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Review

Artificial transmembrane ion transporters as potential therapeutics

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SUMMARY

Various artificial transmembrane transporters, designed to function through mobile carrier or channel mechanisms, have been developed in the past decade. With the aid of structural manipulation and by employing either discrete chemical entities or self-assembled nanostructures, progress has been made in achieving the selective recognition and transmembrane transport of key ions. The ability to perturb intracellular pH or disrupt intracellular ion homeostasis makes transmembrane ion transporters of interest as potential therapeutics that might see use as cancer treatments or as antibacterial agents. In this review, recent progress in the area of artificial transmembrane ion transporter research is summarized with an emphasis on applications involving anticancer research and antibiotic applications. The examples chosen for highlights are meant to be illustrative of key themes involving synthetic ion transport rather than comprehensive. Nevertheless, it is anticipated that this review will provide a useful entry point for the general reader and set the stage for further progress in the area.

INTRODUCTION

Cellular membranes serve as barriers for the exchange of most substrates into and out of cells. However, the exchange of solutes between cells and their extracellular milieu is crucial for maintaining the normal operation of biological systems.[1–3] Small neutral compounds, such as water and carbon dioxide, can cross the phospholipid membranes relatively easily by diffusion. On the other hand, the transmembrane exchange of charged species, including most physiologically important anions and cations, relies on the assistance of proteins that function as either ion carriers or transmembrane ion channels.[4–6] In healthy cells, cooperative feedback between various ion channels serves to maintain precisely the ion gradients across biological membranes and, as a result, desired intracellular ion concentrations crucial for normal biological processes.[7–9] Failure to maintain ion homeostasis is typically manifest in terms of severe pathological consequences. For instance, dysfunction of Cl^- channels is associated with cystic fibrosis, a disease correlated with higher-than-normal intracellular chloride anion concentrations.[10] In principle, cystic fibrosis and other disorders traced to disruptions in ion transport could be controlled by treating patients with functioning chloride anion channels. However, the limited availability, poor stability, and structural complexity of natural ion channels have so far precluded their use in these and other practical applications. Artificial transmembrane transporters, simpler synthetic molecular systems that mimic the salient properties of natural ion channels, could provide a means to overcome these limitations (so-called channel replacement therapy).[11–16] Appropriate molecular design might

The bigger picture

Artificial transmembrane ion transporters are attractive in the context of drug discovery since, in principle, they may be used as novel therapeutics by promoting apoptosis (programmed cell death), interfering with autophagic processes, or inducing an antibiotic response via disrupting cellular ion homeostasis or dissipating intracellular pH gradients. Considerable effort has been devoted into the design of artificial transporters with selective ion recognition and transport. However, only limited progress has been made in terms of translating fundamental research involving transport mechanisms into systems that function *in vitro*. In this review, we summarize recent progress in the area of artificial ion transporters with a focus on their application as potential therapeutics. The associated discussion of accomplishments and remaining challenges is designed to hasten the day when research on artificial transmembrane ion transporters is translated into *bona fide* clinical benefits.

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allow selective recognition and adaptable ion transport to be achieved for a range of ionic targets, including such physiologically important species as chloride, potassium, and sodium. In this context, both discrete molecular systems (so-called unimers) and self-assembled nanostructures have been the subject of study.[17–22] In addition to targeting ion transport-related disorders, artificial ion carriers and channels (collectively referred to as transporters) show promise for inducing apoptosis in cancer cells by perturbing the intracellular pH or disrupting cellular ion homeostasis.[23] In this review we summarize progress made in the construction of synthetic ion transporters (channels and carriers) with a focus on their potential use as anticancer agents. A limited treatment of antibiotic opportunities afforded by synthetic ion transporters is also included.

Over the past decades, enormous progress has been made in terms of understanding cancer. Nevertheless, cancer remains the second largest cause of death worldwide. Currently, systemic treatments, including anticancer drugs (chemotherapy, hormone, and biological therapies), are the modalities of choice in many instances. However, the indiscriminate destruction of normal cells, the toxicity of conventional chemotherapeutic drugs, as well as the development of multi-drug resistance, provide an incentive to seek new treatments based on new mechanisms of action. Artificial transmembrane ion transporters, which can induce apoptosis (so-called programmed cell death) in cancer cells by changing the intracellular pH or disrupting intracellular ion homeostasis, could provide one such alternative approach.[24–30] Moreover, it is possible that by combining ion transporters with other therapeutic modalities, drug resistance could be effectively overcome.[31]

It is also important to appreciate that the therapeutic benefits associated with perturbing ion homeostasis are not limited to cancer. Bacteria are also attractive targets. As the result of their ability to induce an ion imbalance and to disrupt bacterial membranes, several artificial transmembrane ion transporters have shown promise as antibacterial motifs in recent years.[32,33] For instance, polyether ionophores, complex natural products that can transport cations across biological membranes, often display antimicrobial activity, a finding that has inspired the design and synthesis of artificial ion transporters as antibacterial agents.[34] Since a variety of ion transporters have been reported to date, the discussion will be classified according to the type of ions involved, with particular emphasis being given to systems that show promise as transporters of chloride, potassium, and sodium. This focus reflects not only the physiological importance of these ions but also the fact that they have been the target of most artificial ion transport development work reported to date. Additionally, the examples chosen in this review represent a selection of anion transporters reported in the literature that have been studied most extensively in biological systems.

METHODS AND TECHNIQUES

To provide the readers with an introductory guideline to this field, a brief summary of synthetic ion transporters is given in this section. The term ion carrier applies to a transporter that shuttles across the bilayer and, in doing so, brings about the translocation of ions (Figure 1). In contrast, ion channels are scaffolds that can mediate ion transport across membrane bilayers without undergoing appreciable motion and without disturbing the lipid bilayer supra-structures. Synthetic ion channels include both unimolecular channels and self-assembled channels. Unimolecular synthetic ion channels are typically macrocyclic molecules that are long enough to insert into lipid bilayer membranes and serve as guides for transmembrane ion transport.

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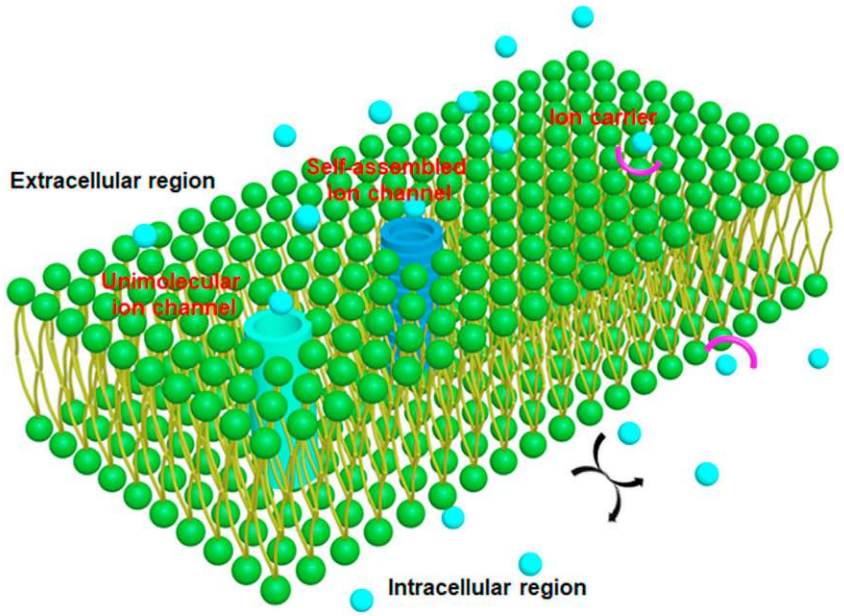

Figure 1. Cartoon illustration of ion carrier and ion channel (unimolecular and self-assembled ion channel)

An advantage of unimolecular channels is their stability, reflecting the fact that they comprise single molecules that remain intact inside lipid bilayers. However, the synthesis of unimolecular channels is often difficult and time consuming. Therefore, the majority of synthetic ion channels reported to date have been created *in situ* via the controlled self-assembly of monomers within the lipid bilayers. However, constructing channels with stable structures and selective binding sites via the rational self-assembly of smaller building blocks remains a challenge.

To differentiate an ion channel from a carrier-type ion transporter, black lipid membrane (BLM) experiments in planar bilayers are typically performed (Figure 2A).[35] Dynamic "open-closed" conductance is generally taken as evidence of ion channel formation (Figure 2B). Moreover, some key parameters of the ion channels could be inferred from these studies, such as the conductance g, the lifetime τ_1, and the open probability P_o. The construction of so-called Hill plots is another tool used to distinguish carrier- from channel-based transport mechanisms.[36] The slope of a Hill plot is equal to the Hill coefficient for the interactions between the agent in question and the membrane under study. A Hill plot with a slope greater than 1.0 is taken as an indication that there is a synergistic concentration dependence (channel mechanism), whereas a slope less than 1.0 indicates competitive concentration dependence (carrier mechanism) (Figure 3A). These limiting regimes are generally interpreted in terms of channel and carrier mechanisms, respectively. However, it is to be appreciated that Hill analyses of synthetic ion channels are not fully developed, and appropriate caution must be exercised in terms of drawing concrete mechanistic conclusions.

A synthetic ion transporter involved in mediating the transport of only a single ion or molecule across a lipid membrane is termed as uniporter, and the process is referred to as uniport. Transporters that move two molecules or ions in the same direction across the membrane are called symporters. If two molecules are moved in opposite

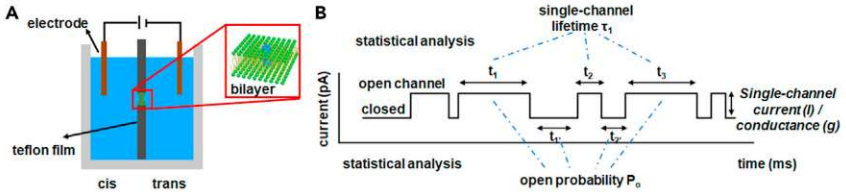

Figure 2. (A) Standard configuration of a black lipid membrane (BLM) experiment.
(B) Key parameters obtained from single-channel currents. Reprinted with permission from Matile and Sakai,[35] copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

directions across the bilayer, the process is called antiport. Transporters involved in the movement of specific ions are called ionophores. If the action of a transporter in moving ions across a membrane results in a net change in charge, the process is described as electrogenic; if there is no net change in charge, the transport is described as electroneutral.

The primary method used to characterize putative ion transporters under cell-free conditions is the liposomal assay.[37] In general, fluorescence spectroscopy is used to monitor fluorophore-containing large unilamellar vesicles (LUVs). Depending on the design of the setup and the fluorescent probes employed, such analyses can provide insights into the influx or efflux of various types of ions. Often, LUVs are prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and then encapsulated with either the pH-sensitive ratiometric fluorescent dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) or the halide-sensitive fluorophore lucigenin (Figure 3B). The fluorescence intensities of HPTS and lucigenin are calibrated to the pH and chloride concentrations, respectively. This allows the optical-based monitoring of H^+ or Cl^- transport processes. Other methods for monitoring changes in ion concentrations, such as NMR spectroscopy and ion-selective electrodes, have also been used in liposomal transport assays.

To test whether a synthetic compound can act as an ion transporter in cells, its ability to induce cytosolic ion concentration changes needs to be evaluated. Taking a potential chloride transporter as an example, changes in Cl^- concentration in the intracellular matrix are typically monitored using a chloride-selective fluorescent probe, such as *N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE), which undergoes fluorescence quenching in the presence of chloride ions. Therefore, upon preincubation of cells with an effective transporter or synthetic channel, quenching of MQAE fluorescence would be expected due to a mediated increase in the intracellular Cl^- concentration.[38] A related strategy involves the use of Fischer rat thyroid epithelial (FRT) cells that express a mutant yellow fluorescent protein (YFP) whose fluorescence is sensitively quenched by chloride ions.[39] Based on the level of fluorescence quenching, the extent of Cl^- transport activity in cells can be estimated. FRT cells expressing a mutant YFP have the advantage that changes in the cellular chloride ion concentration induced by low levels of endogenous Cl^- channels/carriers can be detected. Thus, engineered cells provide useful tools for determining whether a putative synthetic transporter promotes chloride anion influx.

An increase in cytosolic chloride concentrations can induce cell death. Thus, the effect of a synthetic chloride anion transporter on cell viability can be assessed using a standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

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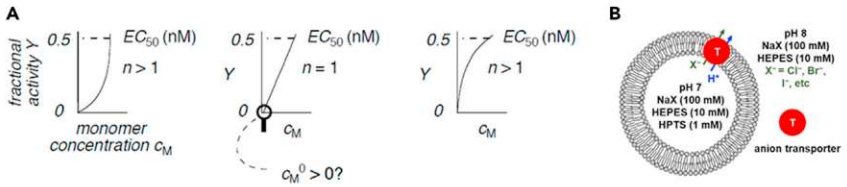

Figure 3. (A) Typical Hill plot for synthetic ion transporters. Reprinted with permission from Matile and Sakai,[35] copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.
(B) Standard configuration of the so-called HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid) assay. Reprinted with permission from Wu et al.,[37] copyright 2018 American Chemical Society.

assay.[40] Apoptosis in cells can be determined by treating cells with a mixture of fluorescein-labeled annexin V and propidium iodide (PI).[41] Transporters with apoptosis-inducing activity will give rise to positive annexin V binding and PI uptake. Because the loss of mitochondrial membrane potential is a hallmark of apoptosis, this event can be determined using a membrane-potential-sensitive probe JC-1. Cells undergoing apoptosis display an increase in the JC-1 green fluorescence and a decrease in red fluorescence.[42] The observation of DNA fragmentation in cells provides additional evidence for apoptosis.[43] DNA fragmentation can be monitored using DNA electrophoresis, nucleus image analysis after staining with a DNA-binding fluorescent dye or a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Taken in concert, these systematic studies can provide robust support for the conclusion that a putative synthetic Cl^- transporter induces apoptosis.

ARTIFICIAL TRANSMEMBRANE ION TRANSPORTERS

Selective and regulated transmembrane ion exchange across lipid bilayers mediated by ion transporters plays a crucial role in various biological processes. Transmembrane ion channels are essential for cell proliferation and appear to play a role in the development of cancer. This was initially noted in the case of potassium channels but has been suggested for other cation channels and chloride channels.[44] Thus, over the past decades, there has been considerable effort devoted to creating artificial ion transporters that can help understand and mimic the functions of natural ion channels. In fact, a number of synthetic ion transporters, designed to promote the through-membrane transport of specific ions, such as chloride, sodium, and potassium, have been developed to date.[45–48] Self-assembled nanostructures created from molecular subunits through noncovalent interactions, such as hydrogen bonding, π-π donor-acceptor interactions, metal coordination, etc., have also been widely created as ion transporters.[49–51] In most cases, classic ion-binding motifs, including crown ethers and so-called cation- or anion-π slides, have been incorporated into synthetic ion transporters.[52–56] Unimolecular transmembrane channels and carriers have also been exploited based on a variety of acyclic and macrocyclic hosts, such as squaramides, pillararenes, calixarenes, cucurbiturils, and calixpyrroles.[57–62]

Artificial transmembrane Cl^- transporters

Typical intracellular and extracellular chloride concentrations are 5–15 mM and 110 mM, respectively.[63] Naturally occurring chloride (Cl^-) ion transporters (e.g., channels) that can mediate the transmembrane transport of Cl^- have been reported to play significant roles in important biological processes, including maintaining ion homeostasis, regulating intracellular pH; they are also important in the context of controlling electrical excitability. Artificial chloride transporters that circumvent

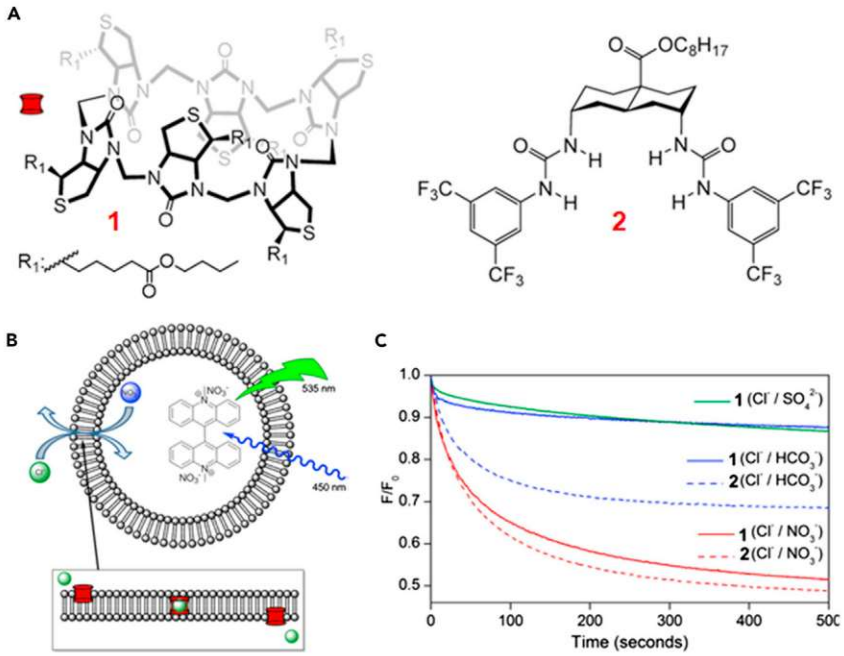

Figure 4. (A) Chemical structures of biotin[6]uril hexaester 1 and bis-urea transporter 2.
(B) Schematic representation of the lucigenin assay used to monitor the chloride anion transport activity of **1**.
(C) Exchange of chloride for different anions by **1** (solid lines) and a control transporter **2** (dashed lines). Reprinted with permission from Lisbjerg et al.,[66] copyright 2015 American Chemical Society.

the associated exchange equilibrium can disrupt critical pH gradients, perturb ion homeostasis, and induce apoptosis of cells.[64] In the case of cancer, this may serve to circumvent classic cancer drug resistance mechanisms.[65] Many artificial transmembrane chloride transporters have been reported to date. Nevertheless, achieving selective chloride anion transport remains a challenge. In this section, we will focus on selected recent studies involving both chloride anion carriers and channels. To provide a succinct overview of the current state-of-the art, the examples of chloride anion carriers chosen for this highlight include both antiporters and symporters. For a similar reason, both unimolecular channels and self-assembled chloride anion channels are discussed.

Artificial transmembrane Cl^- carriers

Antiporters. In 2015, Pittelkow and co-workers reported a class of macrocyclic anionophores, biotin[6]uril hexaesters. Compound **1**, shown in Figure 4A, serves as a representative example.[66] Receptor **1** was found to mediate the through-membrane transport of Cl^- efficiently as a result of presumed CH···anion hydrogen bonding interactions, whereas displaying minimum HCO_3^- transport activity. ^1H NMR spectroscopy and isothermal titration calorimetry (ITC) were used to study the underlying anion binding behavior. The binding affinity of receptor **1** for Cl^- was about two orders of magnitude greater than for HCO_3^-. A lucigenin assay was used to evaluate the ability of the biotin[6]uril hexaester **1** to act as Cl^- transporter (Figure 4B). When POPC vesicles were loaded with a $NaNO_3$ solution, effective Cl^- exchange was observed. However, when $NaNO_3$ was replaced by $NaSO_4$, compound **1** did not transport Cl^- effectively. On this basis, it was inferred that this biotin[6]uril hexaester acts as an antiporter that exchanges Cl^- for NO_3^-.

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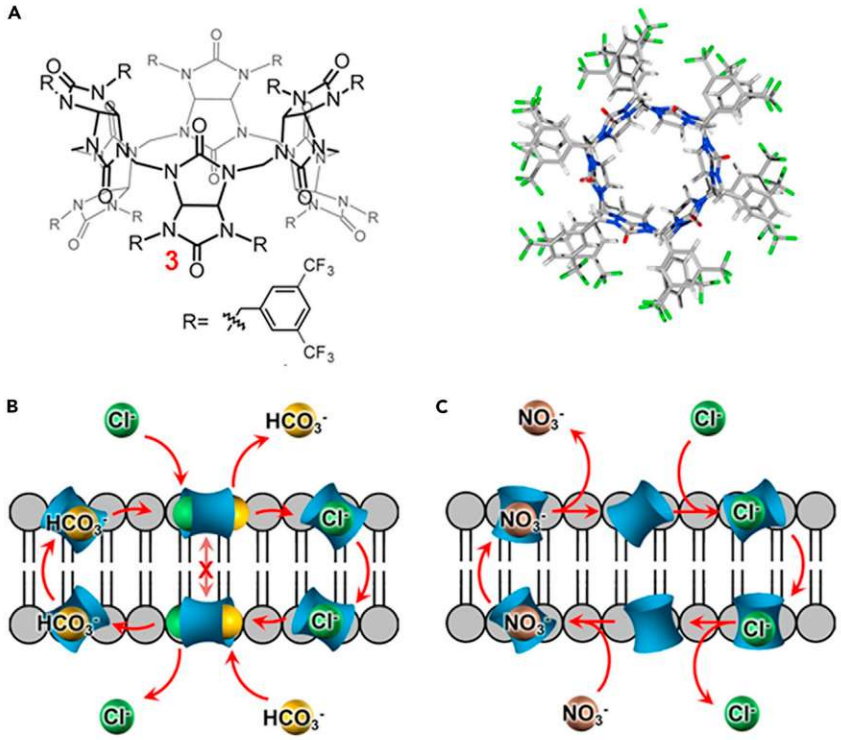

Figure 5. (A) Chemical and X-ray crystal structures of bambus[6]uril 3.
(B and C) Schematic representation of (B) the Cl^-/HCO_3^- and (C) Cl^-/NO_3^- antiport processes mediated by **3**. reproduced with permission from Valkenier et al.,[67] copyright 2019 cell Press.

Transport of HCO_3^- promoted by **1** was also studied by means of the lucigenin assay. As shown in Figure 4C, the rate of fluorescence decay is nearly indistinguishable from that for sulfate counter-transport. This led to the inference that the membrane is impermeable to HCO_3^-. This stands in contrast to what was seen in the case of the bis-urea transporter **2**, which was found to promote Cl^-/HCO_3^- exchange. This work underscores the promise that biotin[6]uril hexaesters hold as selective transmembrane chloride anion carriers.

Šindelář and co-workers have reported another class of macrocyclic anion carriers, bambus[6]uril **3** (Figure 5A), which can efficiently mediate the transport of Cl^- and HCO_3^- through model membranes.[67] The lucigenin assay was used to evaluate the ability of **3** to serve as a Cl^- transporter against a $NaNO_3$ or $NaHCO_3$ gradient. The Cl^- transport rate was found to be two orders of magnitude higher with $NaHCO_3$ than $NaNO_3$. The authors attributed the higher exchange rates of Cl^- to the fact that bambusuril **3** binds the Cl^- and HCO_3^- anions equally well, which can facilitate the fast transport of these two ions through the membrane (Figure 5B). Conversely, the high affinity of bambusuril **3** for NO_3^- was thought to inhibit Cl^- transport (Figure 5C). This work demonstrated the potential of bambusurils as anion transporters and underscores the important role that the relative and absolute anion binding affinities play in regulating anion transport in the case of synthetic anion transporters.

Symporters. The two systems discussed earlier maintain electroneutrality across the membrane as the result of anion exchange. A contrasting approach involves transport systems that are capable of generating a transmembrane ion gradient

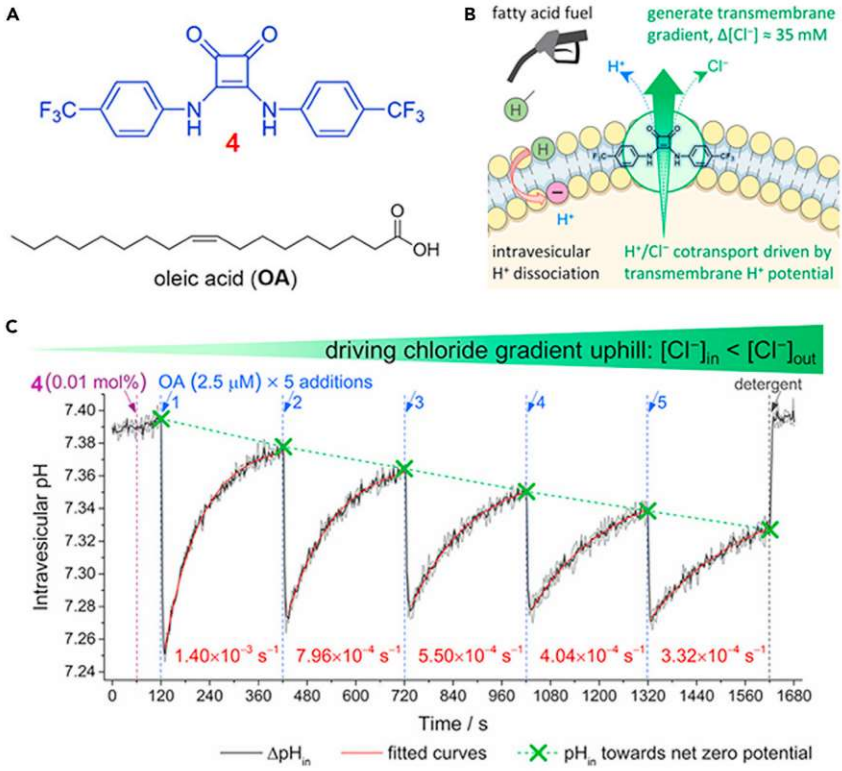

Figure 6. (A) Chemical structures of squaramide 4 and oleic acid (OA).
(B) Schematic illustration of **OA** fueled transmembrane chloride transport mediated by **4**.
(C) Plot of intravesicular pH monitored by HPTS assay, with **4** added at t = 60 s and OA (2.5 μM) at t = 120 s and at ensuing 5 min time intervals to a total of five additions. Reprinted with permission from Howe and Gale,[69] copyright 2019 American Chemical Society.

across lipid membranes. In 1992, Hamilton and co-workers demonstrated that upon addition of oleic acid (OA) to phospholipid vesicles, the pH (pH_{in}) inside the vesicles decreased, thereby generating a transmembrane pH gradient.[68] In 2019, Gale and co-workers reported that by harnessing the transmembrane pH gradient established by OA, appropriately chosen synthetic chloride anion transporters could promote H^+/Cl^- cotransport, thereby generating a transmembrane Cl^- gradient in LUVs.[69] As shown in Figure 6A, squaramide **4** is an excellent chloride anionophore and can mediate H^+/Cl^- cotransport. Upon addition of OA to LUVs, the intracellular pH decreased, thus generating a pH gradient as illustrated in Figure 6B. However, the addition of squaramide **4** dissipates the pH gradient leading to the efflux of Cl^- so as to maintain charge balance across the lipid bilayer. In the presence of chloride transporter **4**, multiple pulsed additions of OA (at 5 min time intervals) resulted in the alternating generation and dissipation of pH gradients (Figure 6C). It is likely that many other artificial ion transporters could be employed in a similar manner, thus allowing for the creation of new ion pumping systems where an external "fuel" is used to drive transmembrane ion transport.

In 2019, Gale and co-workers also reported a voltage-switchable artificial membrane transport system based on macrocycle **5**.[70] Macrocycle **5** is a Cl^- receptor that binds Cl^- with strong affinity and high selectivity (Figure 7A). Here, an HPTS base-pulse assay was used to demonstrate H^+/Cl^- symport activity and to confirm the Cl^-

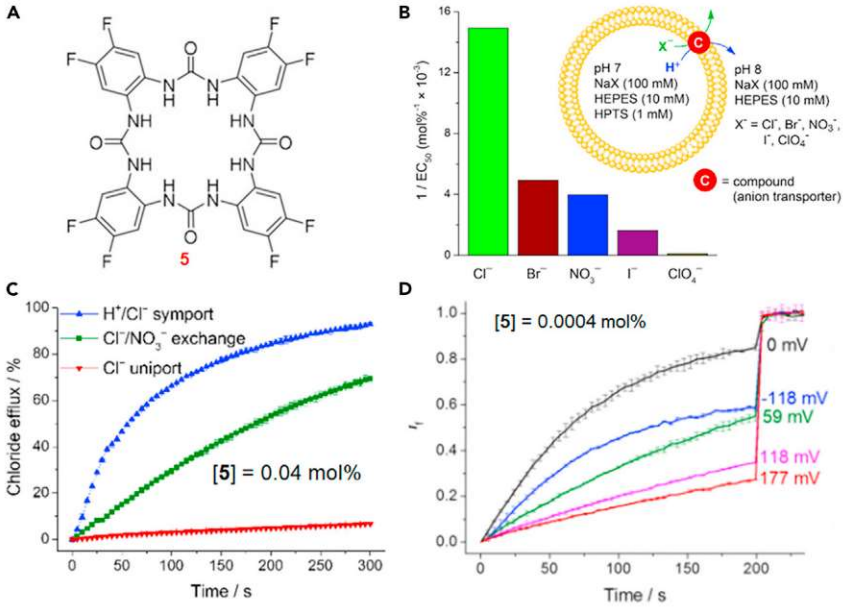

Figure 7. (A) Chemical structure of macrocycle 5.
(B) Relative activity of **5** as a substance capable of promoting H^+/anion symport.
(C) Comparison of the ability of **5** to perform these functions, on the basis of an ion-selective electrode assay.
(D) Voltage-dependence on the transport activity of **5** in an HPTS assay. Reprinted with permission from Wu et al.,[70] copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

selectivity (Figure 7B). Surprisingly, as shown in Figure 7C, macrocycle **5** showed no Cl^- uniport activity across lipid membranes, leading to the conclusion that macrocycle **5** itself cannot diffuse through the membranes, as would be necessary to achieve transport of an ion on its own (so-called uniport). The voltage-dependent H^+/Cl^- symport features of **5** were examined. As shown in Figure 7D, the H^+/Cl^- symport promoted by macrocycle **5** could be switched off by imposing a positive membrane potential; it was also attenuated when a negative potential was applied. The voltage-switchable ion transport demonstrated in this study marks an important step forward in creating smart transport substances that may be able to interact selectively with bacterial or cancer cells by targeting their abnormal membrane potentials.

Artificial transmembrane Cl^- channels

Unimolecular Cl^--channel. Pillar[*n*]arenes, a relatively new class of macrocyclic hosts endowed with symmetric pillar-like structures, show promise as unimolecular channels for the through-membrane transport of small molecules and ions.[71] For instance, in 2013, Hou and co-workers demonstrated that pillar[*n*]arenes (*n* = 5, 6) functionalized with short peptides on both rims could transport amino acids across lipid bilayers efficiently, presumably as the result of forming unimolecular channels.[72] Most natural channels that can transport amino acids are competitively blocked by Cl^-, with only a few examples being reported that can transport both species.[73] In contrast to what is typically seen in nature, Hou and colleagues demonstrated that pillararenes **6x**, **6y**, and **7x** promote the concurrent transport of Cl^- and glycine across egg yolk L-α-phosphatidylcholine (EYPC) membranes (Figure 8). This leads to the suggestion that these receptors may act as unimolecular chloride anion channels, in addition to serving as amino acid transporters.

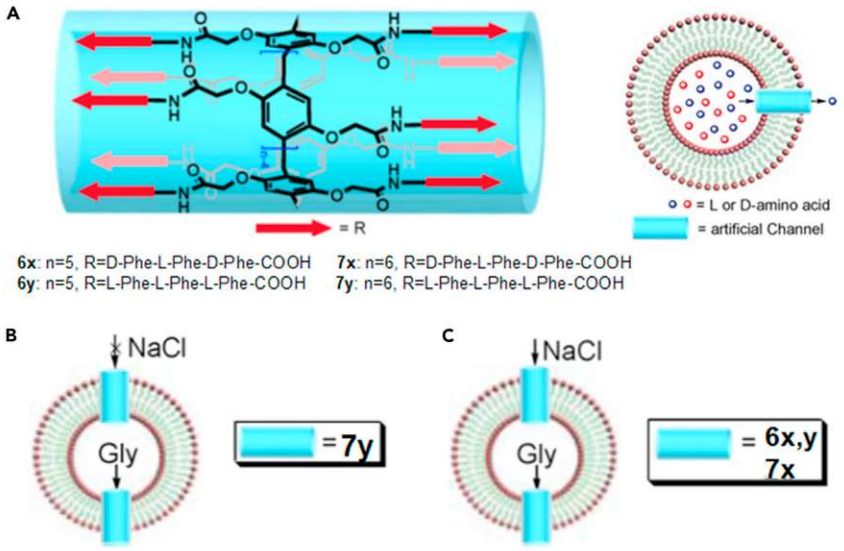

Figure 8. (A) Chemical structures of pillararenes 6x–6y and 7x–7y.
(B and C) Schematic representations of (B) the Cl^- transport suppression observed for **7y** and (C) but not for **6x**, **6y**, and **7x** in the presence of Gly. Reproduced with permission from Chen et al.,[72] copyright 2013 American Chemical Society.

Self-assembled Cl^--channels. In 2010, Gokel and co-workers reported a series of isophthalamide analogs that transport chloride across dioleoylphosphatidyl choline (DOPC) vesicular memmbranes.[74] Among them, 4,4′-dinitropicolinanilide **8** (Figure 9A) showed features characteristic of ion channels. This stood in contrast to most of the other previously tested isophthalamide analogs, which were found to act as mobile carriers. This research team demonstrated that **8** self-assembles to form transmembrane channels through intermolecular π-π donor-acceptor interactions (Figure 9B). Fluorescence spectral studies of **8** in the presence of DOPC vesicles revealed excimer-like emission features, findings that were taken as evidence that **8** underwent self-assembly within the lipid membranes. Additionally, a Hill coefficient of >1 was noted at relatively high concentrations of **8**, as would be expected for Cl^- transport mediated by ion channels. As shown in Figure 9C, the authors proposed a model wherein face-to-face stacking of the triarenes leads to the formation of the proposed channels. This study highlights how self-assembly of suitable low molecular weight units can produce functioning synthetic ion channels.

Gong and co-workers extended the self-assembly strategy to fabricate synthetic ion channels from rigid oligoamide macrocycles **9-H** and **9-NH_2** (Figure 10).[75] They suggested that by modifying the inner cavities of the tubular sub-structures with functional motifs, selective ion transport could be achieved. Fluorescence spectral studies of **9-H** in $CHCl_3$ revealed excimer emission, as would be expected that macrocycle **9-H** forms face-to-face stacks. Further evidence for the presumed self-assembly behavior came from atomic force microscopy. By monitoring the proton transport across a POPC membrane, it was concluded that **9-NH_2** is more effective for mediating Cl^- transport than **9-H**. As inferred from computationally derived electrostatic potential maps, the cavity of the channel self-assembled from **9-NH_2** is more positive than that produced from **9-H**, a difference that is expected to enhance interactions with the targeted chloride anions. The K^+/Cl^- transport selectivities of **9-H** and **9-NH_2** were also evaluated. The permeability ratios (P_{K+}/P_{Cl-}) were calculated

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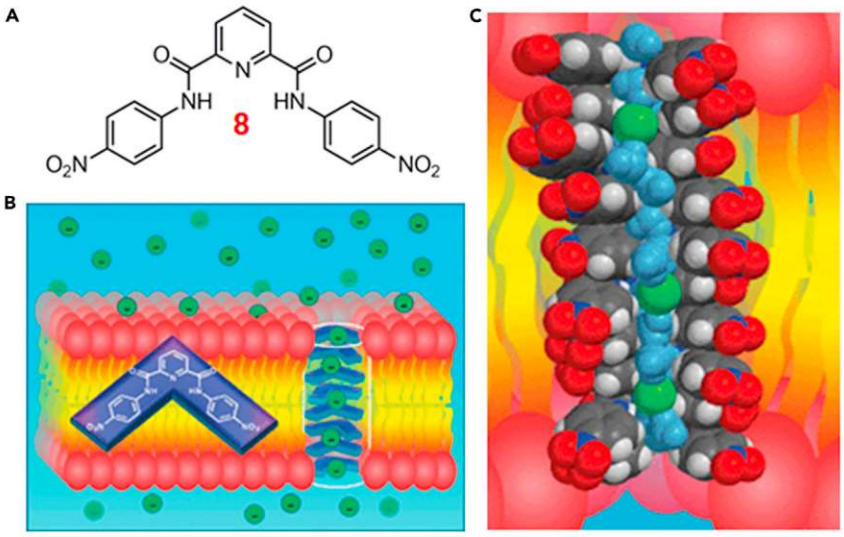

Figure 9. (A) Chemical structure of 4,4′-dinitropicolinamide 8.
(B) Illustration of the self-assembled ion channel consisting of compound **8**.
(C) Cutaway view of a hypothetical stacked channel formed via the aggregation of **8**. Reproduced with permission from Yamnitz et al.,[74] copyright 2010 Royal Society of Chemistry.

to be 9 and 3.5 for the channel formed from **9-H** and **9-NH_2**, respectively. This difference reflects a higher selectivity in the case of **9-NH_2**. This study is notable not just for the transport activity of substances but also for providing a potentially generalizable approach to the design of functional synthetic ion channels with tailored selectivities.

Nanochannels. Nanochannels represent another type of synthetic ion channels. Their high stability, facile modifications, and flexible nature make them attractive as mimics of natural ion channels.[76] In 2016, Jiang and co-workers reported a biomimetic voltage-gated nanochannel that was found to mediate the selective transport of chloride (Figure 11).[77] The authors showed that the native channel, consisting of chloride-responsive molecules, exists in an "off" state. However, when Cl^- enters the nanochannel and is complexed with the chloride-responsive elements, the nanochannel becomes negatively charged, thereby leading to the co-accumulation of cations within the nanochannel. This switches the nanochannel to an "on" state. Under constant voltage conditions, Cl^- is released from the inside nanochannel and transported to the outside nanochannel, the event that switches the channel back to the original "off" state. Due to the presence of the chloride-responsive elements, selective Cl^- transport is seen in the presence of potentially competing halide anions. This study, which relies on the use of a functionalized nanochannel, could provide an attractive and readily generalized approach to creating artificial transmembrane ion transporters.

Artificial transmembrane K^+ transporters

K^+ channels are widely distributed in various types of cells, including cancer cells. It is apparent that tumor cells express K^+ channels differently from healthy cells, and that K^+ channels modulate key aspects of cancer, including proliferation and migration.[78] Therefore, K^+ channels are attractive targets for the development of possible cancer treatments. In this regard, artificial transmembrane K^+ channels that promote a

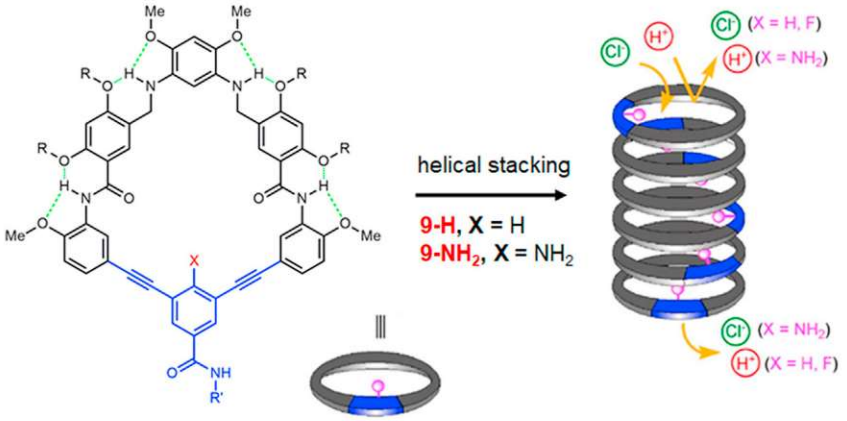

Figure 10. Chemical structures of oligoamide macrocycles 9-H and 9-NH_2 and the proposed self-assembled ion channel

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change in cellular K^+ concentrations have been explored. To date, crown ethers, barrel-stave systems, G-quadruplexes, and amphiphilic peptides have been used to construct K^+ transporters.[79–82] In this section, we present several representative examples of synthetic K^+ transporters, highlighting both self-assembled and unimolecular K^+ channels.

Self-assembled K^+-channels

Crown ethers have a storied history in the context of preparing self-organized K^+ channels. For instance, in 2006 Barboiu and co-workers reported a series of functional crown ethers that can self-assemble into robust K^+ channels.[83] As shown in Figure 12, compound **10**, a benzo-18-crown-6-ether bearing a linear alkyl tail attached via a ureido-ethylamide linker, can form channel-type supra-structures stabilized via hydrogen bonding interactions. Single-crystal X-ray diffraction analysis revealed an antiparallel arrangement of **10** within an overall columnar array in the solid state. The distance between each pair of adjacent crown ethers is 4.8 Å, which is even larger than the 3.3 Å distance reported in a previously reported K^+-(15-crown-5)$_2$ sandwich complex.[84] This led to the suggestion that K^+ might be a more favorable ion for binding to **10** than its smaller congener, Na^+. In accord with these design expectations, compound **10** displayed an order of magnitude higher transport rate for K^+ over Na^+. This study provided key support for the contention that the combination of crown ethers and self-association can allow for the construction of artificial K^+ ion channels.

Despite the success mentioned earlier, synthetic channels that permit selective K^+ transport are still rare. In 2017, Zeng and co-workers reported a family of ion transporting motifs, including **11**, which could be used to prepare highly selective K^+ channels (Figure 13A).[85] The authors demonstrated that these functionalized crown ethers can stack to form tubular channels as the result of presumed hydrogen bonding interactions. The best K^+ transport efficacy was seen for the channel formed from **11** with a K^+/Na^+ selectivity of 14.7. The corresponding EC_{50} value (i.e., effective concentration of channels required to achieve 50% K^+ transport at 200 s) was 6.2 μM. Comparative studies using analogs of **11** provided support for the conclusion that the side chains, H-bonding motifs, and crown ethers all play an important role in regulating the function of this set of synthetic transmembrane K^+ channels.

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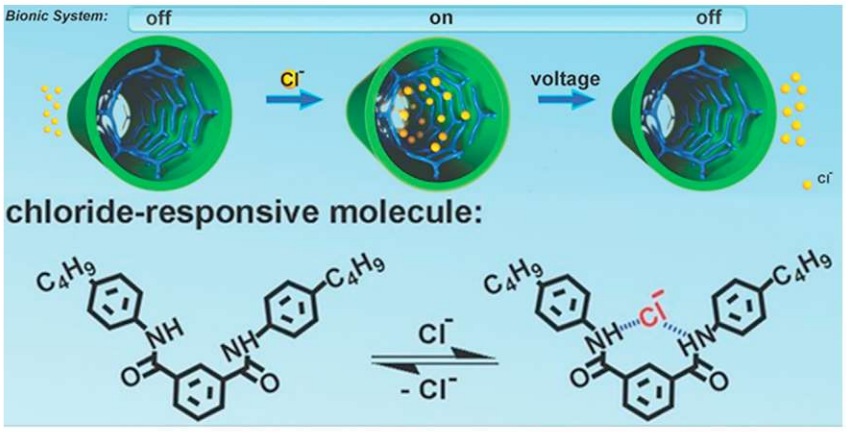

Figure 11. Schematic illustration of a biomimetic voltage-gated chloride nanochannel and chemical structure of a chloride-responsive molecule

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Thus, this work served to highlight some of the design principles that may be exploited to generate artificial K^+ channels.

Unimolecular K^+-channels

To create improved selective K^+ transporters, in 2014 Hou and co-workers appended peptides containing Arg residues onto both rims of a pillararene to afford systems that acted as voltage-gated K^+ channels (Figure 14A).[86] The voltage responsive behavior of pillararenes **12-1**<**12-4** was studied by means of patch-clamp experiments in a planar lipid bilayer. When a negative membrane potential was applied to the system, pillararenes were inserted into the membrane and enabled the transport of K^+ (Figure 14B). It is worth noting that pillararene **12–2** could be reversibly inserted into and displaced from the membrane by changing the applied potential from −100 to +100 mV, thereby switching the transmembrane transport of K^+ on and off. Similar reversibility was not seen for compounds **12–3** and **12–4**, behavior ascribed to their presumed strong aggregation within the hydrophobic interior of the membrane. Nevertheless, this study illustrates a new strategy for creating artificial voltage-gated channels.

Artificial transmembrane Na^+ transporters

Na^+ exchange across membranes by relatively simple carriers or complex protein channels is a very important process in living cells.[87] Investigations using artificial transmembrane Na^+ transporters could contribute to our understanding of the mechanisms of action of their natural counterparts. In addition, artificial transmembrane Na^+ transporters are of interest in the context of developing potential treatments for various diseases. However, the number of reported Na^+ transporters is limited. Moreover, most of the systems studied to date function as artificial channels. In this section, we review recent efforts to create synthetic Na^+ channels.

Self-assembled Na^+-channels

In 2000, Davis and co-workers reported that G-quartets formed by guanine derivatives self-assemble into columnar aggregates, known as G-quadruplexes via π-π donor-acceptor interactions, to generate an apparent ion channel.[88] However, the kinetic instability of G-quartets (dynamic equilibrium between guanine "monomer" and self-assembled G-quadruplex) provided a serious obstacle to the creation of ion channels based on G-quartets. In 2006, Davis and co-workers reported an elegant

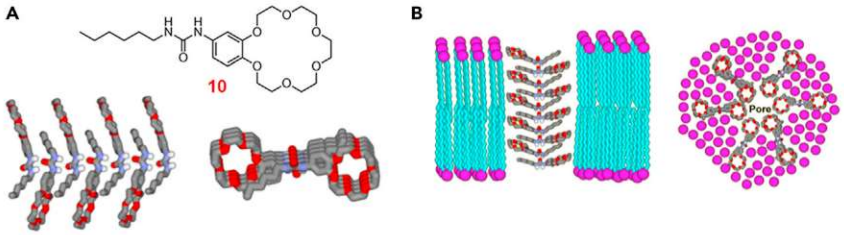

Figure 12. (A) Chemical and single-crystal X-ray structures of crown-ether-derivative 10.
(B) Lateral view (left) of the double-barreled model and top view of the toroidal model (right) of the proposed organization of **10** within the bilayer. Reprinted with permission from Cazacu et al.,[83] copyright 2006 American Chemical Society.

covalent capture strategy to construct stable self-assembled transmembrane channels.[59] Here, after G-quadruplex formation, olefin metathesis using Grubbs catalyst was used to transform the otherwise unstable supramolecules into stable unimolecular ion channels (Figure 15A). The authors showed that the metathesis reaction was effective only when carried out in the presence of lipid bilayers (Figure 15B). The ability of the metathesis product **14** to function as a transmembrane ion channel was evaluated using a standard base-pulse assay (Figure 15C). When pH-responsive dye HPTS-loaded liposomes were suspended in the isotonic corresponding buffer, upon the addition of aqueous NaOH solution, a rapid increase in the internal pH of the liposomes was observed in the presence of **14**, as well as a control compound gramicidin (Figure 15C). In contrast, no change in the pH was seen when **13** was added to HPTS-loaded liposomes. The cross-linked unimolecular G-quadruplex **14** proved active in Na^+ transport, whereas both the constituent monomer **13** and the noncovalent (and non-crosslinked) assembly proved inactive. It is likely that the self-assembly and post-synthetic covalent modification strategy embodied in this study can be exploited to generate useful transmembrane ion channels.

Unimolecular Na^+-channels

Polytheonamide B (Figure 16), which is composed of 48 amino acid residues, is arguably the largest nonribosomal peptide.[89] Extensive NMR studies revealed that polytheonamide B adopts a β-helix structure with a diameter of 4 Å and a length of about 45 Å. These features make polytheonamide B attractive as a unimolecular transmembrane ion channel. Recently, Inoue and co-workers reported the design and synthesis of a polytheonamide mimic **15** and its use as an artificial ion channel.[90] The microenvironment of **15** when embedded within a lipid bilayer was analyzed by absorption spectroscopy. On this basis, it was suggested that the C-terminal residue is located at the head group area of the lipid bilayer, an orientation considered to be a prerequisite for ion transport. Mimic **15** exhibited cytotoxicity against the p388 mouse leukemia cell line (IC_{50} = 12 nM). This finding led to the conclusion that, although simplified, compound **15** maintains the activity-determining structural features of polytheonamide B. It was found that peptide **15** displays effective H^+ and Na^+ channel-like activity without disrupting the membrane in which it was embedded. Thus, this rationally designed ion channel highlights a novel strategy that could be utilized to fabricate unimolecular channels with specific capabilities.

ARTIFICIAL TRANSMEMBRANE ION TRANSPORTERS AS INDUCERS OF CANCER APOPTOSIS

Cancer remains a major contributor to mortality and continues to impose a huge economic burden on society.[91–94] Since various cellular ion channels are

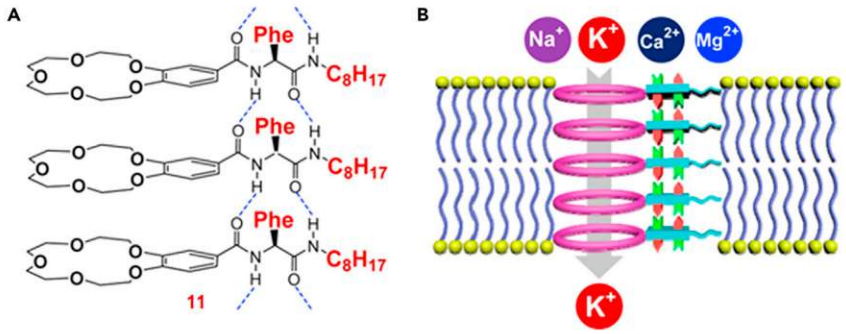

Figure 13. (A) Chemical structure of compound 11 and illustration of the proposed stacking mode.

(B) Cartoon illustration of the selective K^+ transport promoted by **11**. Reprinted with permission from Ren et al.,[85] copyright 2017 American Chemical Society.

deregulated in cancer, many studies have been performed to understand their roles in tumors. In parallel, a variety of artificial transmembrane ion carriers and channels have been created and tested to see if they have anticancer activity. In this section, for the sake of illustration, the focus will be on different ion carrier- and channel-based mechanisms as well as responsive ion transporters.

Chloride transporters

Prodigiosins (cf. Figure 17 for a representative example) are a class of secondary metabolites noted for their red color and anti-proliferative activity.[95] Due to their ability to act as chloride anion transporters and their high anticancer activity, a considerable body of effort has been devoted to assess the biological activity and to understand the mechanisms of prodigiosins, as well as searching for analogs with better bioactivity. In 2005, the chloride-transport ability of a series of structurally related compounds was studied by Sessler and co-workers, including prodigiosin **16**, its analogs **19** and **20**, and dipyrromethenes **17** and **18** (Figure 17).[96] It was found that the order of chloride-transport activity was **16** $\geq$ **19** $\approx$ **17** > **18** $\geq$ **20**. The anticancer activity of the analogs was evaluated using PC3 human prostate cancer and A549 human lung cancer cell lines. The order of antiproliferative activity of the compounds was generally consistent with the order of anion transport activity. This correlation led the authors to propose that this set of compounds mainly function as H^+/Cl^- symporters. This study provided important early support for the notion that anion transporters might serve in due course as anticancer therapeutics.

Because the pyrroles in prodigiosins allow for Cl^- binding via NH–Cl hydrogen bonding interactions, two pyrrole-based receptors, the diamide-strapped calix[4]pyrroles **21** and **22**, were tested to see if they would act as Cl^- transporters and induce cancer cell death via apoptosis (Figure 18A).[97] Analysis of X-ray crystal structures of the complexes showed that Cl^- binds within the calixpyrroles **21** and **22** but Na^+ to the outside calixpyrroles. A liposomal model study revealed that **21** and **22** displayed Cl^-/Na^+ transport activity. In addition, the Cl^- transport mediated by **21** and **22** was significantly enhanced in the presence of monensin (a known Na^+ ionophore). Transporters **21** and **22** displayed anticancer activities against various cancer cell lines with low micromolar IC_{50} values. To obtain insights into the determinants of cytotoxicity displayed by **21** and **22**, their ability to alter intracellular ion concentrations was assessed. Both carriers **21** and **22** were found to increase the intracellular Cl^- and Na^+ concentrations without affecting intracellular K^+ and Ca^{2+} concentrations (Figures 18B and 18C). However, co-treatment of cells with

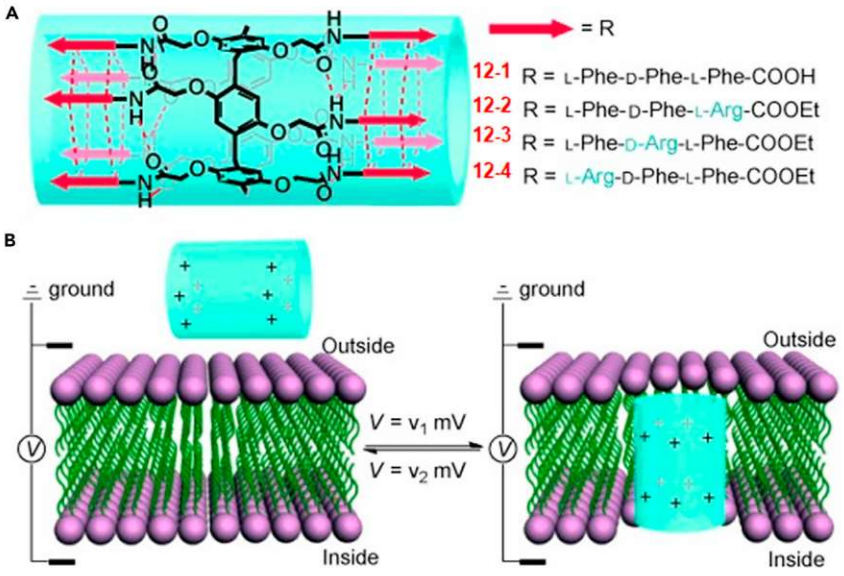

Figure 14. (A) Chemical structures of pillararenes 12-1 < 12-4.
(B) Schematic representation of the voltage-driven channel inserting into and leaving a lipid bilayer. Reproduced with permission from Si et al.,[86] copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

either **21** or **22** and amiloride (a blocker of the natural Na^+ channels) led to decreases in the intracellular Cl^- concentration, leading to the conclusion that in the presence of these carriers, Na^+ ions would enter cells in part through natural sodium channels. The cell death activities of **21** and **22** were greatly reduced in Cl^--free or Na^+-free buffer, supporting the proposition that the observed cell death induced by **21** and **22** results from increases in intracellular Na^+ and Cl^- concentrations. Mechanistic studies of the cell death promoted by **21** and **22** revealed that these receptors increase the levels of intracellular reactive oxygen species, a trigger that results in the release of cytochrome c from the mitochondria and activation of a caspase-dependent apoptotic pathway (Figure 18D). However, receptors **21** and **22** did not activate the apoptosis-inducing factor (AIF)-associated apoptotic pathway (Figure 18E). Later studies suggested that increases in the intracellular Cl^- and Na^+ concentrations promoted by receptors **21** and **22** induce osmotic stress, which eventually promotes caspase-dependent apoptosis.[27] This study is notable in that it provides an understanding of how ion transporters can induce apoptosis and, as such, set the stage for the further development of synthetic ion transport systems for biomedical applications.

Subsequent to the studies mentioned earlier, a set of chloride-selective ion channels were reported by Talukdar and co-workers in 2016. Specifically, a supramolecular barrel-rosette self-assembled through a hydrogen bond network of vicinal diol moieties present at the ends of the monomeric bis-diol **23** acted as an effective chloride anion channel (Figure 19).[98] The Cl^- transport activity of **23** was assessed by means of an HPTS base-pulse fluorescence assay. This system was found to be very efficient (EC_{50} = 2.7 μM), a finding ascribed to its lipophilicity, i.e., log*P* = 5.58, where log*P* refers to the octanol–water partition coefficient, with log*P* = 5 being considered optimal for insertion into lipid membranes. Selective transport of Cl^- over other anions was also observed. Electric conductance experiments using planar lipid

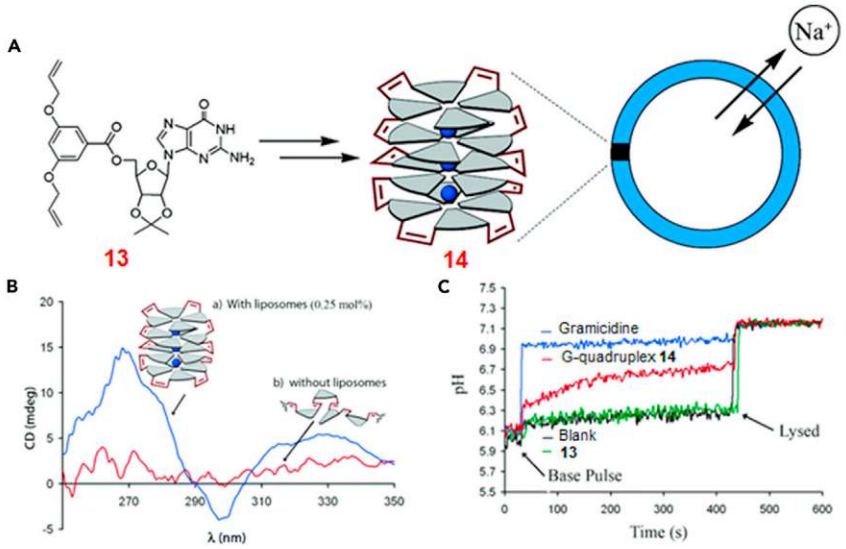

Figure 15. (A) Synthetic routes from 13 to G-quadruplex 14.
(B) CD spectra of unimolecular G-quadruplex **14** (0.05 mM) in 10 mM sodium phosphate (pH 6.4) with (blue) and without (red) EYPC liposomes.
(C) Transport of Na^+ as determined using a pH gradient assay. Reproduced with permission from Kaucher et al.,[59] copyright 2006 American Chemical Society.

membranes provided support for the conclusion that ion channels were being formed (Figure 19B). It was also found that **23** increases the intracellular Cl^- concentration and induces cell death in several cell lines. Furthermore, a cell image analysis using a mitochondrial-membrane-potential-sensitive JC-1 probe revealed that **23** causes a relative increase in the green fluorescence of JC-1, a finding taken as evidence of a loss in mitochondrial membrane potential in cells treated with **23** (Figure 19C). This, in turn, leads to production of reactive oxygen species, release of cytochrome c into the cytosol, and apoptotic cell death. This study provides further support for the notion that artificial Cl^- channels with apoptosis-inducing activity can serve as anticancer agents.

In 2018, Zeng and co-workers reported 1D columnar structures self-assembled from lipophilic monopeptides with N-terminal FMOC (fluorenylmethoxycarbonyl) groups, for instance, **Fmoc-Phe-C4**, as shown in Figure 20A.[99] They showed that the resulting constructs acted as synthetic ion channels. Analysis of the single-crystal X-ray diffraction structure of **Fmoc-Phe-C4** revealed that each of the substituents R_1 and R_2 and the Fmoc group stack above one another in the putative channel network (Figure 20A). On this basis, Zeng and co-workers proposed that changing the Fmoc group to a Cl^--binding motif, such as a tetrafluoroiodobenzyl group, would give rise to artificial Cl^- channels mediated via halogen bonding.[100] As shown in Figure 20B, several such derivatives were synthesized in an effort to optimize the transport activity and selectivity of the resulting putative channels. Based on the results of a pH-sensitive HPTS assay, **A10**, **L8**, and **L10** promoted Cl^- transport with a concomitant pH discharge (Figure 20D). Further tests were then performed, leading the authors to propose that OH^-/Cl^- antiport was the dominant transport mechanism. In particular, **A10** displayed relatively high Cl^- selectivity over other anions, including Br^- and I^-. The anticancer activity of the channels was evaluated *in vitro* using a human breast cancer cell line. The best cell growth inhibition was seen in the case of

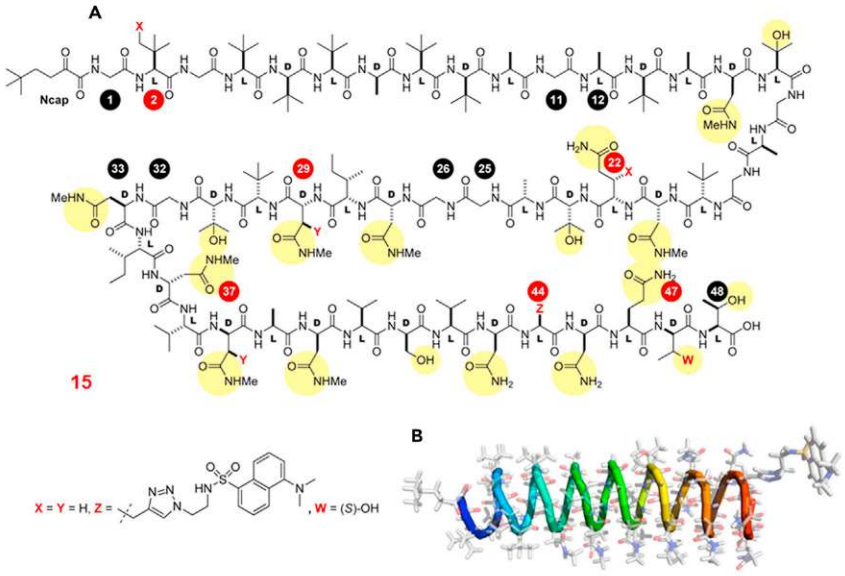

Figure 16. (A) Chemical structure of dansylated polytheonamide mimic 15.
(B) Computer-generated β-helical structure model of **15**. Reproduced with permission from Itoh et al.,[90] copyright 2012 American Chemical Society.

A10 (IC_{50} value of 20 μM for **A10** at [NaCl] = 6.4 g L^{-1}), which proved competitive with the anticancer agent cisplatin measured under similar conditions (IC_{50} = 37 μM). It is likely that the design principles embodied in these systems can be extended to create new types of ion-selective transport channels and potential therapeutics.

In 2017, the first synthetic Cl^- transporters (Figure 21) that both promote apoptosis and affect autophagy in cells were reported.[25] Specifically, the squaramide-based Cl^- transporters facilitated the transport of chloride anions in the order of **S1(4)** > **S3** > **S4** ~ **S2** >> **S5** ~ **S6** in liposomal models. However, they proved ineffective as Na^+ transporters in liposomes. In cells, **S1**, **S3**, and **S4** effectively increased the intracellular Cl^- and Na^+ concentrations without affecting intracellular K^+ and Ca^{2+} concentrations. However, **S2**, **S5**, and **S6** had little or no effect on Cl^- and Na^+ transport. Importantly, the effective ion transporters **S1**, **S3**, and **S4** displayed good cell death activities. Again, the ability of **S1**, **S3**, and **S4** to promote cell death was greatly attenuated in Cl^--free and Na^+-free buffers. In addition, **S1**, **S3**, and **S4** induced caspase-dependent apoptotic cancer cell death without affecting the AIF-dependent apoptotic process. In particular, transporter **S1** disrupted autophagy by decreasing the lysosomal Cl^- concentration concomitantly with an increase in lysosomal pH from 4.5 to ca. 7.0, thereby leading to impairment of the function of lysosomes. Recently, it was found that **S1(4)** promoted apoptosis as the result of the osmotic stress caused by increases in the intracellular Cl^- and Na^+ concentrations.[27] These studies provided key experimental evidence in support of the proposition that synthetic ion transporters can both disrupt autophagy and induce apoptosis.

Ion transporters have been combined with traditional anticancer therapy (e.g., photodynamic therapy, PDT) to both effect autophagy suppression and promote apoptosis induction.[24] For example, Zhang and co-workers designed and

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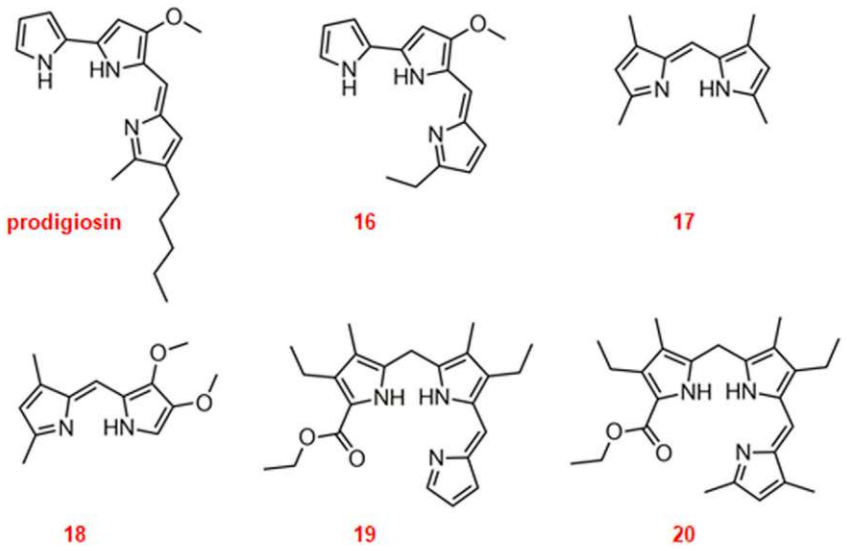

Figure 17. Chemical structures of prodigiosin and its analogs
Reproduced with permission from Sessler et al.,[96] copyright 2005 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

synthesized an ATP-stimulated Cl^- transport nanosystem wherein a squaramide (SQU) Cl^- transporter **4** is encapsulated into a porphyrinic porous coordination network (PCN) (Figure 22A).[101] In the presence of ATP, produced within tumors, SQU@PCN breaks up apart due to metal-ATP interactions. This leads to the release of the SQU, resulting in transport of Cl^- across the cellular and lysosomal membranes (Figure 22B). The authors proposed that the associated influx of Cl^- disrupts intracellular ion homeostasis and further induces apoptosis in cancer cells. Additionally, SQU-mediated Cl^- transport concomitant with efflux of H^+ across the lysosomal membrane serves to increase the lysosomal pH thus suppressing autophagy (Figure 22C), which, in turn, improved the efficacy of light-induced cell killing (Figure 22D) by a typical PDT photosensitizer, tetrakis (4-carboxyphenyl) porphyrin (TCPP). This pioneering work illustrates a novel use for ion transporters in the context of cancer therapy.

Potassium transporters

Growing evidence supports the suggestion that potassium channel dysregulation is associated with key aspects of cancer and that targeting potassium channels is thus a viable therapeutic approach.[78,102] It has been suggested that K^+ efflux causing a reduction of the intracellular K^+ concentration may be an important factor for apoptosis induction.[103] Hence, increasing interest has been devoted to developing artificial K^+-transporters that can perturb cellular K^+ ion homeostasis and thus, presumably, induce apoptosis. To date, crown ethers have been extensively exploited in this context because their affinity for potassium cations may be readily tuned. Indeed, crown ether-based potassium transporters have been demonstrated to act as synthetic apoptosis inducers. However, the apoptotic mechanism by which these systems presumably perturb potassium homeostasis has not been fully elucidated. Progress along these lines has come from a recent report by Kim and coworkers who provided evidence of endoplasmic reticulum (ER) stress-mediated apoptosis (Figure 23).[104] This study involved a set of 18-crown-6 conjugated helical polypeptides **AIP1–AIP4** (AIP, apoptosis-inducing polypeptide) that were modified

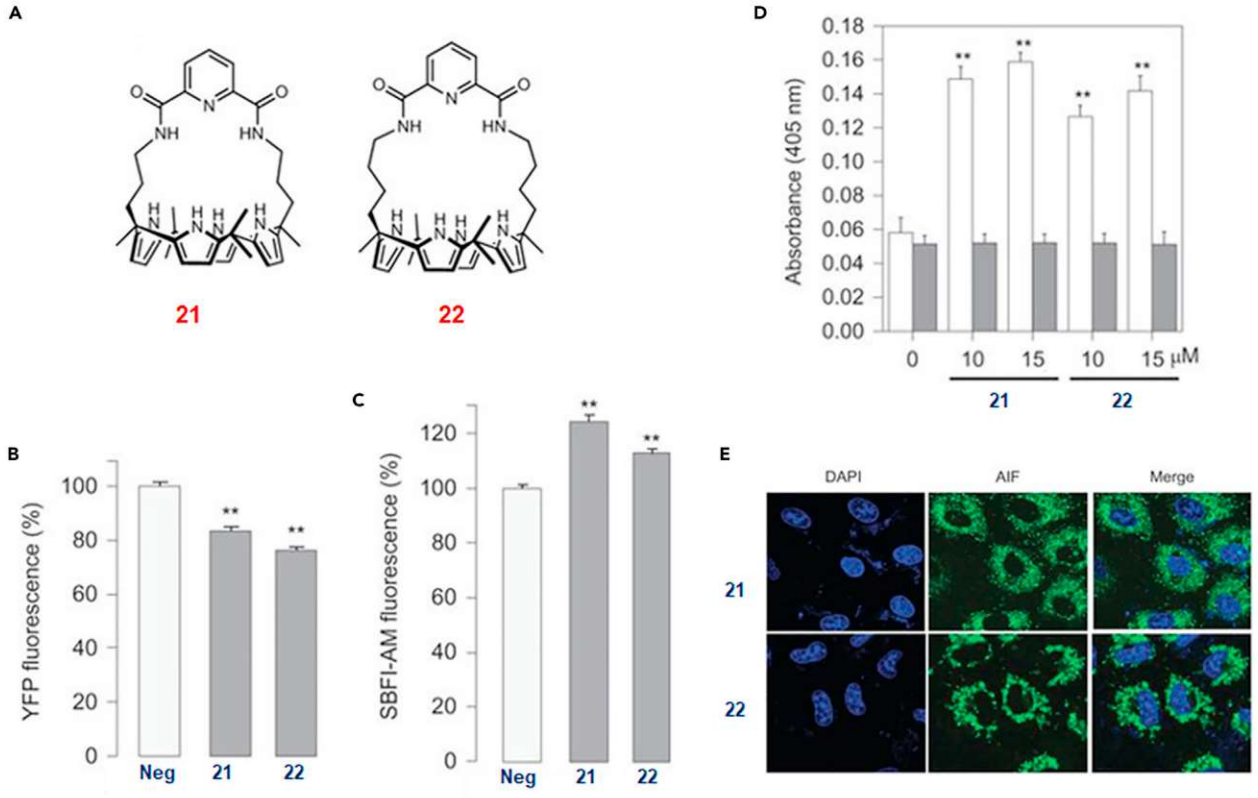

Figure 18. (A) Chemical structures of diamide-strapped calix[4]pyrroles 21 and 22.

(B) FRT cells expressing a mutant YFP were incubated with **21** or **22** for 2 h and the fluorescence intensity of YFP was measured to determine the effect of **21** and **22** on the intracellular chloride ion concentration.

(C) A549 cells pretreated with the Na^+-selective probe, sodium-binding benzofuran isophthalate accetoxymethyl ester (SBFI-AM), for 1.5 h were incubated with **21** or **22**. The SBFI-AM fluorescence was then measured to examine changes in the intracellular sodium ion concentrations.

(D) The caspase activities of lysates of HeLa cells treated with **21** or **22** for 18 h were measured using a colorimetric peptide substrate for caspases, Ac-DEVD-pNA (pNA, *p*-nitroaniline), in the absence (white bar) or presence (gray bar) of Ac-DEVD-CHO (a known inhibitor of caspases).

(E) HeLa cells, treated with **21** or **22** for 18 h, were immunostained using an anti-AIF antibody. The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). Reproduced with permission from Ko et al.,[97] copyright 2014 Nature Publishing Group.

with tetramethylammonium groups and hydrocarbon chains to enhance interactions with plasma membranes (Figure 23B). These polypeptides displayed good potassium transport activities with high selectivity. A single-channel analysis provided evidence for an ion carrier mechanism rather than ion channel formation. The authors demonstrated that **AIP1–AIP4** can disturb K^+ ion homeostasis, thereby activating Ca^{2+} channel proteins that transport Ca^{2+} into the cytosol. Additionally, the ER stress marker proteins were activated through their phosphorylation, indicating induction of ER stress promoted by **AIP1–AIP4**. The authors further demonstrated the generation of ER-stress-mediated apoptosis by using immunoblot assays. As shown in Figure 23C, apoptosis-related proteins were detected from the immunoblot assays of the harvested tumor tissues. *In vivo* experiments revealed that **AIP** could efficiently suppress tumor proliferation in a xenograft mouse tumor model.

To develop new synthetic potassium transporters, amino acid-bearing scaffolds have been introduced. Specifically, Yang and co-workers reported a synthetic K^+ transporter **24** by using an α-aminoxy acid monomer that effected the site-selective K^+/H^+ exchange across mitochondrial and lysosomal membranes in living cells.[105]

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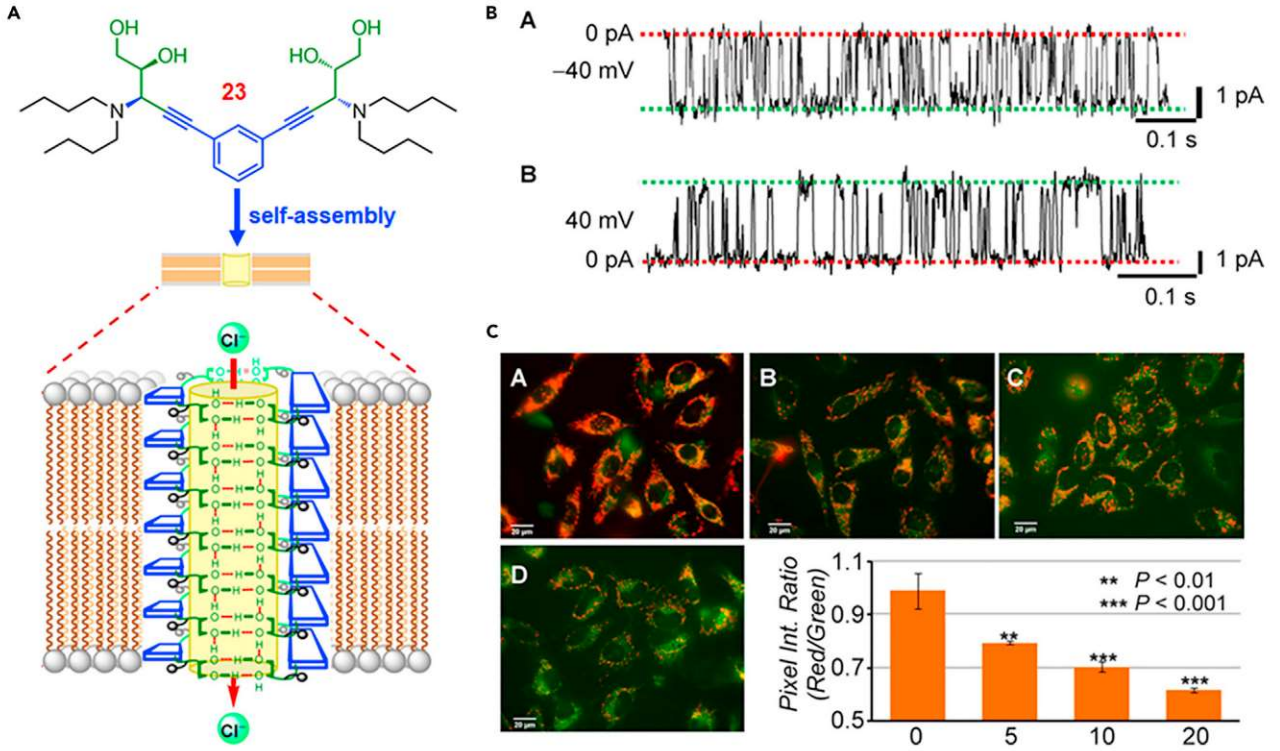

Figure 19. (A) Chemical structure of compound 23 and schematic illustration of the self-assembled transmembrane chloride channel.
(B) Single-channel current traces recorded at −40 (A) and +40 mV (B), holding potentials in 1 M symmetrical KCl solution.
(C) Live cell imaging of HeLa cells treated with (A) 0, (B) 5, (C) 10, and (D) 20 μM of **23** for 24 h followed by staining with JC-1 dye. Shown are merged images of red and green fluorescence images of treated cells. Reproduced with permission from Saha et al.,[98] copyright 2016 American Chemical Society.

As shown in Figure 24, transporter **24** binds K^+ through a combination of electrostatic interactions and chelation. It then releases K^+ as the result of protonation once within the acidic intermembrane environment. On the basis of real-time confocal fluorescence imaging, the authors concluded that transporter **24** could modulate K^+/H^+ fluxes across both the mitochondrial and lysosomal membranes. Transporter **24** was found to induce a sudden increase in mitochondrial superoxide ($O_2^{\bullet-}$) levels and give rise to morphological changes in the mitochondria. These triggered changes were ascribed to K^+/H^+ exchange across the mitochondrial membrane. The authors tested the effect of transporter **24** on drug-resistant ovarian cancer stem cells (CSCs) and observed both apoptosis induction and autophagy suppression. Thus, this work sets the stage for the development of cancer treatments that are predicated on mimicking the function of K^+ channels.

Very recently, Gale, Sessler, Shin, and co-workers reported the first K^+/Na^+ exchangers **25–27** that induce apoptosis of cancer cells (Figure 25A).[106] Analyses of single-crystal X-ray diffraction and NMR spectra of hemispherand-strapped calix[4]pyrroles **25–27** indicate that the Na^+ or K^+ cations are complexed by the hemispherand straps in the receptos and chloride anions are bound to the calix[4]pyrrole subunit. These ion-pair receptors efficiently exchange Na^+/K^+ in the presence of Cl^- in liposomal models (Figure 25B). The presence of Cl^- may increase binding of Na^+ or K^+ cations to the receptors and has the effect of neutralizing the charge of the complex. The ion transport activity of strapped calix[4]pyrrole-based transporters in cells

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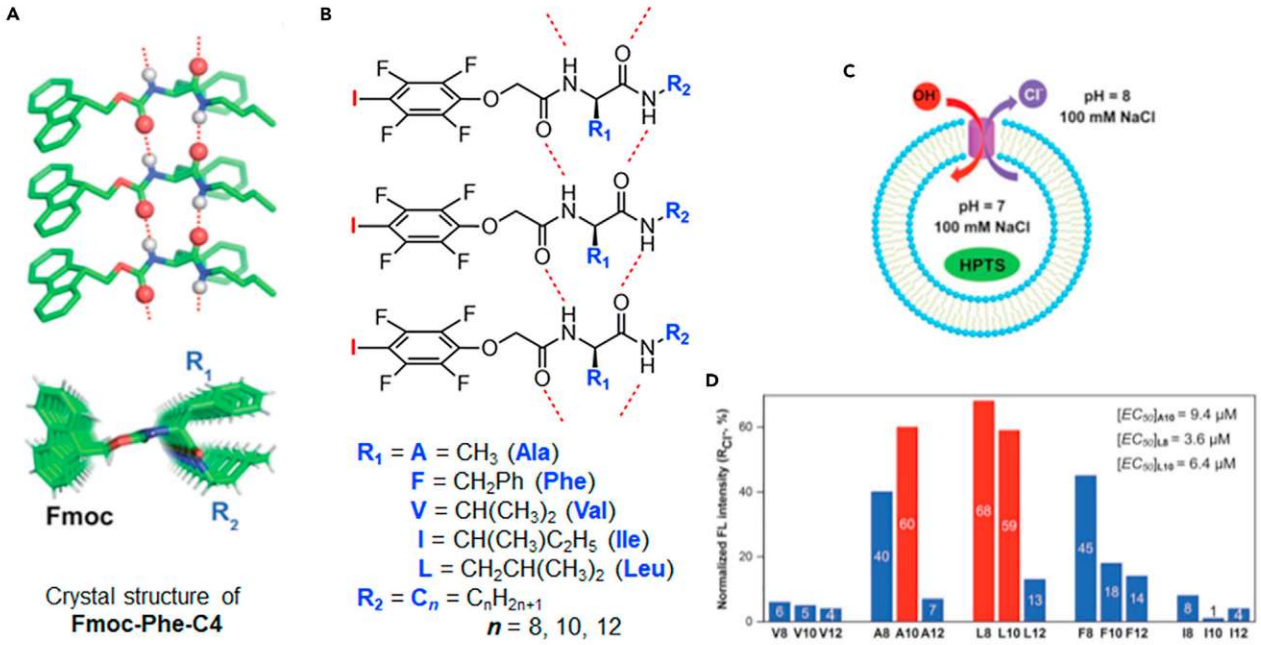

Figure 20. (A) Crystal structure of Fmoc-Phe-C4, illustrating the formation of the 1D columnar ensemble.
(B) Molecular design of the chloride anion-transporting channel library with systematically tunable R_1 and R_2 groups.
(C) Schematic illustration of the HPTS assay used for the Cl^- transport studies conducted using a pH gradient of 7 to 8. d) Normalized transport activities obtained over 5 min at 10 μM for all channel molecules. Reproduced with permission from Ren et al.,[100] copyright 2018 Royal Society of Chemistry.

was further evaluated. These synthetic transporters promoted K^+ efflux and Na^+ influx without significantly affecting the intracellular Cl^- concentration in cells, thus acting effectively as K^+/Na^+ exchangers *in vitro*. The authors further demonstrated that transporters **25–27** induced ER-stress-associated apoptotic cell death by perturbing intracellular cation homeostasis without affecting autophagy (Figure 25C). Moreover, since **25–27** mediate Na^+ influx and K^+ efflux in cells, they do not promote a substantial increase in intracellular ion concentrations and thus do not induce osmotic stress. Thus, these transporters are of interest as potential chemical tools that could prove useful in modulating intracellular cation concentrations and promoting apoptosis via modes of action that differ from most other approaches used to trigger programmed cell death.

Responsive ion transporters

Although synthetic ion transporters show promise as potential cancer therapeutics, they also have limitations. Included among these is a lack of selectivity for cancer cells over normal cells. In this regard, the design and synthesis of ion transporters that can induce cancer cell apoptosis selectively is appealing. Recently, Talukdar and co-workers developed a novel ion transport system that can be activated by the higher intracellular glutathione (GSH) concentrations typically present in cancer cells.[107] These researchers exploited the 2,4-nitrobenzene-sulfonyl (DNS) protected 2-hydroxyisophthalamide **28** depicted in Figure 26A. In the presence of GSH, the DNS group can be efficiently cleaved to release the membrane active 2-hydroxyisophthalamide **29**. Systematic biophysical studies confirmed that **29** could form channels within test membranes and transport ions as M^+/Cl^- ion pairs (where, $M^+ = Li^+$, Na^+, K^+, Rb^+, and Cs^+) (Figure 26B). Planar bilayer conductance measurements were carried out and taken as evidence that ion channels are formed from

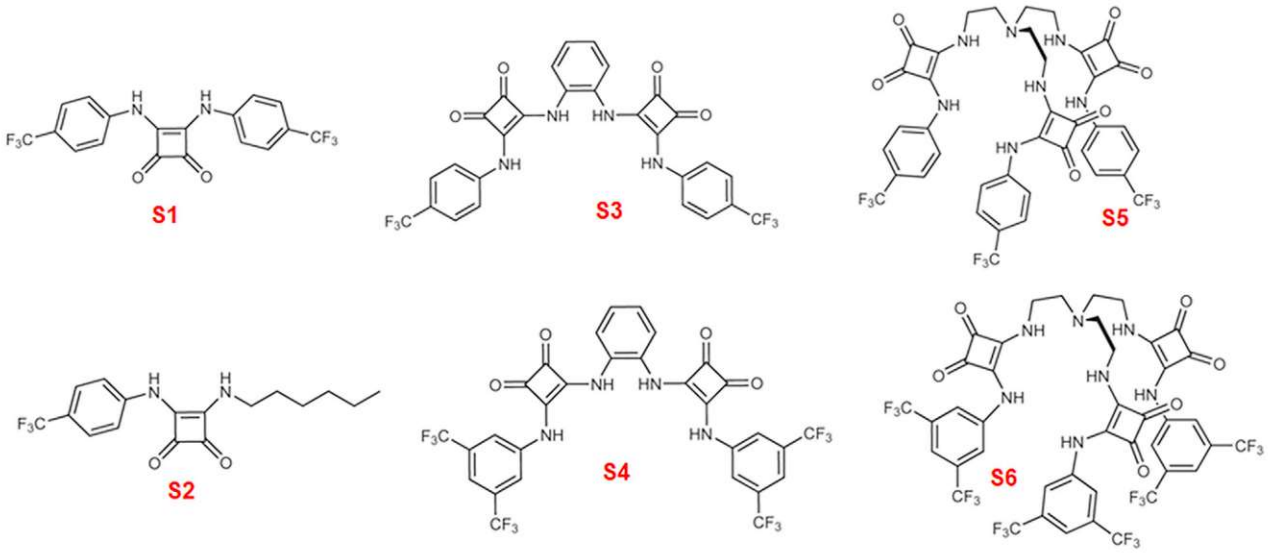

Figure 21. Chemical structures of squaramide-based ion transporters S1–S6
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29 (Figure 26C). An MTT assay was used to determine the viability of MCF-7 cells upon incubation with compound **28**. High cytotoxicity was observed (IC_{50} = *ca* 0.5–1.0 μm). It is worth noting that the membrane active compound **29** was observed to be less toxic than **28**, a result interpreted in terms of the superior cellular uptake efficiency for the DNS protected compound **28**. Additional cellular studies revealed that compound **28** could increase ROS levels, which, in turn, could reduce intracellular GSH levels, altered the mitochondrial outer membrane permeability (MOMP), and mediated cytochrome c release to activate caspase-9 and cleave PARP (poly(ADP-ribose) polymerase), all of which are hallmarks of apoptosis.

Gale and co-workers have recently reported gold complexes of 2,6-bis(benzimidazole-2-yl)pyridines as switchable anion transporters (Figure 27).[108] Complexation of 2,6-bis(benzimidazole-2-yl)pyridines with gold(III) blocks the anion binding site in these receptors. However, in the presence of a reducing agent such as GSH, the gold ion is reduced and liberated from the receptors to generate free anion transporters. Complex **P8** was shown to be non-cytotoxic in non-cancerous cell lines (HEK293 and MCF10A) but highly cytotoxic in SW260 cells (as was the precursor compound **P2**). These observations were attributed to higher GSH concentrations in the cancerous cells that serve to switch on anion transport by reducing and decomplexing the blocking gold ion.

ARTIFICIAL TRANSMEMBRANE ION TRANSPORTERS AS INDUCERS OF POTENTIAL ANTIBIOTICS

Antibiotic-resistant bacteria are an increasing public health threat. Particularly problematic is the rapid dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA).[109] In 2016, Roelens, Sessler, Gale, and co-workers reported a series of aminopyrrolic receptors (Figure 28) that could effectively transport Cl^- and inhibit the growth of clinical strains of *Staphylococcus aureus in vitro*.[110] The chloride anion transport properties of their putative carriers were evaluated using a phospholipid bilayer. High levels of Cl^- transport activity were observed in descending order for compounds **40**, **41**, **38**, **36**, and **39**. By changing the external solutions, for

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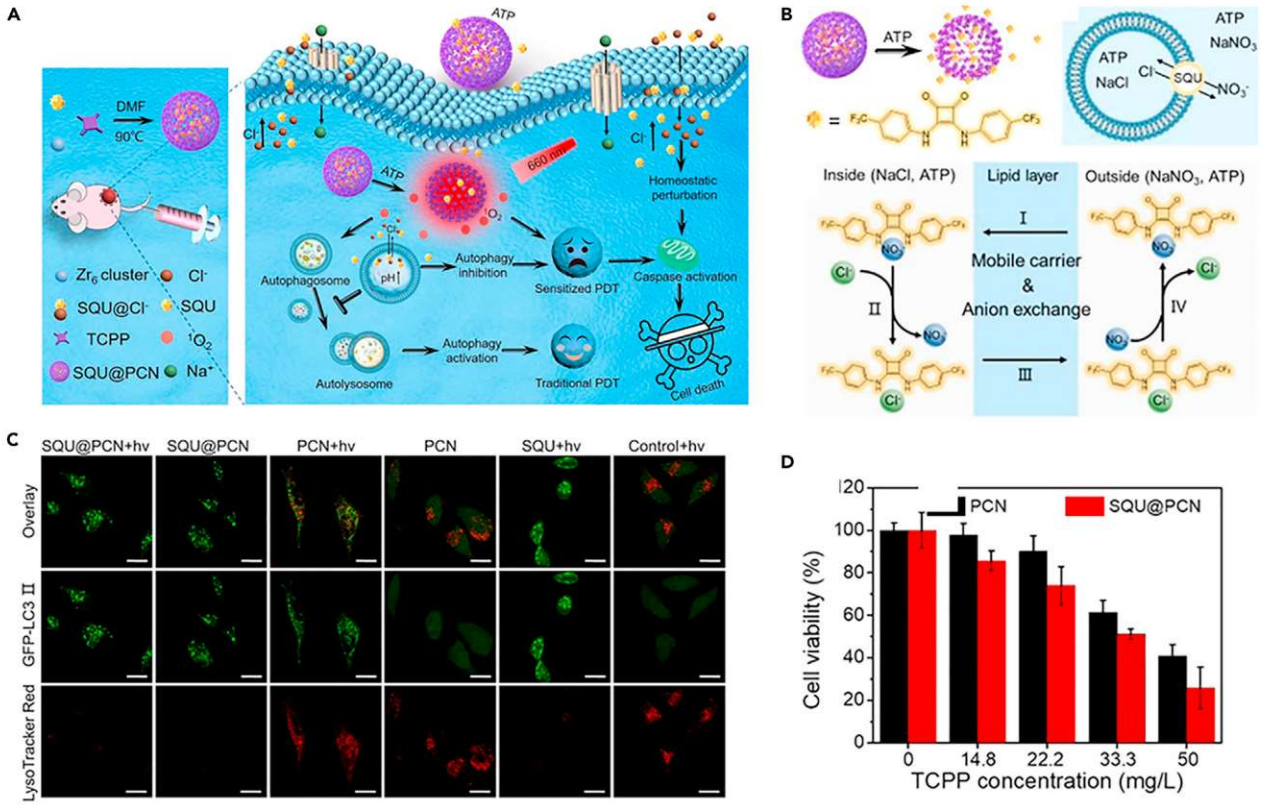

Figure 22. (A) Schematic illustration of SQU@PCN preparation and the tumor cell death process promoted by SQU@PCN.
(B) Proposed mechanism of SQU@PCN-mediated chloride transport.
(C) Study of autolysosome formation by means of a confocal laser scanning microscope (CLSM) with HeLa cells expressing GFP-LC3 (GFP, green fluorescent protein, and LC3, microtubule-associated light chain 3 protein) after treatment with different samples. The lysosome was stained with LysoTracker Red.
(D) *In vitro* cytotoxicity of SQU@PCN and PCN against HeLa cells under NIR irradiation in Cl^--free HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer. Reproduced with permission from Wan et al.,[101] copyright 2019 American Chemical Society.

example, SO_4^{2-}, NO_3^-, or HCO_3^- (as the sodium salts), the authors came to the conclusion that the receptors used in this study were functioning primarily as cation–anion cotransporters. Good antibiotic activity against Gram-positive *S. aureus* was observed. The most active aminopyrrole compounds proved to be in ascending order **38**, **39**, **33**, **36**, **40**, **41**. Although an early study, this report provided important support for the suggestion that ion transporters could be developed as rationally designed antibiotic agents.

Lugdunin, a nonribosomal cyclic peptide, is known to have highly cytotoxic activity against methicillin-resistant *S. aureus*.[111,112] Recently, Grond and co-workers reported a study of lugdunin **44** and its analogs prepared by optimized synthetic process (Figure 29).[113] On the basis of structure–activity relationship analyses, the authors determined the essential structural motifs associated with antimicrobial activity; these included an alternating amino acid architecture, the tryptophan and leucine units, and the *N*-unsubstituted thiazolidine ring. The influence of lugdunin and its analogs on the transmembrane potential of *S. aureus* cells was evaluated, and a full correlation between membrane depolarization and the minimum inhibitory concentration (MIC) values for *S. aureus* was obtained, from which a

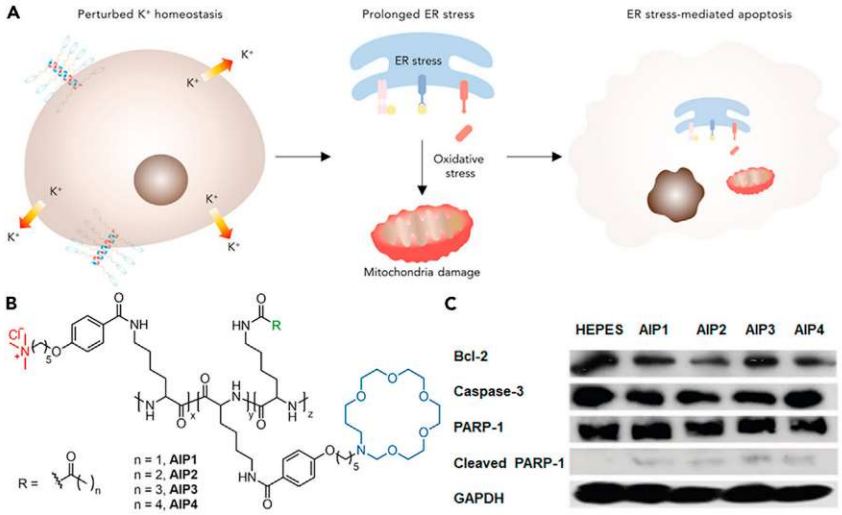

Figure 23. (A) AIPs induce ER-mediated apoptosis by disturbing potassium homeostasis.
(B) Representative structures of **AIPs**, **AIPn**, (n = 1–4).
(C) Immunoblot assays of the harvested tumor tissues for apoptosis-related proteins. Reproduced with permission from Lee et al.,[104] copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

mode of action involving impairment of membrane integrity or ion leakage/transport was inferred. It was concluded that lugdunin did not destabilize the membrane but rather acted to affect proton translocation based on the results of an HPTS assay. Lugdunin was found to mediate effective proton transport even at low concentrations (e.g., 50 nM). However, whether lugdunin transports protons as a mobile transporter or via self-assembly to form an ion channel is still an open question.

For synthetic ion transporters to act as effective antibacterial agents, it is important that they insert into bacterial membranes. In 2017, Hou and co-workers reported a unimolecular tubular channel based on a Trp-containing pillararene **45** (Figure 30A).[114] This system was found to insert into the lipid membrane of Gram-positive bacteria but not into the membranes of mammalian cells as inferred from conductivity studies (Figures 30B–30D). Excellent antimicrobial activity was seen against Gram-positive bacteria. In contrast, **45** showed no antimicrobial activity against Gram-negative bacteria and a low hemolytic toxicity in mammalian cells. The selective membrane insertion features seen in **45** point toward the eventual use of appropriately designed transmembrane ion channels to treat certain antibiotic infections.

MOLECULAR MACHINES FOR ION TRANSPORT

Notwithstanding the promise inherent in synthetic transmembrane ion transporters, other strategies have been explored to make lipid membranes more permeable and to induce programmed cell death. In this context, molecular machines whose conformations can be controlled in response to an outside stimulus are of interest. In certain cases, they have been exploited as smart cell membrane "openers," a feature that makes them attractive as potential anticancer agents.

In 2016, Tour and co-workers reported molecular motors bearing dye units (cyanine for **46**, boron dipyrromethene (BODIPY) for **47**) that were designed to insert into lipid

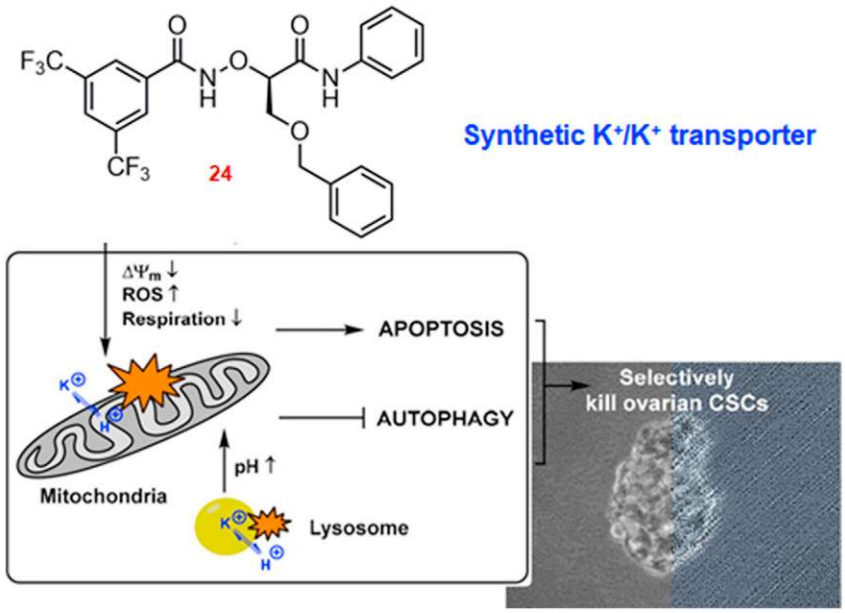

Figure 24. Figure 24. Illustration of the K^+/H^+ transport behavior of compound 24 in the context of mitochondria and lysosomes

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membranes and to "drill holes" into the membranes upon activation with UV light (Figures 31A and 31B).[115] The ability of these molecular motors to promote the irradiation-induced opening of lipid membranes was first tested using synthetic lipid vesicles. Molecular motor **46** and a BODIPY dye were co-encapsulated into a bilipid vesicle. Upon UV irradiation, a large decrease in the BODIPY-based fluorescence intensity of the vesicles was observed by confocal fluorescence microscopy. Control experiments confirmed that such a decrease can be ascribed to the nanomechanical disruption of

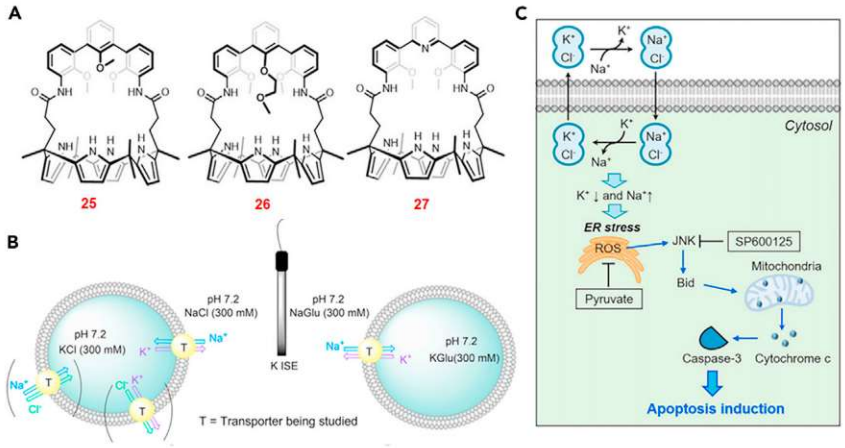

Figure 25. (A) Chemical structures of hemispherand-strapped calix[4]pyrrole ion-pair receptors 25–27.

(B) The K^+ ISE-based assays used to study K^+/Na^+ antiport in presence or absence of chloride. (C) Proposed mechanism of action underlying apoptosis induced by the synthetic ion-pair receptors. Reproduced with permission from Park et al.,[106] copyright 2021 Cell Press.

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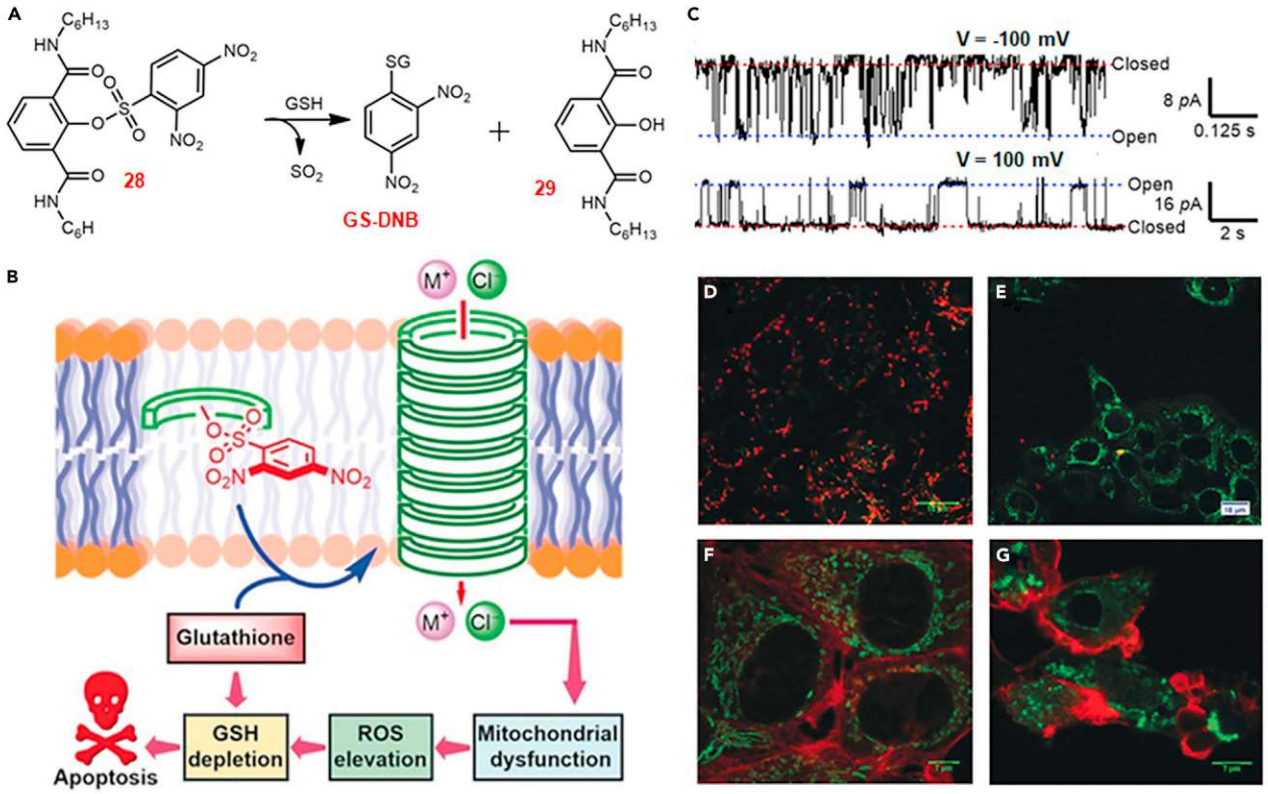

Figure 26. (A) GSH-mediated cleavage of the DNS group from 28, which serves to release 29.
(B) Schematic representation of channel activation inside cell by intracellular GSH and induction of apoptosis.
(C) Measurement of ionic conductance across the planar lipid bilayer membrane of **28** (10 mM) at −100 and at 100 mV in symmetrical KCl solutions (1.0 M).
(D and E) Confocal fluorescence microscopy images of MCF-7 cells treated with (D) 0 and (E) 1 mM of **28** followed by staining with the JC-1 dye.
(F and G) Confocal fluorescence microscopy images of MCF-7 cells treated with (F) 0 and (G) 1 mM of **28** followed by immunostaning with cytochrome c antibody (green). Phalloidin (red) was used to spot the boundaries. Reproduced with permission from Malla et al.,[107] copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

the vesicle bilipid membranes by the photoactivated motion of molecular motor **46**. Furthermore, the authors studied nanomechanical action on mouse embryonic fibroblast (NIH 3T3) cells by using confocal microscopy aided by a super-resolution technique called phase modulation nanoscopy. As shown in Figure 31C–A, molecular motor **47** (green fluorescence for the pendant cy5 dye) can enter the cell and localizes in the mitochondria (stained by MitoTracker Red). Conversely, as shown in Figure 31C–B, molecular motor **46** (red fluorescence for the pendant BODIPY dye) displays cell-surface localization and does not overlap with the lysosome (stained by LysoTracker Green). In addition, small aggregates can be observed inside cytoplasm after incubating molecular motor **46** with cells after 4 h, indicating a time-dependent localization pattern. Control experiments confirmed the active uptake of the molecular motors by endocytosis. Upon UV irradiation, the internalization of molecular motor **46** was significantly accelerated. As shown in Figure 31C–C, with gradual UV irradiation for 150 s, this molecular motor was observed to cross the cellular membrane and be internalized into cells. Controlled time- and UV-exposure-dependent experiments provided support for the suggestion that molecular motor **46** aggregates inside the cytoplasm lead to enhancement of the fluorescent intensity within the cytoplasm, as shown in Figure 31C–D. Further experiments performed with other molecular motors indicated a

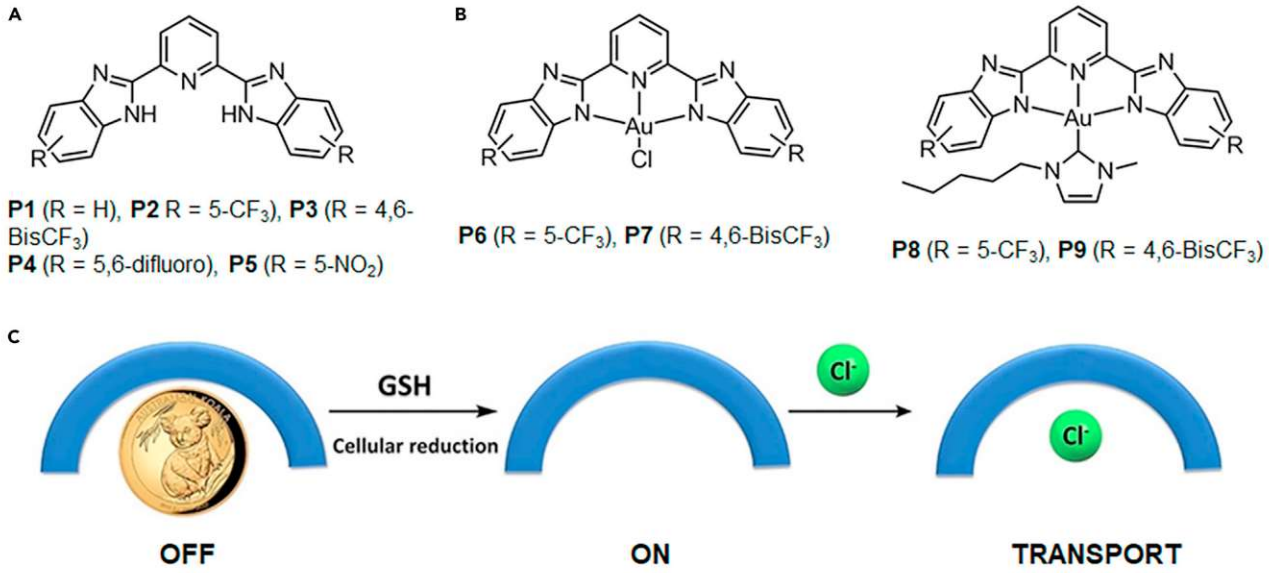

Figure 27. (A) Structure of ON anion transporters P1–P5.
(B) Structures of OFF anion transporter gold complexes **P6–P9**.
(C) GSH-mediated activation of switchable chloride anion transporter. Reproduced with permission from Fares et al.,[108] copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

UV-light-induced perturbation of the cell membrane and promotion of cell death (necrosis).

In a subsequent report, Tour and co-workers presented a set of molecular nanomachines (MNMs) that could be activated by near-infrared (NIR) light via a two-photon excitation process.[116] The use of NIR light was noted as being advantageous in that it is less prone to damage cells as reflected in its preferential use in the context of PDT. Although at an early stage of development, this research highlights the potential utility of molecular machines as devices that can open holes in cell membranes.

A variety of other molecular devices are known that can render more permeable cell membranes or mediate transmembrane ion transport. For instance, in 2016, Tian and co-workers reported an artificial molecular shuttle, **[2]rotaxane**, that can transport ions efficiently across EYPC membranes.[117] As shown in Figure 32, this system consists of an alkyl ammonium thread and a dibenzo-24-crown-8-based macrocyclic wheel bearing a benzo-18-crown-6 subunit as the putative K^+ carrier. The authors proposed that by inserting the molecular shuttle into the lipid membrane, K^+ transport could be accelerated via a shuttling-type mechanism. The ability of **[2]rotaxane** to transport K^+ was evaluated using a well-established fluorescence assay with large, unilamellar lipid vesicles entrapping a pH-sensitive fluorescent probe, 8-hydroxypyrene-1,3,6-trisulfonic acid (LUVs⊃HPTS). These studies revealed that the ion transport activity of **[2]rotaxane** is concentration-dependent (EC_{50} value = 1.0 μM). In accord with the design expectations, selectivity for K^+ was seen as follows: $K^+ > Rb^+ \geq Cs^+ > Na^+ > Li^+$. In order to elucidate the mechanism of the ion transport, a voltage clamp measurement was further performed across the planer bilayer membrane. This study led to the suggestion that ion transport reflects insertion of **[2]rotaxane** into the membrane as a monomeric unit. This study provides a

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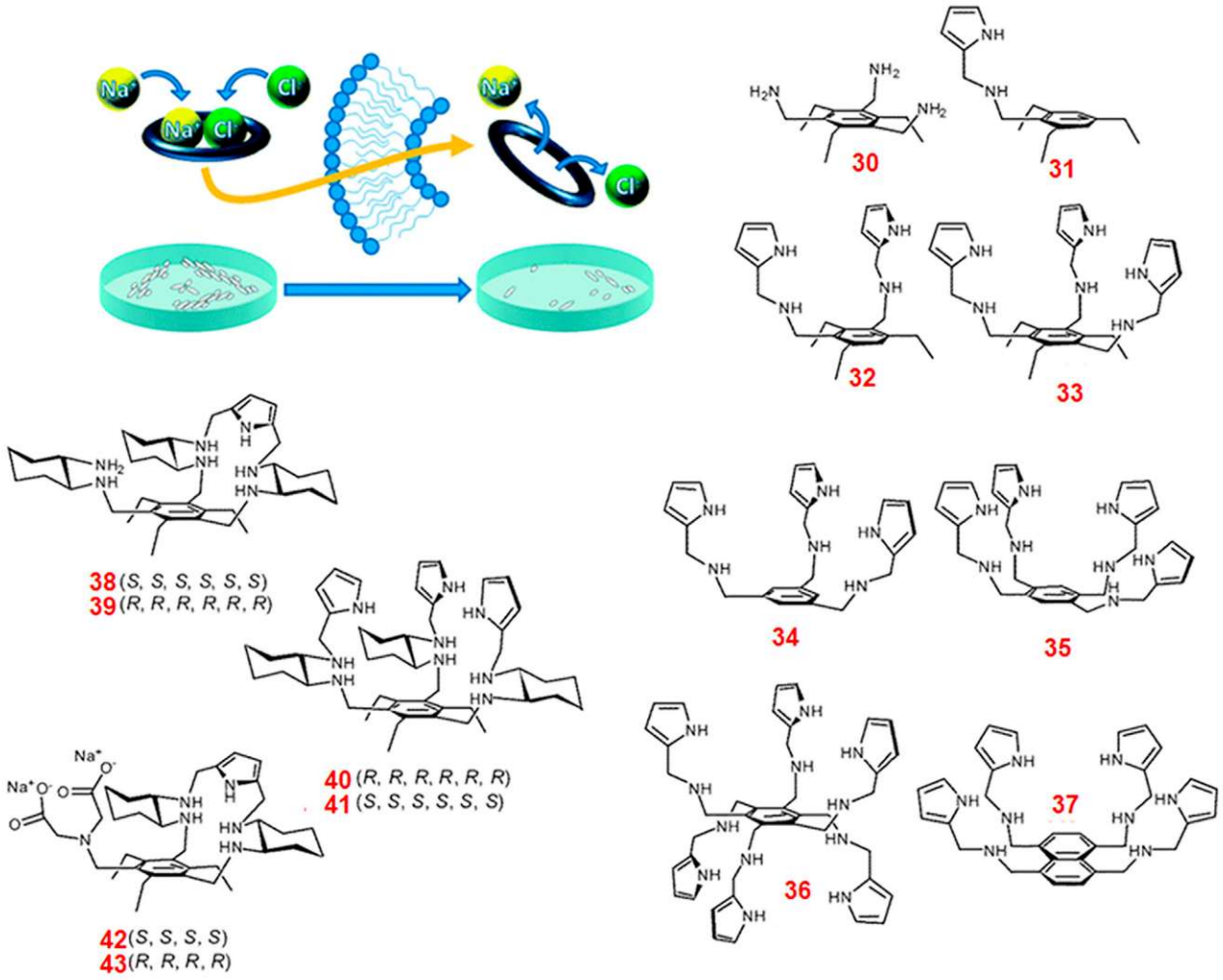

Figure 28. Chemical structures of aminopyrrolic receptors and illustration of the ability to inhibit the growth of *S. aureus*
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working example of a novel strategy that could be generalized to provide ion transporters while illustrating a new application for molecular machines capable of supporting transmembrane shuttling.

More recently (2019), Zeng and co-workers proposed a molecular swing approach to transport that was expected to incorporate the advantages of both ion carriers and ion channels.[118] Their molecular swings, **MS-C5** and **MS-C6** (Figure 33A), were designed to incorporate four essential structural elements referred to as lag bolts (lipid anchors), cross-beams, a flexible rope, and a swing seat, respectively. The authors proposed that the crown ether would move ions across the lipid membrane due to its ability to complex sodium or potassium (Figure 33B). Both **MS-C5** and **MS-C6** were found to possess an extraordinary capacity to transport Na^+ and K^+ as inferred from HPTS assays involving egg yolk l-α-phosphatidylcholine (EYPC)-based LUVs. The authors concluded that H^+/M^+ (M = Na or K) antiport was the main transport mechanism. The antiproliferative activity of **MS-C5** and **MS-C6** was assessed. The IC_{50} values of **MS-C5** and **MS-C6** toward a human primary glioblastoma cell

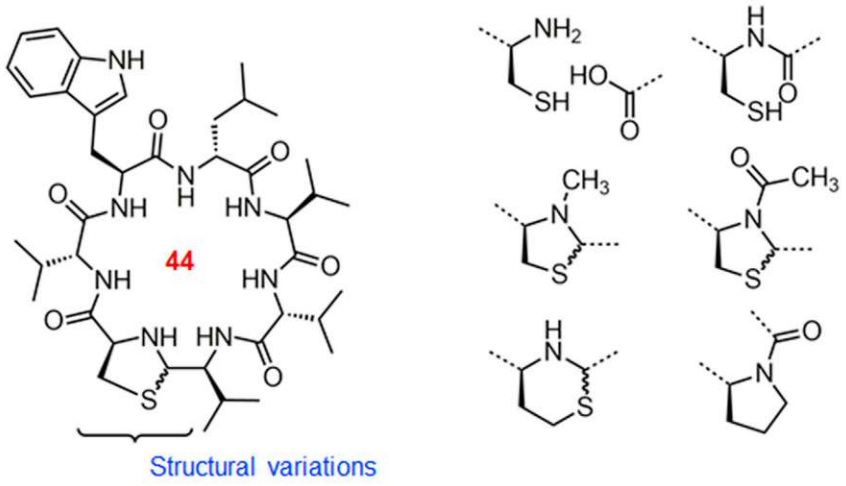

Figure 29. Chemical structures of lugdunin 44 and its analogs
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line (U-87 MG) were determined to be 60 ± 1.4 μM and 45 ± 0.6 μM, respectively. Although these activities are relatively low, they do serve to underscore the potential utility of this class of molecular machines as ion transporters.

CONCLUSIONS AND FUTURE PERSPECTIVES

As illustrated by the selected examples reviewed here, a variety of artificial ion transporters have been developed with the goal of achieving ion recognition and through-membrane ion transport. To date, particular emphasis has been placed

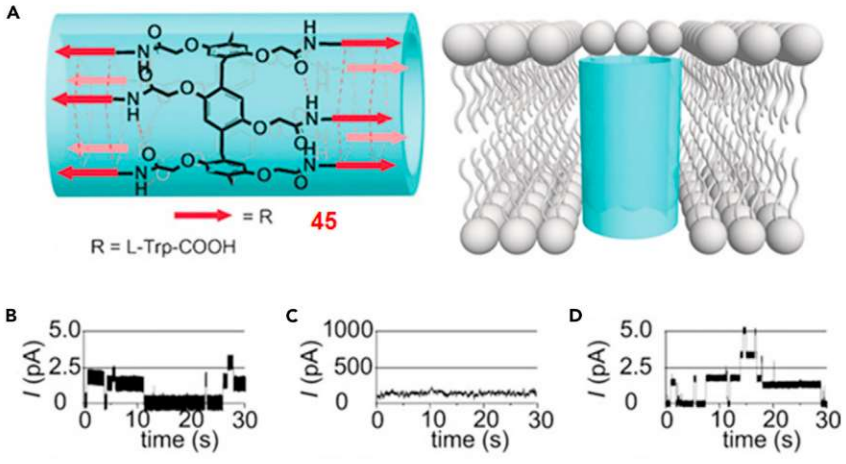

Figure 30. (A) Chemical structure of Trp-containing pillararene 45 and schematic diagram of the unimolecular transmembrane channel proposed to exist within a lipid bilayer.
(B–D) Current traces of **45** at a concentration of (B) 0.1 μM in dipalmitoylphosphatidylglycerol (DPPG)/dipalmitoylphosphatidylethanolamine (DPPE) bilayers, (C) 0.2 mM in DPPG/DPPE bilayers, and (D) 0.2 mM in diphytanoyl phosphatidylcholine (diPhyPC) bilayers at +100 mV in aqueous KCl (1.0 M). Reproduced with permission from Zhang et al.,[114] copyright 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

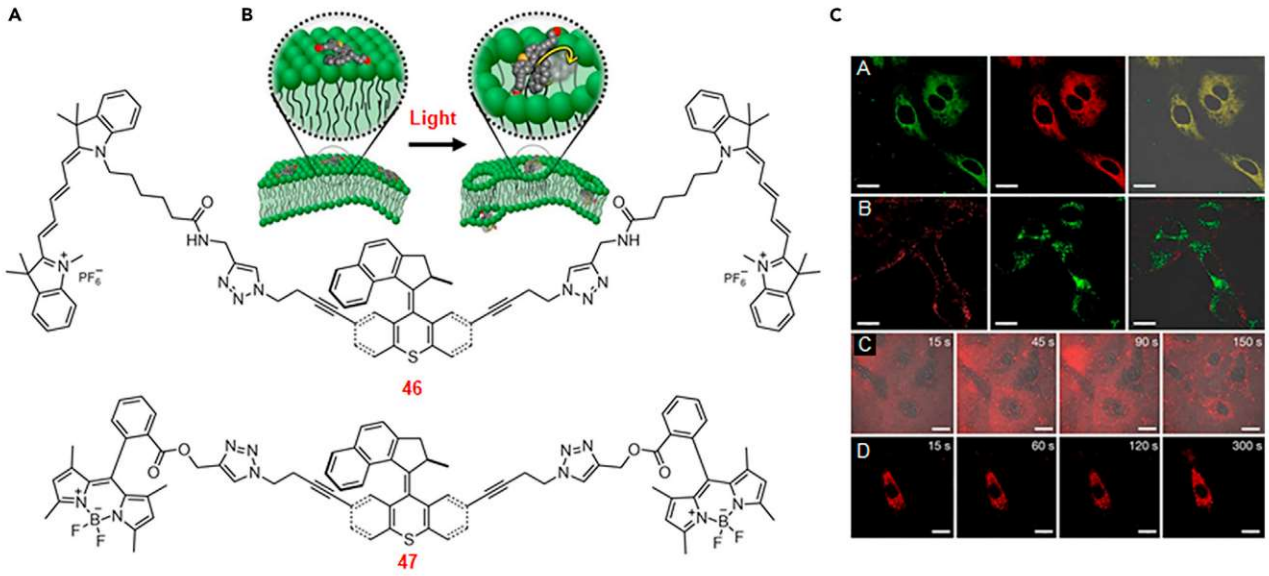

Figure 31. (A) Chemical structures of molecular motors 46 and 47.

(B) Schematic diagram of a molecular machine atop a cell membrane (left). The membrane is then opened by UV-activated nanomechanical action (indicated by the yellow arrow; right).

(C) UV activation was used to promote the nanomechanical-induced entry of **46** and **47** into NIH 3T3 cells: A. CLSM images of nanomachine **47** in the absence of UV activation. B. Nanomachine **46** in the absence of UV activation. C. Merged transmission images showing the time-dependent internalization of **46** observed upon UV activation.

(D) Fluorescent images showing the time-dependent dispersion of intracellular aggregates of **46** formed over the course of 1 h while being subject to a sequence of incubation and wash cycles, followed by UV activation of the motor for the times noted in each image. Reproduced with permission from García-López et al.,[115] copyright 2016 Nature Publishing Group.

on systems effective for the transport of chloride, potassium, and sodium ions, as well as protons. Ion transporters have been reported that can disrupt pH gradients or ion homeostasis. Many of these systems appear promising in terms of promoting an anticancer effect *in vitro* or inhibiting bacterial growth. Taken together, the synthetic transporters discussed in this review are attractive because they operate by

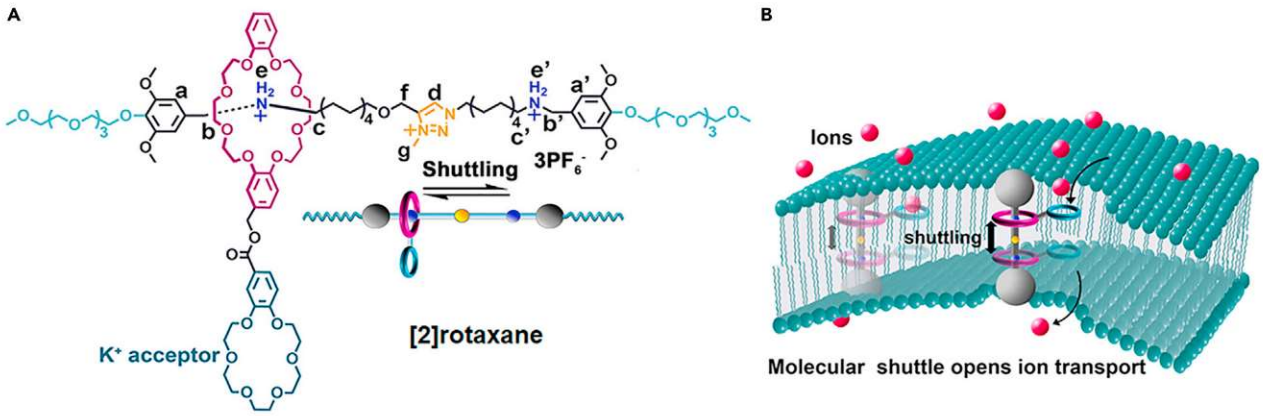

Figure 32. (A) Chemical structure of molecular shuttle [2]rotaxane.

(B) Proposed mechanism of K^+ transport across lipid bilayers. Reproduced with permission from Chen et al.,[117] copyright 2016 American Chemical Society.

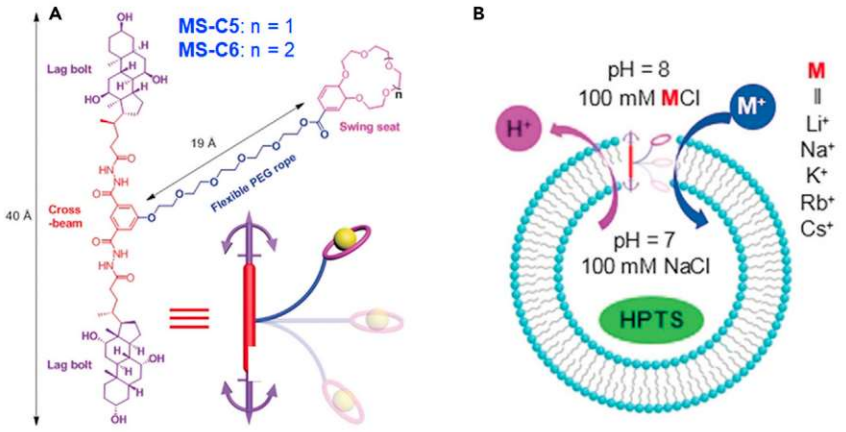

Figure 33. (A) Chemical structures of molecular swings MS-C5 and MS-C6.
(B) Schematic illustration of the HPTS assay used to evaluate the ion-transport activity of these swings. Reproduced with permission from Ren et al.,[118] copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

mechanisms that are orthogonal to traditional chemotherapies. However, it is likely that more effective systems will need to be developed before this promise is fully realized.

Challenges that researchers in the ion transport area face include the following: the design of synthetic ion transporters with well-defined and stable architectures that provide for high transport efficiency and selectivity.[119–121] This may require the exploitation of new architectures, ranging from more precisely designed monomeric transporters to complicated self-assembled nanostructures and molecular machines. Another potential barrier to the development of synthetic transporters, particularly as potential pharmaceuticals, is the fact that most known systems are lipophilic and generally non-selective in terms of their cell targeting. Achieving efficient site targeting, as well as responsive transport within those sites, is another challenge. Specifically, designing novel artificial ion transporters that interact only with cancer cells or that target bacteria selectively while not interacting with normal cells is an exciting frontier in the area of design.

The combination of ion transporters with other therapeutic modalities is also of particular interest and may allow the realization of synergistic anticancer or antimicrobial effects.[122,123] Treatments, such as chemotherapy, radiotherapy, and immunotherapy, warrant exploration in this regard. It is likely, but not yet codified by experiment, that perturbations in pH gradients or ion homeostasis will serve to amplify the therapeutic performance of various established treatments by increasing oxidative stress or by abetting other cell killing mechanisms. Ultimately, artificial ion transporters may allow obstacles, such as resistance and recurrence that plague both patients and medical practitioners, to be overcome. It is also important to appreciate that suitably designed artificial ion transporters will contribute to an increased fundamental understanding of how naturally occurring transmembrane channels function, thus allowing us to treat at the source, as it were, the predicates for a variety of transport-related human diseases. Thus, we think the area of synthetic transmembrane transport is one that is ripe with promise.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.Y., G.C.Y., F.H., and J.L.S; writing – original draft, J.Y., and G.C.Y.; writing – review & editing, J.Y., G.C.Y., J.L.S., P.A.G., I.S., and F.H.

REFERENCES

1. Szostak, J.W., Bartel, D.P., and Luisi, P.L. (2001). Synthesizing life. Nature *409*, 387–390.
2. Dubyak, G.R. (2004). Ion homeostasis, channels, and transporters: an update on cellular mechanisms. Adv. Physiol. Educ. *28*, 143–154.
3. Bröer, S. (2008). Amino acid transport across mammalian intestinal and renal epithelia. Physiol. Rev. *88*, 249–286.
4. Gamper, N., and Shapiro, M.S. (2007). Regulation of ion transport proteins by membrane phosphoinositides. Nat. Rev. Neurosci. *8*, 921–934.
5. Wang, X., Zhang, X., Dong, X.P., Samie, M., Li, X., Cheng, X., Goschka, A., Shen, D., Zhou, Y., Harlow, J., et al. (2012). TPC proteins are phosphoinositide-activated sodium-selective ion channels in endosomes and lysosomes. Cell *151*, 372–383.
6. Guo, J., Zeng, W., Chen, Q., Lee, C., Chen, L., Yang, Y., Cang, C., Ren, D., and Jiang, Y. (2016). Structure of the voltage-gated two-pore channel TPC1 from Arabidopsis thaliana. Nature *531*, 196–201.
7. Sheppard, D.N., Rich, D.P., Ostedgaard, L.S., Gregory, R.J., Smith, A.E., and Welsh, M.J. (1993). Mutations in CFTR associated with mild-disease-form Cl^- channels with altered pore properties. Nature *362*, 160–164.
8. Choi, J.Y., Muallem, D., Kiselyov, K., Lee, M.G., Thomas, P.J., and Muallem, S. (2001). Aberrant CFTR-dependent HCO_3^- transport in mutations associated with cystic fibrosis. Nature *410*, 94–97.
9. Vaughan-Jones, R.D., Spitzer, K.W., and Swietach, P. (2009). Intracellular pH regulation in heart. J. Mol. Cell. Cardiol. *46*, 318–331.
10. Gadsby, D.C., Vergani, P., and Csanády, L. (2006). The ABC protein turned chloride channel whose failure causes cystic fibrosis. Nature *440*, 477–483.
11. Ghadiri, M.R., Granja, J.R., and Buehler, L.K. (1994). Artificial transmembrane ion channels from self-assembling peptide nanotubes. Nature *369*, 301–304.
12. Sakai, N., and Matile, S. (2013). Synthetic ion channels. Langmuir *29*, 9031–9040.
13. Gokel, G.W., and Negin, S. (2013). Synthetic ion channels: from pores to biological applications. Acc. Chem. Res. *46*, 2824–2833.
14. Fyles, T.M. (2007). Synthetic ion channels in bilayer membranes. Chem. Soc. Rev. *36*, 335–347.
15. Barboiu, M., and Gilles, A. (2013). From natural to bioassisted and biomimetic artificial water channel systems. Acc. Chem. Res. *46*, 2814–2823.
16. Montenegro, J., Ghadiri, M.R., and Granja, J.R. (2013). Ion channel models based on self-assembling cyclic peptide nanotubes. Acc. Chem. Res. *46*, 2955–2965.
17. Carmichael, V.E., Dutton, P.J., Fyles, T.M., James, T.D., Swan, J.A., and Zojaji, M. (1989). Biomimetic ion transport: a functional model of a unimolecular ion channel. J. Am. Chem. Soc. *111*, 767–769.
18. Baumeister, B., Sakai, N., and Matile, S. (2000). Giant artificial ion channels formed by self-assembled, cationic rigid-rod β-barrels. Angew. Chem. Int. Ed. *39*, 1955–1958.
19. Gokel, G.W., and Murillo, O. (1996). Synthetic organic chemical models for transmembrane channels. Acc. Chem. Res. *29*, 425–432.
20. Li, X., Wu, Y.D., and Yang, D. (2008). α-Aminoxy acids: new possibilities from foldamers to anion receptors and channels. Acc. Chem. Res. *41*, 1428–1438.
21. Fyles, T.M. (2013). How do amphiphiles form ion-conducting channels in membranes? Lessons from linear oligoesters. Acc. Chem. Res. *46*, 2847–2855.
22. Sisson, A.L., Shah, M.R., Bhosale, S., and Matile, S. (2006). Synthetic ion channels and pores (2004-2005). Chem. Soc. Rev. *35*, 1269–1286.
23. Davis, J.T., Gale, P.A., and Quesada, R. (2020). Advances in anion transport and supramolecular medicinal chemistry. Chem. Soc. Rev. *49*, 6056–6086.
24. Hosogi, S., Kusuzaki, K., Inui, T., Wang, X., and Marunaka, Y. (2014). Cytosolic chloride ion is a key factor in lysosomal acidification and function of autophagy in human gastric cancer cell. J. Cell. Mol. Med. *18*, 1124–1133.
25. Busschaert, N., Park, S.H., Baek, K.H., Choi, Y.P., Park, J., Howe, E.N.W., Hiscock, J.R., Karagiannidis, L.E., Marques, I., Félix, V., et al. (2017). A synthetic ion transporter that disrupts autophagy and induces apoptosis by perturbing cellular chloride concentrations. Nat. Chem. *9*, 667–675.
26. Smith, B.A., Daschbach, M.M., Gammon, S.T., Xiao, S., Chapman, S.E., Hudson, C., et al. (2011). In vivo cell death mediated by synthetic ion channels. Chem. Commun. *47*, 7977–7979.
27. Park, S.H., Park, S.H., Howe, E.N.W., Hyun, J.Y., Chen, L.J., Hwang, I., Vargas-Zuñiga, G., Busschaert, N., Gale, P.A., Sessler, J.L., and Shin, I. (2019). Determinants of ion-transporter cancer cell death. Chem *5*, 2079–2098.
28. Rodilla, A.M., Korrodi-Gregório, L., Hernando, E., Manuel-Manresa, P., Quesada, R., Pérez-Tomás, R., and Soto-Cerrato, V. (2017). Synthetic tambjamine analogues induce mitochondrial swelling and lysosomal dysfunction leading to autophagy blockade and necrotic cell death in lung cancer. Biochem. Pharmacol. *126*, 23–33.
29. Kim, D.H., Rozhkova, E.A., Ulasov, I.V., Bader, S.D., Rajh, T., Lesniak, M.S., and Novosad, V. (2010). Biofunctionalized magnetic-vortex microdiscs for targeted cancer-cell destruction. Nat. Mater. *9*, 165–171.
30. Alfonso, I., and Quesada, R. (2013). Biological activity of synthetic ionophores: ion

transporters as prospective drugs? Chem. Sci. *4*, 3009–3019.

31. Li, H., Valkenier, H., Thorne, A.G., Dias, C.M., Cooper, J.A., Kieffer, M., Busschaert, N., Gale, P.A., Sheppard, D.N., and Davis, A.P. (2019). Anion carriers as potential treatments for cystic fibrosis: transport in cystic fibrosis cells, and additivity to channel-targeting drugs. Chem. Sci. *10*, 9663–9672.

32. Leevy, W.M., Donato, G.M., Ferdani, R., Goldman, W.E., Schlesinger, P.H., and Gokel, G.W. (2002). Synthetic hydraphile channels of appropriate length kill Escherichia coli. J. Am. Chem. Soc. *124*, 9022–9023.

33. Brea, R.J., Reiriz, C., and Granja, J.R. (2010). Towards functional bionanomaterials based on self-assembling cyclic peptide nanotubes. Chem. Soc. Rev. *39*, 1448–1456.

34. Kevin, D.A., II, Meujo, D.A., and Hamann, M.T. (2009). Polyether ionophores: broad-spectrum and promising biologically active molecules for the control of drug-resistant bacteria and parasites. Expert Opin. Drug Discov. *4*, 109–146.

35. Matile, S., and Sakai, N. (2012). The characterization of synthetic ion channels and pores. In Analytical Methods in Supramolecular Chemistry, Second Edition, C.A. Schalley, ed. (Weinheim, Germany: Wiley-VCH), pp. 711–742.

36. Litvinchuk, S., Bollot, G., Mareda, J., Som, A., Ronan, D., Shah, M.R., Perrottet, P., Sakai, N., and Matile, S. (2004). Thermodynamic and kinetic stability of synthetic multifunctional rigid-rod β-barrel pores: evidence for supramolecular catalysis. J. Am. Chem. Soc. *126*, 10067–10075.

37. Wu, X., Howe, E.N.W., and Gale, P.A. (2018). Supramolecular transmembrane anion transport: new assays and insights. Acc. Chem. Res. *51*, 1870–1879.

38. Verkman, A.S., Sellers, M.C., Chao, A.C., Leung, T., and Ketcham, R. (1989). Synthesis and characterization of improved chloride-sensitive fluorescent indicators for biological applications. Anal. Biochem. *178*, 355–361.

39. Jayaraman, S., Haggie, P., Wachter, R.M., Remington, S.J., and Verkman, A.S. (2000). Mechanism and cellular applications of a green fluorescent protein-based halide sensor. J. Biol. Chem. *275*, 6047–6050.

40. Van Meerloo, J., Kaspers, G.J., and Cloos, J. (2011). Cell sensitivity assays: the MTT assay. Methods Mol. Biol. *731*, 237–245.

41. Williams, D.R., Ko, S.K., Park, S., Lee, M.R., and Shin, I. (2008). An apoptosis-inducing small molecule that binds to heat shock protein 70. Angew. Chem. Int. Ed. *47*, 7466–7469.

42. Salvioli, S., Ardizzoni, A., Franceschi, C., and Cossarizza, A. (1997). JC-1, but not DIOC6(3) or rhodamine 123, is a reliable fluorescent probe to assess $\Delta\Psi$ changes in intact cells: implications for studies on mitochondrial functionality during apoptosis. FEBS Lett *411*, 77–82.

43. Hengartner, M.O. (2000). The biochemistry of apoptosis. Nature *407*, 770–776.

44. Kunzelmann, K. (2005). Ion channels and cancer. J. Membr. Biol. *205*, 159–173.

45. Benke, B.P., Aich, P., Kim, Y., Kim, K.L., Rohman, M.R., Hong, S., Hwang, I.C., Lee, E.H., Roh, J.H., and Kim, K. (2017). Iodide-selective synthetic ion channels based on shape-persistent organic cages. J. Am. Chem. Soc. *139*, 7432–7435.

46. Lang, C., Li, W., Dong, Z., Zhang, X., Yang, F., Yang, B., et al. (2016). Biomimetic transmembrane channels with high stability and transporting efficiency from helically folded macromolecules. Angew. Chem. Int. Ed. *55*, 9723–9727.

47. Burns, J.R., Seifert, A., Fertig, N., and Howorka, S. (2016). A biomimetic DNA-based channel for the ligand-controlled transport of charged molecular cargo across a biological membrane. Nat. Nanotechnol. *11*, 152–156.

48. Haynes, C.J.E., Zhu, J., Chimerel, C., Hernández-Ainsa, S., Riddell, I.A., Ronson, T.K., et al. (2017). Blockable $Zn_{10}L_{15}$ ion channels through subcomponent self-assembly. Angew. Chem. Int. Ed. *56*, 15388–15392.

49. Talukdar, P., Bollot, G., Mareda, J., Sakai, N., and Matile, S. (2005). Synthetic ion channels with rigid-rod π-stack architecture that open in response to charge-transfer complex formation. J. Am. Chem. Soc. *127*, 6528–6529.

50. Danial, M., Tran, C.M.N., Jolliffe, K.A., and Perrier, S. (2014). Thermal gating in lipid membranes using thermoresponsive cyclic peptide–polymer conjugates. J. Am. Chem. Soc. *136*, 8018–8026.

51. Jung, M., Kim, H., Baek, K., and Kim, K. (2008). Synthetic ion channel based on metal–organic polyhedra. Angew. Chem. Int. Ed. *47*, 5755–5757.

52. Pfeifer, J.R., Reiss, P., and Koert, U. (2006). Crown ether–gramicidin hybrid ion channels: dehydration-assisted ion selectivity. Angew. Chem. Int. Ed. *45*, 501–504.

53. Kumpf, R.A., and Dougherty, D.A. (1993). A mechanism for ion selectivity in potassium channels: computational studies of cation-pi interactions. Science *261*, 1708–1710.

54. Vargas Jentzsch, A., Hennig, A., Mareda, J., and Matile, S. (2013). Synthetic ion transporters that work with anion–π interactions, halogen bonds, and anion–macrodipole interactions. Acc. Chem. Res. *46*, 2791–2800.

55. Behera, H., and Madhavan, N. (2017). Anion-selective cholesterol decorated macrocyclic transmembrane ion carriers. J. Am. Chem. Soc. *139*, 12919–12922.

56. Yuan, L., Shen, J., Ye, R., Chen, F., and Zeng, H. (2019). Structurally simple trimesic amides as highly selective anion channels. Chem. Commun. *55*, 4797–4800.

57. Busschaert, N., Kirby, I.L., Young, S., Coles, S.J., Horton, P.N., Light, M.E., et al. (2012). Squaramides as potent transmembrane anion transporters. Angew. Chem. Int. Ed. *51*, 4426–4430.

58. Si, W., Xin, P., Li, Z.T., and Hou, J.L. (2015). Tubular unimolecular transmembrane channels: construction strategy and transport activities. Acc. Chem. Res. *48*, 1612–1619.

59. Kaucher, M.S., Harrell, W.A., and Davis, J.T. (2006). A unimolecular G-quadruplex that functions as a synthetic transmembrane Na^+ transporter. J. Am. Chem. Soc. *128*, 38–39.

60. Su, G., Zhang, M., Si, W., Li, Z.T., and Hou, J.L. (2016). Directional potassium transport through a unimolecular peptide channel. Angew. Chem. Int. Ed. *55*, 14678–14682.

61. Jeon, Y.J., Kim, H., Jon, S., Selvapalam, N., Oh, D.H., Seo, I., et al. (2004). Artificial ion channel formed by cucurbit[n]uril derivatives with a carbonyl group fringed portal reminiscent of the selectivity filter of K^+ channels. J. Am. Chem. Soc. *126*, 15944–15945.

62. Kim, D.S., and Sessler, J.L. (2015). Calix[4]pyrroles: versatile molecular containers with ion transport, recognition, and molecular switching functions. Chem. Soc. Rev. *44*, 532–546.

63. Sonawane, N.D., Thiagarajah, J.R., and Verkman, A.S. (2002). Chloride concentration in endosomes measured using a ratioable fluorescent Cl^- indicator: evidence for chloride accumulation during acidification. J. Biol. Chem. *277*, 5506–5513.

64. Saha, T., Hossain, M.S., Saha, D., Lahiri, M., and Talukdar, P. (2016). Chloride-mediated apoptosis-inducing activity of bis(sulfonamide) anionophores. J. Am. Chem. Soc. *138*, 7558–7567.

65. Zhang, S., Wang, Y., Xie, W., Howe, E.N.W., Busschaert, N., Sauvat, A., Leduc, M., Gomes-da-Silva, L.C., Chen, G., Martins, I., et al. (2019). Squaramide-based synthetic chloride transporters activate TFEB but block autophagic flux. Cell Death Dis. *10*, 242.

66. Lisbjerg, M., Valkenier, H., Jessen, B.M., Al-Kerdi, H., Davis, A.P., and Pittelkow, M. (2015). Biotin[6]uril esters: chloride-selective transmembrane anion carriers employing C-H···anion interactions. J. Am. Chem. Soc. *137*, 4948–4951.

67. Valkenier, H., Akrawi, O., Jurček, P., Sleziaková, K., Lízal, T., Bartik, K., et al. (2019). Fluorinated bambusurils as highly effective and selective transmembrane Cl^-/HCO_3^- antiporters. Chem *5*, 429–444.

68. Kamp, F., and Hamilton, J.A. (1992). pH gradients across phospholipid membranes caused by fast flip-flop of un-ionized fatty acids. Proc. Natl. Acad. Sci. USA *89*, 11367–11370.

69. Howe, E.N.W., and Gale, P.A. (2019). Fatty acid fueled transmembrane chloride transport. J. Am. Chem. Soc. *141*, 10654–10660.

70. Wu, X., Small, J., Cataldo, A., Withecombe, A., Turner, P., and Gale, P.A. (2019). Voltage-switchable HCl transport enabled by lipid headgroup transporter interactions. Angew. Chem. Int. Ed. *58*, 15142–15147.

71. Xue, M., Yang, Y., Chi, X., Zhang, Z., and Huang, F. (2012). Pillararenes, a new class of macrocycles for supramolecular chemistry. Acc. Chem. Res. *45*, 1294–1308.

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72. Chen, L., Si, W., Zhang, L., Tang, G., Li, Z.T., and Hou, J.L. (2013). Chiral selective transmembrane transport of amino acids through artificial channels. J. Am. Chem. Soc. *135*, 2152–2155.

73. Carpenter, V.K., Drake, L.L., Aguirre, S.E., Price, D.P., Rodriguez, S.D., and Hansen, I.A. (2012). SLC7 amino acid transporters of the yellow fever mosquito Aedes aegypti and their role in fat body TOR signaling and reproduction. J. Insect Physiol. *58*, 513–522.

74. Yamnitz, C.R., Negin, S., Carasel, I.A., Winter, R.K., and Gokel, G.W. (2010). Dianilides of dipicolinic acid function as synthetic chloride channels. Chem. Commun. *46*, 2838–2840.

75. Wei, X., Zhang, G., Shen, Y., Zhong, Y., Liu, R., Yang, N., Al-mkhaizim, F.Y., Kline, M.A., He, L., Li, M., et al. (2016). Persistent organic nanopores amenable to structural and functional tuning. J. Am. Chem. Soc. *138*, 2749–2754.

76. Hou, X., Zhang, H., and Jiang, L. (2012). Building bio-inspired artificial functional nanochannels: from symmetric to asymmetric modification. Angew. Chem. Int. Ed. *51*, 5296–5307.

77. Liu, Q., Wen, L., Xiao, K., Lu, H., Zhang, Z., Xie, G., Kong, X.Y., Bo, Z., and Jiang, L. (2016). A biomimetic voltage-gated chloride nanochannel. Adv. Mater. *28*, 3181–3186.

78. Huang, X., and Jan, L.Y. (2014). Targeting potassium channels in cancer. J. Cell Biol. *206*, 151–162.

79. Gokel, G.W., and Mukhopadhyay, A. (2001). Synthetic models of cation-conducting channels. Chem. Soc. Rev. *30*, 274–286.

80. Matile, S. (2001). En route to supramolecular functional plasticity: artificial β-barrels, the barrel-stave motif, and related approaches. Chem. Soc. Rev. *30*, 158–167.

81. Davis, J.T., and Spada, G.P. (2007). Supramolecular architectures generated by self-assembly of guanosine derivatives. Chem. Soc. Rev. *36*, 296–313.

82. Voyer, N. (1996). The development of peptide nanostructures. Top. Curr. Chem. *184*, 1–37.

83. Cazacu, A., Tong, C., van der Lee, A., Fyles, T.M., and Barboiu, M. (2006). Columnar self-assembled ureido crown ethers: an example of ion-channel organization in lipid bilayers. J. Am. Chem. Soc. *128*, 9541–9548.

84. Sheldrick, W.S., and Poonia, N.S. (1986). Coordination chemistry of alkali and alkaline earth cations. X-ray structural analysis of bis (benzo-15-crown-5) potassium nitrate monohydrate. Journal of Inclusion Phenomena *4*, 93–98.

85. Ren, C., Shen, J., and Zeng, H. (2017). Combinatorial evolution of fast-conducting highly selective K^+-channels via modularly tunable directional assembly of crown ethers. J. Am. Chem. Soc. *139*, 12338–12341.

86. Si, W., Li, Z.T., and Hou, J.L. (2014). Voltage-driven reversible insertion into and leaving from a lipid bilayer: tuning transmembrane transport of artificial channels. Angew. Chem. Int. Ed. *53*, 4578–4581.

87. Smith, N.J., and Solovay, C.F. (2017). Epithelial Na^+ channel inhibitors for the treatment of cystic fibrosis. Pharm. Pat. Anal. *6*, 179–188.

88. Forman, S.L., Fettinger, J.C., Pieraccini, S., Gottarelli, G., and Davis, J.T. (2000). Toward artificial ion channels: a lipophilic G-quadruplex. J. Am. Chem. Soc. *122*, 4060–4067.

89. Hamada, T., Matsunaga, S., Yano, G., and Fusetani, N. (2005). Polytheonamides A and B, highly cytotoxic, linear polypeptides with unprecedented structural features, from the marine sponge, Theonella swinhoei. J. Am. Chem. Soc. *127*, 110–118.

90. Itoh, H., Matsuoka, S., Kreir, M., and Inoue, M. (2012). Design, synthesis and functional analysis of dansylated polytheonamide mimic: an artificial peptide ion channel. J. Am. Chem. Soc. *134*, 14011–14018.

91. Sawyers, C. (2004). Targeted cancer therapy. Nature *432*, 294–297.

92. Fan, W., Yung, B., Huang, P., and Chen, X. (2017). Nanotechnology for multimodal synergistic cancer therapy. Chem. Rev. *117*, 13566–13638.

93. Xie, J., Lee, S., and Chen, X. (2010). Nanoparticle-based theranostic agents. Adv. Drug Deliv. Rev. *62*, 1064–1079.

94. Reya, T., Morrison, S.J., Clarke, M.F., and Weissman, I.L. (2001). Stem cells, cancer, and cancer stem cells. Nature *414*, 105–111.

95. Fürstner, A. (2003). Chemistry and biology of roseophilin and the prodigiosin alkaloids: a survey of the last 2500 years. Angew. Chem. Int. Ed. *42*, 3582–3603.

96. Sessler, J.L., Eller, L.R., Cho, W.S., Nicolaou, S., Aguilar, A., Lee, J.T., et al. (2005). Synthesis, anion-binding properties, and in vitro anticancer activity of prodigiosin analogues. Angew. Chem. Int. Ed. *44*, 5989–5992.

97. Ko, S.K., Kim, S.K., Share, A., Lynch, V.M., Park, J., Namkung, W., Van Rossom, W.V., Busschaert, N., Gale, P.A., Sessler, J.L., and Shin, I. (2014). Synthetic ion transporters can induce apoptosis by facilitating chloride anion transport into cells. Nat. Chem. *6*, 885–892.

98. Saha, T., Gautam, A., Mukherjee, A., Lahiri, M., and Talukdar, P. (2016). Chloride transport through supramolecular barrel-rosette ion channels: lipophilic control and apoptosis-inducing activity. J. Am. Chem. Soc. *138*, 16443–16451.

99. Ren, C., Ng, G.H.B., Wu, H., Chan, K.H., Shen, J., Teh, C., Ying, J.Y., and Zeng, H. (2016). Instant room-temperature gelation of crude oil by chiral organogelators. Chem. Mater. *28*, 4001–4008.

100. Ren, C., Ding, X., Roy, A., Shen, J., Zhou, S., Chen, F., Yau Li, S.F.Y., Ren, H., Yang, Y.Y., and Zeng, H. (2018). A halogen bond-mediated highly active artificial chloride channel with high anticancer activity. Chem. Sci. *9*, 4044–4051.

101. Wan, S.S., Zhang, L., and Zhang, X.Z. (2019). An ATP-regulated ion transport nanosystem for homeostatic perturbation therapy and sensitizing photodynamic therapy by autophagy inhibition of tumors. ACS Cent. Sci. *5*, 327–340.

102. Shieh, C.C., Coghlan, M., Sullivan, J.P., and Gopalakrishnan, M. (2000). Potassium channels: molecular defects, diseases, and therapeutic opportunities. Pharmacol. Rev. *52*, 557–594.

103. Yu, S.P., Canzoniero, L.M., and Choi, D.W. (2001). Ion homeostasis and apoptosis. Curr. Opin. Cell Biol. *13*, 405–411.

104. Lee, D., Lee, S.H., Noh, I., Oh, E., Ryu, H., Ha, J., et al. (2019). A helical polypeptide-based potassium ionophore induces endoplasmic reticulum stress-mediated apoptosis by perturbing ion homeostasis. Adv. Sci. *6*, 1801995.

105. Shen, F.F., Dai, S.Y., Wong, N.K., Deng, S., Wong, A.S.-T., and Yang, D. (2020). Mediating K+/H+ transport on organelle membranes to selectively eradicate cancer stem cells with a small molecule. J. Am. Chem. Soc. *142*, 10769–10779.

106. Park, S.-H., Hwang, I., McNaughton, D.A., Kinross, A.J., Howe, E.N.W., He, Q., et al. (2021). Synthetic Na^+/K^+ exchangers promote apoptosis by disturbing cellular cation homeostasis. Chem. https://doi.org/10.1016/j.chempr.2021.08.018.

107. Malla, J.A., Umesh, R.M., Yousf, S., Mane, S., Sharma, S., Lahiri, M., et al. (2020). A glutathione activatable ion channel induces apoptosis in cancer cells by depleting intracellular glutathione levels. Angew. Chem. Int. Ed. *59*, 7944–7952.

108. Fares, M., Wu, X., Ramesh, D., Lewis, W., Keller, P.A., Howe, E.N.W., et al. (2020). Stimuli-responsive cycloaurated 'OFF-ON' switchable anion transporters. Angew. Chem. Int. Ed. *59*, 17614–17621.

109. Walsh, T.R. (2018). A one-health approach to antimicrobial resistance. Nat. Microbiol. *3*, 854–855.

110. Share, A.I., Patel, K., Nativi, C., Cho, E.J., Francesconi, O., Busschaert, N., et al. (2016). Chloride anion transporters inhibit growth of methicillin-resistant Staphylococcus aureus (MRSA) in vitro. Chem. Commun. *52*, 7560–7563.

111. Zipperer, A., Konnerth, M.C., Laux, C., Berscheid, A., Janek, D., Weidenmaier, C., Burian, M., Schilling, N.A., Slavetinsky, C., Marschal, M., et al. (2016). Human commensals producing a novel antibiotic impair pathogen colonization. Nature *535*, 511–516.

112. Zhang, S., De Leon Rodriguez, L.M., Leung, I.K.H., Cook, G.M., Harris, P.W.R., and Brimble, M.A. (2018). Total synthesis and conformational study of callyaerin A: anti-tubercular cyclic peptide bearing a rare rigidifying (Z)-2, 3-diaminoacrylamide moiety. Angew. Chem. Int. Ed. *57*, 3631–3635.

113. Schilling, N.A., Berscheid, A., Schumacher, J., Saur, J.S., Konnerth, M.C., Wirtz, S.N., et al. (2019). Synthetic lugdunin analogues reveal essential structural motifs for antimicrobial action and proton translocation capability. Angew. Chem. Int. Ed. *58*, 9234–9238.

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114. Zhang, M., Zhu, P.P., Xin, P., Si, W., Li, Z.T., and Hou, J.L. (2017). Synthetic channel specifically inserts into the lipid bilayer of Gram-positive bacteria but not that of mammalian erythrocytes. Angew. Chem. Int. Ed. *56*, 2999–3003.

115. García-López, V., Chen, F., Nilewski, L.G., Duret, G., Aliyan, A., Kolomeisky, A.B., Robinson, J.T., Wang, G., Pal, R., and Tour, J.M. (2017). Molecular machines open cell membranes. Nature *548*, 567–572.

116. Liu, D., García-López, V., Gunasekera, R.S., Greer Nilewski, L.G., Alemany, L.B., Aliyan, A., Jin, T., Wang, G., Tour, J.M., and Pal, R. (2019). Near-infrared light activates molecular nanomachines to drill into and kill cells. ACS Nano *13*, 6813–6823.

117. Chen, S., Wang, Y., Nie, T., Bao, C., Wang, C., Xu, T., Lin, Q., Qu, D.H., Gong, X., Yang, Y., et al. (2018). An artificial molecular shuttle operates in lipid bilayers for ion transport. J. Am. Chem. Soc. *140*, 17992–17998.

118. Ren, C., Chen, F., Ye, R., Ong, Y.S., Lu, H., Lee, S.S., et al. (2019). Molecular swings as highly active ion transporters. Angew. Chem. Int. Ed. *58*, 8034–8038.

119. Wu, X., and Gale, P.A. (2021). Measuring anion transport selectivity: a cautionary tale. Chem. Commun. *57*, 3979–3982.

120. Wu, X., Judd, L.W., Howe, E.W., Withecombe, A.M., Soto-Cerrato, V., Li, H., et al. (2016). Nonprotonophoric electrogenic Cl^- transport mediated by valinomycin-like carriers. Chem *1*, 127–146.

121. Clarke, H.J., Howe, E.N.W., Wu, X., Sommer, F., Yano, M., Light, M.E., Kubik, S., and Gale, P.A. (2016). Transmembrane fluoride transport: direct measurement and selectivity studies. J. Am. Chem. Soc. *138*, 16515–16522.

122. Patel, M.B., Garrad, E., Meisel, J.W., Negin, S., Gokel, M.R., and Gokel, G.W. (2019). Synthetic ionophores as non-resistant antibiotic adjuvants. RSC Adv. *9*, 2217–2230.

123. Carreira-Barral, I., Rumbo, C., Mielczarek, M., Alonso-Carrillo, D., Herran, E., Pastor, M., et al. (2019). Small molecule anion transporters display in vitro antimicrobial activity against clinically relevant bacterial strains. Chem. Commun. *55*, 10080–10083.