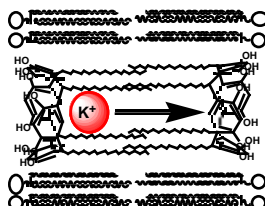


Artificial Ion Channels

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ABSTRACT



This review describes the design and properties of artificial ion channels. The structures utilized by different researchers illustrate the numerous approaches toward mediating ion transport across membranes. The structural aspects and function of different artificial channels are discussed.

Living organisms depend on the transport of ions across cell membranes. Many essential biological processes are driven by concentration gradients between the interior and exterior of cells. The breakdown of ATP for energy is driven by $\text{Na}^+\text{-K}^+$ ATPase via a concentration gradient developed by transmembrane ion transport.¹ The acetylcholine receptor in the post-synaptic neuron of nerve and muscle cells passes a signal to the next neuron by Na^+ transport through an ion channel in the receptor.² A rare example of an anion channel is the chloride-bicarbonate exchanger essential for CO_2 transport from tissues to the lungs.³

that then carries the ion across the lipid bilayer and releases it on the other side. The channel transport mechanism occurs through a structured pore that spans the lipid bilayer, allowing for ion flow across the membrane.⁴

Gramicidin is the most well characterized membrane ion channel. It has a low molecular weight and contains D-amino acids. The channel consists of two gramicidin units (shown in red and blue in Figure 2) held in a head-to-head association by six hydrogen bonds.⁵

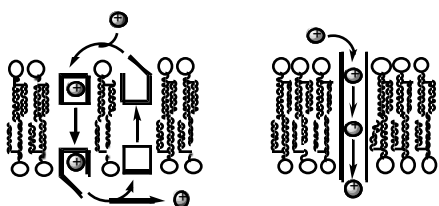


Figure 1. The carrier (left) and channel (right) mechanisms of ion transport across membranes.

There are two mechanisms by which ions are transported across membranes (Figure 1). The carrier mechanism involves the encapsulation of an ion by a membrane-soluble ionophore

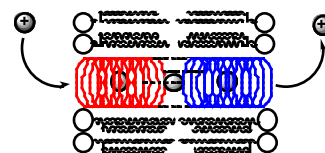


Figure 2. Structure of the Gramicidin A channel.⁵

Due to the many studies on its structure and function, gramicidin is often cited as a model for the development of artificial ion channels. The design and synthesis of artificial channels is intended to both increase our understanding of natural ion channels and provide possible avenues to novel non-natural antimicrobials. Although research over the last decade has provided a wealth of knowledge concerning the

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structure and function of transmembrane protein channels, the transport mechanisms and factors affecting ion selectivity are still largely unknown.⁶

Characteristics of artificial ion channels. In reviewing artificial ion channels, a few key features obviate themselves as necessary for channel structure. The ion channel, once assembled, must span the bilayer membrane. Typical membrane thicknesses range from 25 Å to 50 Å. Secondly, molecules that make up the channel should be amphiphilic, with polar and hydrophobic segments. This amphiphilicity directs the "head groups" to the external aqueous environment, while the hydrophobic region anchors itself in the membrane. Lastly, many artificial channels consist of simple and repetitive building blocks.

A structural characteristic that is more difficult to control is the channel's pore diameter. The pore size can affect ion selectivity, e.g. K⁺ over Na⁺, or allow for molecules to pass based on size exclusion.

Detection of ion channels. Both liposomal and planar membranes are used to detect transport via a channel mechanism.⁴ Various techniques have been employed to study the ion transport rate across membranes. The pH stat method measures protons ejected from liposomes due to the influx of metal cations during base titration. The observation of chromophoric change of a water-soluble UV/Vis- or fluorescent-active dye upon complexation with influxing metal ions inside liposomes can also be employed to study transport rates. Finally, NMR is a valuable tool. By adding a shift reagent, such as Dy(III) or Gd(III), to the extravesicular solution, metal nuclei (e.g. Na⁺) outside the liposomes will have different chemical shifts than encapsulated nuclei, since the shift reagents cannot pass into the liposomes.⁴

It is also critical to discriminate between the channel and carrier mechanisms. A comparison of ion transport rates between the membrane's gel and liquid states is one method of deciphering the two mechanisms. Ion carriers are inhibited in the gel state due to slower diffusion across the membrane. Channels, however, are unaffected by a liquid-to-gel state change. Convincing evidence for the channel mechanism can be obtained by observation of single-channel current. In an electric field, ion flow creates a current that can be detected. Blocking agents can stop the ion flow, thus supporting the observation of the channel event.⁴

In the beginning. The preparation of a non-peptide ion channel was first reported in 1982.⁷ This, along with subsequent reports, however, lacked the critical proof of channel formation. In 1988, Lear and DeGrado⁸ reported the synthesis of peptides showing single-channel currents. One peptide channel studied by Lear and DeGrado is H₂N-(Leu-Ser-Ser-Leu-Leu-Ser-Leu)₃-CONH₂ (**1**).

Peptide **1** forms α -helical structures, four of which come together to form the channel. The single-channel current trace for **1** (Figure 3) indicates a current of 10 pA and an open-state lifetime of 3-8 ms. The lifetime was invariable over

transmembrane voltages of 25-175 mV. For comparison, the acetylcholine receptor has an open-state lifetime of 23 ms.⁹

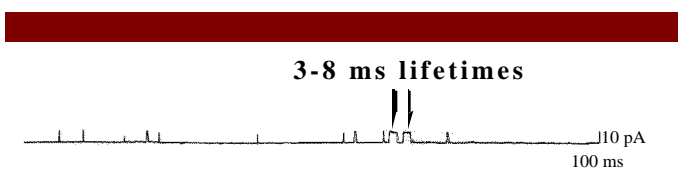


Figure 3. Single-channel current trace for ion channel **1**. (Adapted from ref. 8)

By measuring the conductances of HCl, LiCl, NaCl, KCl, and CsCl mediated by **1**, it was found that the experimental values were proportional to the conductances of the corresponding ions in aqueous solution. This correlation indicates a cation-selective channel. The pore diameter formed by **1** was estimated by testing cations of different size. Protonated tris-hydroxymethylaminomethane has a diameter of 7 Å and passed through the channel. The glucosammonium cation, with a diameter of 10 Å, was impermeable. The channel diameter was estimated to be 8 Å.

Reports of non-peptide artificial channels began to appear in 1992. Artificial channels are either assembled from multiple building blocks or comprised of a single molecule. These two different approaches are discussed below.

ASSEMBLED CHANNELS

Kobuke. In 1992, Kobuke reported the first non-peptide ion channel.¹⁰ Using a simple design, Kobuke synthesized the amphiphilic ion pair **2** shown in Figure 4. The carboxylates form the core of the channel and the ammonium cations form a hydrophobic outer wall embedded in the membrane. The ion pair's length is 24 Å and spans the lipid monolayer, i.e. one half of the distance through the membrane. The channel formed from **2** was both cation selective and voltage dependent.

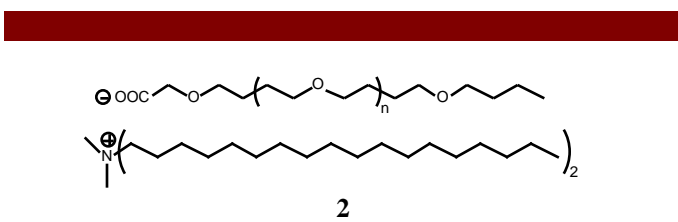


Figure 4. The first non-peptide ion channel.¹⁰

Changing gears to a resorcin[4]arene-based design (Figure 5) led Kobuke to discover the first K⁺-selective non-peptide channel.¹¹ Two molecules of **3** form an active structure

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resembling that of Gramicidin A.⁵ Replacing the R groups on the resorcin[4]arene with cholic acid derivatives gave channels with longer open state lifetimes without affecting the K⁺-selectivity.¹²

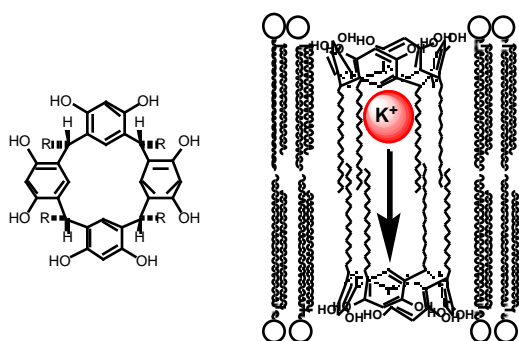


Figure 5. Structure of Kobuke's resorcin[4]arene half-channel **3** (R = (CH₂)₁₆CH₃) (left) and the active channel structure (right).¹¹

Ghadiri. Ghadiri postulated that an even number of alternating D and L amino acids in a cyclic peptide would allow the ring to exist in a low-energy flat conformation. In this conformation, all amide functionalities lie perpendicular to the plane of the ring.¹³ The peptide rings then stack by β -sheet formation between the layers.

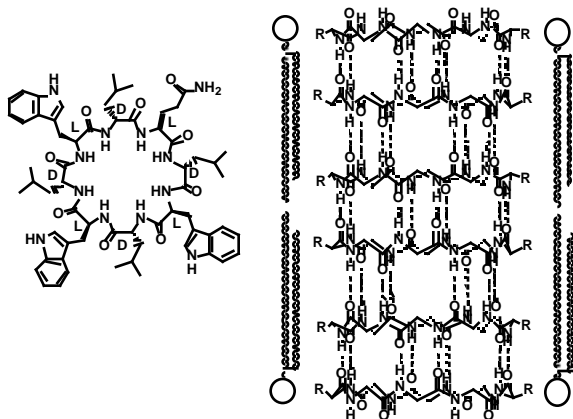


Figure 6. Ghadiri's cyclic peptide **4** (left) and structure of the membrane-spanning self-assembled ion channel (right, R = CH(CH₃)₂, other side chains omitted for clarity).¹³

Ghadiri synthesized the peptide *cyclo*[-(Trp-D-Leu)₃-Gln-D-Leu] (**4**). Leucine was selected to provide a hydrophobic surface to anchor the peptide in the membrane while the indole ring of Trp could make up the polar core. In an aqueous

liposomal suspension, the peptide inserts into the membrane and self-assembles to form a channel. The channel activity was demonstrated using a pH gradient with an encapsulated fluorescent dye. The structure of **4** and the self-assembled nanotube are shown in Figure 6.

The nanotube structure has awesome potential. Since the channel is formed by self-assembly of identical molecules, the structure should adapt to any membrane thickness. Also, the pore diameter can be adjusted by varying the number of amino acids in the ring. A cyclic decapeptide with an internal diameter of 10 Å successfully transported glucose across a lipid bilayer.¹⁴

Matile. Matile's design is based on a substituted rigid rod structure formulated to mimic the structure of the macrolide antibiotic amphotericin B. The backbone is comprised of oligo(*p*-phenylenes) and acts as the hydrophobic portion of the channel aggregate.¹⁵ Polar substituents make up the interior of the cavity. The length of eight phenylenes is long enough to span the bilayer membrane and proved to form channel structures. Figure 7 shows the general structure of the octa(*p*-phenylene) compounds.

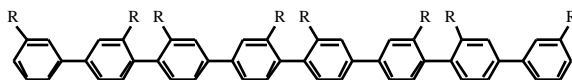


Figure 7. Matile's oligo(phenylene) rod structure.¹⁵

Cation transport was monitored by the fluorescence change of a dye encapsulated in the liposome. Ion transport by an oligo(*p*-phenylene) (R = OCH₂CH(OH)CH₂OH) showed a proton selectivity relative to Na⁺ and K⁺.

By appending short amino acid chains (R = OCH₂C(O)-Leu-NH₂) to the oligo(*p*-phenylene), Matile found that leucine chains interlock via formation of β -sheets, giving a " β -barrel" structure capable of ion transport.¹⁶

SINGLE MOLECULE CHANNELS

Fyles. Fyles took a single molecule approach towards ion channel development. His design contained a central connector unit with radiating wall units. These wall units bore polar head groups.¹⁷ The structure shown in Figure 8 is an example of Fyles' model. The glucose serves as the polar head group, a macrocyclic tetraester serves as the wall unit and the central connector is an 18-crown-6. The most effective wall structures had both a hydrophilic and hydrophobic side for channel formation in the membrane.

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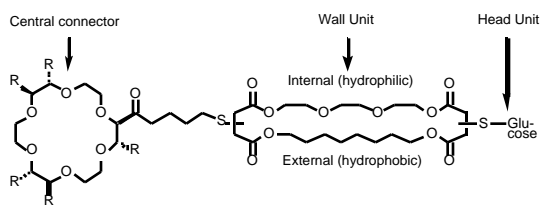


Figure 8. Structure of Fyle's single-molecule ion channel.¹⁷

These compounds functioned as ion channels, transporting Li^+ , Na^+ , K^+ , Rb^+ , and Cs^+ across a phospholipid bilayer.¹⁸ All cation fluxes were determined by the pH stat method. For these compounds to function as ion channels, the vicinal groups (wall units) attached to the crown ether ring must be trans. Compounds with cis-stereochemistry act as carriers. In a recent report, Fyles has demonstrated similar ion channel activity with a compound that has an acyclic wall unit.¹⁹

Lehn. Lehn's "Bouquet" approach also uses a single molecule to comprise the channel. These bouquet molecules consist of a central macrocycle, 18-crown-6 or a α -cyclodextrin, linked to multiple poly(ethyleneoxide) or polymethylene chains.^{20,21} One such compound (**5**) is shown in Figure 9. Both Na^+ influx and Li^+ eflux were observed by NMR using Li^+ -filled liposomes. The channel was shown to function as an antiport, exchanging the cations in a one-for-one manner.

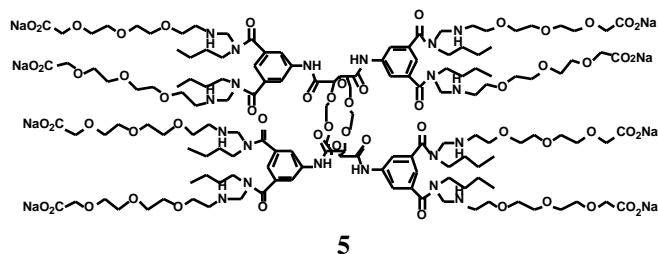


Figure 9. An example of Lehn's bouquet molecules.²¹

Gokel. Gokel's model follows the general structure: "sidearm-crown-spacer-crown-spacer-crown-sidearm". The first compound designed and synthesized (**6**) is shown in Figure 10.²² Cation flux was observed first using a liposome-encapsulated pyranine dye, the Na^+ salt of which is fluorescent. With a low pH outside the liposome, the presence of a channel compound caused a proton influx, protonating the

dye and resulting in a disappearance of fluorescence. The observation of cation transport was reinforced by ^{23}Na NMR studies. A channel mechanism was confirmed by single-channel current measurements.²³

An interesting result came when the polymethylene sidearm of **6** was replaced with a poly(ethylene oxide) chain. The proton flux through the channel was greatly retarded. A larger number of donor atoms was expected to enhance the transport rate. The explanation given for this unexpected result was that the oxygens bound the cations too strongly inside the channel, thus slowing their movement. In channels, a large number of coordinating groups is undesirable for this reason.

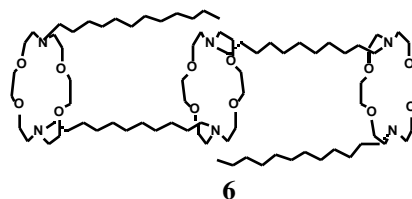


Figure 10. Gokel's channel compound in its predicted conformation.²²

Summary and Discussion. There are numerous approaches to the design of artificial ion channels. Commonly incorporated groups into channel pores include crown ether rings, poly(ethyleneoxide) chains, and OH functionalities, all of which are intimately involved in ion transport. Membrane anchors have been varied from polymethylenes to aromatics to steroids. Designed channel structures have consisted of aggregated amphiphilic ion pairs, dimerized amphiphiles or half-channels, and bolaamphiphiles, which can function as unimolecular channels. There are also backbone crown ether stacks and nanotubes formed by the self-assembly of cyclic peptides. Numerous other approaches have resulted in channels with unique structures and properties.²⁴

Ion selectivity is a property of all channels, cation selectivity being the most common. Selectivity among cations, e.g. K^+ over Na^+ , is rarely observed. Insight into the structural features necessary for ion selectivity can provide a basis for the manipulation of artificial channel function.

Some artificial channel designs are intended to structurally mimic natural channels, such as Gramicidin A and amphotericin B, while others are based on novel scaffolds. Ion channels in natural systems are specifically tailored to function under physiological conditions, and therefore represent excellent motifs on which to base artificial structures. Novel approaches are, however, based on supramolecular constructs and may provide scaffolds useful for the study of natural channels with unknown active structures and in the design of therapeutic agents.

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