed voltage gating is significant, probably because of the multiplied asymmetry in the bundles. The same approach was reproduced soon afterwards in the Kobuke group with asymmetric cholate scaffolds **19**.[19] Cholate is a classical motif in synthetic channels because it is a readily accessible facial amphiphile that can span almost one leaflet of a bilayer membrane and can be easily modified. It will be discussed again in the context of ligand-gated assembly of cholates **20** into the metal-organic channel **21** (see below).

Whereas the approaches by Kobuke and Fyles use fixed terminal charges to introduce the asymmetry needed for voltage gating, we have been interested to achieve the same by dipole-potential interactions with neutral scaffolds. One year after Tom Fyles, push-pull rods **22** have been introduced as general scaffold to build voltage-gated ion channels.[20] Several donors and acceptors have been tested at both termini. Sulfide donors are most attractive because they can be converted into sulfone acceptors. This allows us, in principle, to turn macrodipoles along rigid-rod scaffolds on and off in situ. For transport, crown ethers or peptides are placed along the scaffold, the latter for the parallel self-assembly into artificial push-pull β-barrels (Figure 5).

Molecular Recognition and Catalysis

The creation of synthetic ion channels that respond to chemical stimulation is of highest importance because they are the basis of all applications toward sensing and catalysis. Except for an early study from the Fuhrhop group, this topic became accessible with the availability of artificial β-barrels. The first report on blockage of a synthetic ion channel with support from single-channel conductance experiments appeared in 2000, describing the inactivation of a giant β-barrel pore with a DNA duplex.[14] Functional studies are complemented by structural studies in support of the formation of the transmembrane inclusion complex **14** (Figure 5). Since then, many examples have been realized with the same motif, including α-helix recognition and aromatic donor-acceptor complexes. Molecular recognition in inclusion complexes within artificial β-barrel pores turns out to be much more effective than association at the entrance of the barrel. Transmembrane pores offer the unique advantage to measure the depth of molecular recognition within an inclusion complex.[21] For voltage-dependent blockage, artificial β-barrels of the general structure **15** are equipped with different peptide strands. With this significant synthetic effort, active sites can be contracted toward the middle of the ion-conducting pathway. This active-site contraction increases the voltage dependence of molecular

recognition. According to the Woodhull equation, the depth of molecular recognition within the synthetic pores is about 1 nm, that is as expected from their structures.

With evidence for binding in hand, the extension to sensing and catalysis was the obvious next step. The first realized approach is biosensing with synthetic pores. In biosensors, the selectivity of enzymes is used to detect an analyte in a complex mixture from the supermarket or the hospital. For biosensing with synthetic ion channels, this enzymatic signal generation has to consume or produce substrates or products that activate or inactivate the pore. This was first realized for the detection of the activity of aldolases, glycosyltransferases, phosphatases and kinases with artificial β-barrels analogous to **15**.[22] Biosensing with synthetic pores was then applied to the detection of sucrose, lactose, lactate, citrate and glutamate in the context of an artificial tongue, and to the detection of polyphenols, phytate, IP_7 and cholesterol. Covalent capture strategies to amplify signals for otherwise undetectable analytes have been introduced, and other sensing principles such as differential sensing or the aptamer version of immunosensing have been realized over the years.

The second main application of molecular recognition by synthetic ion channels is catalysis. The catalytic pore **15** was reported first in 2001 to catalyze the hydrolysis of ester **16** into product **17**. The catalytic pore shows turnover and follows Michaelis-Menten kinetics.[23] A significant K_M = 4.1 μM confirms that, contrary to catalysis in solution, a quite substantial ground-state stabilization is needed to catch the substrate on the way through the catalytic pore. To overcompensate this ground-state stabilization, a transition-state stabilization of -56 kJ/mol is more than sufficient.[23] This value corresponds to an outstanding catalytic proficiency of 7.1×10^9 M^{-1}. The voltage dependence of catalysis reveals steering effects for both substrate binding and product release, long-range effects related to enzymatic catalysis that can only be studied with catalytic pores. Subsequent studies demonstrate the supramolecular nature of the catalytic pore[1] and the influence of ionic strength and lateral membrane pressure. However, the promise to observe chemical transformations within synthetic catalytic pores by conductance experiments on the single-molecule level remains to be confirmed.

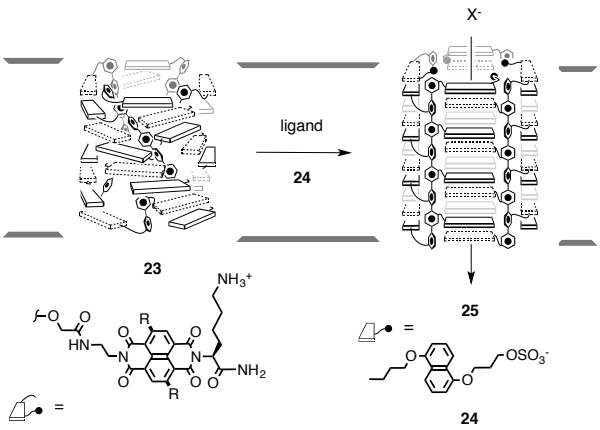

Figure 6. Ligand-gated synthetic ion channels with π-stack architectures. R = H (colorless) or isopropylamino (blue).

Ligand gating, that is the activation of synthetic ion channels by molecular recognition, was more difficult to realize. The first example, reported in 2004, focuses on the binding of amphiphiles to the hydrophilic outer surface of artificial β-barrel pores of the general structure **15** to enable their partitioning into lipid bilayer.[24] This example is particularly nice because ligand gating is coupled with blockage. Namely, once inserted into the membrane by ligand recognition at the outer barrel surface, the active pore can be cleanly blocked by α-helix recognition at the inner barrel surface. However, biomimetic ligand gating should not operate by partitioning. A ligand-gated synthetic ion channel should exist in the membrane in a closed state and then, in response to molecular recognition, undergo a conformational change to yield the open active pore (Figure 6).[25] In the first ligand-gated synthetic ion channel, the closed architecture **23** is a tetrameric helical π-stack. The twisting into a closed helix was designed to occur because of the mismatch of the repeat distance of 5 Å of the *p*-octiphenyl scaffold and the 3.4 Å repeat of a face-to-face π-stack formed by naphthalenediimides (NDIs) attached along the scaffold. The intercalation of π-basic dialkoxynaphthyl ligands **24** increases the repeat distance in the stack and thus causes the opening of the closed helix into barrel-stave architecture **25** with a central ion channel. The experimental results are in support of

these high expectations. Ligand gating occurs with high sensitivity (EC_{50} = 13.7 μM) and cooperativity (n = 6.5). The obtained ion channels show a low, anion-selective single-channel conductance that is consistent with the expected barrel-stave architecture **25**. This system was used later on to combine synthetic ion channels with artificial photosynthesis. All that had to be done was to use π-stacks that 1) can absorb visible light and 2) can transport electrons and holes. This was easily achieved by introducing electron-donating alkylamino groups R into the core of the NDIs. The resulting stacks have blue color, are capable of symmetry-breaking photo-induced charge separation into NDI radical anions and cations, and convert photonic energy into transmembrane proton gradients.

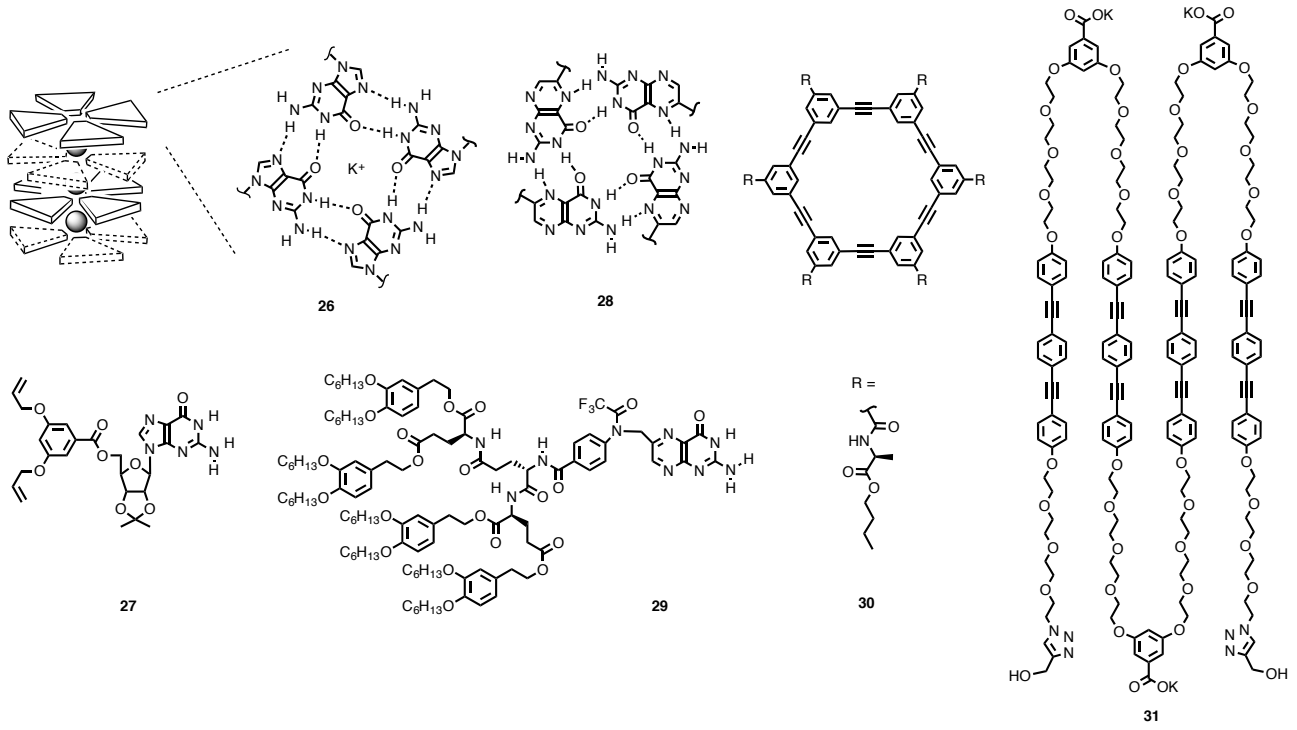

Figure 7. Synthetic ion channels with π-stack architectures composed of G- and folate quartets as well as shape-persistent covalent *m*-OPE macrocycles and *p*-OPE oligomers.

A more recent, highly innovative approach to ligand gating uses organometallic chemistry. The heavy-metal approach to synthetic ion channels appeared 2008 in the literature and will be discussed later on. To apply organometallics to ligand gating, the group of Simon Webb in Manchester equipped the classical cholate scaffold in **20** with a pyridine at ring A and a hydrophilic tail at ring D

(Figure 5).[26] Coordination of two pyridines to palladium in the middle of the membranes should then cause the dimerization of monomers **20** into transmembrane scaffolds **21**. Because of the facial amphiphilicity of cholate (see above), these metal-organic scaffolds should in turn self-assemble into transmembrane bundles with a central ion channel. Transport activity does indeed increase upon addition of palladium. Removal of Pd^{2+} with hexathia-18-crown-6 closes the metal-organic channel. Binding of the biotins at the surface of the membrane causes vesicle aggregation and inactivates the ligand-gated ion channels.

π-Stacks

In 2006, π-stacks were established as fundamentally new structural motif to build synthetic ion channels (Figure 7). This is particularly interesting because π-stacks can transport also electrons. They are thus attractive for applications of synthetic ion channels in artificial photosystems. For example, electron/anion antiport can and has been use to build electroneutral photosystems capable of multiple turnovers without membrane polarization.

Early studies on ligand gating with π-stack **23** demonstrate that π-stacks can provide access to other important functions (see above, Figure 6).[25] π-Stacks really moved into focus with a report from the Davis group in early 2006 on G-quartet ion channels **26** (Figure 7).[27] G-quartets are cyclic tetramers formed by guanine derivatives around potassium cation templates that also support the π-stacking of G-quartets on top of each other. The idea to use hydrophobic guanosines such as **27** to build G-quartet ion channels has been around for some time. However, the relatively poor stability of G-quartets formed by monomeric guanosine derivatives made this vision more difficult to realize than expected. The solution came from an elegant covalent capture strategy conceived in the Davis group. To stabilize the large, transmembrane architecture **26** reminiscent of an ion channel, terminal olefins are placed at the periphery of monomers **27**. Grubbs cross metathesis after self-assembly in lipid bilayer membranes then transforms the unstable supramolecules into unimolecular ion channels. According to circular dichroism (CD) spectroscopy analysis, G-quartets containing oligomers are only

obtained when metathesis is applied in the presence of lipid bilayers. The crosslinked G-quartet architecture **26** is active in two different Na transport assays in vesicles, whereas monomer **27** is inactive. In the following years, the G-quartet approach continued to be useful to build larger pores with G-quartets serving as scaffolds.

The appearance of G-quartet ion channels coincided with that of folate quartet channels **28** from the group of Takashi Kato in Tokyo.[2] Like G-quartets, folate derivatives form cyclic tetramers that stack on top of each other. Contrary to G-quartets, folate quartets are stable enough to form also without templation from central cations during the assembly process. The formation of the more stable folate-quartet channels should thus be possible without the covalent capture. In monomer **29**, a hydrophobic dendron is attached to insulate the folate quartet from the surrounding membrane with a self-organizing hydrogen-bonded network and a lipophilic surface. The formation of folate quartets **28** in bilayer membranes causes the appearance of distinct, small single-channel currents, indicating the existence of at least two different active structures with slightly different conductance (Figure 1B). The folate channel **28** is cation selective with a fairly pronounced and clean Eisenman I topology (Cs>Rb>K>Na>Li). CD spectra of the active structure confirm the formation of folate quartets.

Last year, a very elegant synthetic ion channel with an innovative, beautiful π-stack architecture has been reported by the group of Bing Gong.[28] Different to the preceding quartets, the Gong channel is built from covalent, shape-persistent *m*-oligophenylethynyl (OPE) macrocycles **30**. Comparable to G-quartets **26** and cyclopeptides **12**, these shape-persistent, planar macrocycles and the foldamer helices formed by their linear and longer homologs have been studied for many years in different contexts, and their application to synthetic ion channels was only a question of time. Transmembrane π-stacks are stabilized by surrounding self-organizing hydrogen-bonded networks and a lipophilic surface to interact with the bilayer membrane. The inner surface facing the central channel of the stack formed by **30** is completely hydrophobic. As in biology, hydrophobic channels are of interest to transport water. With π-stacked **30**, a small single-channel conductance of 5.8 pS is detectable in the presence of potassium. However, a spectacular H/K permeability ratio of ~2000 is in strong support of hydrogen-bonded chains of water along the hydrophobic channel. According to light scattering

experiments, the channel formed by stacked macrocycles **30** mediates the osmotic swelling and shrinking of vesicles. This carefully characterized system thus contributes, together with the pillararenes **9** from Jun-Li Hou and complementary systems from the groups of Percec and Barboiu, to an increasingly rich collection of potential water channels. Significance and challenges regarding this young field have been stated above.

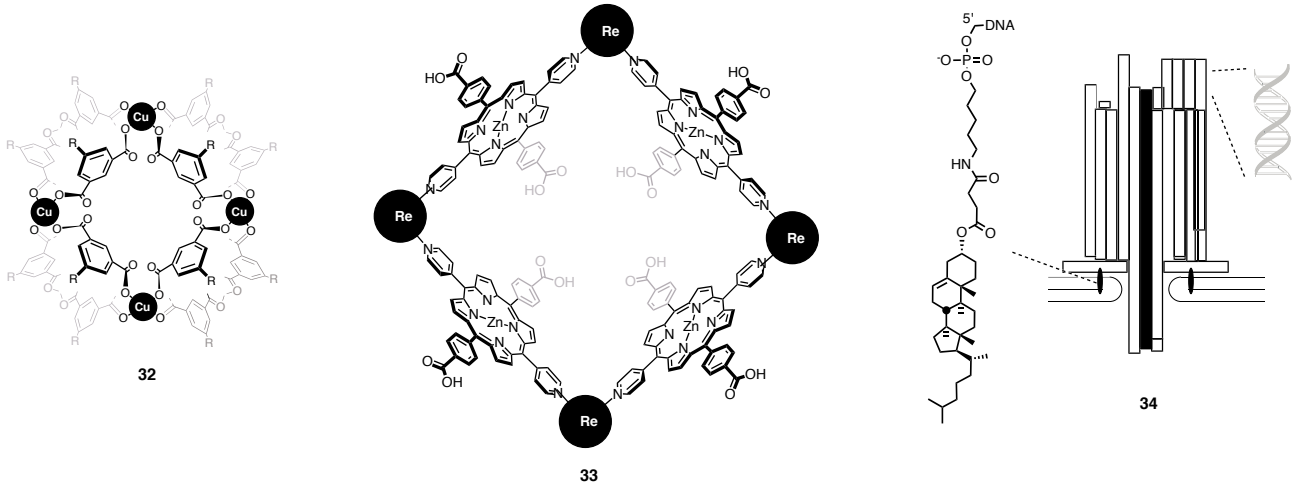

Figure 8. Synthetic ion channels obtained from innovative interdisciplinary approaches: Metal-organic scaffolds and DNA origami.

A different, very recent approach to transmembrane π-stack architectures comes from the group of Kazushi Kinbara in Sendai.[15] In the very long oligomers **31**, four short *p*-oligophenylethynyls are separated by oligoethyleneglycol solubilizers with negatively charged carboxylates in the middle (Figure 7). These oligomers are designed to fold into transmembrane structures that look like helix bundles. Fluorescence depth quenching confirms that the vertical OPE stacks are located in the middle of the membrane. These OPE stacks are essential for activity, monomers and analogs with reduced triple bonds are inactive. The formation of ohmic, short-lived and quite homogenous single channels is observed in planar bilayer conductance experiments. This lability and a Hill coefficient $n = 4$ made the authors conclude that the active structure is a tetramer of **31**, that is a supramolecular

bundle of a covalent bundle, a motif very well known from nature. An interesting Eisenman selectivity sequence XI with a preference for lithium has been reported. Contrary to π-architectures with G-quartets, folate quartets or *m*-OPEs, the stack of linear *p*-OPEs in the Kinbarra system are oriented parallel to the lipid tails in the membranes. Similar transmembrane bundles of conjugated oligomers have been reported for *p*-oligophenyls, oligo-naphthalenediimides and oligo-perylenediimides.

Metal-Organic Channels

Synthetic ion channels with metal-organic scaffolds have been introduced in 2008. At that time, metal-organic frameworks (MOFs) already attracted massive interest as porous materials that could eventually be used to absorb and store hydrogen, CO_2, and so on. The success of MOFs in the solid state suggested that similarly porous metal-organic architectures could be used to create synthetic ion channels. Cations have been used before in architectures containing G-quartets or metalloporphyrins. Real heavy-metal channels were proposed first in an inspired report from the Fyles group, proposing to apply the classical coordination squares from Fujita and Stang to the construction of synthetic ion channels. However, the topic finally took off in 2008 with reports from the groups of Simon Webb and from Kimoon Kim in Pohang, South Korea.

The palladium-gated ion channel **21** has already been described in the context of ligand gating (Figure 5).[26] In brief, coordination chemistry is used to bring amphiphilic cholates in the two leaflets of a bilayer membrane together, and the obtained metal-organic scaffolds self-assemble into transmembrane ion channels. The realized use of transmembrane metal-organic scaffolds is particularly valuable because ligand gating, one of the most demanding function, is realized at the same time (see above).

The metal-organic polyhedron (MOP) **32** appeared at the same time (Figure 8).[29] This stable, neutral cuboctahedron is easily accessible from isophthalates and $Cu(OAc)_2$. The hydrophilic interior

with diameter of 13.8 Å can be reached from all sides through coordination squares with a diameter of 6.6 Å. In planar bilayers, isophthalate MOPs **32** generate small, ohmic, long-lived and cation-selective single-channel currents. As with Kobuke's pioneering calixarene channels **8** (Figure 3),[10] cation-π interactions in Kim's MOPs **32** have been suggested to determine the cation selectivity topology.

Among the synthetic ion channels with metal-organic scaffolds that followed their discovery in 2008, a very recent example from the group of Paolo Tecilla in Trieste stands out.[16] In the metal-organic pore **33**, this innovative strategy is used to address two central challenges in the field, that is to synthesize large architectures and to stabilize large empty space within these structures (Figure 8). The approach builds on a design from the Kobuke group to connect supramolecular porphyrin macrocycles with carboxylic acid dimers. In the Kobuke system, the porphyrin macrocycles are formed by the classical coordination of peripheral pyridines to zinc cations in the porphyrin core. Not so in the Tecilla system. Here, peripheral pyridines are coordinated to Re(I) to form stable porphyrin tetramers with large pores in the middle. Hill coefficients $n = 1$ suggest that the dimers needed to span the membrane are thermodynamically stable. Very high activities were found in fluorogenic vesicles. Moreover, the large metal-organic pores **33** can be blocked by large enough PAMAM dendrimers.

DNA Origami

As with MOFs, the recent application of DNA origami represents a conceptually innovative approach that transplants a hot topic from another discipline into the field of synthetic ion channels.[17] This intertopical crossfertilization is most exciting. DNA origami has been introduced recently as a revolutionary approach to fold planes of crosslinked DNA duplexes into virtually any large structure of free choice. Early examples include 40 nm sized boxes that can be opened, closed and locked. DNA origami thus addresses one of the central challenges faced with synthetic ion channels and

pores, that is the availability of general strategies to synthesize large architectures. Naturally, as with synthetic peptides (Figure 4), one can question if an ion channel constructed from DNA duplexes really qualifies as synthetic ion channel. Nevertheless, the results obtained with functional DNA origami are absolutely fantastic.

The DNA origami pore **34** was designed to look like α-hemolysin (Figure 8). α-Hemolysin is a biological β-barrel toxin that is most popular in the field because it forms stable pores that are ideal for applications to stochastic sensing on the single-molecule level.[30] The giant architecture **34** is composed of co-axial DNA duplexes, cholesterol tails attached at one end of the bundle to assure binding to the membrane, and a small barrel extending from there to span the bilayer membrane.[17] The main difference to α-hemolysin is that the transmembrane pore has a hydrophilic outer surface that should cause the membrane to roll up into micellar defects to surround the pore with a hydrophilic contact.

This conceptually quite simple approach gave marvelous results. The synthesized architectures are large enough for direct imaging with electron microscopy. The same is possible for the binding to vesicles, the obtained images show the DNA origami sitting on the bilayer membrane as designed. In planar bilayers, DNA origami **34** produces the large, long-lived ohmic single-channel currents that are expected from the diameter of the designed pore. Most importantly, the passage of single-stranded DNA through the origami pore **34** can be observed as a short blockage event on the single-channel level. The extent of blockage depends on the length of the DNA blocker, blockage duration on the duplex or quadruplex terminus placed to slow down the translocation. The importance of these results, although they will have to be confirmed, cannot be overestimated. It is the first time that blockage has been detected on the single-channel level with synthetic pores, and the perspective to do organic chemistry in the broadest sense within DNA origami pores is fascinating.

Perspectives

Looking back over three decades, it is exciting to realize how the field of synthetic ion channels and pores has matured. In general, the most significant insights could be secured when fundamental principles of ion-channel biophysics could be addressed with the grand principles of organic chemistry, and *vice versa*. This is true for the generation of single-channel currents, voltage gating, ligand gating and blockage on the one hand and their application to study molecular recognition, sensing, catalysis and artificial photosystems on the other hand. Big breakthroughs occurred reliably at maximal crossfertilization with hot topics beyond precedence in nature. Examples include MOFs, DNA origami, organocatalysis or biosensing. More practical applications in materials sciences, biology and medicine exist, but they are not the most important driving force for past and future development of the field and thus covered only en passant in this review.

The most important challenge today concerns synthetic organic chemistry. Synthetic ion channels and pores that can do "more than just punch holes" are consistently large architectures. Synthetic strategies to prepare large functional systems - without overwhelming efforts and without too much help from biological approaches - are remarkably rare. The examination of promising approaches from other fields such as MOFs or DNA origami is so exceptionally important for exactly this reason.

Progress with the organic synthesis of large architectures will be the key to clean up the basics, that is to learn how to predictably and reliably generate single-channel currents that respond to physical as well as chemical stimulation. This is the heart of the matter. In single-channel experiments, the binding of a blocker to a channel can be seen as a drop in conductance to the level describing the host-guest complex, and a return to the conductance level of the free open channel upon release of the blocker (Figure 1C). Although much material has accumulated in this direction, the detection of single-channel blockage has been reported only for DNA origami[17] and, in one case and irreversibly, also for rigid-rod β-barrels.[14] Both are large architectures.

Lessons from biological and bioengineered pores illustrate that with predictable access to responsive single-channel currents, the grand principles of organic chemistry can be explored in the

broadest sense on the single-molecule level. Pioneering work from the Bayley group in Oxford documents applicability of single-channel blockage to the stochastic sensing of single analytes, the sequencing of single genes, and the detection of chemical reactions.[30] The holy grail, maybe more from a personal point of view, is catalysis within synthetic pores. The vision of substrates entering a synthetic pore on one side and being ejected transformed at the other side of the membrane is fascinating. Preliminary results confirm that long-range steering effects - a bit as in enzymes - will be operational. The ultimate objective will be to study single reactive intermediates in catalytic pores. However, this topic is very demanding, is unexplored with biological and bioengineered pores and has not progressed since the initial introduction of synthetic catalytic pores.[23]

Progress with the organic synthesis of larger architectures will also enable more systematic studies on ion selectivity. Studies of this intrinsic property with synthetic ion channels are so far more eclectic than systematic. Milestone achievements such as the calixarenes **8** and MOPs **32** with potassium selectivity or pillararenes **9** and π-stacks **30** with proton selectivity have been mentioned above from different points of view. However, most current studies on ion selective transport focus on transporters other than ion channels, mainly ion carriers. This is understandable considering that the ion selectivity of synthetic ion channels is particularly difficult to explore. The fundamental challenge is that the tight ion binding needed for high selectivity slows down the fast transport needed with ion channels. The biological solution of the problem to combine selectivity with speed is to place multiple ion-binding sites in line. Several synthetic transport systems for cooperative ion hopping across the membrane have been reported, including classical crown ether motifs (e.g., **4**, **6**, **7**, Figure 3) and hydrogen-bonded chains, cation-π slides and anion-π slides established along rigid-rod scaffolds. However, as far as pure synthetic ion channels are concerned, systematic studies on ion hopping with selectivity topologies from single-channel permeability ratios remain as another important challenge for the future.

All applications beyond single-channel measurements are not unique for synthetic ion channels and can be realized with any synthetic transport system. This does not mean that they are less relevant. In the contrary, ion-selective transporters, antibiotics, channel-replacement therapeutics, delivery

systems, water purification systems, sensors, photosystems, catalysts, and the like: The perspectives beyond single-channel measurements are about as broad, as significant and as entertaining as it gets.

Acknowledgment

We warmly thank all present and past coworkers and collaborators who have contributed to this research. For financial support, we thank the University of Geneva, the European Research Council (ERC Advanced Investigator), the National Centre of Competence in Research (NCCR) Chemical Biology and the Swiss NSF.

(1) Litvinchuk, S.; Bollot, G.; Mareda, J.; Som, A.; Ronan, D.; Shah, M. R.; Perrottet, P.; Sakai, N.; Matile, S. Thermodynamic and Kinetic Stability of Synthetic Multifunctional Rigid-Rod β-Barrel Pores: Evidence for Supramolecular Catalysis. *J. Am. Chem. Soc.* **2004**, *126*, 10067-10075.

(2) Sakai, N.; Kamikawa, Y.; Nishii, M.; Matsuoka, T.; Kato, T.; Matile, S. Dendritic Folate Rosettes as Ion Channels in Lipid Bilayers. *J. Am. Chem. Soc.* **2006**, *128*, 2218-2219.

(3) Tabushi, I.; Kuroda, Y.; Yokota, K. A,B,D,F-Tetrasubstituted β-Cyclodextrin as Artificial Channel Compound. *Tetrahedron Lett.* **1982**, *23*, 4601-4604.

(4) Kennedy, S. J.; Roeske, R. W.; Freeman, A. R.; Watanabe, A. M.; Besch, H. R. Jr. Synthetic Peptides Form Ion Channels in Artificial Membranes. *Science* **1977**, *196*, 1341-1342.

(5) Kobuke, Y.; Ueda, K.; Sokabe, M. Artificial Non-Peptide Single Ion Channels. *J. Am. Chem. Soc.* **1992**, *114*, 7618-7622.

(6) Neevel, J. G.; Nolte, R. Ion Transport Across Vesicle Bilayers Mediated by an Artificial Channel Compound. *Tetrahedron Lett.* **1984**, *25*, 2263-2266.

(7) Carmichel, V. E.; Dutton, P.; Fyles, T.; James, T.; Swan, J.; Zojaji, M. Biomimetic Ion Transport: A Functional Model of a Unimolecular Ion Channel. *J. Am. Chem. Soc.* **1989**, *111*, 767-769.

(8) Nakano, A.; Xie, Q.; Mallen, J.; Echegoyen, L.; Gokel, G. W. Synthesis of a

Membrane-Insertable, Sodium Cation Conducting Channel: Kinetic Analysis by Dynamic ^{23}Na NMR. *J. Am. Chem. Soc.* **1990**, *112*, 1287-1288.

(9) Voyer, N.; Robitaille, M. A Novel Functional Artificial Ion Channel. *J. Am. Chem. Soc.* **1995**, *117*, 6599-6600.

(10) Tanaka, Y.; Kobuke, Y.; Sokabe, M. A Non-Peptidic Ion Channel with K^+ Selectivity. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 693-694.

(11) Si, W.; Chen, L.; Hu, X.-B.; Tang, G.; Chen, Z.; Hou, J.-L.; Li, Z.-T. Selective Artificial Transmembrane Channels for Protons by Formation of Water Wires. *Angew. Chem. Int. Ed.* **2011**, *50*, 12564-12568.

(12) Lear, J. D.; Schneider, J. P.; Kienker, P. K.; DeGrado, W. F. Electrostatic Effects on Ion Selectivity and Rectification in Designed Ion Channel Peptides. *J. Am. Chem. Soc.* **1997**, *119*, 3212-3217.

(13) Ghadiri, M. R.; Granja, J. R.; Buehler, L. K. Artificial Transmembrane Ion Channels from Self-Assembling Peptide Nanotubes. *Nature* **1994**, *369*, 301-304.

(14) Sakai, N.; Baumeister, B.; Matile, S. Transmembrane B-DNA. *ChemBioChem* **2000**, *1*, 123-125.

(15) Muraoka, T.; Shima, T.; Hamada, T.; Morita, M.; Takagi, M.; Tabata, K. V.; Noji, H.; Kinbara, K. Ion Permeation by a Folded Multiblock Amphiphilic Oligomer Achieved by Hierarchical Construction of Self-Assembled Nanopores. *J. Am. Chem. Soc.* **2012**, *134*, 19788-19794.

(16) Boccalon, M.; Iengo E.; Tecilla, P. Metal-Organic Transmembrane Nanopores. *J. Am. Chem. Soc.* **2012**, *134*, 20310-20313.

(17) Langecker, M.; Arnaut, V.; Martin, T. G.; List, J.; Renner, S.; Mayer, M.; Dietz, H.; Simmel, F. C. Synthetic Lipid Membrane Channels Formed by Designed DNA Nanostructures. *Science* **2012**, *338*, 932-936.

(18) Fyles, T. M.; Loock, D.; Zhou, X. A Voltage-Gated Ion Channel Based on a Bis-Macrocyclic Bolaamphiphile. *J. Am. Chem. Soc.* **1998**, *120*, 2997.

(19) Goto, C.; Yamamura, M.; Satake, A.; Kobuke, Y. Artificial Ion Channels Showing Rectified Current Behavior. *J. Am. Chem. Soc.* **2001**, *123*, 12152-12159.

(20) Winum, J.-Y.; Matile, S. Rigid Push-Pull Oligo(*p*-Phenylene) Rods: Depolarization of Bilayer Membranes with Negative Membrane Potential. *J. Am. Chem. Soc.* **1999**, *121*, 7961-7962.

(21) Baudry, Y.; Pasini, D.; Nishihara, M.; Sakai, N.; Matile, S. The Depth of Molecular Recognition: Voltage-Sensitive Blockage of Synthetic Multifunctional Pores with Refined Architecture. *Chem. Commun*. **2005**, *40*, 4798-4800.

(22) Das, G.; Talukdar, P.; Matile, S. Fluorometric Detection of Enzyme Activity with Synthetic Supramolecular Pores. *Science* **2002**, *298*, 1600-1602.

(23) Sakai, N.; Sordé, N.; Matile, S. Synthetic Catalytic Pores. *J. Am. Chem. Soc.* **2003**, *125*, 7776-7777.

(24) Gorteau, V.; Perret, F.; Bollot, G.; Mareda, J.; Lazar, A. N.; Coleman, A. W.; Tran, D.-H.; Sakai, N.; Matile, S. Synthetic Multifunctional Pores with External and Internal Active Sites for Ligand Gating and Noncompetitive Blockage. *J. Am. Chem. Soc.* **2004**, *126*, 13592-13593.

(25) Talukdar, P.; Bollot, G.; Mareda, J.; Sakai, N.; Matile, S. Synthetic Ion Channels with Rigid-Rod π-Stack Architecture that Open in Response to Charge-Transfer Complex Formation. *J. Am. Chem. Soc.* **2005**, *127*, 6528-6529.

(26) Wilson, C. P.; Boglio, C.; Ma, L.; Cockroft, S. L.; Webb, S. J. Palladium(II)-Mediated Assembly of Biotinylated Ion Channels. *Chem. Eur. J.* **2011**, *17*, 3465-3473.

(27) Kaucher, M. S.; Harrell, W. A. Jr,, Davis, J. T. A Unimolecular G-Quadruplex that Functions as a Synthetic Transmembrane Na^+ Transporter. *J. Am. Chem. Soc.* **2006**, *128*, 38-39.

(28) Zhou, X.; Liu, G.; Yamato, K.; Shen, Y.; Cheng, R.; Wei, X.; Bai, W.; Gao, Y.; Li, H.; Liu, Y.; Liu, F.; Czajkowsky, D. M.; Wang, J.; Dabney, M. J.; Cai, Z.; Hu, J.; Bright, F. V.; He, L.; Zeng, X. C.; Shao, Z.; Gong, B. Self-Assembling Subnanometer Pores with Unusual Mass-Transport Properties. *Nat. Commun.* **2012**, *3*, 949.

(29) Jung, M.; Kim, H.; Baek, K.; Kim, K. Synthetic Ion Channel Based on Metal–Organic

Polyhedra. *Angew. Chem. Int. Ed.* **2008**, *47*, 5755-5757.

(30) Gu, L.-Q.; Braha, O.; Conlan, S.; Cheley, S.; Bayley, H. Stochastic Sensing of Organic Analytes by a Pore-Forming Protein Containing a Molecular Adapter. *Nature* **1999**, *398*, 686-690.