Part I
Self-Assembly and Nanoparticles: Novel Principles

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1
Self-Assembled Artificial Transmembrane Ion Channels

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1.1
Overview

Natural ion channels are large, complex proteins that span lipid membranes and allow ions to pass in and out of cells. These multimeric channel assemblies are capable of performing the complex tasks of opening and closing in response to specific signals (gating) and allowing only certain ions to pass through (selectivity). This controlled transport of ions is essential for the regulation of both intracellular ion concentration and the transmembrane potential. Ion channels play an important role in many biological processes, including sensory transduction, cell proliferation, and blood-pressure regulation; abnormally functioning channels have been implicated in causing a number of diseases [1]. In addition to large ion channel proteins, peptides (e.g., gramicidin A) and natural small-molecule antibiotics (e.g., amphotericin B and nystatin) form ion channels in lipid membranes.

Due to the complexity of channel proteins, numerous research groups have during recent years been striving to develop artificial analogues [2–7]. Initially, the synthetic approach to ion channels was aimed at elucidating the minimal structural requirements for ion flow across a membrane. However, more recently the focus has shifted to the development of synthetic channels that are gated, providing a means of controlling whether the channels are open or closed. Such artificial signal transduction could have broad applications to nanoscale device technology. In this chapter we will present examples of some strategies used in the development of artificial ion channels, and describe some techniques commonly used to evaluate their function.

1.1.1
Non-Gated Channels

The artificial ion channels described to date can be divided into two major classes. The first class consists of non-gated channels, which are synthetic compounds

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that simply form transmembrane pores. The second class comprises gated channels that incorporate a means of regulating ion flow across a membrane. A number of strategies have been used in the development of non-gated artificial channels, ranging from the assembly of monomers to form transmembrane pores to the use of single molecules capable of spanning the entire thickness of a lipid bilayer. The monomeric channels generally have more well-defined structures than those formed through aggregation.

1.1.1.1 Aggregates

For ion channels produced through the aggregation of amphiphilic molecules, monomers must first assemble in each leaflet of a lipid bilayer to form a pore with a hydrophilic interior. When aggregates in each leaflet of the bilayer align, a transmembrane channel is formed (Figure 1.1A). Examples of amphiphilic molecules that display this behavior include an oligoether-ammonium/dialkyl phosphate ion pair **1** [8] and a sterol-polyamine conjugate **2** [9] (Figure 1.1B). Artificial ion channels have also been generated through the stacking of cyclic monomers. Both cyclic β^3-peptides **3** [10] and D,L-α-peptides **4** [11] form transmembrane pores. The activities of these peptide-based aggregate ion channels are comparable to that of the natural channel-forming peptide gramicidin A.

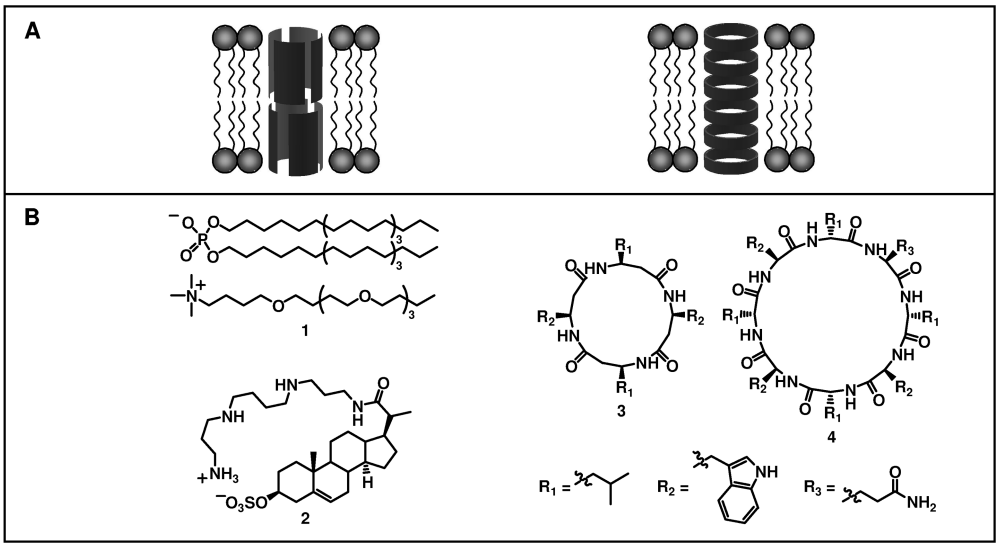

Fig. 1.1 (A) Schematic representations of artificial ion channels assembled through the aggregation of amphiphilic monomers and the stacking of cyclic peptides. (B) Compounds that aggregate to form transmembrane ion channels.

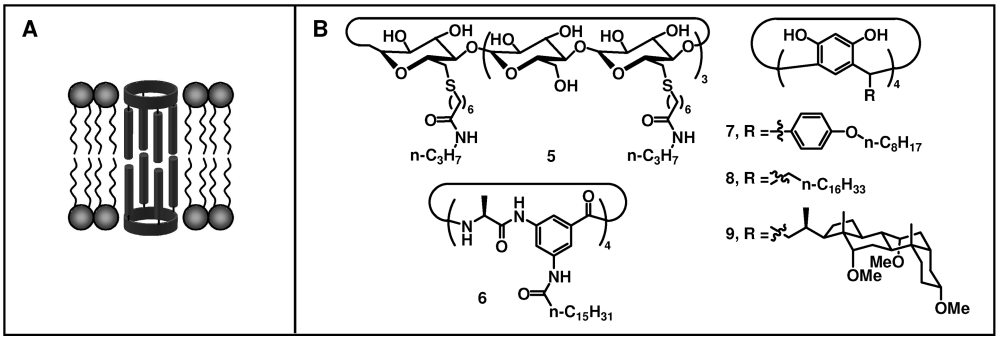

Fig. 1.2 (A) Schematic representation of a transmembrane channel formed through the dimerization of pore-forming monomers. (B) Compounds that form ion channels through dimerization.

1.1.1.2 **Half-Channel Dimers**

A common approach to designing synthetic ion channels has been to functionalize a pore-forming macrocycle with lipophilic groups such as alkyl chains or cholic acid. When these molecules insert into each leaflet of the bilayer and align, the macrocycles act as pores at each membrane surface, while the lipophilic groups serve as channel walls (Figure 1.2A). A variety of macrocycles have been utilized in the construction of half-channel molecules, including β-cyclodextrin **5** [12], cyclic peptides **6** [13], and resorcinarenes **7–9** [14–17] (Figure 1.2B).

1.1.1.3 **Monomolecular Channels**

Using a similar strategy to that described above for the assembly of half-channel dimers, a monomolecular channel **10** has been reported that comprises β-cyclodextrin with oligobutylene glycol chains attached to one face [18] (Figure 1.3A,B). In this case, the macrocycle provides a pore at the surface of the membrane, but the chains are sufficiently long so that a single molecule spans the entire thickness of the bilayer. This monomolecular channel was reported to have a Na^+ transport activity that was 36% that of gramicidin A.

Alternatively, monomolecular ion channels have been designed such that a single macrocycle resides near the center of the bilayer, while the attached lipophilic chains radiate outward toward the membrane surfaces (Figure 1.3A). Examples of molecules reported to function in this manner include a β-cyclodextrin with oligoethers attached to both the primary and secondary faces **11** [19], a calixarene-cholic acid conjugate **12** [20], as well as crown ethers functionalized with cholesterol **13** [21], bola-amphiphiles **14** [22], or oligoethers **15** [23] (Figure 1.3B). The activity of the calixarene-cholic acid conjugate **12** was found to be approximately 73% that of the channel-forming antibiotic amphotericin B.

Artificial single-molecule ion channels that incorporate multiple pore-forming crown ether macrocycles include a peptide-crown ether conjugate **16** [24] and a

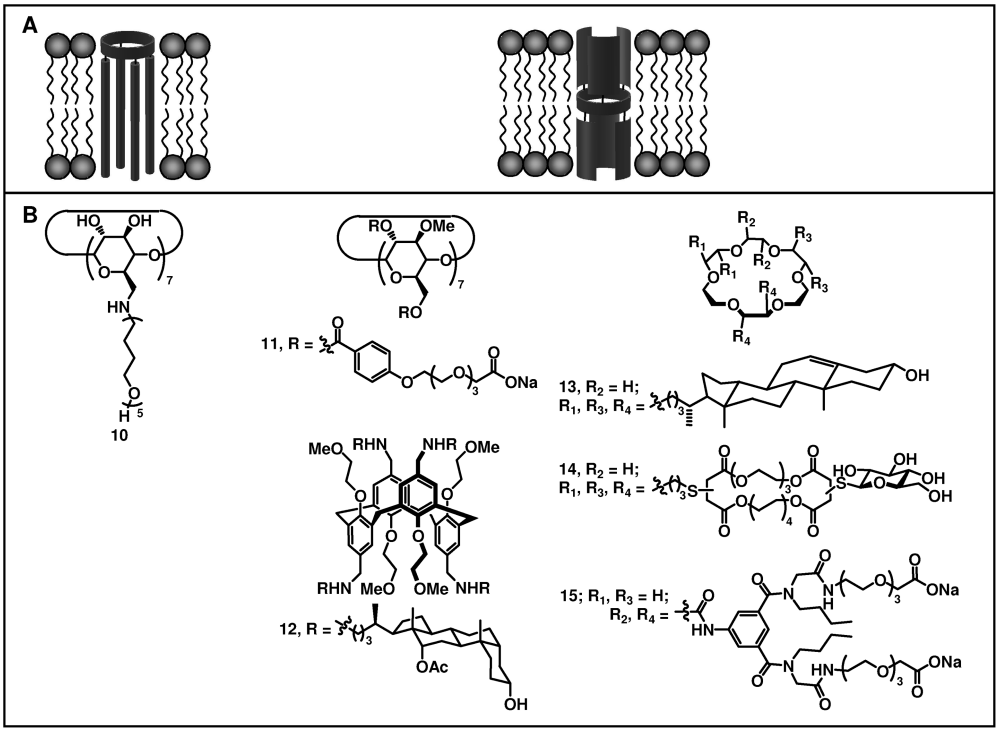

Fig. 1.3 (A) Schematic representations of monomolecular ion channels incorporating a single macrocycle. (B) Structures of monomolecular ion channels that incorporate a single macrocycle.

tris(macrocycle) hydraphile channel **17** [25] (Figure 1.4A,B). Attaching crown ethers to a helical peptide scaffold provided a channel that allowed ions to pass through a series of macrocycles as they traversed the membrane. In the hydraphile channel, distal crown ethers are thought to serve as pore openings at the membrane surfaces, while the central azacrown ether stabilizes ions as they pass across the bilayer. It was reported that the hydraphile channel was 28% as active as gramicidin D.

Although common, the incorporation of macrocycles is not a prerequisite for monomolecular channel formation. An amphiphilic molecule **18** incorporating a number of lysine and cholic acid groups as well as a *p*-phenylene diamine linker served as a monomeric transmembrane channel [26] (Figure 1.4A,B).

1.1.2 Gated Channels

While the previous examples demonstrate that artificial channels can promote transmembrane ion transport, they do not provide a means of controlling

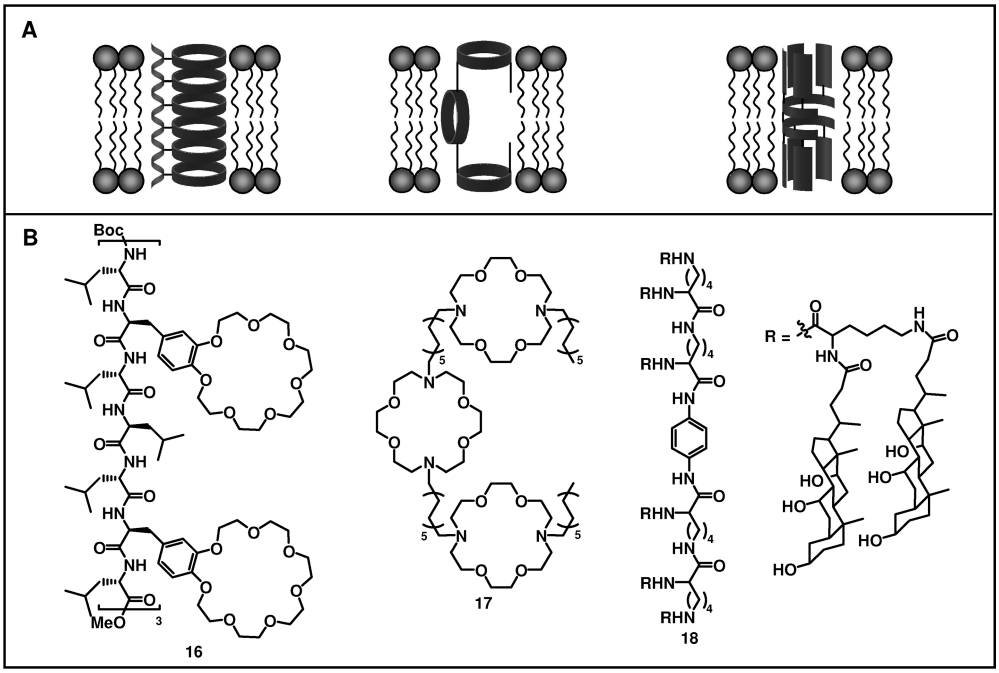

Fig. 1.4 (A) Schematic representations of monomolecular ion channels. (B) Compounds that serve as monomolecular ion channels.

whether the channel is open or closed. Signal-activated synthetic channels bring the field one step closer to mimicking the function of natural ion channels, as active channels are only formed in the presence of a specific signal. As with natural channels, a variety of methods can be used to gate these synthetic analogues, including light, voltage, and ligand activation.

1.1.2.1 **Light-Gated Channels**

Although not a stimulus for natural channels, light has been used to control transmembrane ion transport through a synthetic channel. This was accomplished by incorporating an azobenzene group into an oligoether carboxylate-alkylammonium ion pair **19** [27] (Figure 1.5B). With a *trans*-azobenzene unit present, the ion pair aggregates promoted transmembrane ion transport (Figure 1.5A). However, upon isomerization to the *cis*-azobenzene, single channel currents were no longer detected, indicating channel blockage.

1.1.2.2 **Voltage-Gated Channels**

An early example of a voltage-gated channel relied on the use of an alkylammonium-oligoether phosphate ion pair **20** with an overall negative charge (Figure 1.6B). These ion pairs assemble into half-channels in each leaflet of a

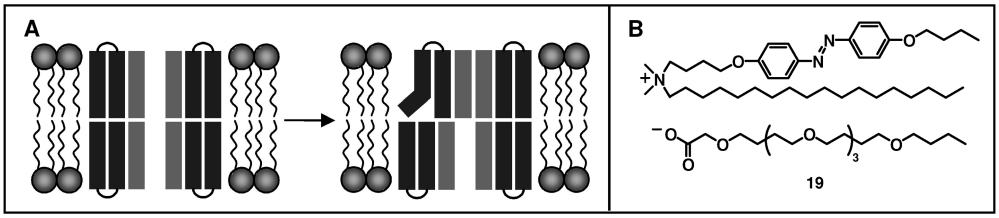

Fig. 1.5 (A) Schematic representation of a light-gated ion channel.
(B) Structure of ion pairs that assemble into a light-gated ion channel.

bilayer (Figure 1.6A). When aggregates in each leaflet align to form transmembrane channels, there are typically unequal numbers of negatively charged monomers in each half channel, resulting in an overall molecular dipole [28]. Depending on the orientation of this molecular dipole, an applied voltage either stabilizes or destabilizes the assemblies, providing voltage-dependent ion transport.

Similar voltage-gated channels were constructed using membrane-spanning monomers with molecular dipoles (Figure 1.6A). Both, a bis-macrocycle bolaamphiphile **21** with a carboxylic acid and a succinic acid on opposite ends [29] and a bis-cholic acid compound **22** with a carboxylic acid on one end and a phosphoric acid group on the other [30], assemble into voltage-gated channels (Figure 1.6B).

The use of charged monomers is not a prerequisite for achieving voltage-gated transport through artificial channels. Using a peptide-dialkylamine conjugate **23** that dimerizes in lipid bilayers, a chloride-selective channel was developed that demonstrated voltage-dependent gating [31] (Figure 1.7A,B). A second example of a channel incorporating uncharged monomers utilizes tripeptide-functionalized *p*-octiphenyl rods with a methoxy group on one end and a methyl

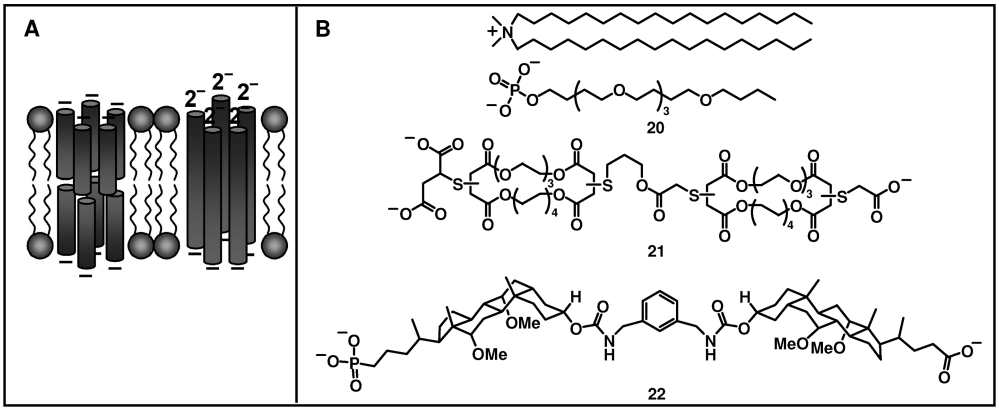

Fig. 1.6 (A) Schematic representations of voltage-gated ion channels.
(B) Compounds that assemble into voltage-gated ion channels.

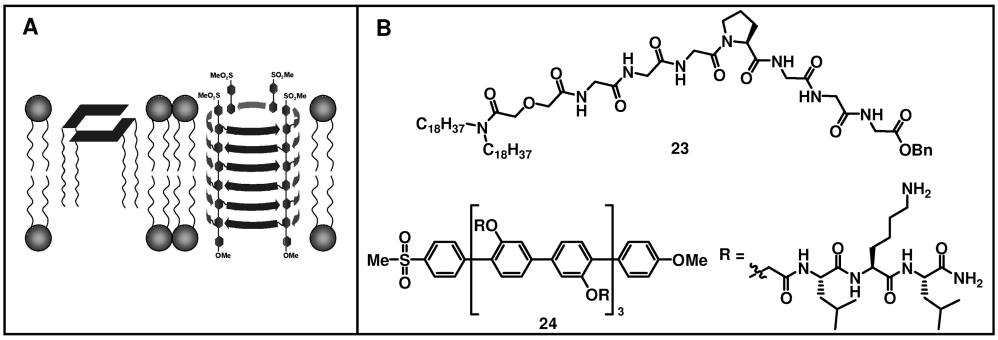

Fig. 1.7 (A) Schematic representations of voltage-gated ion channels. (B) Uncharged compounds that assemble into voltage-gated ion channels.

sulfone on the other **24** [32]. These *p*-octiphenyl rods with axial dipoles displayed voltage-dependent β-barrel assembly.

1.1.2.3 Ligand-Gated Channels

A number of ligand-gated artificial ion channels have been reported. In an early example of a ligand-activated channel, polyhistidine and copper ions were utilized to organize transmembrane assemblies of iminodiacetate-functionalized oligophenylenes **25** [32] (Figure 1.8A,B). This assembly was found to have a K^+ transport activity comparable to that of amphotericin B.

A second example of ligand gating relies on the formation of charge-transfer complexes to open the channel [33]. In this case, *p*-octiphenylene rods functionalized with naphthalenediimide groups **26** initially assembled into π-helices which act as closed ion channels (Figure 1.9A,B). Upon the addition of a dialkoxynaphthalene **27** that intercalated between the naphthalenediimide groups, charge-

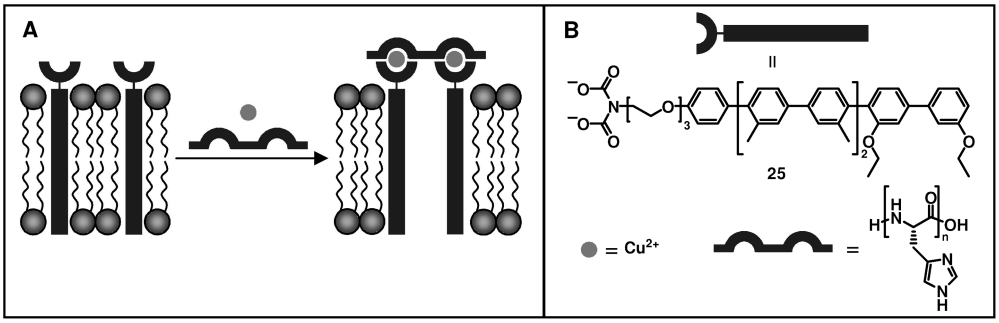

Fig. 1.8 (A) Schematic representation of a ligand-gated ion channel. (B) Components of the ligand-gated ion channel.

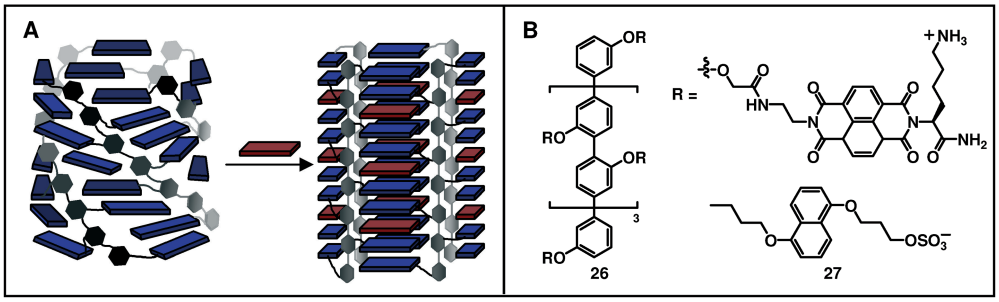

Fig. 1.9 (A) Schematic representation of a ligand-gated ion channel. The intercalation of dialkoxynaphthalene molecules (red) between naphthalenediimide groups (blue) causes the channel to open. (B) Components of the ligand-gated ion channel.

transfer complexes formed, leading to untwisting of the assemblies and an opening of the channels.

The addition of a ligand may not only lead to the formation of open channels; rather, it can also cause the blockage of artificial ion channels. This type of blockage gating has been demonstrated with a cucurbituril-based channel that is blocked by acetylcholine [34] as well as with a β-barrel pore blocked by polyglutamate [35].

1.2 Methods

As natural ion channels act in cell membranes, cell membrane mimics are used to assess the activity of artificial ion channels. Either planar lipid bilayers or spherical lipid bilayers called vesicles, or liposomes, are utilized in ion transport experiments.

1.2.1 Planar Bilayers

Planar bilayer clamp studies provide a means of establishing that a synthetic compound acts as a transmembrane ion channel [36, 37]. The set-up for these experiments involves preparing a bilayer membrane across a small hole in a hydrophobic partition between two chambers containing an electrolyte solution (Figure 1.10A). An electric potential is established across the lipid bilayer by inserting electrodes into the solution chambers; the current passing between these electrodes is then monitored using a bilayer clamp instrument. As the bilayer itself acts as a good insulator, step changes in the conductance represent ion transport through transmembrane channels.

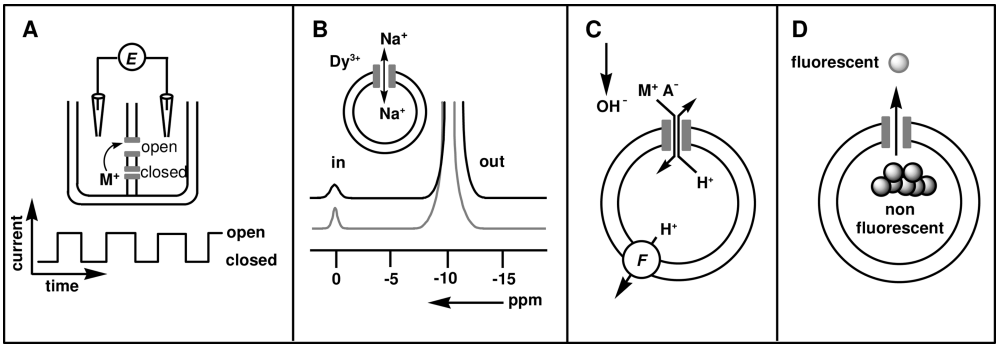

Fig. 1.10 (A) Schematic representations of the apparatus used in planar bilayer experiments and the type of data generated. (B) Depiction of the exchange of Na^+ ions inside vesicles and how it affects the ^{23}Na NMR spectra. (C) Representation of the flow of ions in and out of vesicles during a pH-stat experiment. (D) Depiction of an experiment using a concentration-sensitive fluorescent dye to monitor ion transport.

1.2.2 Vesicles

Vesicles, or spherical lipid bilayers enclosing an aqueous space, are also used to assess the ability of synthetic compounds to act as artificial ion channels. Vesicles can be prepared by a number of different methods, including sonication, extrusion, and detergent dialysis [38]. Dynamic light scattering and electron microscopy allow the size distribution and morphology of vesicles to be assessed. Common techniques used to monitor ion transport across vesicle bilayers include ^{23}Na NMR, pH-stat, pH- or environment-sensitive fluorescent dyes, and ion-selective electrodes.

1.2.2.1 ^{23}Na NMR

For ^{23}Na NMR experiments [39–42], large unilamellar vesicles are prepared in a NaCl solution. Addition of a dysprosium tripolyphosphate shift reagent changes the chemical shift of the Na^+ in the external solution [43]. In the presence of an active channel, the Na^+ ions inside and outside the vesicles exchange, leading to line broadening of the ^{23}Na NMR signals (Figure 1.10B). This line broadening is directly proportional to the rate of transmembrane Na^+ transport.

1.2.2.2 pH-Stat

For a typical pH-stat experiment [44], vesicles are prepared in a pH 6.6 buffer and the external solution is subsequently adjusted to pH 7.6. Following the addition of the proton carrier carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) to ensure rapid proton transport across the vesicle membranes, a metal sulfate solution is added to establish an opposing cation gradient (Figure 1.10C).

Upon addition of the channel, proton efflux occurs and a solution of base is added to maintain the pH at 7.6. The amount of base needed to maintain the pH is related to the activity of the channel.

1.2.2.3 Fluorescence

A variety of fluorescent dyes can be entrapped in vesicles to provide information regarding the activity of ion channels (Figure 1.10D). Fluorescent probes utilized include the pH-sensitive dye 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) [45–47], the concentration-sensitive dye 5(6)-carboxyfluorescein (CF) [48, 49], the potential-sensitive dye safranin O [50], and the fluorophore/quencher pair 8-aminonaphthalene-1,3,6-trisulfonate (ANTS)/*p*-xylenebis(pyridinium) bromide (DPX) [51].

1.2.2.4 Ion-Selective Electrodes

Channel activity can also be assessed using an ion-selective electrode to monitor the amount of a certain ion (such as Na^+ or Cl^-) released from vesicles [52, 53].

1.3 Outlook

Significant progress has been made in the field of artificial ion channels since the first example was reported in 1982. To date, a variety of strategies have been utilized in the construction of ion channels that mimic the function of those found in nature. These artificial channels have found applications in molecular recognition [54], sensing enzymatic reactions [32], as artificial enzymes [55], and in biosensors [56]. In addition, a few synthetic channels have exhibited antibacterial activity [57, 58].

Despite this progress, certain obstacles remain in the drive to achieve truly biomimetic ion transport. One issue that must be addressed is the regulation of transmembrane ion transport through these synthetic channels. While considerable progress has been made in the development of gated channels, there is still a need for artificial channels with well-defined structures that can be opened and closed repeatedly, and in a reliable manner.

References

1 Ashcroft, F. M. (2000) *Ion Channels and Disease*. Academic Press, San Diego, CA.

2 Matile, S., Som, A., Sordé, N. (2004) Recent synthetic ion channels and pores. *Tetrahedron* **60**, 6405–6435.

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

4 Koert, U. (2004) Synthetic ion channels. *Bioorg. Med. Chem.* **12**, 1277–1350.

5 Fyles, T. M., van Straaten-Nijenhuis, W. F. (1996) Ion channel models. In: Reinhoudt, N. D. (Ed.), *Comprehensive*

Supramolecular Chemistry. Elsevier, Oxford, Vol. 10, pp. 53–77.
6 Hector, R. S., Gin, M. S. (2005) Signal-triggered transmembrane ion transport through synthetic channels. *Supramol. Chem.* **17**, 129–134.
7 Koert, U., Al-Momani, L., Pfeifer, J. R. (2004) Synthetic ion channels. *Synthesis* 1129–1146.
8 Kobuke, Y., Morita, K. (1998) Artificial ion channels of amphiphilic ion pairs composed of oligoether-ammonium and hydrophobic carboxylate or phosphates. *Inorg. Chim. Acta* **283**, 167–174.
9 Merritt, M., Lanier, M., Deng, G., Regen, S. L. (1998) Sterol-polyamine conjugates as synthetic ionophores. *J. Am. Chem. Soc.* **120**, 8494–8501.
10 Clark, T. D., Buehler, L. K., Ghadiri, M. R. (1998) Self-assembling cyclic β^3-peptide nanotubes as artificial transmembrane ion channels. *J. Am. Chem. Soc.* **120**, 651–656.
11 Ghadiri, M. R., Granja, J. R., Buehler, L. K. (1994) Artificial transmembrane ion channels from self-assembling peptide nanotubes. *Nature* **369**, 301–304.
12 Tabushi, I., Kuroda, Y., Yokota, K. (1982) A,B,D,F-tetrasubstituted β-cyclodextrin as artificial channel compound. *Tetrahedron Lett.* **23**, 4601–4604.
13 Ishida, H., Qi, Z., Sokabe, M., Donowaki, K., Inoue, Y. (2001) Molecular design and synthesis of artificial ion channels based on cyclic peptides containing unnatural amino acids. *J. Org. Chem.* **66**, 2978–2989.
14 Wright, A. J., Matthews, S. E., Fischer, W. B., Beer, P. D. (2001) Novel resorcin[4]arenes as potassium-selective ion-channel and transporter mimics. *Chem. Eur. J.* **7**, 3474–3481.
15 Chen, W.-H., Nishikawa, M., Tan, S.-D., Yamamura, M., Satake, A., Kobuke, Y. (2004) Tetracyano-resorcin[4]arene ion channel shows pH dependent conductivity change. *Chem. Commun.* 872–873.
16 Tanaka, Y., Kobuke, Y., Sokabe, M. (1995) A non-peptidic ion channel with K^+ selectivity. *Angew. Chem. Int. Ed.* **34**, 693–694.
17 Yoshino, N., Satake, A., Kobuke, Y. (2001) An artificial ion channel formed by a macrocyclic resorcin[4]arene with amphiphilic cholic acid ether groups. *Angew. Chem. Int. Ed.* **40**, 457–459.
18 Madhavan, N., Robert, E. C., Gin, M. S. (2005) A highly active anion-selective aminocyclodextrin ion channel. *Angew. Chem. Int. Ed.* **44**, 7584–7587.
19 Pregel, M. J., Jullien, L., Canceill, J., Lacombe, L., Lehn, J.-M. (1995) Channel-type molecular structures. Part 4. Transmembrane transport of alkali-metal ions by 'bouquet' molecules. *J. Chem. Soc. Perkin Trans. 2*, 417–426.
20 Maulucci, N., De Riccardis, F., Botta, C. B., Casapullo, A., Cressina, E., Fregonese, M., Tecilla, P., Izzo, I. (2005) Calix[4]arene-cholic acid conjugates: a new class of efficient synthetic ionophores. *Chem. Commun.* 1354–1356.
21 Pechulis, A. D., Thompson, R. J., Fojtik, J. P., Schwartz, H. M., Lisek, C. A., Frye, L. L. (1997) The design, synthesis and transmembrane transport studies of a biomimetic sterol-based ion channel. *Bioorg. Med. Chem.* **5**, 1893–1901.
22 Fyles, T. M., James, T. D., Kaye, K. C. (1993) Activities and modes of action of artificial ion channel mimics. *J. Am. Chem. Soc.* **115**, 12315–12321.
23 Jullien, L., Lehn, J.-M. (1988) The "chundle" approach to molecular channels: synthesis of a macrocycle-based molecular bundle. *Tetrahedron Lett.* **29**, 3803–3806.
24 Otis, F., Voyer, N., Polidori, A., Pucci, B. (2006) End group engineering of artificial ion channels. *New J. Chem.* **30**, 185–190.
25 Murillo, O., Watanabe, S., Nakano, A., Gokel, G. W. (1995) Synthetic models for transmembrane channels: structural variations that alter cation flux. *J. Am. Chem. Soc.* **117**, 7665–7679.

26 Chen, W.-H., Shao, X.-B., Regen, S. L. (2005) Poly(choloyl)-based amphiphiles as pore-forming agents: transport-active monomers by design. *J. Am. Chem. Soc.* **127**, 12727–12735.

27 Kobuke, Y., Ohgoshi, A. (2000) Supramolecular ion channel containing *trans*-azobenzene for photocontrol of ionic fluxes. *Colloids Surf., A* **169**, 187–197.

28 Kobuke, Y., Ueda, K., Sokabe, M. (1995) Totally synthetic voltage dependent ion channel. *Chem. Lett.* 435–436.

29 Fyles, T. M., Loock, D., Zhou, X. (1998) A voltage-gated ion channel based on a bis-macrocyclic bolaamphiphile. *J. Am. Chem. Soc.* **120**, 2997–3003.

30 Goto, C., Yamamura, M., Satake, A., Kobuke, Y. (2001) Artificial ion channels showing rectified current behavior. *J. Am. Chem. Soc.* **123**, 12152–12159.

31 Gokel, G. W., Schlesinger, P. H., Djedovič, N. K., Ferdani, R., Harder, E. C., Hu, J., Leevy, W. M., Pajewska, J., Pajewski, R., Weber, M. E. (2004) Functional, synthetic organic chemical models of cellular ion channels. *Bioorg. Med. Chem.* **12**, 1291–1304.

32 Sakai, N., Mareda, J., Matile, S. (2005) Rigid-rod molecules in biomembrane models: from hydrogen-bonded chains to synthetic multifunctional pores. *Acc. Chem. Res.* **38**, 79–87.

33 Talukdar, P., Bollot, G., Mareda, J., Sakai, N., Matile, S. (2005) Ligand-gated synthetic ion channels. *Chem. Eur. J.* **11**, 6525–6532.

34 Jeon, Y. J., Kim, H., Jon, S., Selvapalam, N., Oh, D. H., Seo, I., Park, C.-S., Jung, S. R., Koh, D.-S., Kim, K. (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.

35 Gorteau, V., Bollot, G., Mareda, J., Pasini, D., Tran, D.-H., Lazar, A. N., Coleman, A. W., Sakai, N., Matile, S. (2005) Synthetic multifunctional pores that open and close in response to chemical stimulation. *Bioorg. Med. Chem.* **13**, 5171–5180.

36 Fyles, T. M., Loock, D., van Straaten-Nijenhuis, W. F., Zhou, X. (1996) Pores formed by bis-macrocyclic bola-amphiphiles in vesicle and planar bilayer membranes. *J. Org. Chem.* **61**, 8866–8874.

37 Murillo, O., Suzuki, I., Abel, E., Murray, C. L., Meadows, E. S., Jin, T., Gokel, G. W. (1997) Synthetic transmembrane channels: functional characterization using solubility calculations, transport studies, and substituent effects. *J. Am. Chem. Soc.* **119**, 5540–5549.

38 Torchilin, V. P., Weissig, V. (2003) *Liposomes*. Oxford University Press, New York, NY.

39 Riddell, F. G., Hayer, M. K. (1985) The monensin-mediated transport of sodium ions through phospholipid bilayers studied by ^{23}Na-NMR spectroscopy. *Biochim. Biophys. Acta* **817**, 313–317.

40 Espínola, C. G., Delgado, M., Martín, J. D. (2000) Synthetic flux-promoting polyether models: cation flux dependence on polyoxyethylene chain length. *Isr. J. Chem.* **40**, 279–288.

41 Murillo, O., Watanabe, S., Nakano, A., Gokel, G. W. (1995) Synthetic models for transmembrane channels: structural variations that alter cation flux. *J. Am. Chem. Soc.* **117**, 7665–7679.

42 Pregel, M. J., Jullien, L., Lehn, J.-M. (1992) Towards artificial ion channels: transport of alkali metal ions across liposomal membranes by ''bouquet'' molecules. *Angew. Chem. Int. Ed.* **31**, 1637–1640.

43 Gupta, R. K., Gupta, P. (1982) Direct observation of resolved resonances from intra- and extracellular sodium-23 ions in NMR studies of intact cells and tissues using dysprosium(III)tripolyphosphate as paramagnetic shift reagent. *J. Magn. Reson.* **47**, 344–350.

44 Fyles, T. M., James, T. D., Kaye, K. C. (1993) Activities and modes of action

of artificial ion channel mimics. *J. Am. Chem. Soc.* **115**, 12315–12321.

45 Kano, K., Fendler, J. H. (1978) Pyranine as a sensitive pH probe for liposome interiors and surfaces: pH gradients across phospholipid vesicles. *Biochim. Biophys. Acta* **509**, 289–299.

46 Clement, N. R., Gould, J. M. (1981) Pyranine (8-hydroxy-1,3,6-pyrenetrisulfonate) as a probe of internal aqueous hydrogen ion concentration in phospholipid vesicles. *Biochemistry* **20**, 1534–1538.

47 Menger, F. M., Davis, D. S., Persichetti, R. A., Lee, J.-J. (1990) Synthetic flux-promoting compounds. Exceeding the ion-transporting ability of gramicidin. *J. Am. Chem. Soc.* **112**, 2451–2452.

48 Carmichael, V. E., Dutton, P. J., Fyles, T. M., James, T. D., Swan, J. A., Zojaji, M. (1989) Biomimetic ion transport: a functional model of a unimolecular ion channel. *J. Am. Chem. Soc.* **111**, 767–769.

49 Das, G., Onouchi, H., Yashima, E., Sakai, N., Matile, S. (2002) Binding of organic anions by synthetic supramolecular metallopores with internal Mg^{2+}-aspartate complexes. *ChemBioChem* **3**, 1089–1096.

50 Sakai, N., Gerard, D., Matile, S. (2001) Electrostatics of cell membrane recognition: structure and activity of neutral and cationic rigid push-pull rods in isoelectric, anionic, and polarized lipid bilayer membranes. *J. Am. Chem. Soc.* **123**, 2517–2524.

51 Das, G., Matile, S. (2002) Transmembrane pores formed by synthetic *p*-octiphenyl *β*-barrels with internal carboxylate clusters: regulation of ion transport by pH and Mg^{2+}-complexed 8-aminonaphthalene-1,3,6-trisulfonate. *Proc. Natl. Acad. Sci. USA* **99**, 5183–5188.

52 Weber, M. E., Schlesinger, P. H., Gokel, G. W. (2005) Dynamic assessment of bilayer thickness by varying phospholipids and hydraphile synthetic channel chain lengths. *J. Am. Chem. Soc.* **127**, 636–642.

53 Pajewski, R., Ferdani, R., Pajewska, J., Djedovič, N., Schlesinger, P. H., Gokel, G. W. (2005) Evidence for dimer formation by an amphiphilic heptapeptide that mediates chloride and carboxyfluorescein release from liposomes. *Org. Biomol. Chem.* **3**, 619–625.

54 Baudry, Y., Bollot, G., Gorteau, V., Litvinchuk, S., Mareda, J., Nishihara, M., Pasini, D., Perret, F., Ronan, D., Sakai, N., Shah, M. R., Som, A., Sordé, N., Talukdar, P., Tran, D.-H., Matile, S. (2006) Molecular recognition by synthetic multifunctional pores in practice: are structural studies really helpful? *Adv. Funct. Mater.* **16**, 169–179.

55 Som, A., Matile, S. (2002) Rigid-rod *β*-barrel ion channels with internal ''cascade blue'' cofactors – catalysis of amide, carbonate, and ester hydrolysis. *Eur. J. Org. Chem.* **22**, 3874–3883.

56 Husaru, L., Schulze, R., Setiner, G., Wolff, T., Habicher, W. D., Salzer, R. (2005) Potential analytical applications of gated artificial ion channels. *Anal. Bioanal. Chem.* **382**, 1882–1888.

57 Fernandez-Lopez, S., Kim, H.-S., Choi, E. C., Delgado, M., Granja, J. R., Khasanov, A., Kraehenbuehl, K., Long, G., Weinberger, D. A., Wilcoxen, K. M., Ghadiri, M. R. (2001) Antibacterial agents based on the cyclic D,L-*α*-peptide architecture. *Nature* **412**, 452–455.

58 Leevy, W. M., Gokel, M. R., Hughes-Strange, G. B., Schlesinger, P. H., Gokel, G. W. (2005) Structure and medium effects on hydraphile synthetic ion channel toxicity to the bacterium *E. coli*. *New J. Chem.* **29**, 205–209.