Synthetic ion channels in bilayer membranes

Thomas M. Fyles

Received 30th August 2006
First published as an Advance Article on the web 24th October 2006
DOI: 10.1039/b603256g

Natural ion channels are large protein complexes that regulate key functions of cells. Supramolecular chemists have been able to take hints from Nature to design and prepare completely synthetic ion channel systems that reproduce many of the fundamental functions of natural channels. This *tutorial review* introduces the field to non-specialists. It examines the design, synthesis, incorporation, and characterization of synthetic ion channels in bilayer membranes, and points to potential applications of synthetic ion channels.

All cells are surrounded by a closed membrane that defines the cell relative to its environment. On the inside of the membrane are all the functions that make up cellular life; the outside is the rest of the world. Even within cells, membranes play key roles to sequester biochemical products and processes and to regulate the biochemical business of living. Biological membranes are semipermeable as a result of the barrier properties of the lipid bilayer that is found in all membranes. Lipid bilayers allow relatively free diffusion of small nonpolar molecules but are highly impermeable to polar solutes and biomacromolecules such as proteins or DNA. Retaining macromolecules makes excellent sense since all cells need to retain their essential biochemical machinery, but polar solutes from the environment, such as sugars, amino acids, and ions, are also essential to the workings of the cell, yet cannot pass through a bilayer membrane on their own. The solution to this problem, undoubtedly one of the key steps in the early evolution of cellular life, is provided by ion transporters.

Consider the energy of an ion, such as a sodium cation, as it moves across a bilayer membrane. The bilayer is composed of lipids arranged in a two-molecule sandwich about 4 nm thick. The polar head groups of the lipids contact water while the "meat" of the sandwich is provided by the hydrocarbon tails of the lipids. The Na^+ ion on one side of the membrane is well solvated by water, but as it moves into the membrane, the water needed for these stabilizing interactions is lost and the ion is increasingly destabilized. The magnitude of the energy barrier for this process is comparable to the desolvation energies of the ion, and can reach many hundreds of kJ mol^{-1}; free diffusion *via* direct ion desolvation rarely happens. The role of an ion transporter is to replace the ion–water interactions with alternative stabilizing intermolecular interactions between the ion and the transporter. As the ion moves into the membrane within the transporter, it experiences only minor destabilization and can traverse the membrane without surmounting a high energy barrier. In essence, an ion transporter is a catalyst of translocation; it facilitates ionic diffusion through the membrane but is not consumed in the process.[1]

Ion transport through membranes is a supramolecular function. It was recognized from the dawn of supramolecular chemistry that the nature of transmembrane ion transport requires the formation of specific stabilizing intermolecular interactions at the expense of lost interactions with solvent. As such, the well-known supramolecular principles of preorganization and complementarity between the transporter and its intended guest ion govern the selectivity and efficacy of the transporter. Much of the early emphasis in supramolecular chemistry was on ion carriers in which the transported ion is encapsulated within the carrier and diffuses through the membrane with the moving carrier.[2] Even though such systems reproduce many of the functions of ion transporters in natural bilayer membranes, carrier-mediated transport is inherently slow and does not play a prominent role in natural systems that transport simple ions.[2]

Ion transport in Nature occurs *via* ion channels and ion pumps (Fig. 1).[3,4] Ion channels are *passive transporters*; ions flow through the transporter driven by the concentration gradient of the ion or driven by an imposed electrochemical gradient. Ion pumps are *active transporters*; ions flow through the transporter against their concentration gradient thereby creating electrochemical gradients across membranes. The energy required for the pump is provided by an external energy source such as the hydrolysis of ATP. Together, the ion channels and ion pumps in cells acquire nutrients from the

Department of Chemistry, University of Victoria, Victoria BC, Canada, V8W 3P6 E-mail: tmf@uvic.ca; Fax: +1 250 721 7147; Tel: +1 250 721 7150

Tom Fyles

Tom Fyles did his doctoral work with C. C. Leznoff at York University and spent 1977–1979 as a post-doctoral fellow with Jean-Marie Lehn in Strasbourg. He then joined the University of Victoria where he is now Professor of Chemistry. His research interests in membranes, transport, and supramolecular chemistry were kindled by two seminars: a 1974 presentation by D. J. Cram on his amino acid "resolving machine" and a 1975 seminar by J.-M. Lehn on crowns, cryptands, and catalysis.

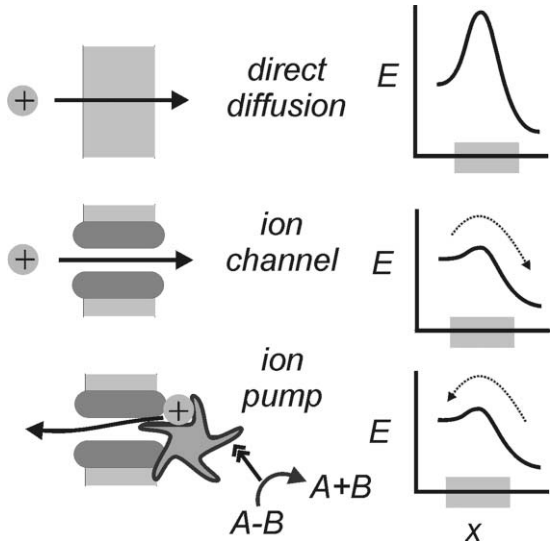


Fig. 1 Cartoon of ion transport through a membrane by direct diffusion (top), or catalyzed by an ion channel (middle). Both processes are driven by the concentration gradient of the transported ion. Ion pumps use reaction free energy changes (A–B bond breakage) to drive the transported ion against its concentration gradient (bottom).

environment, secrete waste to the environment, regulate cell volume, and underpin the electrical signalling of nerves. Both channels and pumps are highly selective, and the selectivity derives from the supramolecular principles enunciated above. Channels and pumps are distinguished from carriers in the relative motions of the transporter and the ion; ions move past a membrane-bound channel or pump (like cars moving in a tunnel), while ions and their carriers move together through the membrane (like cars on a ferry).

Natural ion channels and pumps are large protein complexes consisting of a central channel portion that spans the membrane, and additional regions on one or both sides of the membrane that control access to the channel region. High resolution structures of portions of channel proteins have only emerged within the past decade and there are still relatively few channels characterized structurally.[3,4]

The ion conducting portion of natural ion channels is typically a bundle of four to seven protein helices that span the membrane. At the mouth of the channel, the bundle is held open in an hourglass shape to allow ready access and rapid diffusion of hydrated ions though an aqueous "vestibule". Ion selectivity arises in a constricted volume between the helices in which the water of ionic solvation is replaced by polar interactions with functional groups on the transporter. For high flux channels, this selectivity filter region is quite short (1–1.5 nm long) with a diameter sized to require significant ion desolvation. The molecular recognition of ions within the selectivity filter has precisely the characteristics one would expect for molecular recognition of the ions: carbonyl oxygens act as donors to cations such as sodium, hydrogen-bonding hydroxyl groups act as donors to chloride ions, close control of binding site size and topology allows size discrimination between ions, and judiciously placed charges provide electrostatic control of selectivity. The ion conducting portions of channel proteins are typically surrounded with an outer layer of protein helices which are solvated by the membrane lipids. Ion channel protein complexes are large structures: diameters of 5–7 nm and lengths of 10–15 nm are typical and dwarf the 0.2–0.3 nm diameter ions they transport. They are also remarkably effective molecular catalysts and can selectively transport up to 10^8 ions per second.[1]

Supramolecular chemists have long been inspired by the functional sophistication of naturally occurring ion channels and ion pumps and we have created a rich variety of biomimetic ion transport systems in an effort to replicate transport functions using small molecules and synthetic compounds.[2] This effort is motivated partly from curiosity about how the biochemical apparatus functions on a molecular level; insights from smaller systems are expected to inform and constrain mechanistic proposals about natural transporters. Another motivation is the realization that natural transporters perform a number of cellular processes that would be very useful in a technological or pharmaceutical context. As discussed below, there are recent examples that show the antimicrobial activity of synthetic ion channels. Other potential applications, such as drug delivery, lie close to the biochemical systems that inspire them. Others, such as ion channel sensors, are more remotely bio-inspired.

An emphasis on the functional characteristics of artificial membrane transporters necessarily requires a structural framework for the artificial transporter. In addition to natural ion channels, the structures of other membrane-active small molecules from Nature that act as ion transporters can provide structural insights for artificial transporter designs. Cyclic ion carriers such as valinomycin, and acyclic carrier ionophores such as the polyether antibiotic lasalocid A provide convergent donors for encapsulated ions. This is closely akin to the binding of cations in crown ethers and cryptands and the transport functions of the natural and synthetic carrier compounds are closely similar.[5]

A more potent inspiration for the design of artificial ion channels is found in the channel-forming peptide gramicidin (Fig. 2).[6,7] Gramicidin is a pentadecapeptide containing alternating D- and L-amino acids. In membranes and nonpolar solvents it forms a β-helix, with a hydrophilic interior and a lipophilic exterior bearing the amino acid side chains. The helix length is roughly half the thickness of a bilayer membrane, so, within a bilayer, two gramicidin molecules form an end-to-end dimer stabilized by six intermolecular hydrogen bonds. The resulting structure creates a water-filled tunnel through the membrane that is an efficient channel for alkali cations. The gramicidin channel therefore embodies an appealing structure that directly relates to its function as a hollow tube for the passage of cations.

The polyene antibiotic amphotericin also forms channels in sterol-containing bilayer membranes[8] and provides an alternative small-molecule model to relate channel structure to function (Fig. 2). The amphotericin monomer has dual amphiphilic character: the mycosamine head group will orient the molecule in a bilayer membrane with this polar group in contact with the aqueous phase and the polyene tail in contact with the lipid hydrophobic region. Several monomers in this orientation then aggregate to create a water-filled tube lined by the hydroxyl groups on the edge of the amphotericin

 This journal is © The Royal Society of Chemistry 2007

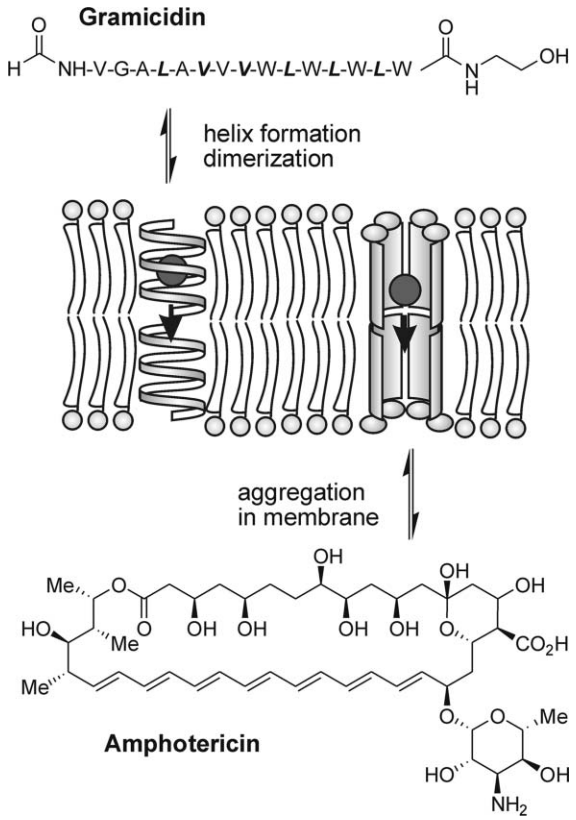


Fig. 2 Examples of ion channels formed by naturally occurring compounds. The peptide gramicidin forms a head-to-head helical dimer that opens a tubular channel within a bilayer membrane (D-amino acids are indicated in italic). The polyene antibiotic amphotericin forms an aggregate channel surrounding an aqueous pore.

macrocycle. A single amphotericin molecule is only about as long as half the thickness of a bilayer membrane, so two such aggregates associate in an end-to-end fashion to create a membrane-spanning channel. Although this structure is less well characterized than the gramicidin channel, it nonetheless provides a clear structural suggestion for the creation of functional artificial ion channels.

These two structural paradigms provide basic design criteria for artificial ion channels. Firstly, a membrane-spanning structure is required. The overall thickness of a lipid bilayer membrane is about 4 nm, with a hydrophobic core thickness of about 3–3.5 nm. Secondly, the active membrane-spanning structure must enclose a significant volume for the passage of the ion. As a result, the active structures have molecular weights in excess of 3–4 kDa. Both the dimensions and the molecular weights are large by the standards of small molecule synthesis. Thirdly, the interior of the channel is hydrophilic. In gramicidin the ions are largely desolvated during passage, but the larger amphotericin aggregate allows the passage of hydrated ions. Whatever strategy is chosen for an artificial system, the structure must provide stabilizing contacts for an ion in transit. These can be direct transporter–ion interactions, or more simply transporter–solvated ion interactions. Fourthly, the channel must embed itself into a bilayer membrane through hydrophobic contacts with the hydrocarbon regions of the lipids. As a consequence, artificial ion channels are surfactants. There is a balance between simple detergent action of the synthetic compound that will lead to membrane disruption and eventually to membrane lysis, and the more regular and defined behaviour expected of an ion channel. A way to limit this type of non-specific disruption is to create structures that have a shape that is compatible with the lamellar nature of the bilayer membrane. Compounds that are roughly columnar in aspect, and that will fit into the membrane by direct displacement of a lipid molecule without further structural disruption, are likely to lead to channel behaviour rather than detergent behaviour.

These descriptive design criteria are poorly predictive, particularly for the selection of synthetic targets, or for the selection between competing candidates. As a result, much of the work in the synthetic ion channels area has been driven by more mundane considerations such as the projected length of the synthesis, the availability of suitable starting materials, and ways in which a single synthesis strategy can yield related products with little additional effort.

Even with these constraints, chemists have produced a remarkable range of active ion channels. As recently as a decade ago, it was possible to review this area comprehensively,[9] but an explosion of interest and innovation has now made that dubious task impossible. Recent reports are extensively discussed in excellent specialist reviews,[10–12] symposia-in-print,[13] and in other reviews in this issue. The focus of this review is on the supramolecular aspects of ion channels: on the molecular recognition of ions by channels and the ways in which transporters facilitate ion transport; on the self-assembly of supramolecular aggregates that form ion channels; on the molecular recognition and insertion of channels into membranes and the ways in which inactive structures convert to active structures; and on the overall energetics that govern this very complex molecular environment. At the risk of excessive simplification, the channel systems discussed will fall into two broad classes: tubular channels, in which a relatively well-defined tubular structure akin to the gramicidin channel is the principal design element, and aggregate channels, in which the central design element is the association of components in a manner similar to the amphotericin aggregate channel. To a large extent this distinction simply reflects the scale and complexity of the synthetic effort required; the synthesis of small molecules for aggregates is simpler than the synthesis of larger tubular structures.

How are ion channels studied?

There are two main experimental approaches to the study of synthetic ion channels: vesicles or planar bilayer membranes. Vesicles (or liposomes) are prepared by dispersion of lipids in an aqueous buffer to form spherical closed-shell structures ranging from below 20 nm to above 1 μm in diameter and bounded by one or more layers of bilayer membrane.[14] The internal aqueous volume is therefore isolated from the external medium. Vesicles are generally robust structures that can entrap a wide variety of species in the interior compartment. They are readily purified by gel permeation or centrifugation, and can be prepared from a very wide range of lipid

compositions. Characterization techniques for determining the size distribution of the sample, the overall lipid concentration, and the number of bilayers (uni- *versus* multi-lamellar vesicles) are simple and well-developed.[14]

Ion transporters are catalysts of translocation; they accelerate the diffusion of ions from one side of a membrane to the other. Consequently, any kinetic technique that can distinguish ionic concentrations on one or both sides of the bilayer can be used for the determination of transport activity in vesicles. The interior volume of typical vesicles is of the order of femto- to attolitres, and the gain or loss of a relatively small number of transported ions is sufficient to produce a very large change in the internal ionic concentration. Conversely, release of this small amount of entrapped material into a large external volume can result in only relatively small changes, which require sensitive detection methods. One solution to these basic considerations is the use of pH- or ion selective-fluorescence dyes to report the internal ionic composition.[11] An example using the pH-sensitive pyranine dye HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid) is illustrated in Fig. 3. Vesicles were prepared in the presence of HPTS in a dilute buffer and the excess external dye was removed by gel filtration during purification. The transporter was added to the vesicles and, at the point indicated, a pH-gradient was established by addition of base to the external solution. The collapse of the pH-gradient is reported by the change in fluorescence of the entrapped indicator as a change in the ratio of fluorescence due to the acid and conjugate-base forms of the dye. Ratiometric methods such as this provide additional assurance that the transporter has not simply ruptured the vesicles. Data of this type can be treated to give rate constants as in any other conventional kinetic measurement. A wide range of dyes is available and this type of method is very widely used.

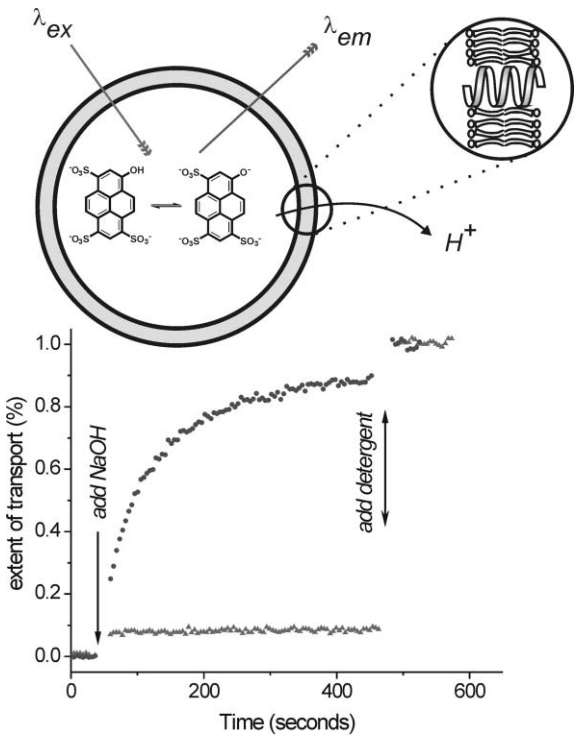


Fig. 3 A method to measure ion transport across a vesicle membrane. The entrapped fluorescent dye reports the internal pH of the vesicle in response to a shift in the external pH. Vesicles containing an active transporter (upper curve) show a shift in the internal pH; no change is found in the absence of a transporter (lower curve).

Sodium NMR spectroscopy can be used to determine the translocation rate through a line-shape analysis method.[10] A suitable paramagnetic relaxation agent, either outside or entrapped in the vesicle, produces a chemical shift difference between sodium ions inside and outside of the vesicle. Addition of an active transporter allows exchange between the two populations, with a corresponding change in the line-width and peak shape of the signal due to the internal sodium. Although an analysis of the signal can give a value of the exchange rate constant, it is more common to simply determine relative activity of a range of compounds based on the line-width. Ion-selective electrodes for specific ions such as chloride or protons are very sensitive and specific, so direct measurement of concentration or the rate-of-change *via* a pH-stat kinetic method can also be used.

Planar bilayers can be formed across a small hole in a hydrophobic barrier between two aqueous electrolyte solutions. The bilayer is formed by ''painting'' a small amount of lipid in a nonpolar solvent such as decane across the opening, and then further gentle brushing or other manipulation produces a region of a bilayer held in the middle of an annulus of solvent.[1] ''Solvent free'' planar bilayer membranes can be formed by passing the orifice through a monolayer of lipid at the air–water interface. Although these techniques might seem foreign to chemists, they are remarkably reliable and the membranes produced have reproducible properties (resistivity, capacitance). Bilayer membranes are ideal for the study of the ionic conductance of ion channels using a technique known as a voltage clamp, in which a constant transmembrane potential is applied and the resultant current changes are monitored as a function of time (Fig. 4). The membrane itself is a good resistor, so, in the absence of an open channel, very little current is observed. When a single ion channel opens, the ionic flux in response to the applied potential produces a current in the range of pico- to nanoamps. The ''opening'' appears as a step-change in the conductance of the membrane. When an active channel ceases to function, the ionic current falls

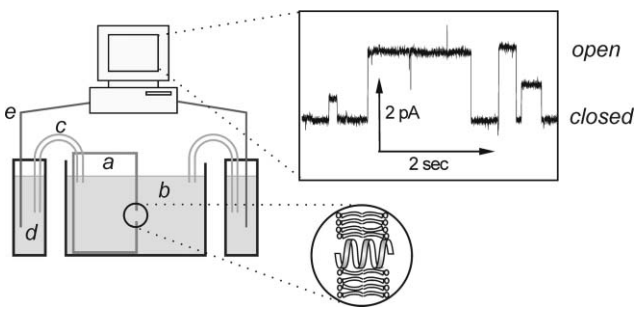


Fig. 4 Schematic of a voltage clamp experiment. A cuvette (a), with a small hole cut into one face, is immersed in an electrolyte (b) and the bilayer is formed by painting lipid across the hole. Electrical contact with Ag/AgCl reference electrodes (e) in a reference electrolyte (d) is *via* Agar salt-bridges (c). A potential is applied and the current measured as a function of time. Channels appear as abrupt transitions.

This journal is © The Royal Society of Chemistry 2007

abruptly back to the baseline of the unaltered bilayer membrane. This step-change behaviour is a unique and unmistakable signature of a single ion channel.

The voltage clamp technique observes single ion channels individually, and a distribution of properties between different individual channel openings is common. The collective properties of channels are assessed by statistical techniques to determine the specific conductance of the channel, its open probability, the voltage-dependence of the conductance, and the ionic selectivity of the transport.

The voltage clamp technique was developed for naturally occurring channels which typically exhibit a single conductance state and an open duration in the 10–500 msec range. Fortunately, many synthetic ion channels behave in a directly comparable fashion and so can be analyzed using established techniques. However, not all synthetic channels behave as the ideal case illustrated in Fig. 4 and the study of synthetic ion channels has greatly expanded the descriptive natural history of ion channels. Examples are known where the channel "never" closes, where the conductance varies (decays or increases) during the course of a single opening, or where the channel exhibits a large number of independent conductance levels. All of these are functional ion channels; all of them illustrate alternative ion transport mechanisms that are no longer evident in modern biochemical systems.

Tubular ion channels

One of the best-characterized synthetic ion channels is formed by a series of tris-macrocycles dubbed hydraphiles related to the active parent **1a** (Fig. 5).[15] The design initially envisaged that all three aza-crown ethers would stack co-facially to create a transmembrane tube for cation conduction. Although the central macrocycle is essential for high activity, a number of structural variants with smaller central crown ethers are also active, thus it is unlikely that the cation in transit passes through the central macrocycle; rather, donor groups in the central relay stabilize the cation in transit without an explicitly tubular structure. The active structure, sketched in Fig. 5, is supported by a number of types of evidence. The fluorescence spectrum of the dansyl derivative **1d** suggests that the dansyl group is in an environment with a polarity intermediate between methanol and ethanol. This would correspond to the ester-rich region of the bilayer below the phosphatidyl head groups (so-called mid-polar region).[15] Fluorescence quenching experiments with lipids bearing a spin-label at different depths further support a location of the dansyl near the bilayer surface and well away from the bilayer mid-plane. Hydraphile channels within bilayer membranes do not aggregate, as shown from the first-order concentration dependence of fluorescent energy transfer from the methyl indole derivative **1c** to the dansyl derivative **1d**; dimers or higher aggregates would have shown a more complex concentration dependence of energy transfer. Finally, the benzyl derivative **1b** allows a Hammett analysis of the effect of donor–acceptor properties of the benzyl lariat ether on the channel activity determined from ^{23}Na NMR linewidth experiments. The results are consistent with a lariat-ether type interaction of the channel with the transported cation.[15]

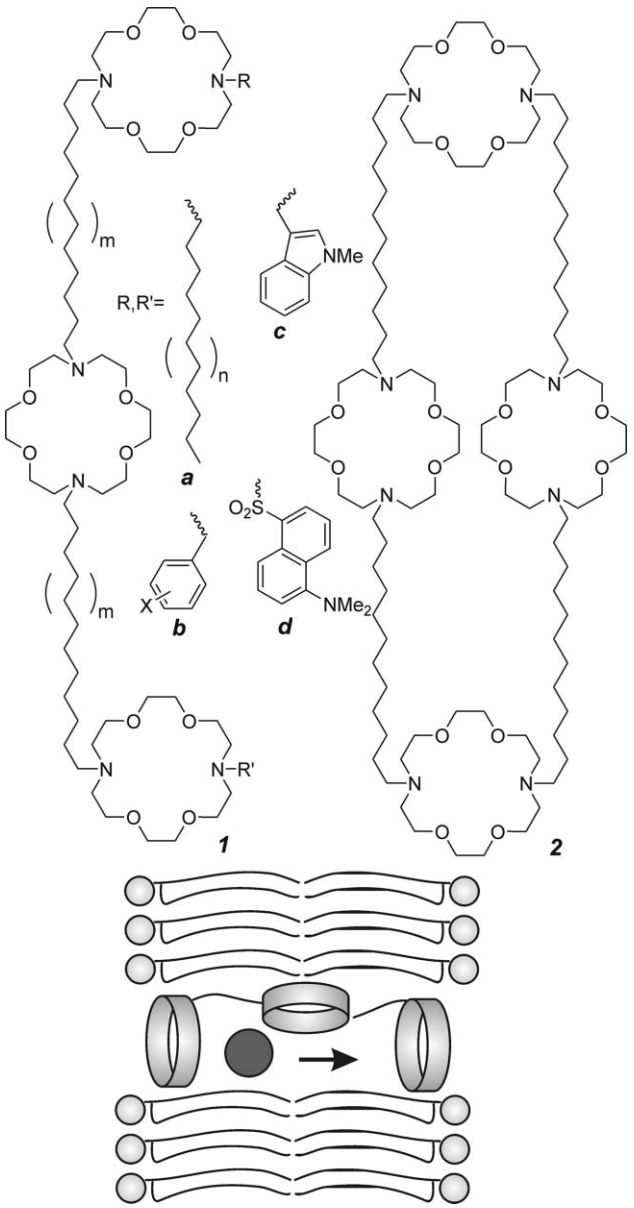


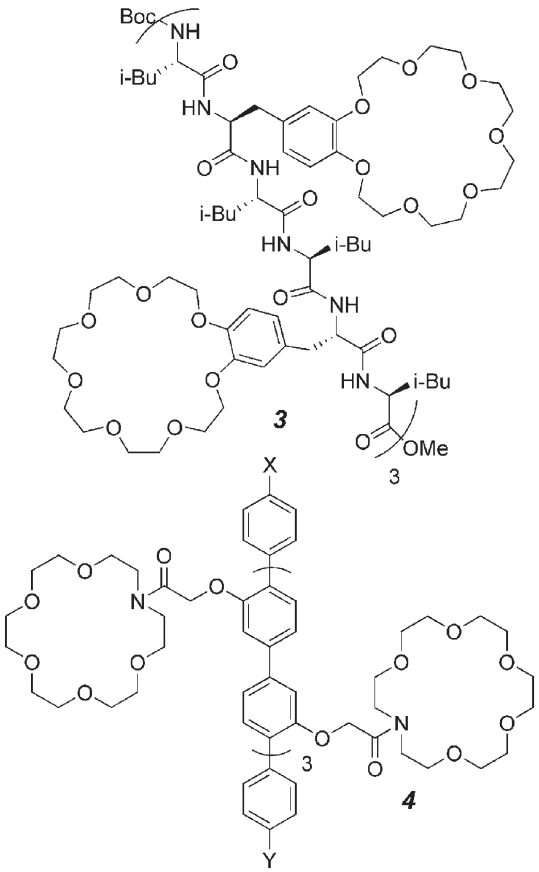
Fig. 5 Hydraphile channels. The three crown ether units define a tubular channel across the bilayer membrane with two macrocyclic portals and a mid-plane relay.

The covalently linked macropentacycle **2** is about three-fold more active than **1a** or **1d**.[16] On one level this could be seen as confirming evidence for the tubular structure sketched in Fig. 5. However, amide-containing precursors of **2** also show enhanced activity. If direct cation complexation were an essential component of the translocation then the amido-crown ethers should show significantly reduced activity due to the reduced number of good cation donors. The data is collectively better interpreted as supporting a channel in which water and partially hydrated cations interact with the available donors of the transporter through both Lewis base–cation and Lewis base–water–cation interactions. The enhanced activity of **2** and its amido-precursors is therefore due to a larger number of donor atoms placed deep within the bilayer.

The hydraphiles are biologically active and, more significantly, their activity can be correlated with their ability to form ion channels. Using a patch-clamp technique, in which the ionic permeability of the membrane of a living cell was directly measured, compound **1a** was shown to enhance ionic permeability while smaller fragments of **1a** failed to elicit any response.[17] The effect of added **1a** on the cell is to depolarize the membrane, thereby eliminating a metabolic driving force for the cell. Hydraphiles are toxic to both Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria, but is the toxicity really related to the formation of ion channels? The answer appears to be "yes". A series of compounds related to **1b**, in which the number of spacing methylene chains between the aza-crown ethers varied from eight to twenty, shows the expected peak in transport activity at about fourteen methylene units. The toxicity of the compounds varies along the same series with a peak toxicity at the same overall length.[18] The most toxic compound (**1b**, $m = 14$) inhibits the growth of *E. coli* at the remarkably low concentration of 2 μM.

The co-facial organization of crown ethers is a compelling structural motif for tubular ion channels, and there have been many examples reported, particularly in the early literature.[9] Compounds **3** and **4** illustrate two different successful approaches that produce active materials. Compound **3** uses the helix-forming propensity of oligo-leucine as a scaffold to enforce an α-helical superstructure. The peptide also includes the crown ether modified phenylalanine derivative in the [*i*, *i* + 4] positions of the helix. This spacing will place the crowns directly atop one another along one edge of the helix, forming the desired tubular stack. This also creates an amphipathic helix that is expected to act as a membrane disrupting agent. Compound **3** appears to be inactive as a disrupting agent, relative to a shorter oligomer containing only four crown ethers.[19] It does show single-channel activity in planar bilayers and is active in vesicle assays. Compound **4** contains octiphenyl as the scaffold for the arrangement of the pendant crown ethers. Here the length and linearity are controlled by the scaffold, but the face-to-face positioning of the crown ethers is uncontrolled except through the restricted rotations of the octiphenyl. Derivatives of **4** in which the X, Y groups are neutral (–SMe, $–SO_2Me$) are relatively ineffective as ion channels, as assessed by a vesicle assay using HPTS (pyranine dye; Fig. 3), compared to derivatives bearing a changed head group ($–SCH_2CH_2NH_3^+$).[11] The activity further depends on the polarization of the membrane; this issue will be examined in a later section.

The octiphenyl scaffold is central to the formation of another class of tubular channels called rigid-rod β-barrels (Fig. 6).[11] The octiphenyl bears eight short sections of peptide that drive self-association with the formation of a β-sheet structure by anti-parallel interdigitation of the peptide strands at the [*i*, *i* + 3] positions of the octiphenyl. The inter-ring octiphenyl torsion angles direct the closure of a precursor oligomer into the formation of a discrete β-barrel. The β-sheet structure places the side chains of alternate amino acids on the opposite sides of the sheet. Thus, the sheet from **5a** has the two leucine residues on one face, and the lysine residue on the other. The more restricted volume inside the barrel places the



second and fourth amino acid side chains of compounds **5a–f** on the inside of the β-barrel where they line the channel. The overall architecture is highly suited to insertion into a bilayer membrane: the exterior is hydrophobic, the interior is water filled, and the overall length of 3.4 nm is well matched to the hydrophobic core thickness of the bilayer. The overall internal dimensions are large: the long-lived (minutes) channels formed by **5a** have a conductance of 3.6 nS suggesting an apparent internal diameter of 2.5 nm. The diameter is controlled by the charge repulsions between the side chains: the neutral histidine channel **5b** has a conductance of 0.7 nS, consistent with the diameter expected of a cyclic tetramer (0.52 nm). The diameter is also controlled by the length of the peptide side chain; the extended histidine-containing compound **5c** shows a conductance of 1.2 nS, corresponding to an apparent internal diameter of 0.7 nm. The smaller structures give shorter-lived openings (seconds for **5b**; milliseconds for **5c**). In this system as well, differential substitution leads to an overall dipole and sensitivity to sign of the membrane polarization (**5f** see below).

The large internal diameter of these channels allows the transport of large ionic species, and at the same time, the internal functionality of the barrel can be used to control the transport.[20] For example, the large cationic β-barrel **5a** is blocked by oligonucleotides in the B-DNA conformation. The smaller arginine-containing channel **5d** is blocked by single nucleotides such as UDP; this produces a system in which the channel can be switched off by an enzymatic process that produces UDP. The potential of this strategy as the basis of a "naked-eye" sugar sensor system has been demonstrated using

 This journal is © The Royal Society of Chemistry 2007

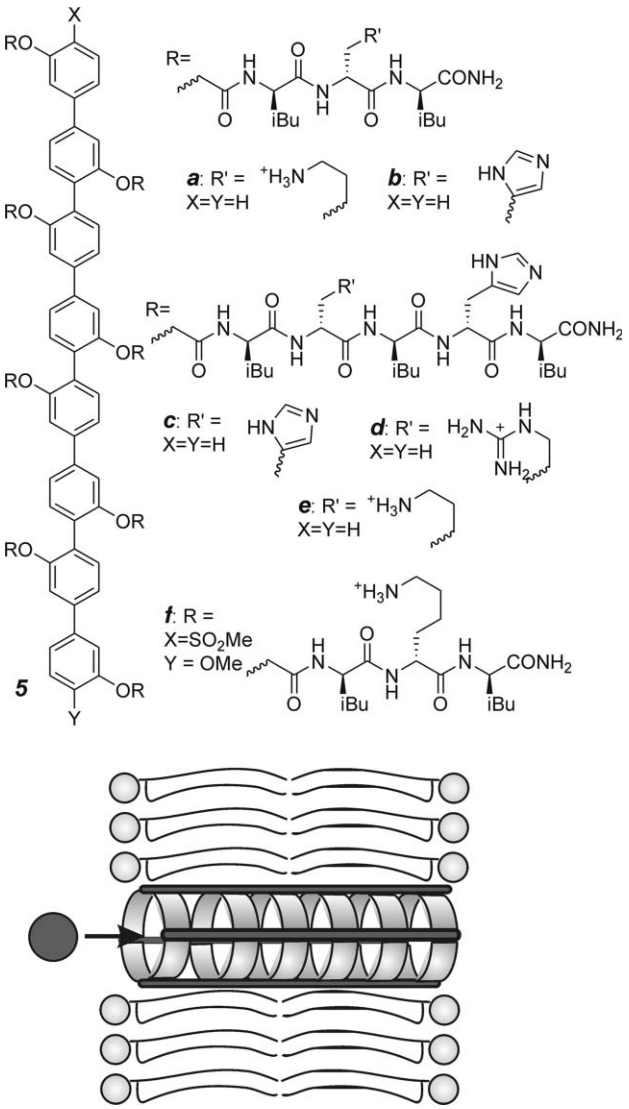


Fig. 6 Octiphenyl β-barrel channels. The peptide strands interdigitate to form an anti-parallel β-sheet structure with the nonpolar side chains on the outside and the polar side chains on the inside of the barrel.

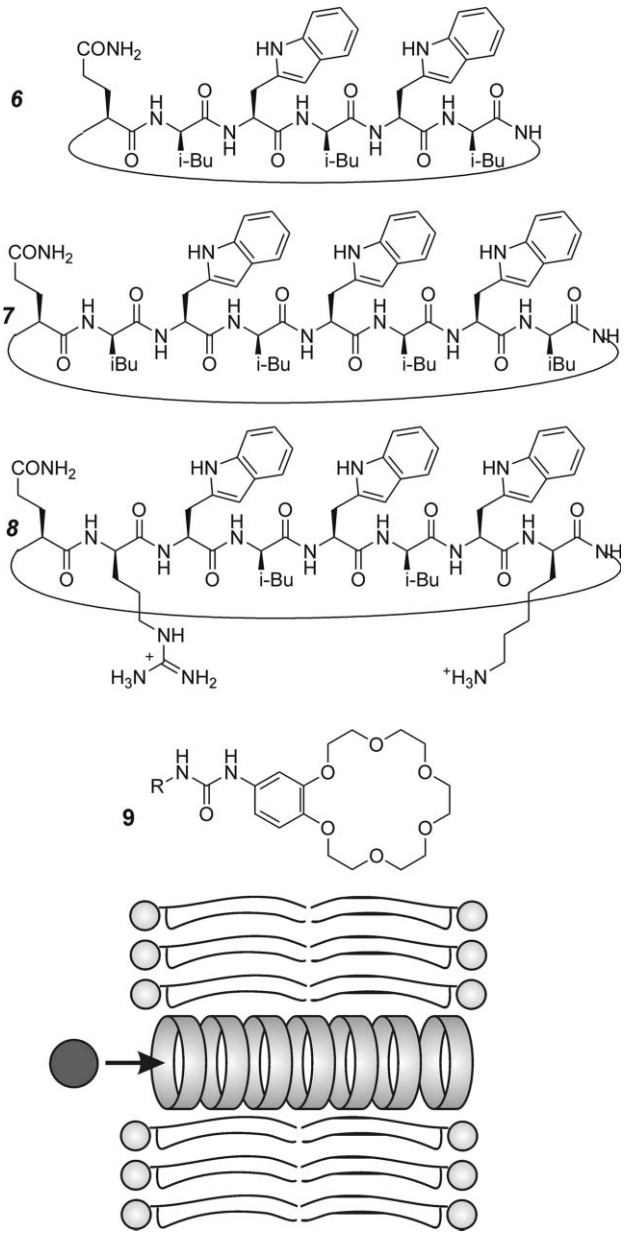


Fig. 7 Channels from hydrogen-bonded stacks of macrocycles. Cyclic peptides with alternating D,L- amino acids associate in a face-to-face fashion *via* multiple hydrogen bonds (**7**, **8**) or face-to-face stacks of crown ethers are supported by a chain of ureido hydrogen bonding (**9**).

5e. This channel is blocked by ATP but is significantly less blocked (factor of 20-fold) by ADP. Transport activity is detected by efflux of entrapped carboxyfluorescein (CF) from vesicles. Inside the vesicle the CF concentration is high and CF fluorescence is self-quenched, but, as CF diffuses through the channel, the fluorescence increases dramatically; pore unblocking therefore results in significant fluorescence increase. It is thus possible to use the channel activity to detect kinase activity as occurs in the phosphorylation of glucose by ATP. Increasing concentrations of glucose result in enhanced fluorescence as increasing amounts of ATP are consumed, resulting in increasing numbers of un-blocked channels. In conjunction with invertase to hydrolyze sucrose to glucose, the sugar concentrations of soft drinks can be determined.[21] The inside of these β-barrel channels provides a dense array of charges and functional groups. General-acid/general-base catalysis of ester hydrolysis inside the pores is possible using the imidazole functional groups in channel **5d**, as demonstrated by the hydrolysis in transit of the acetate ester of HPTS (pyranine dye; Fig. 3).[20]

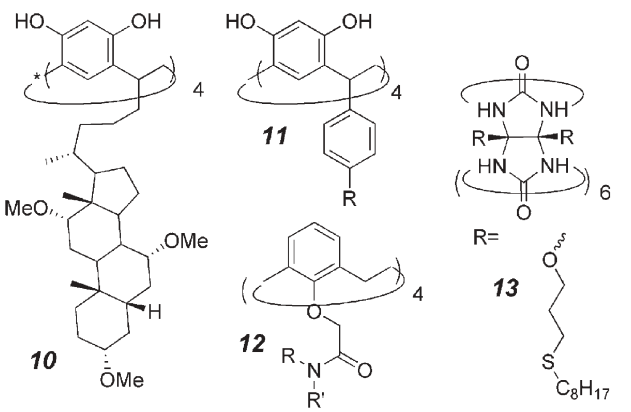
Another tubular channel system maintained by peptide hydrogen bonding is illustrated in Fig. 7. This system utilizes cyclic octa- and decapeptides formed from alternating D- and L- amino acids. This arrangement allows the same intermolecular hydrogen bonding pattern as in a β-sheet but the cyclic peptide structure directs the hydrogen-bonding interactions above and below the mean plane of the macrocycle, which in turn disposes the side-chains into equatorial positions around the macrocycle. The net result is a face-to-face stack of macrocycles, a so-called peptide nanotube.[22] The ion channels formed by compounds **6** and **7** consist of stacks of about six

monomer units and have specific ionic conductance values consistent with the internal diameters of 0.7 and 1.0 nm respectively. The decapeptide **7** has more conformational flexibility than the octapeptide **6**, with the result that the mean open lifetime of **7** is eight-fold shorter than that of **6**.[23] These channels show strong Na^+ over Cl^- selectivity comparable to the much more restricted gramicidin channel, yet the decapeptide **7** is able to transport glutamate ion in vesicles and in planar bilayer membranes. The glutamate–chloride selectivity cannot be solely due to size discrimination and must also reflect an internal stabilization afforded to the glutamate zwitterions in transit that is absent or reduced for chloride.[23]

Cyclic peptides **6–8** are potent antibacterial agents and show marked activity against both Gram-positive (*B. subtilis*, *Staphylococcus aureus*) and Gram-positive (*Streptococcus pneumoniae*, vancomycin-resistant *Enterococcus faecalis*) bacterial species.[24] The antimicrobial action is rapid, suggesting that the toxicity is related to membrane depolarization rather than a receptor-mediated site of action. The proposed depolarization was directly observed in intact *S. aureus* cells, and compound **8** showed *in vivo* antibiotic efficacy in mice challenged with methacillin-resistant *S. aureus*.

Another strategy for face-to-face stacking of macrocycles using a hydrogen-bonding scaffold to form a channel is demonstrated by the ureido-crown ether **9**.[25] In nonpolar solvents, head-to-tail hydrogen bonding of the ureas leads to an extended oligomeric structure that in the solid-state shows parallel sacks of face-to-face macrocycles. In planar bilayer membranes this system produces a rich assortment of channel conductance states. Some of the states are related to tubular stacks as illustrated in Fig. 7; others are much larger and are akin to the toroidal pores formed by other simple amphiphiles (see below).

Many other systems containing macrocyclic components have been reported[9]; compounds **10–13** are some recent examples of this type.[11] Does the ion pass through the macrocycle during transport across the membrane? In the case of the resorcinarene channels **10** and **11**, or the calix[4]arene channel **12** the answer must be "no", as these components make up the restrictive portals that entrap guests within carcerands. Channels are reliably formed by these compounds, but a tubular structure is very unlikely; the aggregate structures discussed below are more probable. Larger macrocycles, such as the cucurbit[6]uril derivative **13**, are more likely candidates for a cation-through-macrocycle trajectory.[26] Cation transport by **13** shows an anomalous selectivity sequence of $Li^+ > Cs^+ \sim Rb^+ > K^+ > Na^+$, which is the opposite of the binding selectivity sequence for the parent cucurbit[6]uril. The sequence suggests that the macrocycle acts as a selectivity filter that retards the transport of the best bound cations.



Aggregate ion channels

An alternate structural motif for ion channels is provided by the aggregate channels formed by amphotericin. The amphotericin channel involves both the antibiotic and sterols arranged in two half-channel sections within the two bilayer leaflets. The design of synthetic compounds that could mimic this very complex hierarchical self-assembly is a daunting task which is dramatically simplified through the use of larger membrane-spanning subunits (Fig. 8). In this case, the edge-to-edge aggregation is the only type required as the inter-leaflet interactions are replaced by covalent linkages. Compounds which are active as aggregates are typically bolaamphiphiles (two-headed amphiphiles) that are sufficiently long to adopt a membrane-spanning structure within the bilayer.

The progression of structures from the poly-macrocycle **14** to the acyclic oligoester **17** illustrates the co-evolution of mechanistic appreciation of the factors that control orientation and aggregation in bilayer membranes and the progressive simplification of the required syntheses. Compound **14** was designed as an explicit example of a tubular channel and shows activity comparable to gramicidin in vesicle experiments.[9] The design and synthesis plan allows structural variations in the head groups, and in the ester and crown ether macrocycles, and after considerable synthetic effort, a series of structure–activity relationships was explored. One key question is the role of the central macrocycle, and the series was replicated with a bridging tartaric acid unit in place of the crown ether (**15**). These compounds are simpler to make, and showed about the same level of activity and ionic selectivity as the "parent" compound **14**.[9] Clearly the central crown ether serves only to hold the structure together and has a limited functional role. Further truncation and simplification lead *via* **16**[27,28] to the linear ester **17**.[29] Compound **17** is indistinguishable from **16a** with respect to its activity in vesicles or in planar bilayer membranes and shows simpler and more reliable channel formation than parent structure **14**.

The channels formed by **15–17** are due to aggregation within the membrane to the level of dimers or trimers. The concentration dependence of cation transport in vesicles shows an apparent kinetic order of 2 or higher for many examples. Voltage clamp studies are consistent with the view that the monomers are inactive, and that the only active channels involve dimers or larger oligomers.[9] Unlike the peptide examples discussed above, there are few self-complementary functional groups in **15–17** to drive the aggregation. On the contrary, the head group carboxylates are deprotonated at the pH of the transport experiments, so there is an inherent repulsion between monomers.

Aggregation is principally driven by the properties and organization of the lipids acting on the bolaamphiphile as a solute. A bilayer membrane is a liquid-crystalline solvent in

 This journal is © The Royal Society of Chemistry 2007

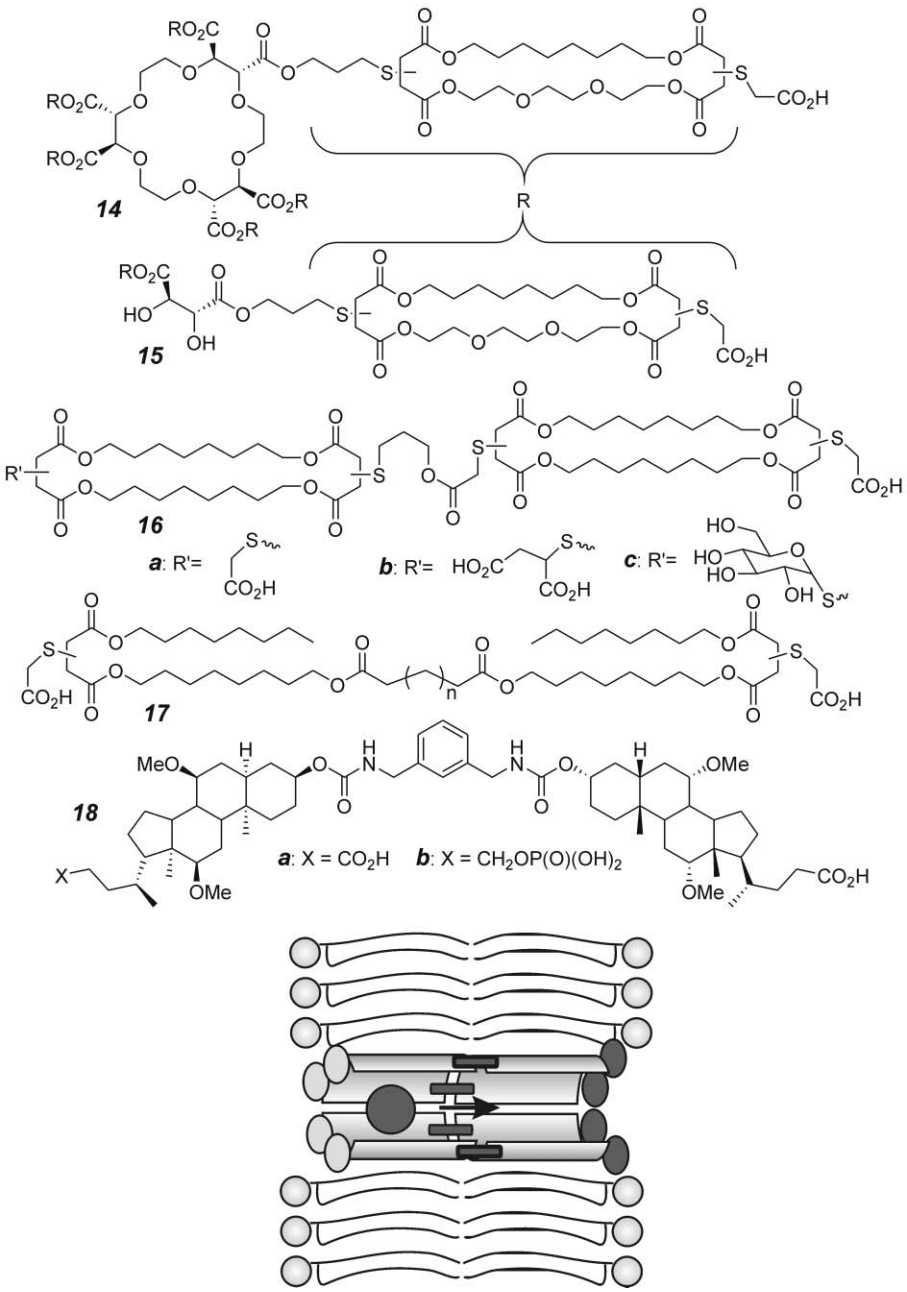


Fig. 8 Aggregate channels from bolaamphiphiles. Membrane-spanning monomers associate edgewise to form a water-filled pore. Dissymmetric channel-forming compounds can produce an asymmetric distribution within the membrane that leads to voltage-gated transport.

which solutes preferentially align with the long axis parallel to the long axis of the individual lipid molecules. Thus, membrane-spanning bolaamphiphiles **15–17** will be oriented with their edges roughly parallel simply as a result of the solvent properties of the lipid. Lipids in vesicles and planar bilayer membranes under the usual experimental conditions are in the gel state. In this state there is considerable fluidity in the hydrocarbon region of the bilayer and a crystal-like array of head groups; at higher temperatures the head group order melts to give a fluid state. Gel-state lipids exclude solutes to achieve a type of phase-separation in which the solutes are confined to regions between more ordered lipid-only regions. These regions are akin to grain-boundaries so diffusion is predominantly restricted to a one-dimensional path. A potential channel-forming compound is therefore oriented, concentrated in a specific region, and has a high probability of encountering a second molecule of channel-forming compound through restricted diffusion. The disordered regions in which the solutes reside are also the regions where the permeability of water is enhanced due to the poor packing between the hydrocarbon tails of the lipids. Channel-forming compounds have polar functionality within the bilayer that will be partly hydrated. When a pair of channel-forming compounds collide edge-to-edge, the water molecules between them can organize, recruit adjacent water molecules from the bilayer mid-plane and from the aqueous solutions, and open a water channel

spanning the bilayer. The flow of hydrated ions is then possible and the channel is detected.

The structures of these aggregates are variable and dynamic, with the result that they impose relatively little steric selectivity on the transport process. Hydrated ions are transported with partial loss of the inner hydration shell so the ionic selectivity typically reflects the dehydration energy sequence from Cs^+ to Li^+ for cations. For a similar reason, most channels of this type are selective for cations over anions as the dehydration energies of simple anions are higher than those of cations of comparable size. Head group charge plays a role as an electrostatic selectivity filter, so ionic head groups discriminate better between cations than neutral or anionic head groups. The internal ether oxygens in **14** and **15** confer relatively small benefit to the channel, but do reduce the aggregation number by providing more sites for water cross-linking; the channels formed by **16a** are larger and more readily recruit additional monomers and rearrange during a channel opening.[27]

Although less structurally defined than tubular channels, aggregate channels can give rise to the same range of transport functions as their better-organized cousins. An example is the voltage-gated channels formed by **16b**. Voltage-gating is the activation or opening of a channel through a change in transmembrane potential; at some (low) applied potential the channel is ''off'', but, as the membrane potential is increased to a higher value, the channels ''open'' and ionic current flows. This is a non-linear current–voltage response curve akin to that exhibited in electronic circuitry by diodes. A related function is rectification, in which ion flow in one direction through a channel is easier than flow of the same ion in the opposite direction under the same driving potential. Voltage-gating requires a non-centrosymmetric channel with a significant molecular dipole. In addition, the channel insertion must occur with an asymmetric distribution of orientation. This is implied by the sketch in Fig. 8, in which the similarly shaded ends of the bolaamphiphile are aligned on one face of the bilayer. In a practical sense this can be achieved through the use of head groups that have widely different hydration energies. Partial dehydration is required for the flip–flop penetration of a head group through a bilayer, so the acetate head group of **16b** or **16c** will pass more readily through a bilayer than the more highly hydrated succinate or glycosidic head groups of **16b**,**c**. The aggregate, once formed, will have an overall dipole. The energy of the dipole, and indirectly the stability of the aggregate, will therefore depend on the sign and magnitude of the applied transmembrane potential. The stabilizing relative orientation of the dipole and electric field vectors will enhance the probability that a channel will open, while the destabilizing orientation will reduce the probability of a channel opening and will reduce the overall current flow.

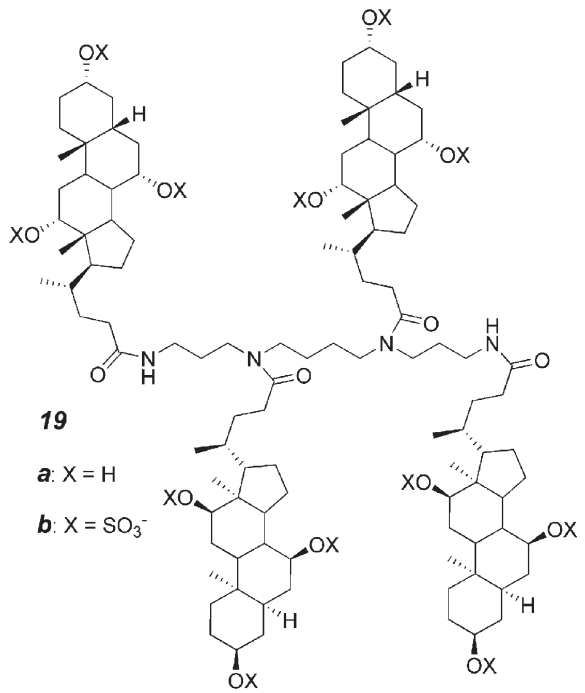
This type of voltage-gating is demonstrated in the series of compounds **16a–c**. Compound **16a** has equivalent head groups so there is no preferential orientation as it inserts into a bilayer; a linear current–voltage curve is obtained for these channels. Compounds **16b** and **16c** have two different head groups, so introduction to one side of a bilayer clamp experiment results in preferential insertion of the acetate end through the membrane. The result for **16b** is a strongly non-linear current–voltage response in which substantial currents are carried at negative applied potentials, but the system is an effective insulator at positive applied potentials.[28] If the orientational asymmetry is lost through introduction of **16b** on both sides of the membrane, or breakage and reformation of the bilayer, the current–voltage response becomes linear. A significant overall dipole is required; compound **16c** inserts correctly, but the overall dipole moment of the aggregate is smaller so no voltage-gating activity is observed. A similar behaviour has been reported for the bis-cholate bolaamphiphiles **18a**,**b**.[30] In this case as well, the symmetric compound **18a** cannot show the voltage-gated response, while the dissymmetric compound **18b** gives a non-linear current–voltage response. The asymmetry of insertion is enhanced by introducing the compound to a polarized membrane.

A third example of a voltage-gated pore is given by the β-barrel formed from the dissymmetric octiphenyl **5f** (Fig. 6).[31] The active β-barrel is likely a tetramer as judged from the single-channel conductance. In the cyclic tetramer, all four octiphenyl units are aligned in the same direction as a direct consequence of the formation of the β-sheet by hydrogen bonding between the peptide side-chains. The overall structure therefore has a larger net dipole than any individual strand. It is unlikely that the small difference in hydration between the two ends of the channel plays a significant role in creating the asymmetric distribution within the bilayer. It is proposed that monomeric **5f** adopts a preferential organization within the bilayer driven by the sign of the membrane potential. The oligomerization in the membrane would also be voltage-dependent as it requires energy to align the dipoles within the barrel. The macroscopic result is a strong non-linear voltage-dependence of the initial formation of the conducting state. In some cases, the formed pore then shows a linear (Ohmic) current–voltage behaviour indicating that the pore itself is not rectifying.

Aggregation to form oligomeric channels, as found with compounds **14–18**, depends on the monomer concentration raised to a power that reflects the stoichiometry (2 for dimers, 3 for trimers *etc*.) In the absence of any specific stabilizing intermolecular interactions, larger aggregates do not form to an appreciable extent since the mass action term is so unfavourable. To form larger aggregates and larger channels, the monomers must associate to some degree. Relative to amphotericin, compounds **15** or **16** are ''pre-associated'' through the bridging covalent bonds between the macrocycles. Compounds **19a**,**b** illustrate another type of covalent ''pre-association'' that leads to ion channel activity. The inspiration for this design is the antibiotic squalamine, a polyamine conjugated steroidal sulfate.[32] The presumed mode of action of this antibiotic is *via* an aggregate pore in which the protonated polyamines line the core of the aggregate and the hydrophobic faces of the steroid face the lipid bilayer. A similar mode of action is found for amphipathic peptide antibiotics.[33] All these naturally occurring antibiotics show good specificity for Gram-negative bacteria, suggesting that synthetic functional mimics might be a potential source of new therapeutic agents. Compound **19a**, and higher dendrimer oligomers bearing six and eight cholate groups, form dimeric channels, suggesting that one molecule occupies one of the bilayer leaflets and the channels only open when a second molecule in the other leaflet

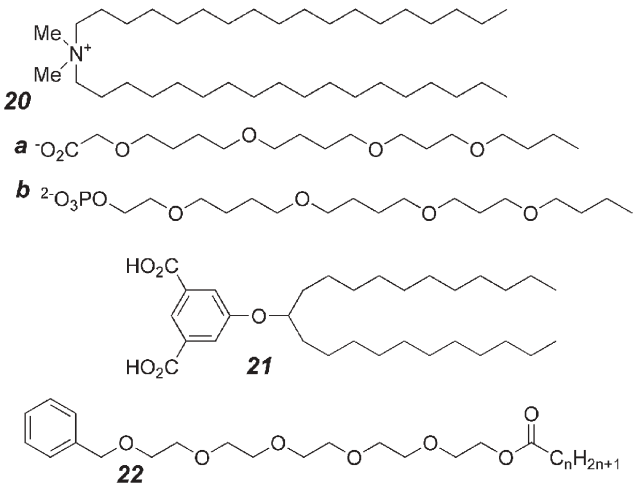
 This journal is © The Royal Society of Chemistry 2007

encounters the first to form an end-to-end dimer.[34] Surprisingly, sulfated derivatives such as **19b** can cross bilayer membranes and have shown good anti-viral activity against HIV.[35]



What is the simplest molecule that will form an ion channel?

Some of the active compounds discussed above are "simple": readily prepared in a few steps, contain few functional groups, low molecular weight. How far can the simplification go and still retain desirable functional characteristics? Many common detergents exhibit channel-like activity at concentrations below their critical micelle concentrations.[11] In general, these channels are transient, irregular, and poorly reproducible. In contrast, the simple compounds **20–22** produce regular channel openings in voltage clamp experiments. These openings have uniform characteristics and, in all functional respects, appear to be like the channels formed from more complex materials.[11] The active structures are elusive. In general, the specific conductance and ionic selectivity suggest that the internal openings of the channels are small, and it is possible that they are aggregates of the type discussed for compounds **15–19**. It is likely that many molecules are involved in the aggregates; the channels formed by the ion pairs salts **20a**,**b** occur in a narrow concentration range and there is evidence that asymmetric aggregates that are voltage-dependent can form as a result of a small imbalance in the numbers of molecules on either side of the membrane. None of these simple compounds are long enough to span a bilayer completely, so it is surprising to note that the activities of **21** and of **22** are strongly dependent upon the hydrocarbon chain lengths. The case of **22** is unusually sensitive to this structural variable, as a derivative with two additional methylene groups in the tail is completely inactive.[36] Relatives of these very low molecular weight compounds offer good prospects for applications in drug delivery as they are simple to prepare and their transport functions can be controlled by pH and by photoisomerization.[11]



A final example of a very active and very simple compound that forms ion channels is given in Fig. 9. The lipophilic ethylenediamine palladium(II) complex **23** was expected to self-assemble with an equivalent amount of 4,4′-bipyridine into a channel with a large metallosupramolecular square portal. That does not occur; rather, **23**, acting on its own, forms very

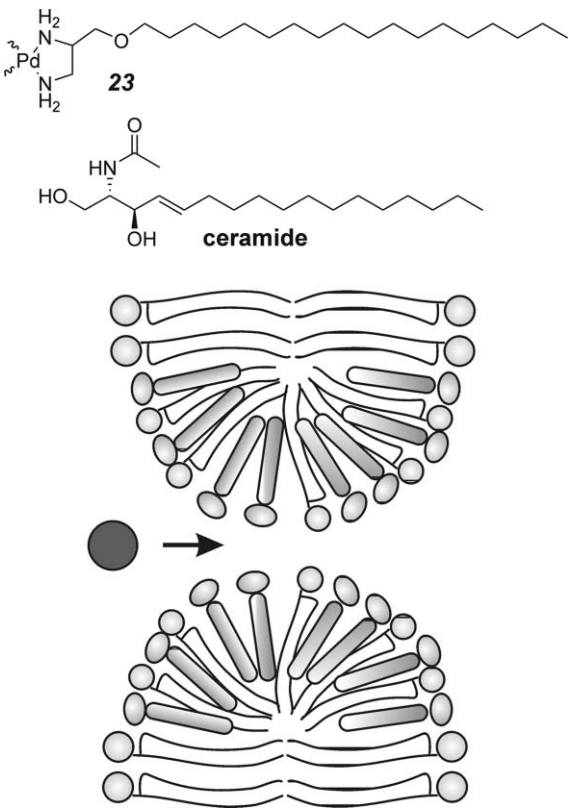


Fig. 9 A toroidal channel formed by single-chain amphiphiles. Transient channels formed by detergents are presumed to have this type of structure. Long-lived channels form when the amphiphiles have stabilizing head-to-head interactions.

large (>1 nm diameter), very long lived (>10 minutes) and very high-conductance channels (nA).[37] From their size and stability characteristics these openings resemble the channels formed by ceramide in which hydrogen-bonding between head groups creates stacks of ceramide that line the faces of a toriodal pore in the membrane.[38] Fig. 9 shows the cross-section of the pore and the requirement that the pore-forming compound stabilize the positive curvature perpendicular to the bilayer plane. Within the plane of the bilayer (not shown) the opposite curvature must be stabilized. There is no specific hydrogen-bonding stabilization possible between the monomers of **23**, but a lipid-bridging, or a lipid–water bridging interaction might serve the same role.

Summary

Membrane transport is a supramolecular function that simultaneously tests our current understanding of supramolecular structure and supramolecular dynamics. On a structural level, the current examples fall short, in some cases dramatically short, of even the simplest ion channels from Nature. But on a functional level, some of the activities and sophistication achieved with synthetic channels is directly and quantitatively comparable to natural channels. These achievements are all the more striking when compared to efforts to mimic enzymes with synthetic systems in which catalytic acceleration factors still fall many orders of magnitude behind natural benchmarks. Catalysis of translocation has proved to be relatively simple to achieve.

As in all catalytic systems, the active structures and the molecular mechanisms of transport remain elusive. This is equally true of natural ion channels for which only a handful of structures are known, and the mechanistic insights they provide are ambiguous and incomplete. Part of the mechanistic challenge is in the scale of the process; ion translocation in a channel occurs over a distance of nanometres and over a time of hundreds of nanoseconds. The body of knowledge from detailed examination of the gramicidin channel over the past three decades[6] shows how much remains to be done for any of the synthetic channels discussed in this review. However, the lack of mechanistic certainty need not inhibit the development of useful applications of artificial ion channels. The many examples discussed above that relate channel-forming ability to antibiotic activity clearly show the potential for the creation of new therapeutic agents. There is equivalent potential for the development of new drug delivery and sensor applications.

Membrane transport systems will continue to provide a fruitful testing ground for the development of supramolecular chemistry for (at least) the next three decades. The progression from molecular recognition of alkali metal cations to self-assembled metallosupramolecular assemblies is a progression in complexity. The problems of the near future will require systems and techniques to confront and study complex systems directly. Membrane transport systems already act on this level: molecular scale events act within, and influence the behaviour of, many hundreds of thousands of other molecules in the system. We have uncovered some ways to control the simple behaviours and we can confidently expect to control even more complex processes and sequences. The underlying principles are clear from our efforts over the past decade: the membrane dominates the energy landscape, and we need to use the bilayer to assemble and guide the complex structures and functions we will wish to create. We are well started; there is plenty to do.

Acknowledgements

Work from the author's laboratory discussed above was supported by the Natural Sciences and Engineering Research Council of Canada, and was energetically pursued by the excellent team of co-workers listed in the references. Horace Luong and Todd Sutherland kindly provided data for Fig. 3 and 4 respectively.

References

1 B. Hille, *Ionic Channels of Excitable Membranes*, Sinauer Associates, Incorporated, Sunderland, 3rd edn, 2001.
2 T. M. Fyles, *Bioorg. Chem. Front.*, 1990, **1**, 72–111.
3 E. Gouaux and R. MacKinnon, *Science*, 2005, **310**, 1461–1465.
4 F. Hucho and C. Weise, *Angew. Chem., Int. Ed.*, 2001, **40**, 3100–3116.
5 G. R. Painter and B. C. Pressman, *Top. Curr. Chem.*, 1982, **101**, 83–110.
6 O. S. Andersen, R. E. Koeppe and B. Roux, *IEEE Trans. Nanobiosci.*, 2005, **4**(1), 10–20.
7 G. A. Woolley and B. A. Wallace, *J. Membr. Biol.*, 1992, **129**, 109–136.
8 D. M. Cereghetti and E. M. Carreira, *Synthesis*, 2006, 914–942.
9 T. M. Fyles and W. F. van Straaten-Nijenhuis, in *Comprehensive Supramolecular Chemistry*, ed. D. N. Reinhoudt, Elsevier Science, Amsterdam/New York, 1996, vol. 10, pp. 53–77.
10 G. W. Gokel and A. Mukhopadhyay, *Chem. Soc. Rev.*, 2001, **30**, 274–286.
11 S. Matile, A. Som and N. Sorde, *Tetrahedron*, 2004, **60**, 6405–6435.
12 U. Koert, L. Al-Momani and J. R. Pfeifer, *Synthesis*, 2004, 1129–1146.
13 U. Koert, *Bioorg. Med. Chem.*, 2004, **12**, 1277.
14 R. R. C. New, in *Liposomes: A Practical Approach*, ed. R. R. C. New, IRL Press, Oxford, 1990, ch. 3, pp. 105–161.
15 G. W. Gokel, R. Ferdani, J. Liu, R. Pajewski, H. Shabany and P. Uetrecht, *Chem.–Eur. J.*, 2001, **7**, 33–39.
16 H. Shabany and G. W. Gokel, *Chem. Commun.*, 2000, 2373–2374.
17 W. M. Leevy, J. E. Huettner, R. Pajewski, P. H. Schlesinger and G. W. Gokel, *J. Am. Chem. Soc.*, 2004, **126**, 15747–15753.
18 W. M. Leevy, M. E. Weber, P. H. Schlesinger and G. W. Gokel, *Chem. Commun.*, 2005, 89–91.
19 Y. Vandenburg, B. D. Smith, E. Biron and N. Voyer, *Chem. Commun.*, 2002, 1694–1695.
20 N. Sakai, J. Mareda and S. Matile, *Acc. Chem. Res.*, 2005, **38**, 79–87.
21 S. Litvinchuk, N. Sorde and S. Matile, *J. Am. Chem. Soc.*, 2005, **127**, 9316–9317.
22 J. D. Hartgerink, T. D. Clark and M. R. Ghadiri, *Chem.–Eur. J.*, 1998, **8**, 1367–1372.
23 J. Sánchez-Queseda, H. S. Kim and M. R. Ghadiri, *Angew. Chem., Int. Ed.*, 2001, **40**, 2503–2506.
24 S. Fernadez-Lopez, H.-S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxen and M. R. Ghadiri, *Nature*, 2001, **412**, 452–455.
25 A. Cazacu, C. Tong, A. van der Lee, T. M. Fyles and M. Barboiu, *J. Am. Chem. Soc.*, 2006, **128**, 9541–9548.
26 Y. J. Jeon, H. Kim, S. Jon, N. Selvapalam, D. H. Oh, I. Seo, C.-S. Park, D.-S. Koh and K. Kim, *J. Am. Chem. Soc.*, 2004, **126**, 15944–15945.
27 T. M. Fyles, D. Loock and X. Zhou, *Can. J. Chem.*, 1998, **76**, 1015–1026.

 This journal is © The Royal Society of Chemistry 2007

28 T. M. Fyles, D. Loock and X. Zhou, *J. Am. Chem. Soc.*, 1998, **120**, 2997–3003.
29 T. M. Fyles and C. Hu, *J. Supramol. Chem.*, 2001, **1**, 207–215.
30 C. Goto, M. Yamamura, A. Satake and Y. Kobuke, *J. Am. Chem. Soc.*, 2001, **123**, 12152–12159.
31 N. Sakai, D. Houdebert and S. Matile, *Chem.–Eur. J.*, 2003, **9**, 223–232.
32 P. B. Savage, *Eur. J. Org. Chem.*, 2002, 759–768.
33 R. M. Epand and H. J. Vogel, *Biochim. Biophys. Acta*, 1999, **1462**, 11–28.
34 J. Zhang, B. Jing and S. L. Regen, *J. Am. Chem. Soc.*, 2003, **125**, 13984–13987.
35 B. Jing, V. Janout, B. C. Herold, M. E. Klotman, T. Heald and S. L. Regen, *J. Am. Chem. Soc.*, 2004, **126**, 15930–15931.
36 T. M. Fyles, R. Knoy, K. Müllen and M. Sieffert, *Langmuir*, 2001, **17**, 6669–6674.
37 T. M. Fyles and C. C. Tong, *New J. Chem.*, 2006, DOI: 10.1039/b610660a.
38 L. Siskind and M. Colombini, *J. Biol. Chem.*, 2000, **275**, 38640–38644.

Save valuable time searching for that elusive piece of vital chemical information.

Let us do it for you at the Library and Information Centre of the RSC.

We are your chemical information support, providing:

- Chemical enquiry helpdesk
- Remote access chemical information resources
- Speedy response
- Expert chemical information specialist staff

Tap into the foremost source of chemical knowledge in Europe and send your enquiries to

library@rsc.org

12120515

RSC Publishing

www.rsc.org/library