FEATURE ARTICLE

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Molecular ferries: membrane carriers that promote phospholipid flip-flop and chloride transport

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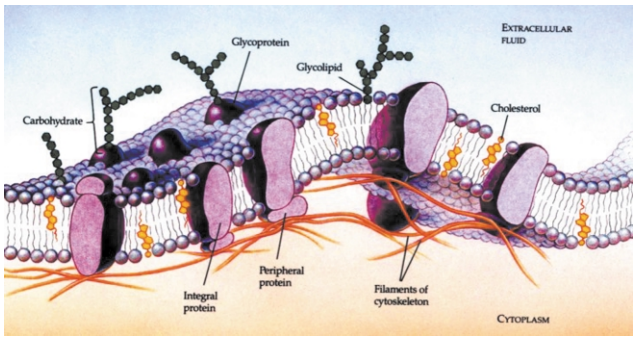
The facilitated transport of ionic or polar solutes through biological membranes is an essential process for cellular life, and a major technical goal of the pharmaceutical industry. Synthetic receptors with affinities for anions are shown to act as molecular ferries and facilitate the movement of chloride ions and salts across vesicle and cell membranes. A process that competes with chloride transport is phospholipid translocation or flip-flop. This has led to the development of synthetic scramblases that can alter the transmembrane distribution of phospholipids and induce biological responses such as membrane enzyme activation. The facilitated translocation of phospholipids with multiply-charged head groups, like phosphatidylserine, is a difficult supramolecular challenge that requires a complementary, multitopic receptor with appropriate amphiphilicity.

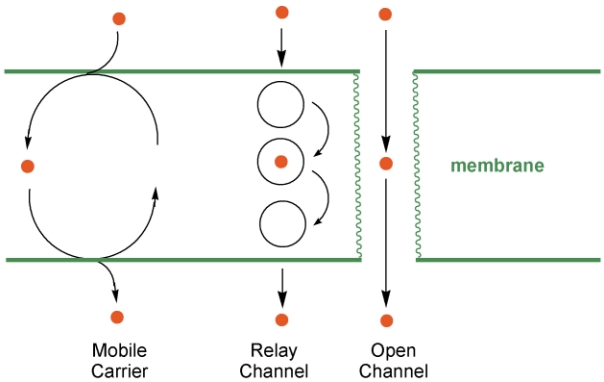
Introduction

A typical mammalian cell is around 3 μm in diameter and is surrounded by a 6 nm wide plasma membrane. From the human perspective, these dimensions are equivalent to a 10 m diameter seminar room surrounded by a shell 20 cm thick. This extraordinarily thin membrane is all that separates the cytosolic space from the extracellular fluid and the lumina of the various organelles. The basic structure of a typical cell membrane is a self-assembled bilayer of polar lipids, non-polar lipids, and proteins (Scheme 1). The ratio by weight of protein to lipid varies from 3.6 in the inner mitochondrial membrane to 0.25 in the lipid-rich myelin membrane.[1] The proteins can act as receptors, enzymes, channels, and/or pumps which endows the membrane with the capability to: (a) alter its flexibility and mechanical strength, (b) control the concentration of ions and molecules in the various cellular spaces, (c) conduct biochemical reactions, and (d) recognize and communicate with other cell membranes.[2] Elucidation of the molecular mechanisms that produce these various membrane functions is the goal of a large number of research laboratories with a wide range of skills and instrumentation. The primary expertise in our laboratory is organic supramolecular chemistry and the specific focus of this article is our recent work on the facilitated transport of anionic solutes through bilayer membranes.



Scheme 1 Typical cell membrane.†

The passage of a charged or polar solute through an unbiased phospholipid bilayer is very slow. The primary barrier is diffusion of the solute through the lipophilic membrane interior. A recent molecular dynamics simulation of unassisted diffusion of Na^+ and Cl^- through a bilayer membrane shows water and phospholipids accompanying the ion.[3] To facilitate transport, cells have evolved to use various supramolecular strategies. Three limiting cases are shown in Scheme 2. The first strategy, which is employed extensively in our work, uses a mobile carrier to associate with the polar solute and form a lipophilic complex which can then diffuse through the membrane, *i.e.*, a



Scheme 2 Membrane transport mechanisms.

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molecular ferry. The next two cases are channel mechanisms. They differ in the extent of interaction between solute and the channel walls. In some cases there is extensive interaction such that the solute can be visualized as briefly occupying specific binding sites as it passes through the channel. Such a relay mechanism can exhibit high solute selectivity. In other cases, the channel is sufficiently large that the solute can pass through as a highly solvated species, and there is only a rough selection based on the size of the solvated solute. Both types of channel systems can promote movement either passively down an electrochemical gradient, or actively in a specific direction. Furthermore, channels can have sophisticated switching mechanisms with chemical, electrical, optical, or mechanical triggers. Transport channels are usually complicated supramolecular assemblies of large transmembrane proteins and it is currently a substantial challenge to understand their mechanisms of action at the molecular level. One of the aims of our research is to design low-molecular-weight mimics of membrane transport systems. These simplified mimics can be used to address fundamental mechanistic questions, or they can be developed into later generation versions that have useful applications.[4]

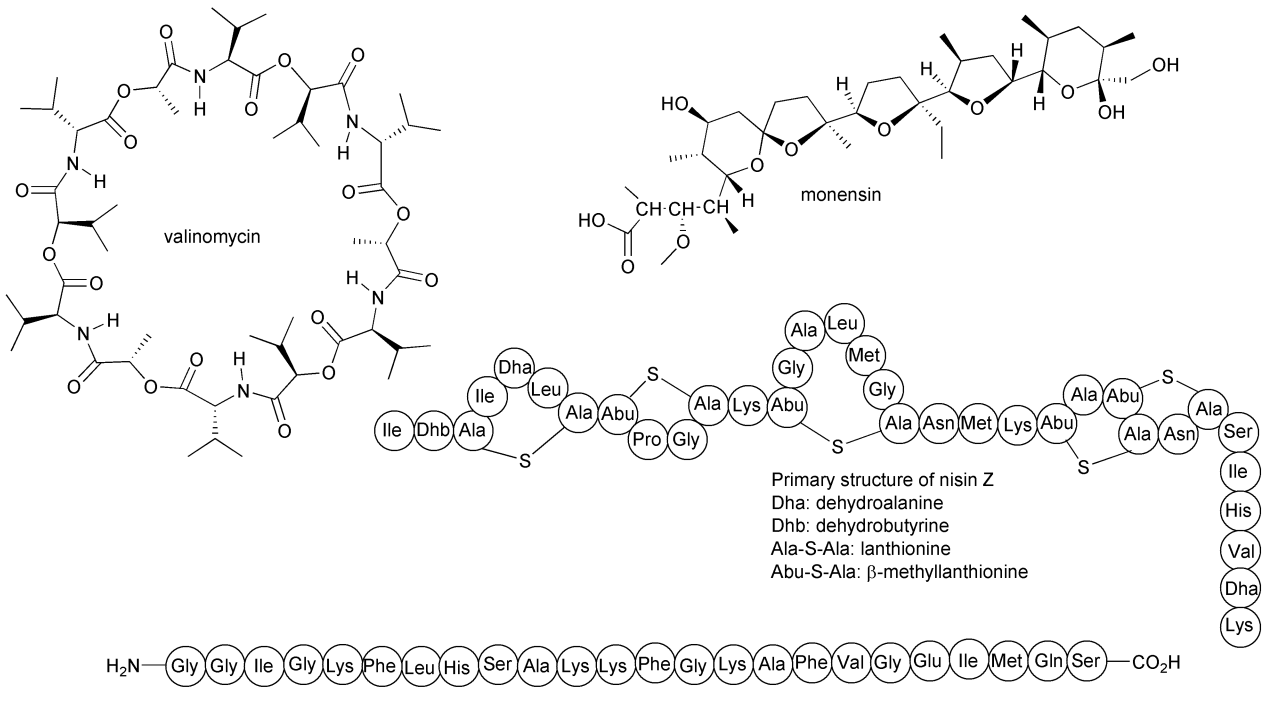
When discussing transport across cell membranes it is important to bear in mind that the membranes are likely to be polarized with electrical and various chemical gradients. These gradients are maintained by the concerted action of more than twenty different endogenous membrane transporters. The most dominant are the ATP-driven ion pumps which control Na^+, K^+, Ca^{2+}, and H^+ gradients. These primary ion gradients are subsequently used by co-transport (simultaneous passage of two or more solutes in the same direction) or antiport (simultaneous passage of two or more solutes in opposite directions) systems as energy sources to move other ions or polar molecules against their respective concentration gradients. Thus in cells, electrochemical gradients can drive membrane transport processes that appear intuitively to be unfavored. For example, consider a nerve cell with a typical membrane potential of −60 mV (inside negative), and a Cl^- concentration gradient of 50 mM internal and 100 mM external. Although there is a concentration gradient promoting Cl^- transport into the cell, it is overwhelmed by the electrical potential such that the free energy for inward Cl^- transport is +4 kJ mol^{-1}! A useful rule of thumb to remember is that at equilibrium, a univalent ion has a ten-fold concentration gradient to match a 60 mV transmembrane electric potential.

Facilitated membrane transport

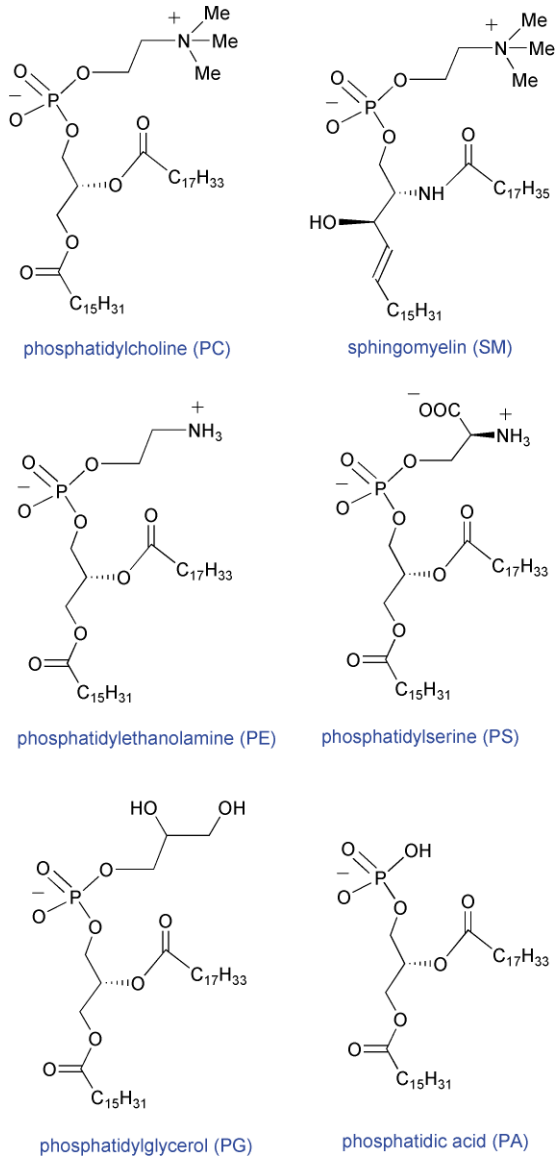
A range of amphiphilic peptides like nisin and magainin (Scheme 3) are known to form channels that allow the leakage of cell contents. The dynamic structure and mechanism of action of these self-assembled channels is a major research topic.[5] A relatively new viewpoint is that the visually appealing barrel stave model may not be very common, and that a more likely permeation process is the carpet-like mechanism.[6] The biological purpose of many of these channel-forming natural products is to kill opposing organisms, and they appear to do so by inducing membrane leakage and depolarization of electrochemical gradients.

Bacteria have also developed low-molecular-weight ionophores such as valinomycin and monensin (Scheme 3) that do not disrupt the membrane integrity, but instead act as mobile transport carriers for metal cations (cationophores).[7] The neutral cationophores transport cations across the mitochondrial membrane which destroys the transmembrane electric potential, and diminshes ATP production by the ATPases. Furthermore, mitochondrial depolarization leads to membrane disruption and release of cytochrome c from the inner membrane surface which activates caspase enzymes that induce programmed cell death (apoptosis). Most cationophores are toxic compounds, and have high activity against many gram-positive bacteria, yeast, and fungi.

It is intriguing to consider why the natural world contains a large number of mobile cation carriers, but practically no analogous mobile anion carriers. There are likely chemical and biological reasons for this situation. From a molecular recognition perspective, cation transport is relatively easy to achieve because there is little binding competition from the few cationic residues that are present on the common phospholipids (Scheme



Scheme 3 Structure of channel-formers nisin and magainin, and the mobile carriers valinomycin and monensin.

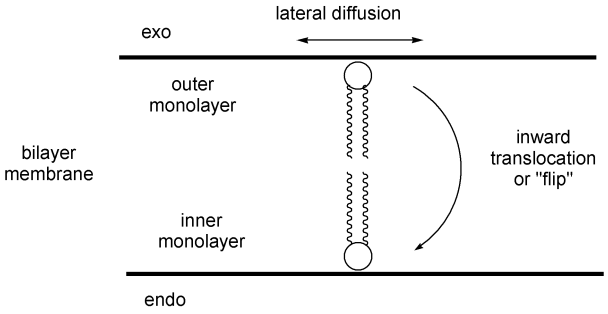


Scheme 4 Structures of the common phospholipids.

4). Conversely, anion transport is a more difficult supramolecular challenge because a putative carrier has to bind the anionic solute at the membrane surface where there is a very high concentration of competing phosphate diester residues. On the other hand, it is known that that the interior of a standard, unperturbed bilayer membrane has a net positive charge so if a mobile ionophore can move an anionic solute past the polar membrane surface then passage through the middle of the membrane should be relatively easy.[8] Nonetheless, in 1999 when we contemplated treating a bilayer membrane with a low-molecular-weight anionophore, we predicted that the most likely supramolecular outcome would be transmembrane movement of the phospholipid head group. This thought experiment inspired us to learn more about phospholipid flip-flop and transmembrane phospholipid distribution.

Phosplipid flip-flop

Unlike lateral diffusion, which is very rapid, the translocation (or flip-flop) of phospholipids across a model bilayer membrane is known to be a very slow process with a half-life of hours to days (Scheme 5).[9] Flip-flop rates in these artificial systems are strongly dependent on the composition of the polar head-group, and less dependent on the length of the acyl chains.[10]



Scheme 5 Phospholipid translocation or flip-flop.

Nonetheless, if an artificial membrane is given enough time it will reach an equilibrated state with a random transmembrane distribution of phospholipids. This is not the case with most biological membranes which have an asymmetric distribution of phospholipids (Fig. 1). The degree of asymmetry varies; the

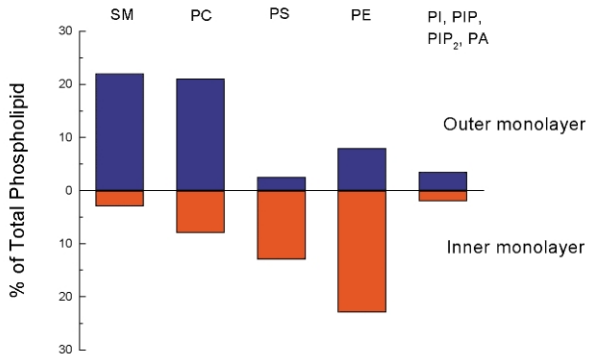
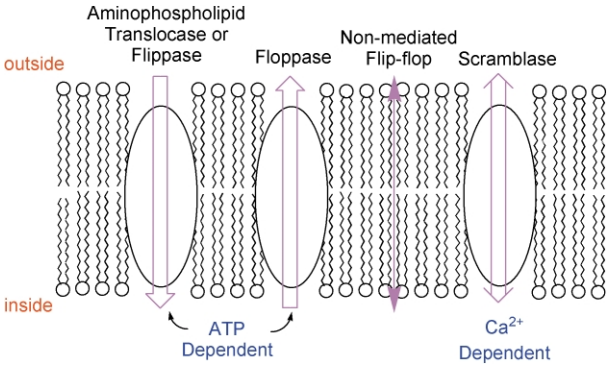


Fig. 1 Distribution of phospholipids across an erythrocyte membrane.

plasma membranes of most eukaryotes maintain a high degree of asymmetry, whereas a lesser degree of transbilayer asymmetry is observed in the membranes of subcellular organelles and bacterial species. The asymmetric distribution of phospholipids is a fundamental feature of normal cell operation. For example, the phosphatidylserine (PS) that is normally localized in the inner monolayer of an animal plasma membrane is vital not only for exocytosis and intracellular fusion processes, but also for lipid–protein interactions and signal transduction pathways.[11–14]

The asymmetric transmembrane distribution of lipids is generated and maintained by a number of phospholipid translocases that vary in lipid specificity, energy requirements, and direction of translocation.[15] For example, the plasma membrane phospholipid asymmetry is maintained by the synchronous action of the aminophospholipid flippase which selectively pumps PS and phosphatidylethanolamine (PE) to the inner monolayer of the membrane, and a nonspecific, less-active floppase which moves phospholipids to the outer monolayer (Scheme 6). Some of the translocase proteins have been isolated, but presently it is not known how translocation works at the molecular level. The literature contains some speculation that translocases operate by forming pores and indeed pore forming compounds are known to promote phospholipid flip-flop.[16] Furthermore, it has recently been shown that translocation of phospholipids with singly charged head groups can be promoted by single transmembrane peptides.[17] Nonetheless, the aminophospholipid flippase is quite head group selective and so the transport mechanism in this, and related systems, must involve a high degree of phospholipid head group recognition. A recent crystal structure of a bacterial Lipid A floppase shows a sophisticated chamber structure that appears to cycle through a dynamic three-stage binding–translocation–release mechanism.[18]

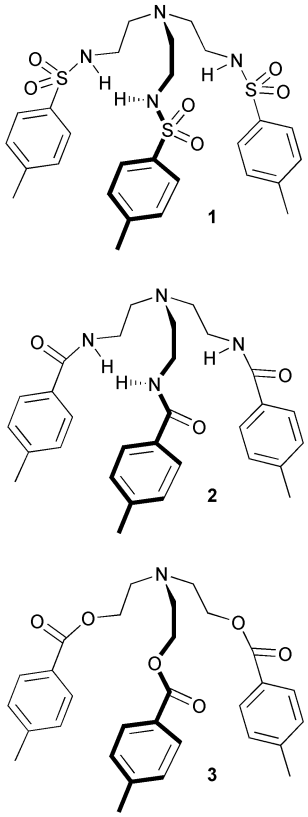


Scheme 6 Enzyme promoted flip-flop in an erythrocyte membrane.

A loss of normal phospholipid asymmetry is often associated with abnormal cell function. For example, the appearance of PS in the outer monolayer of membranes correlates with cell death and clearance by phagocytosis.[19] Inactivation of the aminophospholipid flippase alone is not sufficient to expose PS; a simultaneous Ca^{2+}-dependent nonspecific scrambling process is necessary. PS-exposing apoptotic cells are cleared from the bloodstream by macrophages following a specific recognition event between PS receptors on the macrophage and the externalized PS. Similarly, aging erythrocytes and platelets slowly externalize PS, culminating in engulfment by macrophages.[20] Another consequence of phospholipid randomization is blood coagulation. The binding of blood cells to proteins involved in the coagulation cascade is dependent on PS exposure. The tenase and prothrombin complexes bind to the patches of anionic lipid on the cell surface, and through a series of activation steps the fibrin matrix of the clot is formed.[21]

Thus, we had two initial reasons for developing synthetic tranlocation mimics. The first motivation was to learn what minimal supramolecular requirements would induce translocation of the various phospholipid head groups across a bilayer membrane. The second more practical motivation was to generate a series of synthetic scramblases that would promote the appearance of PS on the surface of cell surfaces, and hopefully produce selective biological and therapeutic responses.

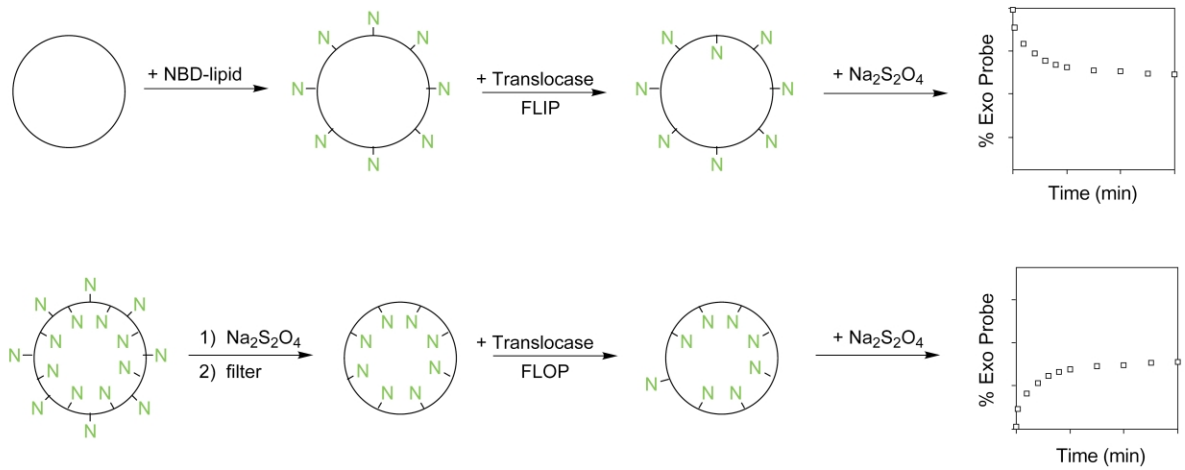
As a starting point, we chose to develop a translocase for phosphatidylcholine (PC), since it has a zwitterionic head group and seemed a more straightforward transport challenge. There is experimental evidence that the primary site of hydration in the PC head group is the phosphate diester residue and not the diffuse tetraalkylammonium cation.[22] We reasoned that if an appropriate uncharged anion receptor could form a hydrogen bonded complex with the phosphodiester, then the lipophilic receptor/head group complex would more readily diffuse through the membrane. We chose to initially examine simple derivatives of tris(aminoethyl)amine (tren), namely sulfonamide **1** and amide **2**, as well as control ester **3** (Scheme 7).[23–25]



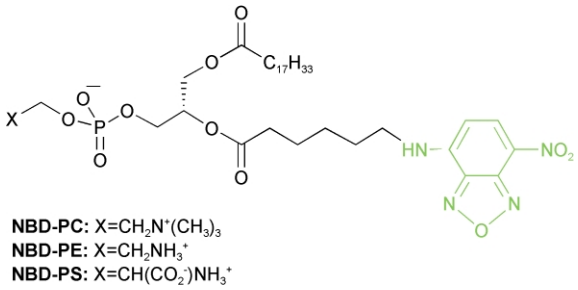
Scheme 7 Tren-based phospholipid scramblase candidates.

Compounds **1** and **2** were previously shown by Reinhoudt and coworkers to act as receptors for anions like dihydrogen phosphate.[26] The compounds also have a nice balance of water solubility and amphiphilicity so they can be readily formulated for transport studies.

We employ the NBD/dithionite quenching assay to measure the rate with which fluorescent NBD-labeled phospholipids are translocated across vesicle membranes (Schemes 8 and 9).[27] The assay starts with surface differentiated vesicles prepared with NBD-lipids (0.5 mol%) in either the membrane outer monolayer (*exo*-labeled) or inner monolayer (*endo*-labeled). Upon treatment with sodium dithionite ($Na_2S_2O_4$), the NBD



Scheme 8 Fluorescent assay for flip-flop.



Scheme 9 NBD-labeled phospholipids.

fluorescence is quenched due to chemical reduction of the nitro group. Vesicle membranes are effectively impermeable to dithionite, therefore, only NBD-lipid located in the outer leaflet is chemically quenched. At any given time, the percentage of NBD-lipid located in the outer monolayer can be determined from the drop in fluorescence intensity when a portion of the vesicles is subjected to dithionite quenching. The system progresses to an equilibrated state with the outer monolayer containing about 60% NBD-lipid. Reported half-lives for inward translocation correspond to the time taken to reach 80% of the NBD-lipid in the outer monolayer

We found that sulfonamide **1** was especially good at promoting NBD-PC translocation, whereas the amide **2** and ester **3** were not effective (Table 1).[23] In light of the success

Table 1 Half-lives (min) for NBD-phospholipid translocation into vesicles

	Scramblase	NBD-PC	NBD-PE	NBD-PS
1		5	5	> 120
2		> 180	> 180	> 180
3		> 180	> 180	> 180

with sulfonamide **1**, the inability of amide **2** to promote translocation was puzzling because it is also a hydrogen bond donor (although less acidic than **1**). Structural insight was gained from X-ray structures of **1** and **2** (Fig. 2).[25] The structure

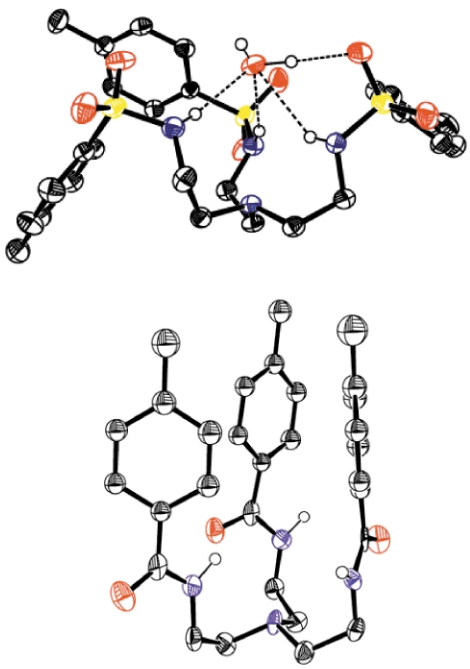
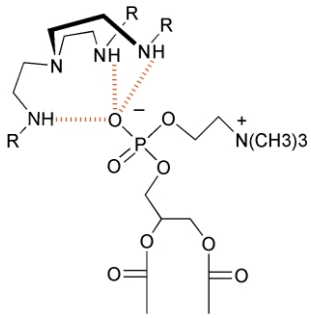


Fig. 2 X-Ray structures of **1**·H_2O (top) and **2** (bottom).

of **1** shows that it is preorganized with an open binding pocket for an anionic residue such as the phosphate diester group in PC, but the X-ray structure of **2** shows that the *anti*-amide bond conformation forces the three aryl groups to block this anion binding pocket. Additional structural and kinetic studies indicate that the supramolecular complex in Scheme 10 is the



Scheme 10 Proposed complex between **1** and PC head group.

species that is translocated through the membrane. The sulfonamide **1** can also transport natural PC across cell membranes. This is most vividly demonstrated by the echinocyte rescue experiment shown in Scheme 11. It is known that

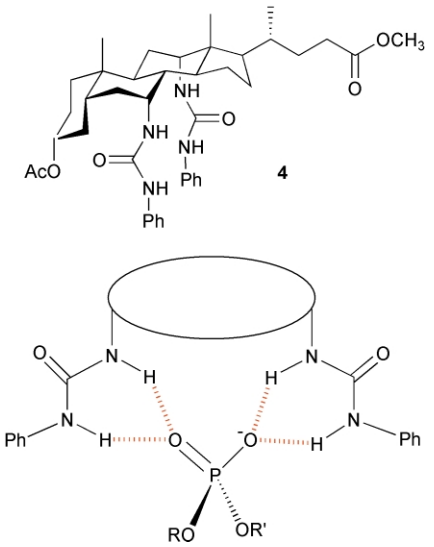
Scheme 11 Echinocyte rescue experiment.

treatment of blood cells with dilaurylphosphatidylcholine (DLPC) induces a change in morphology from discocyte to echinocyte.[28] A return to the discocyte shape occurs as the PC slowly equilibrates across the bilayer. We found that the rate of shape return is strongly enhanced upon addition of sulfonamide **1**, but less so by amide **2**, and hardly at all by ester **3**.[24]

The translocation property of sulfonamide **1** has been used as a biological tool to study the peroxisome proliferator-activated receptor γ (PPARγ).[29] Upon binding specific lipid ligands, PPARγ undergoes a structural rearrangement that releases transcriptional inhibitors and recruits transcriptional co-activators. Oxidized hexadecyl azelaoyl PC (azPC) was observed to increase PPARγ activity. Furthermore, expression of PPAR-responsive genes was observed to increase in the presence of **1**, suggesting that the azPC must translocate through the cell membrane.

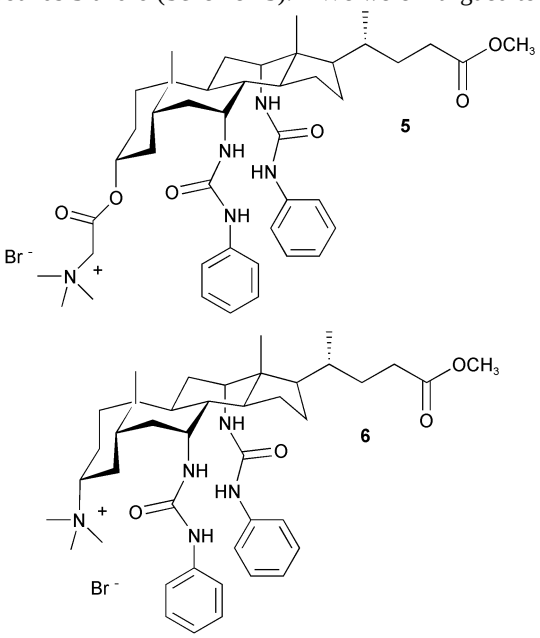
More recently we have embarked on a fruitful collaboration with Professor A. Davis and his coworkers at Bristol University. Together we have synthesized and evaluated the phospholipid translocation abilities of various steroid derivatives that have been structurally altered to have anion binding pockets. In particular, the cholate scaffold is well-suited for membrane transport,‡ because its facial amphiphilicity provides sufficient water solubility, yet the compound can also partition readily into bilayer membranes.[30] Initially we prepared and examined the 3-acetoxybis(urea) **4** and showed that it was about five times more effective at NBD-PC translocation across vesicle and cell membranes than the tren sulfonamide **1**.[31] A combination of kinetic and structrural studies indicated that **4** forms a stoichiometric complex with the PC head group (Scheme 12) which promotes its diffusion across the membrane. However, compound **4** has little effect on the translocation of anionic PS which is the eventual goal of this research.

We needed to design a version of **4** with PS-translocation abilities. We reasoned that at neutral pH, cationic versions of **4** should form charge-neutral complexes with the anionic PS



Scheme 12 Cholate bis(urea) **4** and its supramolecular complex with a phosphate diester.

group, and so we prepared and evaluated structurally related compounds **5** and **6** (Scheme 13).[32] We were intrigued to find



Scheme 13 Cationic cholate derivatives.

that these two compounds have remarkably different translocation abilities. Vesicle translocation experiments showed that analogue **6** can only weakly translocate NDB-labeled phosphatidic acid (PA) and phosphatidylglycerol (PG), anionic phospholipids with singly charged head groups, and cannot translocate NDB-labeled PS, PC and PE, phospholipids with multiply charged head groups (Table 2). On the other hand,

Table 2 Half-lives (min) for NBD-Phospholipid translocation into vesicles.

Scramblase	NBD-PS	NBD-PC	NBD-PE	NBD-PG	NBD-PA
4	30	30	30	< 1	< 1
5	30	20	30	8	< 1
6	> 180	≫180	≫180	120	120
None	≫180	≫180	≫180	> 180	> 180

compound **5** can effectively translocate all types of anionic NBD-labeled phospholipids across vesicle membranes. Moreover, compound **5** can also translocate endogenous PS across erythrocyte membranes. The amount of PS on the erythrocyte surface was measured by flow cytometry analysis in conjunction with fluorescein labeled PS-binding protein Annexin V (Annexin V-FITC).[33] As shown in Fig. 3, the addition of

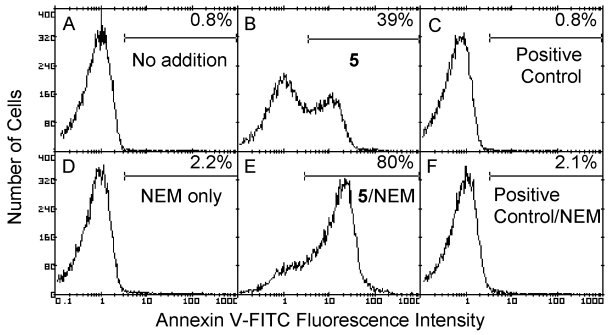
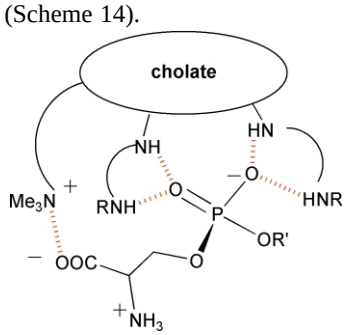


Fig. 3 Flow cytometry analysis of Annexin V-FITC binding to normal or NEM-pretreated erythrocytes.

compound **5** increased the fraction of cells that bound Annexin V-FITC from 1% to 39%. This effect was magnified to 80% when the cells were pretreated with *N*-ethylmaleimide which inhibits the endogenous PS flippase from pumping the PS back to the inner monolayer of the cell membrane.[13]

We believe that the difference in translocation properties between **5** and **6** provides useful information about the difficulty of translocating PS compared to PA or PG. In essence, the difference can be interpreted in terms of the Hofmeister effect, a commonly observed partitioning selectivity that is attributed to differences in solvation.[34] In the case of PA or PG, it is relatively easy to desolvate their singly charged head groups (using compounds **5** or **6** for example) and induce membrane translocation. However, it is intrinsically harder for phospholipids with multiply-charged head groups, like PS, to diffuse through the lipophilic interior of a bilayer membrane. At neutral pH, the PS head group has three charges that sum to a net negative charge (Scheme 4). Translocation of the PS head group is facilitated if some or all of these residues are desolvated.[10] When compound **6** binds to the PS head group, the resulting supramolecular complex is highly amphiphilic and so does not readily translocate. However, the ditopic binding pocket of compound **5** is large enough to simultaneously contact the phosphate and the carboxylate residues, which leads to more effective dehydration of the PS head group and subsequent translocation (Scheme 14).



Scheme 14 Putative ditopic complex between **5** and the PS head group.

The idea that it is easier for a scramblase to translocate a singly charged phospholipid compared to a multiply charged phospholipid is consistent with a recent report that single, membrane spanning helical peptides can translocate PA and PG, but not PS and PC.[17] The hypothesis predicts that PA and PG can be translocated by structurally simple, monotopic receptors that can desolvate their relatively small head groups (even a single proton may suffice[35]). However, larger, multitopic receptors are needed to bind and translocate the multiply-charged PS head group.[36] It seems that an important evolutionary property of PS is the fact that it is intrinsically difficult to

translocate. This makes it easier for cells to maintain asymmetric PS transmembrane distributions which they can then use as platforms for various recognition and signaling processes. Such asymmetries would be significantly harder to preserve with PA or PG as a substitute for PS.

With a functioning PS scramblase in hand we are now in a position to test if it can induce biological activities. To date we have only examined the ability of **5** to affect the blood coagulation process.[32] Specifically, we have measured the effect of **5** to alter the conversion of prothrombin to thrombin on the erythrocyte surface. We find that eyrthocytes treated with **5** can increase the amount of thrombin by a factor of four. Various control experiments indicate that this is due to the increased amount of PS on the cell surface which facilitates assembly of the precursor prothrombinase complex. In the future we will examine other biological processes. For example, it is known that external PS levels can influence phagocytosis, aging, and abnormal adherence, whereas internal PS levels can influence cell signaling, enzyme activation, exocytosis, intracellular fusion, and membrane mechanical stability.[11–15]

Chloride transport

Once we established that mobile anionophores can translocate phospholipids across a membrane, we were curious to see if they can also transport small anions such as Cl^-.‡ Our interest also has a practical motivation since it is possible that mobile anionophores could be used as antibiotics or as Cl^- transporters for diseases such as cystic fibrosis.[37]

As a starting point we chose to examine the ability of cholate **4** to promote the efflux of Cl^- from vesicles.[38] A chloride selective electrode was used to monitor Cl^- levels outside the vesicles; a straightforward assay that gives very reproducible data. We found that compound **4** can promote Cl^- efflux from vesicles, but not the leakage of fluorescent dyes like carboxyfluorescein. The rate of Cl^- efflux was dependent on the identity of the external anion in the order, NO_3^- > HCO_3^- > SO_4^{2-} (Fig. 4), but was independent of cation identity, *i.e.*, Na^+

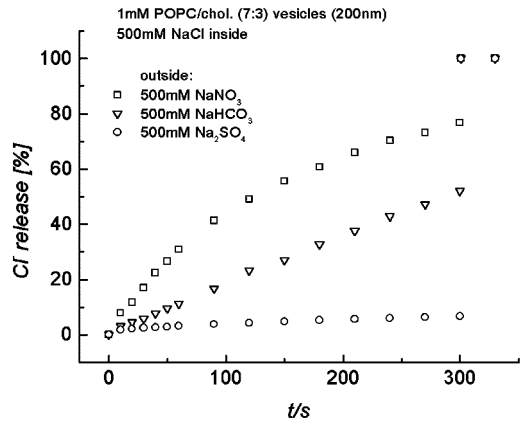
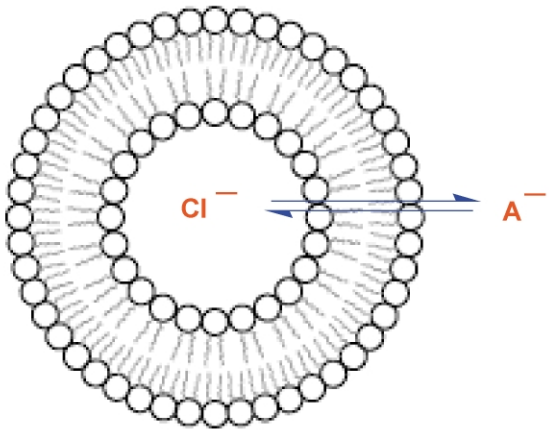


Fig. 4 Cl^- release from vesicles upon addition of **4** as a function of external anion. The vesicles were lysed after 300 s.

~ K^+ ~ Cs^+. This is strong evidence that the transport process is an anion antiport mechanism (Scheme 15) and that **4** acts as a mobile carrier. The compound does not produce defects in the membrane because a vesicle system with Cl^- external and SO_4^{2-} internal leads to a negative potential inside as expected with an antiport mechanism and a transport selectivity of Cl^- over SO_4^{2-}. In terms of potential applications, we have recently discovered analogues of **4** that are able to raise the rate of Cl^- transport across a layer of epithelial cells.[38] The utility of this result is the focus of future studies.

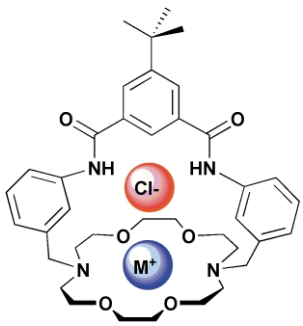


Scheme 15 Anion antiport mechanism.

Chloride salt transport

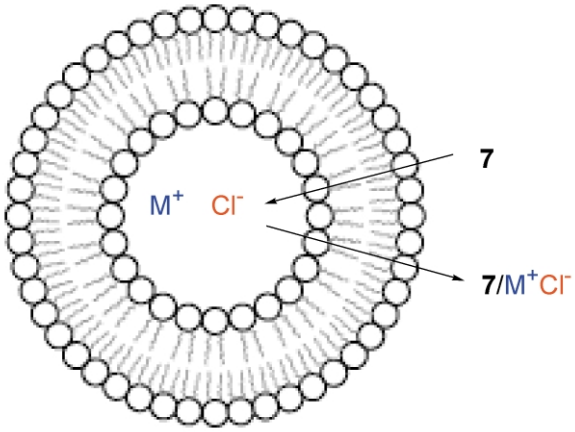
The prodigiosins are a rare example of natural, mobile carriers for Cl^-, and they work by co-transporting H^+/Cl^- in the same direction.[39] A mechanistic consideration of salt co-transporters suggests a number of reasons why they may be quite effective as membrane transport agents. (1) A neutral anionophore must use an anion antiport mechanism or otherwise transport must be driven by a transmembrane potential gradient. Thus, a potential drawback with an antiport process is the requirement for a suitable counter anion for back transport. As seen with cholate **4** the rate of chloride efflux from vesicles is very dependent on the identity of the entering anion (Fig. 4). (2) With a salt co-transporter it is a neutral complex that partitions into the membrane which can be energetically quite favorable. This is related to the reason why weak acids and bases are often good drug candidates; they have high membrane permeability because at neutral pH a small fraction of the material is uncharged and can readily pass through the membrane interior.

Our Cl^- efflux experiments with salt-binding macrobicycle **7** (Scheme 16)[40] show that it is indeed an outstanding Cl^-



Scheme 16 Receptor **7** can bind salts as contact ion-pairs.

transporter and more active than the cholate **4**.[41] Since compound **7** uses a co-transport mechanism (Scheme 17), the rate of Cl^- efflux from vesicles is not dependent on the identity of the external ions (Fig. 5). This mechanistic difference may become important in certain transport applications like drug delivery. For example, if the goal is to use a mobile anionophore to transport an anionic drug into a cell, then an antiporter like **4** may not be effective because the cell cytoplasm does not contain a lipophilic anion that can be simultaneously transported in the reverse direction, or it cannot overwhelm an unfavorable membrane potential. However, this would not likely be the case with salt co-transporter **7**, whose ability to deliver salts into cells should be independent of the identity of cell contents and membrane potential.



Scheme 17 Salt efflux from vesicles using co-transporter **7**.

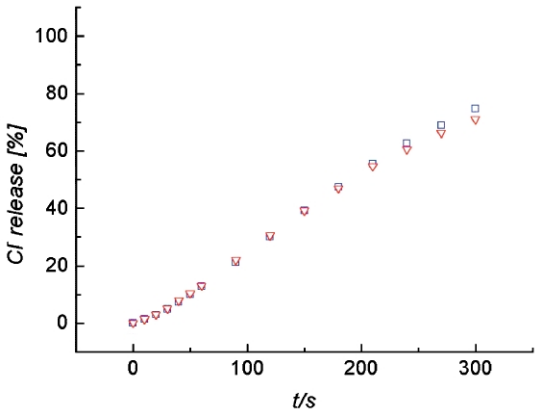


Fig. 5 Cl^- efflux upon addition of **7** to vesicles containing 500 mM of NaCl and dispersed in 375 mM Na_2SO_4 (squares) or 375 mM Cs_2SO_4 (triangles).

Summary

Our work shows that small, anion receptor molecules with appropriate amphiphilicity can bind and transport Cl^- across vesicle and cell membranes. A process that competes with Cl^- transport is facilitated phospholipid translocation or flip-flop, however, it should be possible to enhance either pathway by appropriate molecular design. For example, one way of increasing Cl^- transport is to develop a salt transporter like **7** that operates by a co-transport mechanism. The facilitated translocation of PS, a biologically important phospholipid with a multiply-charged head group, is a difficult supramolecular challenge that requires a complementary, multitopic receptor with appropriate amphiphilicity. Our initial success at thrombin activation makes us hopeful that effective synthetic PS scramblases will induce a range of useful biological and pharmaceutical effects.

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Notes and references

† Taken from *Biology*, 5th edn., ed. N. Campbell, J. Reece and L. Mitchell, Benjamin Cummings, New York, 1999. Reprinted with permission from Pearson Education, Inc.

‡ Synthetic membrane transporters are presently being developed by a number of research groups, and the designs of Regen and Kobuke also employ cholate scaffolds. For a recent summary, see reference 4.

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