Influence of lipids on protein-mediated transmembrane transport

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Abstract

Transmembrane proteins are responsible for transporting ions and small molecules across the hydrophobic region of the cell membrane. We are reviewing the evidence for regulation of these transport processes by interactions with the lipids of the membrane. We focus on ion channels, including potassium channels, mechanosensitive and pentameric ligand gated ion channels, and active transporters, including pumps, sodium or proton driven secondary transporters and ABC transporters. For ion channels it has been convincingly shown that specific lipid-protein interactions can directly affect their function. In some cases, a combined approach of molecular and structural biology together with computer simulations has revealed the molecular mechanisms. There are also many transporters whose activity depends on lipids but understanding of the molecular mechanisms is only beginning.

Key words: membrane; ion channel; transporter

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[1]Abbreviations used: PC, phosphatidylcholine; PE, phophatidylethanolamine; PS, phophatidylserine; PI phosphatidylinositol; PG, phosphatidylglycerol; PA, phosphatidic acid; CL, cardiolipin; DPG, diphosphatidylglycerol; PIP_2 or PI(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; SM, sphingomyelin; APC, amino acid-polyamine-organocation superfamily; EPR, electron paramagnetic resonance; MFS, major facilitator superfamily; MD, molecular dynamics; TCDB, transporter classification database; PDB, protein databank

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1. Introduction

Transport of ions and small molecules across the cell membrane is catalyzed by specialized proteins. Since transmembrane proteins are located within a lipid environment it is an obvious question in how far their function is dependent on or modulated by protein-lipid interactions. Are there any interactions essential to molecular transport beyond simply providing a stabilizing environment for the membrane protein? If so, what is the nature and specificity of these interactions? Are the physical thermo-elastic properties of the membrane sufficient to describe any effects or is a molecular detailed, chemical picture needed? As with many questions in biology, the answer seems to be "all of the above". In this review we set out to gather the evidence for the influence of lipids on the function of channels and transporters. We focus on the transport of matter by ion channels, water pores and primary and secondary transporters and regrettably omit the whole field of transmembrane signalling, i.e. the transport of information. Within the channels and transporters we are taking a broad view and try to gather a range of proteins for which functional experimental data suggests a direct effect of lipid type or bilayer properties on transport; for systems not included the reader is referred to other reviews (Opekarova and Tanner, 2003; Lee, 2004; Nyholm et al., 2007; Marsh, 2008; Lee, 2011). Unlike the situation a few years ago, we now also have crystal structures for many of the proteins themselves or for homologs

and hence it is now becoming possible to develop a molecular picture of the effect of lipids on transmembrane transport.

2. Biological membranes

Biological membranes contain membrane proteins and lipids. Polar (amphipathic) lipids consist of a hydrophilic headgroup and a hydrophobic tail region (van Meer et al., 2008). Most membrane lipids have a zwitterionic or charged headgroup, which largely determines the chemical properties (such as hydrogen bonding capability and charge) and hydrophobic acyl chains, which differ in length and the number of unsaturated bonds. Membrane properties such as the phase and fluidity depend on the balance between acyl chain packing and headgroup interactions. In aqueous solution, many lipid can assemble into membranous structures. Not all lipids can form bilayers, though; membranes in biological systems consist of mixtures of bilayer-forming lipids with other lipids.

The most common lipids found in biological membranes are glycerophospholipids, sphingolipids, sterols, and saccharolipids (classification according to LIPID MAPS (Fahy et al., 2005, 2009)). Glycerophospholipids consist of a glycerol backbone to which two acyl chains are attached at the sn-1 and sn-2 positions whereas a phosphate group is linked at sn-3. Figure 1 shows zwitterionic (net-neutral under physiological conditions) phosphatidylethanolamine (PE) and phosphatidylcholine (PC), anionic phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidic acid (PA), and the doubly phosphorylated PI, phosphatidylinositol 4,5-bisphosphate (PIP_2) with an overall charge of -3. Diphosphatidylglycerol, commonly referred to as cardiolipin (CL), is a charged lipid commonly found in prokaryotes and the inner membrane of mitochondria. Sphingolipids such as sphingomyelin (SM) are a major class of lipids in mammalian cells that can pack more tightly than PC and confer stability to a membrane (van Meer et al., 2008). Saccharolipids contain fatty acid directly linked to a sugar backbone. The outer membrane of Gram-negative bacteria consists predominantly of lipopolysaccharide (LPS), whose membrane component is the saccharolipid lipid A (Raetz et al., 2007).

Although this review focuses on the phospholipid component of the membrane it is worthwhile remembering that most eukaryotic membranes contain about 30–40% sterols such as cholesterol in addition to neutral and charged phospholipids. Sterols on their own do not form bilayers but together with bilayer-forming lipids, they can form a liquid-ordered phase (L_o or l_o) (van Meer et al., 2008). Addition of cholesterol to a pure lipid membrane above the liquid-crystalline phase transition temperature disrupts the liquid crystal so that the reduction in fluidity leads increased membrane stiffness. It also increases acyl chain ordering, which leads to membrane thickening above the phase transition temperature and membrane thinning below (Ipsen et al., 1990). The saturation of the lipid acyl chains can change this behavior and hence in general, cholesterol effects have to be assessed carefully in each case (Nomura et al., 2012).

Membrane composition. All organisms strive to keep their membranes in the liquid-crystalline L_α phase (also known as liquid-disordered L_d or l_d). Below a critical temperature, bilayers form a solid gel phase (L_β or s_o), which does not allow free lateral movement in the plane of the membrane. The transition temperature depends on the type of lipid, the length and saturation of the acyl chains, and the composition of the membrane (van Meer et al., 2008).

The phospholipid composition of some typical membranes are shown in Table 1 (see also e.g. Yorek (1993); Opekarova and Tanner (2003) and the Membrane Protein Lipid Composition Atlas (`http://opm.phar.umich.edu/atlas.php`) for more membranes of different species,

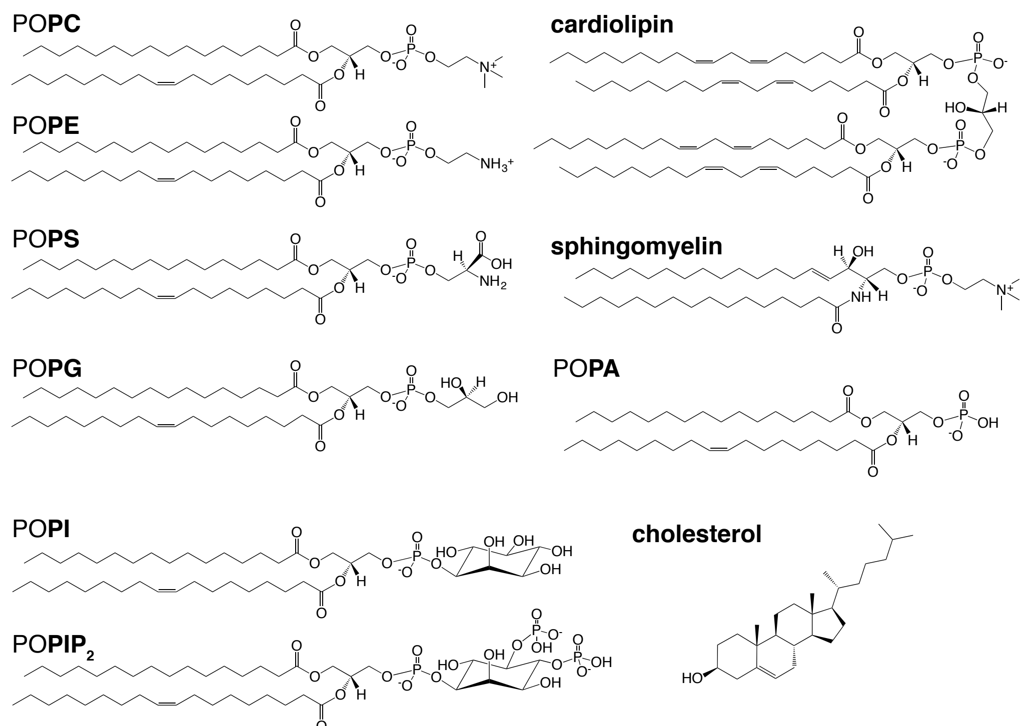

Figure 1: Common types of lipids in biological membranes. A range of phospholipids are shown with the glycerol group acylated with a palmitoyl (16:0, abbreviated as P) and oleoyl (18:1(9Z), O) group in the sn1 and sn2 position. The headgroups are abbreviated as PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIP_2, phosphatidylinositol 4,5-bisphosphate; PA, phosphatidic acid.

Table 1: Phospholipid composition of selected membranes.

organism	membrane/ tissue	% of total phospholipid[a] PC	PE	PI	PS	SM	CL	PG	PA	other	reference
E. coli	plasma[b]	–	75	–	–	–	5	20	–	–	Raetz (1986)
	outer	–	45[†]	–	–	–	–	–	–	55[†]	Raetz (1986)
B. subtilis	plasma	–	12	–	–	–	4	70	–	14	Clejan et al. (1986)
C. glutamicum	plasma, 10°C	–	–	24	–	–	17	53	2	4	Ozcan et al. (2007)[c]
	plasma, 30°C	–	–	33	–	–	25	35	3	4	Ozcan et al. (2007)[c]
	plasma, 40°C	–	–	20	–	–	57	13	2	4	Ozcan et al. (2007)[c]
S. cerevisiae	plasma[d]	17	20	18	34	–	0	–	4	7	Zinser et al. (1991)
	inner mitoch.	40	24	16	4	–	16	–	2	–	Zinser et al. (1991)
	outer mitoch.	46	33	10	1	–	6	–	4	–	Zinser et al. (1991)
spinach[e]	leaves	47	17	9	–	–	1	26	–	–	Wintermans (1960)
corn[f]	leaves	26	14	7	–	–	14	28	–	11	Roughan and Batt (1969)
rabbit[e]	sarcolemma	39	30	7	6	6	1	–	–	6	Philipson et al. (1980)
human[g]	muscle	48	26	9	3	4	5	–	1	3	Yorek (1993)
	heart	22/17[*]	14/12[*]	4	3	6	15	–	–	7	Yorek (1993)
	brain	34	13/20[*]	3	11	14	2	–	–	3	Yorek (1993)
	erythrocytes	31	19/9[*]	1	13	24	2	–	–	1	Yorek (1993)

[a] PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; CL, cardiolipin (diphosphatidylglycerol); PG, phosphatidylglycerol; PA, phosphatic acid; LPS, lipopolysaccharide [b] exponentially grown cells; stationary phase cells are enriched in CL (Cronan, 1968, 2003)
[c] values were estimated from Fig. 6 in Ozcan et al. (2007)
[d] Sterols are the overall majority component, ergosterol:phospholipid 3.31:1 mol/mol (Zinser et al., 1991).
[e] *Spinacea oleracea* [d] *Zea mays* [g] *Oryctolagus cuniculus* [h] *Homo sapiens* [*] plasmalogen (PC/choline plasmalogen or PE/ethanolamine plasmalogen) [†] Total *E. coli* outer membrane contains about 50% lipopolysaccharide (LPS); the inner leaflet consists of about 90% PE while the outer leaflet contains almost exclusively LPS (Raetz, 1986; Yorek, 1993).

organelles and tissues). Bacterial membranes are often rich in PE and PG lipids and contain cardiolipin (CL), which otherwise is mostly found in the inner membrane of mitochondria. Eukaryotic and in particular mammalian membranes tend to be rich in PC lipids, with various compositions of PE, SM, PS, and PI. Highly charged anionic lipids such as the phosphoinositide PI(4,5)P_2 (PIP_2) occur at a level of only about 1% in the phospholipids of eukaryotic membranes but have been shown to have profound effects on transport proteins, as will be discussed below.

Some mammalian membranes such as ones in the nervous system also contain significant levels of plasmalogens, which are glycerophospholipids that attach an alkyl chain at the sn-1 position through a vinyl-ether linkage instead of an ester-linked fatty acid (Braverman and Moser, 2012).

Thermo-elastic properties. Stiffness and fluidity are determined by the membrane composition and external variables such as temperature and pressure.

Pressure profile. The pressure profile along the membrane normal is dominated by the attractive, contracting interactions between the headgroups (including water and ions) and the volume-

exclusion entropic repulsion of the acyl tails (Cantor, 1999).

Hydrophobic thickness. Bulk bilayer properties such as the thickness has been shown to affect transport properties. For example, the activity of the branched-chain amino acid transport system of *Lactococcus lactis* depends on the acyl chain length of the lipids (in 't Veld et al., 1991). Phillips et al. (2009) review the energetics of protein-induced deformations of the membrane, in particular for the mechanosensitive channels. Andersen and Koeppe (2007) review how the bilayer thickness changes the conformational energy landscape of membrane proteins and discuss the lipid bilayer as an allosteric regulator of protein function.

Intrinsic curvature. The intrinsic curvature is the ratio of the diameter of a head group with respect to the tail group of a lipid. One example is when a lipid has a PS or PE headgroup, which are smaller, and a diacyl tail group the overall bilayer will have a negative intrinsic curvature. Brown (2012) and Soubias and Gawrisch (2012) review the importance of balance between curvature and hydrophobic forces in lipid-protein environment. They emphasisizes how intrinsic curvature (e.g. long-range lipid-protein interactions) can affect membrane protein folding, stability, and the ability to transition between conformations. The importance of membrane intrinsic curvature on protein function has been shown to be especially true in the case of GPCR proteins (Botelho et al., 2006; Soubias et al., 2010).

Charge density. Anionic phospholipids such as PS or PI are often enriched in the inner leaflet of membranes, thus leading to a negative charge density. It is likely an oversimplification to assume a homogeneous distribution as microscopic techniques have revealed clusters of PI(4,5)P_2 and/or PS in small membrane domains (van den Bogaart et al., 2011; Fairn et al., 2011). The local surface charge density will influence the targeting of whole polycationic proteins to membrane (Fairn and Grinstein, 2012) and the interaction of cationic domains of proteins with the membrane. The charge density of the lipid headgroup can also influence the local concentration of free ions near the membrane and embedded proteins as well as shift the local pH of the bilayer surface.

3. Lipid-protein interactions

As will be discussed below, the lipid environment affects the stability of a membrane protein and even protein topology, i.e. in which direction a protein is inserted into the membrane. The types of protein-lipid interactions that must be ultimately responsible for lipid-dependence of protein stability and function can be broken down into two different categories: physical properties of the lipid membrane and site-specific interactions.

3.1. Physical properties of the membrane

The physical properties of the membrane can affect membrane proteins through non-specific interactions. Specific changes in lipids alter bulk membrane properties such as the fluidity, bending moduli, or charge density. The membrane environment of a transmembrane protein is thus viewed as a continuum that affects the protein in a mean field manner.

The physical properties of the lipid membrane are an important part of the environment for membrane proteins, especially for integral membrane proteins such as channels and transporters. Properties such as the chemical composition of headgroups, bilayer thickness, surface tension, curvature, or the voltage gradient due to membrane-associated ions can all influence the structure

and mobility of a membrane protein. These effects are non-stochiometric in nature, i.e. they do not depend on a specific lipid-to-protein ratio.

For example, functional reconstitution of the purified, delipidated bacterial (*E. coli* and *B. subtilis*) SecYEG translocase requires anionic phospholipids for activity (van der Does et al., 2000). Non-bilayer lipids such as PE or dioleoylglycerol stimulated translocation, unlike bilayer-forming PE derivatives, suggesting that the lipid shape instead of the amino group of PE is a deciding factor in activating SecY. Highest activity (ca 75% of experiments with native bacterial membranes) was observed for lipid mixtures similar to the native bacterial membranes (30% DOPG and 70% DOPE for *E. coli* and 70% DOPG and 30% DOPE for *B. subtilis*). The requirements of high concentrations of specific lipids and a fixed membrane composition suggests that in this case physical bilayer properties such as the lateral pressure profile or hydrophobic thickness affect the conformations accessible to the active translocase.

3.2. Site-specific interactions

The site-specific interactions are comprised of direct lipid binding at specific locations with transmembrane protein. When considering specific lipid-binding sites, it is important to distinguish the locations where lipid binding occurs and the ratio of bound lipids required for a transmembrane protein to transport its substrate across the bilayer. There are two types of bound forms of a lipid molecule: the annular sites located on the transmembrane protein surface creating a shell of lipid molecules around the protein and the non-annular sites located between transmembrane helices (Lee, 2011). The binding of annular lipids depends on both the length of fatty acid acyl chain and the structure of the polar headgroup region in hotspot regions of the transmembrane protein. The length of the acyl chain distorts the hydrophobic matching or mismatching between the protein and the surrounding bilayer (Cybulski and de Mendoza, 2011), e.g. the role of bilayer thickness on membrane protein function. The structure of the polar headgroup is involved with the binding anionic lipids to hotspot regions on transmembrane proteins. For example, the binding of anionic lipids to non-annular sites on membrane proteins such as the potassium channel KcsA can also be important for function (Marius et al., 2005). In the case of KcsA, it is suggested that the packing preference of transmembrane alpha-helices creates a structure that matches well with the surrounding lipid bilayer, thus allowing lipid and protein to meet without either having to change much. In addition, the intrinsic curvature of the bilayer influences the binding of annular lipids. In the case of rhodopsin, the rhodopsin-membrane environment preferentially accommodates lipids with a PE head group and docosahexanoyl acyl (DHA) tail groups, which introduces a negative intrinsic curvature into the bilayer. Experimental studies have shown that rhodopsin is stabilized by establishing hydrogen bonds with the PE head group and that the transition between different conformational states of rhodopsin is influenced by the headgroup (Botelho et al., 2006; Soubias et al., 2010). Simulations have shown that polyunsaturated DHA tails modulate the direct protein-lipid interactions (Feller et al., 2003; Grossfield et al., 2006).

3.3. Stability and Topology of Membrane Proteins

Phospholipids stabilize the membrane protein structure (Hunte and Richers, 2008). Both functional reconstitution of detergent-extracted membrane proteins and crystallization typically require that a number of lipids stay attached to the protein; finding less harsh detergents that avoid delipidation are an important means to increase the chances of crystallizing membrane proteins (Sonoda et al., 2011). Many crystal structures contain at least parts of annular phospholipids,

indicating that sites exist to which lipids can bind, although typically with low affinity. Tables 2 and 3 collect some examples of channels and transporters that have been co-crystallized with lipids or lipid-like detergent molecules.

The orientation of membrane proteins depends on the anionic lipid content of the membrane (van Klompenburg et al., 1997). Positively charged protein loops are preferably located on the inside of the cell, presumably due to favorable electrostatic interactions (in addition to the negative membrane potential) that bias insertion in according with the positive-inside rule (von Heijne, 1986, 2006).

4. Ion channels and water pores

The cell membrane forms a hydrophobic barrier to polar and charged molecules and ions. Transport requires specialized integral membrane proteins that provide a pathway across the membrane. Ion channels are membrane proteins that allow passive diffusion of ions down their concentration gradient through an aqueous pore. They are typically selective and can be switched between a conducting and a non-conducting state by external signals, a process termed "gating". With the patch clamp technique it is possible to measure ion conductance and gating at the single molecule level so that the effect of the membrane on transport can be accurately quantified.

This sections updates our understanding of the effects of membrane lipids on ion channel structure and function (Tillman and Cascio, 2003), in particular with some of the more recent findings on the role of PIP_2 on ion channels Tucker and Baukrowitz (2008) and our insights into modulation of mechanosensitive (Martinac, 2011) and ligand gated ion channel (Baenziger and Corringer, 2011) function by lipids.

4.1. Potassium channels

K^+-channels are responsible for transporting potassium across the lipid bilayer to regulate functions such as secretion of neurotransmitters, heart beat, skeletal muscle contraction, and transpiration in plant leaves. Potassium channel function is known to be mediated by the surrounding lipid environment and by specific channel-lipid interactions. Atomic resolution crystal structures are available for many of the important classes of potassium channels, facilitating, in conjunction with single channel electrophysiology, an understanding of the underlying molecular mechanisms.

4.1.1. KcsA

The original structure of the KcsA K^+-channel was solved with bound detergent, diacyl glycerol (Zhou et al., 2001). The headgroup of the detergent indicated that a anionic phospholipid might necessary for channel function (Zhou et al., 2001). One of the first dynamic studies of KcsA-lipid dependence was performed using the experimental fluorescence technique of quenching Trp by brominated phospholipids to delineate changes in bilayer thickness and annular, short-range protein-lipid interactions (Williamson et al., 2002). The Trp residues on KscA are located near the membrane surface, which makes it ideal studying the effects of lipid bilayer thickness using fluorescence of brominated phospholipids. Williamson et al showed that Trp was located within a very hydrophobic environment with a bilayer thickness of 37 Å. They also showed that the strongest lipid binding occured using di(C22:1)PC with a relative binding constant of 1.20±0.06. The lipid bilayer thickness determined by Williamson et al was consistent with the Zhou et al KcsA x-ray structure that contained the modeled as a diacylglycerol

Table 2: Experimental structures of ion channels and pores with either detergent or lipid bound.

Protein	PDB ID	Bound Lipid or Detergent
KcsA H^+-gated K-channel	1K4C; 3IGA	lauryl dimethylamine-N-oxide
K_v1.2-K_v2.1 chimera	2R9R	phosphatidylglycerol
K_v2.1-K_v1.2 chimera	3LNM	phosphatidylglycerol
K_{ir}2.2 inward-rectifier K-channel	3SPI; 3SPG	PIP_2
K_{ir}2.2 inward-rectifier K-channel	3SPC	dioctanoylglycerol pyrophosphate
K_{ir}3.1 prokaryotic K_{ir} channel chimera	2QKS	β-nonylglucoside
bacterial cyclic nucleotide-regulated channel	3BEH	lauryl dimethylamine-N-oxide
voltage-gated Na channel	3RVY; 4EKW; 4DXW	phosphocholine
SLAC1 anion channel, TehA homolog	3M71	β-octylglucoside
GLIC prokaryotic pentameric ligand-gated ion channel	3EAM; 3P50	phosphatidylcholine
GluCl anion-selective receptor	3RHW	dodecyl-beta-d-maltoside
AQP0	1YMG	β-nonylglucoside
AQP0	2B6O	phosphocholine
AQP0	3M9I	phosphoethanolamine
AQP1	1J4N	β-nonylglucoside
AQP4	2ZZ9	phosphatidylethanolamine
AQP4	3GD8	β-octylglucoside
AQPZ	1RC2	β-octylglucoside
AQPZ	2ABM	phosphatidylethanolamine, phosphatidylglycerol
AQPZ	3NK5	β-octylglucoside
GlpF glycerol facilitator channel	1FX8; 1LDF	β-octylglucoside
aquaglyceroporin	3C02	β-octylglucoside
P. pastoris aquaporin, Aqp1	2W2E	β-octylglucoside
pentameric formate channel	3KLY	β-octylglucoside
AmtB ammonia channel	1U7G; 2NS1	β-octylglucoside
N. europaea Rh protein	3B9W	β-octylglucoside
Rh C glycoprotein ammonia transporter	3HD6	β-octylglucoside

Table 3: Transmembrane transporter protein crystal structures with either detergent or lipid bound.

Protein	PDB ID	Bound Lipid or Detergent
MexB bacterial multi-drug efflux transporter	2V50	dodecyl-β-D-maltoside
leukotriene LTC4 synthase	2PNO	dodecyl-β-D-maltoside
leukotriene LTC4 synthase	2UUH	dodecyl-α-D-maltoside, palmitic acid, palmitoleic acid
FucP, MFS transporter	3O7Q	β-nonylglucoside
sodium/calcium exchanger NCX	3V5U	1-oleoyl-R-glycerol
concentrative nucleoside transporter	3TIJ	decyl-β-D-Maltopyranoside
UraA uracil/H^+ symporter	3QE7	β-nonylglucoside
AdiC arginine:agmatine antiporter	3L1L	β-nonylglucoside
LeuT leucine transporter	2A65; 2QEI; 3F3A; 2QJU; 3GJD; 3MPN; 3QS4	β-octylglucoside
LeuT leucine transporter	3TT1	2-hydroxymethyl-6-octylsulfanyl-tetrahydro-pyran-3,4,5-triol
BetP betaine transporter	4DOJ	phosphatidylglycerol
aspartate transporter	2NWL	palmitic acid
mitochondrial ADP/ATP carrier	1OKC	3-laurylamido-N,N'-dimethylpropylaminoxyde, 1,2-diacyl-sn-glycero-3-phosphocholine
apical sodium-dependent bile acid transporter $ASBT_{NM}$	3ZUY	phosphatidylethanolamine, lauryl dimethylamine-N-oxide
Riboflavin transporter	3P5N	β-nonylglucoside
thiamin transporter	3RLB	β-nonylglucoside
MalFGK2-MBP maltose uptake transporter	3PV0; 3RLF	phosphatidylglycerol

(Williamson et al., 2002; Zhou et al., 2001). While the optimal membrane thickness was determined, the chemical nature of K^+ channel-phospholipid binding was yet to be understood, e.g. a zwitterionic phosphatidylcholine headgroup with a net neutral charge differs significantly from the phosphatidylglycerol headgroup that has a net negative charge.

An additional fluorescence study was used to determine the chemical preference of the headgroups within the vicinity of the K^+ channel (Marius et al., 2005). They again used the method of Trp quenching by brominated phospholipids, however this time they used it to determine the binding constants for both annular and non-annular lipids. They showed that annular lipid binding sites have a similar binding affinity for anionic lipids versus phosphatidylcholine lipids on the extracellular side of KcsA. In contrast, the intercellular lipid binding affinity is structurally specific as the phosphatidylglycerol binds about three times stronger than the phosphatidylcholine. This is consistent with structure of KcsA as Arg residues reside near the location of the phospholipid headgroup and if the headgroup where phosphatidylcholine it would cause a repulsion resulting in poor lipid packing around the channel. As for the non-annular lipids, Marius et al showed that KcsA exhibits a remarkable selectivity for anionic lipid (Marius et al., 2005). However, it was unknown if it was required all four non-annular sites of the tetramer to be occupied by anionic lipid in order for the channel to open.

In 2008 using fluorescence quenching methods, Marius et al. (2008) showed that only three of the four non-annular sites were required to have anionic lipid bound in order for channel conductance to occur. Moreover, they showed that by increasing the concentration of PG in the bilayer the non-annular binding sites exhibit a weak binding-constant of 0.42 ± 0.06 (in units of mol fraction) for POPG and KcsA (Marius et al., 2008). These studies paved the way for more recent studies, which could begin to characterize the structural specificity of the non-annular sites. Deol et al. (2006) used molecular dynamics simulation to show two arginine residues, Arg-64 and Arg-89, to preferentially interact with and stabilize the phosphatidylglycerol molecule located in the non-annular binding sites by forming hydrogen bonds.

Another method used to understand the non-annular lipid interactions is electron paramagnetic resonance (EPR). For instance, the EPR spectra of nitroxide-labeled lipids can be used to provide information that distinguishes between lipid molecules in the bulk bilayer and lipids associated directly with membrane protein. Bolivar et al. (2012), using a combination of fluorescence quenching experiments and EPR experiments with nitroxide-labeled lipids, showed upon mutating these two arginines to leucines that no change occurs in binding affinity within the non-annular sites. This suggests that these residues are not involved in direct interaction with the lipid. The hydrogen bonding of the NH-group of the peptide bond between residues Thr-85 and Leu-86 was proposed to stabilize non-annular lipids (Bolivar et al., 2012).

4.1.2. Voltage-gated K^+-channels

While the KcsA channel requires anionic non-annular lipids to function, the question remained if voltage-gated potassium channels (K_v) also exhibit similar characteristics. In 2007, two crystallographic structures were solved of chimeric K_v channels (Long et al., 2007). In these structures, the K_v channels have a single non-annular phosphatidylglycerol bound to each of the monomeric units (Long et al., 2007). However, at the time of this review, it has not been established experimentally or computationally whether K_v channels require all four bound lipids to be fully functional.

Interestingly though, recent studies have also shown that some K_v channels depend on PIP_2 for their function. Kruse et al. (2012) showed that phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$)

depletion inhibited $K_v7.1$ and $K_v7/2/7.3$ channel current by more than 90%; in contrast to previous work (Decher et al., 2008; Oliver et al., 2004) they did not observe such PIP_2-dependence in other members of the K_v family (Kruse et al., 2012).

4.1.3. Inward rectifier (K_{ir}) channels

Probably the best example of direct protein-lipid interactions is provided by PIP_2 controlling channel function, where PIP_2 takes on the role of a signalling molecule Hilgemann et al. (2001); Gamper and Shapiro (2007). Here we discuss the inward rectifying potassium (K_{ir}) channels as an example; further channels interacting with PIP_2 are reviewed by Hilgemann et al. (2001) and Gamper and Shapiro (2007).

The family of K_{ir} channels play a role in the regulation of many diverse physiological processes such as membrane excitability, heart rate, vascular tone, insulin release, salt flow, intracellular pH and regulate cell factors such G proteins, phosphatidylinositol 4,5-bisphosphate, and ATP, For the K_{ir} channels, the gating transition is regulated both by pH and the lipid ratio near the channel. The phosphatidylinositol-(4,5)- bisphosphate (PIP_2) binds to the K_{ir} channel and induces pore opening in all known eukaryotic K_{ir} channels (Bichet et al., 2003; Tucker and Baukrowitz, 2008). Using patch clamp, previous experimental studies showed that the depletion of PIP_2 in the membrane lead to a loss of channel activity (Baukrowitz and Fakler, 2000; Kobrinsky et al., 2000; Cho et al., 2005) but the exact site where K_{ir} channel binds PIP_2 remained unknown. Rapedius et al. (2007) showed that $K_{ir}1.1$ channels with a K80V mutation that established a H-bond at the TM1-TM2 helix-bundle crossing dramatically altered the kinetics of PIP_2 activation without affecting PIP_2 binding affinity using Xenopus oocytes and patch clamp experiments. This study was one of the first to determine the apparent PIP_2 binding affinity and predict the vicinity of the PIP_2 binding region on the K_{ir} channel (Rapedius et al., 2007). Thereafter, Nishida et al solved the chimeric $K_{ir}3.1$/K_{ir}Bac1.3 channel structure (Nishida et al., 2007) with a detergent molecule bound to K_{ir} channel. This structure was compared to previous data taken from patch-clamp data for $K_{ir}4.1$/$K_{ir}5.1$ channels and original Rapedius et al homology model to observe the PIP_2 association and helix-bundle crossing behavior (Rapedius et al., 2007). The detergent site was in good agreement with previous studies (Rapedius et al., 2007).

Stansfeld et al. (2009) predicted and identified the actual PIP_2 binding site of the K_{ir} channel using a combination of computational homology modeling, coarse-grained and all-atom MD and experimental mutagenesis data (Fig. 2A). Using the last 0.25 microseconds from their all-atom MD simulations and multi-scale modeling techniques, they predicted that PIP_2 forms H-bonds and binds with the R50, R54, K67, R176, and R206 of the $K_{ir}6.2$ channel; they also observed similar PIP_2 binding behavior for the $K_{ir}1.1$ and $K_{ir}3.1$-K_{ir}Bac1.3 chimeric channels of similar residues aligned with those of the $K_{ir}6.2$ channel (Stansfeld et al., 2009). From these results, it is difficult to make direct quantitative comparisons for this predicted with respect to the lipid binding site of the Nishida et al structure as the structure contains bound detergent molecules (Nishida et al., 2007; Stansfeld et al., 2009). The predicted simulation binding site and the crystal structure detergent binding site were close, as the headgroup of the simulated PIP_2 molecule was laterally displaced within 5 Å in comparison to the detergent headgroup. Hansen et al. (2011) determined a structure of $K_{ir}2.2$ with the PIP_2 bound to both the wild-type and R186A mutant (Fig. 2B). This structural binding site for the PIP_2 lipid is similar, within 2 Å, to the Stansfeld et al simulation PIP_2 binding site (Hansen et al., 2011; Stansfeld et al., 2009).

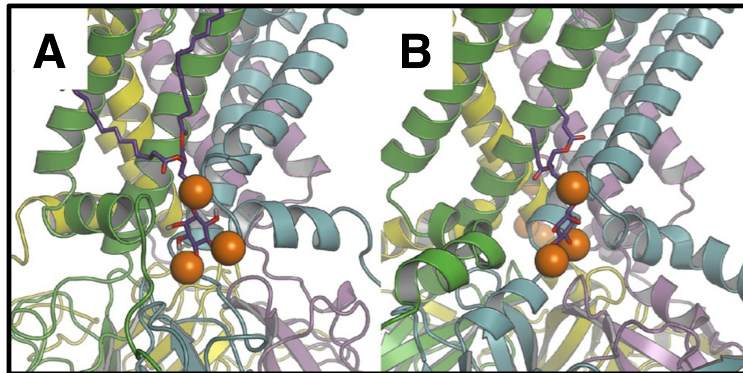

Figure 2: Binding of PIP_2 to the K_{ir} channel. **A**: Predicted binding pose from multi-scale MD simulations (Stansfeld et al., 2009). **B**: Interaction of PIP_2 with the channel in the X-ray crystal structure (Hansen et al., 2011); not all atoms in the acyl chains are resolved. The phosphate atoms are highlighted in orange. (The figure was kindly provided by Dr Phillip Stansfeld)

4.2. Mechanosensitive channels

Mechanosensitive (MS) channels sense changes in membrane tension and enable bacteria to grow under osmotic stress or are important in sensing pressure or gravity in higher organisms (Hamill and Martinac, 2001). Eukaryotic mechanosensitive channels are typically K^+-channels that are sensitive to polyunsaturated fatty acids, mechanical deformation of the membrane, and temperature changes. Their molecular mechanisms are not yet well understood although a recent recent crystal structure of the lipid/mechanosensitive TRAAK (TWIK-related arachidonic acid-stimulated K^+) channel suggests that an amphipathic helix with conserved basic residues could act as a lipid sensor (Brohawn et al., 2012). Much more data are available for bacterial channels. They undergo large structural rearrangement in response to mechanical forces conveyed by proteins or the membrane (Martinac, 2011). The interaction of the membrane with the channel can be well described by continuum models of the membrane (Wiggins and Phillips, 2005)

There are two well-studied bacterial MS channels, MscL and MscS, and the lipid environment surrounding the MS channel has been known to influence channel function. For example, MscL preferentially binds anionic lipids and these anionic lipids cluster near a group of positively charged residues close to the lipid-water interface (Powl et al., 2005). Moreover, Powl et al have shown that the periplasmic side of the channel exhibits very little selectivity for anionic phospholipids while on the cytoplasmic side of the channel three positively charged residues, Arg-98, Lys-99, and Lys-100, have a preferential binding affinity for anionic lipids (Powl et al., 2005, 2008). This preference also is higher for phosphatidic acid over zwitterionic lipid such as phosphatidylcholine and thus changes in the heterogeneity of the bilayer lipid composition affects channel flux (Powl et al., 2005, 2008). The presence of anionic lipid increases the rate of flux of small molecules through the mechanosensitive channel MscL (Powl et al., 2008). Powl et al also investigated the affects of lipid chain length on channel function by measuring the quenching of the fluorescence of Trp residues in MscL (Powl et al., 2007). They showed that acyl chain-length dependence of lipid binding was different on the two sides of MscL and that the cytoplasmic side exhibited a greater dependence on chain-length than the periplasmic side. (Powl et al., 2007). The chain-length was also independent of the molar ratio of lipid/MscL Thus

based on these previous studies (Powl et al., 2005, 2007, 2008) , it suggests that MS channel function is modulated more by direct interaction with the annular phospholipid.

Nomura et al. (2012) studied co-localized MscL/MscS channels in PE:PC liposomes and found that their proximity decreased the threshold tension for opening. Reducing the membrane thickness by varying the acyl chain lengths of the lipids decreased the threshold for MscL more than for MscS, an effect attributed to the fact that the closed-to-open transition of MscL requires a larger conformational change than that of MscS. Conversely, addition of cholesterol increases the activation threshold of MscS much more than that of MscL. Cholesterol increases bilayer stiffness and thickness. The stiffening might impede the MscS conformational change more than the increase in thickness with resulting hydrophobic mismatch affects MscL.

4.3. Aquaporins

Aquaporins (AQP) play a role in the transport of water, small linear alcohols, and gases across the membrane. AQP not only serves as a water pore (channel without any gating functionality) but transports gases across the bilayer as well as acting as an adhesion molecule forming junctions between bilayer surfaces. Aquaporins function both as individual momomers and as a tetramer in the membrane. Aquaporin function has been shown to be fairly insensitive to membrane composition (Zeidel et al., 1994) but the results on aquaporins discussed below establish a promising approach to investigate the general principles underlying lipid-protein interactions.

It has been difficult to determine the specific lipid mediated interactions with AQP or lipids associated specifically with channel function. The initial structures of AQP, specifically the human aquaporin-1, were solved using two-dimensional electron crystallography and did not contain bound lipids (Murata et al., 2000; Ren et al., 2001). This methodology provided insight into the tetrameric architecture and folding of AQP while also characterizing its position within the hydrophobic bilayer environment. These studies also showed that AQP1 could form well-ordered 2D crystals using different lipid environments (Murata et al., 2000; Ren et al., 2001).

Based on the initial experimental data, initial MD simulations of AQP1 were undertaken, placing the channel in either a pure palmitoyl-oleoyl-phosphatidyl-choline (POPC) or a pure palmitoyl-oleoyl-phosphatidyl-ethanolamine (POPE) lipid bilayer environment. These studies confirmed that AQP could function in different bilayers and suggested that there were several major interactions between AQP amino acids and the headgroups of annular lipids (Zhu et al., 2001; Törnroth-Horsefield et al., 2006; Tajkhorshid et al., 2002, 2003; Jensen et al., 2001, 2003; Zhu et al., 2004; de Groot and Grubmüller, 2005). These interactions included salt bridging of Arg or Lys residues with the negatively charged phosphate of the lipids or direct hydrogen bonding of Ser or Asn sidechains with lipid headgroup.

In 2005 the 2D crystals of AQP0 further characterized the tetramer as well as identified the nature of the lipids surrounding the aquaporin. The lipids were modeled as complete or partial dimyristoyl phosphatidylcholine molecules with nine lipids associated with each AQP0 monomer (Gonen et al., 2005). Furthermore, the lipids bridged all contacts between the AQP tetramers, and were characterized as annular lipids that mediate lattice interactions. Gonen et al also hypothesized that AQP0 mutations associated with trafficking might actually prevent proper protein-lipid packing and thus influence the folding or integration of AQP within the bilayer. In 2009, the AQP4 structure was crystallized also with 2D electron crystallography and annular phosphatidylethanolamine lipids were resolved within the structure (Tani et al., 2009). The structure showed five PE bound lipid molecules to each monomeric unit. The lipids are located on the extracellular leaflet and hydrogen bond with the Gly61, Pro139, His151, and Asn153 for

the AQP4 channel in the adjoining bilayer. This structure was one of the first observations of a membrane protein directly interacting with lipids in an adjoining membrane (Tani et al., 2009).

Based on these experimental and computational studies several generalizations can be made about AQPs. In general AQPs do not require specific lipids and do not exhibit high-affinity lipid binding sites, thus allowing for a more heterogeneous lipid composition to drive the overall curvature necessary to mediate junction contacts. As none of the AQP structures with resolved lipids has been solved within a three-dimensional crystal environment and the current 2D electron crystallography structures contain different lipid types, the current hypothesis is that AQPs do not require specific lipids to function or contain high-affinity lipid-binding sites. This non-specific lipid-protein interactions indicates that the heterogeneous lipid composition surrounding the channel provides the bilayer curvature necessary to mediate AQP-based junction contacts and overall channel function. The 2D electron crystallographic structures provide only a snapshot in time and the question remains are these structures representative of the exact binding site adopted by unconstrained lipids surrounding the channel?

Recently, Aponte-Santamaria et al used MD simulations to determine the position and orientation of dimyristoyl-phosphatidylcholine (DMPC) lipids surrounding an AQP0 channel (Aponte-Santamaría et al., 2012). After 100 ns, they demonstrated that the 2D electron crystallographic structures do appropriately describe the behavior and packing of annular lipids surrounding the AQP tetramer by constructing a 3D time-averaged lipid-density map. One use of the simulation generated 3D lipid-density map may be to refine the lipid tail structures in the 2D electron crystallography structure of AQP1. Moreover, from the 3D lipid map, Aponte-Santamaria et al suggest that the property that defines the DMPC lipid position around AQP0 is the behavior of the acyl chains of the surrounding lipid not the head group (Aponte-Santamaría et al., 2012). They also identified seven residues at the AQP0 surface by monitoring the potential energy function during the simulation. Based on those calculations the following residues are believed to interact with lipids electrostatically and through van der Waals contacts: Arg5, Tyr105, Arg113, Arg196, Lys238, Trp10, and Trp202 (Aponte-Santamaría et al., 2012). The residues only form transient bonds, e.g. the residues do not form hydrogen bonds or contacts with a single specific lipid but rather any lipid within its immediate vicinity.

4.4. Pentameric ligand gated ion channels

The pentameric ligand gated ion channels (pLGIC) are a group of cation or anion selective ion channels that are involved in fast electro-chemical synaptic transmission (Taly et al., 2009). As summarized by Baenziger and Corringer (2011), the nicotinic acetylcholine receptor (nAChR) does not tolerate delipidation during purification and is exceptionally sensitive to its lipid environment, requiring both anionic lipids such as PS or PA and neutral lipids such as cholesterol. TM helix M4, which juts out into the membrane, is likely to play a role in receptor-lipid interaction. The C-terminal end of M4 can interact with $\beta6$–$\beta7$ loop, which couples the extracellular ligand binding domain to the transmembrane domain with the ion channel gate. Lipid molecules were seen near M4 in the crystal structure of the bacterial homolog GLIC (Bocquet et al., 2009) (Table 2), suggesting that the conformation of the M4-lipid sensor could be modulated by specific lipid interactions or global membrane properties (Baenziger and Corringer, 2011).

5. Transporters

Ion channels and pores facilitate passive diffusion across the membrane. Transporters use a source of free energy to catalyze active translocation of substrates. Primary transporters hy-

drolyze ATP to ADP to power transport. Most secondary transporters couple energetically uphill substrate movement to the downhill flow of ions stored in the transmembrane Na^+ or proton gradient. Transporters cycle through a range of conformations in order to move substrate across the membrane. The change in protein shape affects the protein-membrane interface and thus it is plausible that the bilayer could have an effect on transport (Andersen and Koeppe, 2007).

This section reviews a range of primary and especially secondary transporters for which lipid-protein interactions have been demonstrated to modulate transport. The transporters are systematically grouped by their transporter classification database (TCDB, `http://www.tcdb.org/`) numbers (Saier et al., 2006, 2009).

In many cases, we do not yet understand the molecular mechanisms by which lipids exert their effect on transporters although, as in the case of ion channels, the spectrum appears to range from indirect to very specific interactions. PIP_2, for instance, has been shown to specifically interact with primary and secondary transporters (Hilgemann et al., 2001; Gamper and Shapiro, 2007).

5.1. Ion pumps

5.1.1. Ca^{2+}-ATPase

The plasmalemmal calcium pump (PMCA) of the erythrocyte membrane is a P-type ATPase (Palmgren and Nissen, 2011) that catalyzes Ca^{2+} efflux and is responsible for keeping the intracellular calcium concentration low. It is physiologically activated by calmodulin but it can also be switched to the same high affinity state for Ca^{2+} by anionic lipids (PS, CL, PI, PA, PIP_2) (Niggli et al., 1981; Enyedi et al., 1987). Neutral phospholipids (PC, SM, PE) had no effect. Because anionic lipids had to be at least 40% of the total phospholipids to have an effect, a concentration rarely encountered in mammalian membranes, it is unlikely that PMCA is regulated by anionic lipids *in vivo*. It has been hypothesized that lipids affect the conformation of the C-terminal calmodulin-binding autoinhibitory domain and thus mimic the effect of either calmodulin binding or trypsin-cleavage of the domain (Niggli et al., 1981). The number of annular lipids of yeast PMCA4 changes from 29 in the autoinhibited state to 14 in the activated state in the presence of either calmodulin or PS (Mangialavori et al., 2012), suggesting that the activated state is more compact inside the membrane. A number of crystal structures of the Ca^{2+}-ATPase of sarcoplasmic reticulum (SERCA) have been published (reviewed in Palmgren and Nissen (2011); Toyoshima and Inesi (2004)) but as they do not contain the calmodulin domain it is difficult to gain insight into the proposed action of anionic lipids.

Unlike PMCA, SERCA was inhibited by PS and PA when the mole fraction of anionic lipid in a PC membrane exceeded 60% (Dalton et al., 1998). Specifically, maximal levels of binding of ATP is reduced below 40% of the native membrane values, without a concomitant reduction in affinity. Anionic lipids did not bind specifically to the protein so one possible explanation offered was that oligomerization of ATPases renders MgATP binding sites inaccessible (Dalton et al., 1998). Complex effects of chain length (and thus membrane thickness) have also been demonstrated, independent of the inhibitory effects of anionic lipids (Dalton et al., 1998).

The structural studies showed that P-type ATPases undergo a large conformational change between the E1 (high Ca^{2+} affinity) and the E2 (low affinity) state (Palmgren and Nissen, 2011; Toyoshima and Inesi, 2004) and hence lipids can influence transport by interfering with the conformational rearrangements as seen in the two following examples.

The Ca^{2+}-free structure of SERCA (Obara et al., 2005) has a PE molecule modelled in between transmembrane helices M2 and M4. Lee (2011) suggest that PE acts as a wedge to inhibit

movements of the helices relative to each other and so explain some of the inhibitory effects of PE on SERCA (Starling et al., 1996).

The rate limiting step of transport of SERCA is modulated by bilayer fluidity (Squier et al., 1988). The experiments showed that rotational mobility of the membrane protein was directly dependent on membrane fluidity. The apparent Arrhenius activation energy of calcium-dependent ATPase activity doubled once the membrane fluidity fell below a threshold value.

5.1.2. Na^+,K^+-ATPase

Cardiac sarcolemmal Na^+,K^+-ATPase (3.A.3.1), another P-type ATPase (Palmgren and Nissen, 2011), is responsible for establishing the Na^+ gradient across the membrane that serves as the energy source for many secondary transporters; around 20% of the cell's energy is spent on powering the Na^+,K^+-ATPase, with up to 60% in brain (Rolfe and Brown, 1997).

The pump was only active when reconstituted in into PC:PS (30:50 by weight) vesicles with a high cholesterol level (20% by weight) (Vemuri and Philipson, 1989). The requirement for cholesterol was specific because a range of close structural cholesterol analogues were not sufficient for activity. The sterol requirement was lost when the exchanger was reconstituted into PC:CL vesicles. In contrast, sarcoplasmic/endoplasmatic reticular Ca^{2+}-ATPase (SERCA) functioned in either PC:PS or PC:CL proteoliposomes without requiring cholesterol (Vemuri and Philipson, 1989). The molecular basis for these lipids requirements remains unclear.

5.2. Exchangers and cotransporters

5.2.1. Sodium-calcium exchanger (NCX1)

The cardiac Na^+-Ca^{2+} exchanger NCX1 (Reeves and Condrescu, 2008), a member of the Ca^{2+}:cation antiporter (CaCA) family (2.A.19), requires anionic phospholipids for activity (Vemuri and Philipson, 1988). NCX1 exists as a homodimer in the membrane (Ren et al., 2008; John et al., 2011) and recently the structure of a bacterial homolog (NCX from *Methanococcus jannaschii*, NCX_{Mj}) was solved (Liao et al., 2012). The reconstitution of the Na^+-Ca^{2+} exchanger NCX1 transporter into pure PC vesicles showed barely any exchange activity as measured by initial transport rate (Vemuri and Philipson, 1988). Higher activity (about 25% of the value when reconstituted in vesicles from native sarcolemmal membranes) was observed for mixed membranes containing phosphatidylserine (PS), cardiolipin (CL), or phosphatidic acid (each approximately 50% by weight) whereas phosphatidylinositol (PI) and phosphatidylglycerol (PG) showed very little enhancement of activity. Addition of cholesterol (ca. 20% by weight, about 35% mol/mol) elevated transport activity to native-like levels for the former three anionic lipids but not for PI or PG. PC was replaced with PE or SM without any sizable effects. Optimal lipid environments such as PC:PS:cholesterol in a 3:5:2 ratio fully activate the NCX1 transporter and render it insensitive to stimulation by other signals (Vemuri and Philipson, 1988). These results suggest that PS, CL and PA can interact with NCX1 unlike PG and PI, perhaps because the negatively charged moiety of the former lipids is less buried in the headgroup than the one of the latter. Cholesterol appears to be essential at concentrations similar to native sarcolemma of about 40% of the total phospholipid fraction (Philipson et al., 1980).

The lipid requirements of the cardiac sarcolemmal Na^+-Ca^{2+} exchanger mirror the optimum membrane composition found for cardiac sarcolemmal Na^+,K^+-ATPase, with the same specific cholesterol requirement and loss of the cholesterol-dependence in PC:CL vesicles (Vemuri and Philipson, 1989). Thus, sterol/protein interactions can depend on the specific phospholipid environment.

In cardiac membranes, NCX1 is directly stimulated by PIP_2 (Hilgemann and Ball, 1996). The transporter contains a positively charged carboxy-terminal domain that was highlighted as as a possible PIP_2 binding site (Hilgemann and Ball, 1996) and PIP_2 was found bound to the transporter although no specific site could be identified (Asteggiano et al., 2001). NCX1 can be inactivated by decreasing intracellular Ca^{2+} or increasing intracellular Na^+ concentrations (Reeves and Condrescu, 2008). Increased levels of PIP_2 eliminate the inactivation (He et al., 2000; Yaradanakul et al., 2007) and He et al. (2000) showed that PIP_2 could bind to the endogenous XIP region following putative TM helix 5 (Iwamoto et al., 1999; Reeves and Condrescu, 2008; Ren et al., 2010). Additional indirect mechanisms of PIP_2-regulation of NCX1 such as effects on trafficking have also been described (Yaradanakul et al., 2007).

5.2.2. Na^+/HCO_3^- cotransporter

The rat bicarbonate transporter NBCe1-A, a homolog of human SLC4 transporters and member of the Anion Exchanger (AE) family 2.A.32 (Romero et al., 2004; Kurtz et al., 2004; Majumdar and Bevensee, 2010), was shown to be stimulated by PIP_2 (Wu et al., 2009). The mechanism of activation is not known and could either be by direct interaction with a stretch of lysines near the carboxyl terminus or via intermediate signalling molecules, perhaps similar to how inositol 1,4,5-trisphosphate indirectly activates pNBC1 (Shirakabe et al., 2006).

5.2.3. Na^+/H^+ exchanger

The Na^+/H^+ exchanger NHE1 (monovalent cation:proton antiporter-1 (CPA1) family, 2.A.36 (Chang et al., 2004)) is stimulated by PIP_2 (Aharonovitz et al., 2000). Two potential PIP_2 binding motifs (Yu et al., 1992) were found in the carboxyl-terminal domain of the transporter and recombinant domains on their own were shown to bind the lipid in vitro. Mutants lacking the putative PIP_2 binding domain showed substantially reduced transport activity and moreover both sites needed to be simultaneously occupied for optimum activity. One hypothesis for the molecular mechanism suggests that PIP_2 sites 1 and 2 must be positioned closely to the inner surface of the plasma membrane to allow transport while free movement (when not bound to PIP_2) or removal of the domain would then autoinhibit the exchanger (Aharonovitz et al., 2000).

5.3. MFS transporters

The major facilitator superfamily (MFS) transporters are the largest evolutionary group of transporters (Chang et al., 2004). They can act either as uniporters, transporting the substrate down its own electrochemical gradient, or as secondary active transporters, utilizing an ion gradient (typically protons in prokaryotes, plants, and fungi and sodium in animals) to drive transport or operating as solute-solute antiporters. All MFS family members share a common architecture that is intricately linked to their function via the alternating access mechanism (Law et al., 2008; Radestock and Forrest, 2011).

5.3.1. LacY

The lactose permease (LacY) of *E. coli*, a member of the sugar porter family (TCDB #2.A.1.1) couples the transport of one proton to the uptake of one sugar molecule (Kaback et al., 1990). It has been studied extensively and lipids have been found to affect it at various stages of its transport cycle.

LacY requires a fluid (liquid-crystalline) membrane for full transport activity. Experiments below the phase transition temperature of the membrane showed an abrupt decrease in β-galactoside

transport activity (Linden et al., 1973; Thilo et al., 1977) Analysis of the transport rate $k = \nu \exp(-\Delta E^{\ddagger}/k_B T)$ revealed that membrane fluidity did not affect the activation energy $\Delta E^{\ddagger}$ of transport but the frequency prefactor ν. This behavior can be qualitatively explained in the light of the structural model of the alternating access mechanism in secondary transporters (Law et al., 2008; Forrest et al., 2011; Radestock and Forrest, 2011; Weyand et al., 2011). A symporter such as LacY can utilize the binding energy of substrates to drive the conformational change in one direction, say from the outward facing conformation to the inward facing one. The switch in the return direction, however, can only occur as a stochastic, thermally activated process because no substrate is transported. A change in membrane fluidity does not affect the energetics but it does change the frequency with which conformational fluctuations can occur that return the transporter to an outward facing conformation. Thus, if the return-leg of the transport cycle is rate limiting then it is quite plausible that membrane fluidity alters the transport rate in the way observed in these early experiments (see Linden et al. (1973); Thilo et al. (1977) and references therein).

LacY requires PE (or PS) for maximal activity (Chen and Wilson, 1984) when inserted into membranes containing anionic lipids such as PG and CL. PE was shown to be required to couple energetic uphill transport to the proton motive force; PC would not support active transport although substrate binding (as measured by the Michaelis constant) or facilitated (passive) diffusion of lactose did not differ significantly between PE and PC (Seto-Young et al., 1985; Bogdanov and Dowhan, 1995). PS and mono- and di-methylated PE could substitute for PE (Chen and Wilson, 1984; Wang et al., 2002), indicating that interactions between the protein and the amine of the headgroup might be important. Molecular dynamics simulations of LacY in a PE membrane identified a specific salt-bridge between a PE lipid headgroup and the charged residues Asp68 and Lys69 in LacY (Lensink et al., 2010) (Fig. 3). These two residues are part of a GXXXD(R/K)XGR(R/K) motif that is highly conserved in MFS transporters (Griffith et al., 1992). Mutational studies had shown that Asp in the fifth position of the motif (Asp68 in LacY) was needed for high transport rates and even a conservative mutation to Glu showed markedly reduced activity (Jessen-Marshall et al., 1995). However, Asp68 did not seem to be required for the transport process itself, i.e. the conformational change of the transporter, because a range of mutants including D68A were competent for H^+-dependent transport albeit at much lower overall rates (Jessen-Marshall et al., 1995).

These results taken together with the data on membrane fluidity seem to make it worthwhile to investigate if the LacY symporter couples protein conformational fluctuations to fluctuations in the membrane (perhaps via Asp68) in order to accelerate the inward-to-outward conformational change of its transport cycle.

The LacY system was also used to study the role of lipids in membrane protein topogenesis. Mapping the topology of LacY in the presence (PE:PG:CL membrane) and absence of PE (PG:CL membrane) revealed that lack of PE resulted in misorientation of the full N-terminal 6-helix bundle with helix 7 residing in the interfacial lipid headgroup region (Bogdanov et al., 2002). Remarkably, addition of PE to the mis-oriented permease restored the ability for proton-driven transport although only helices 3 to 6 flipped orientation and the N-terminus together with helix 1 remained mis-oriented. Addition of PC partially restored the topology but not transport (Wang et al., 2002). Additional studies on a range of other transporters (Zhang et al., 2003, 2005; Vitrac et al., 2011) showed that PE-dependent topological misorientation depends on the presence of negatively charged residues on the protein surface. Under physiological membrane conditions, positively charged residues bias the protein domain to insert in such a way that these residues face the cytoplasm, an effect known as the "positive-inside rule" (von Heijne, 1986,

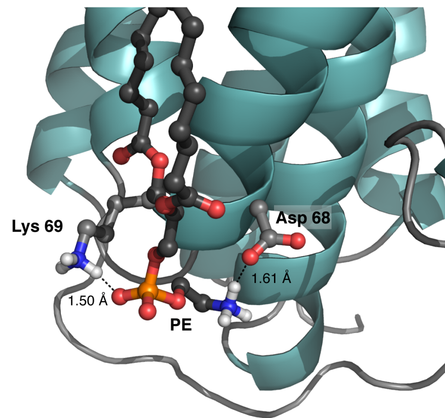

Figure 3: A tight coupling between a POPE lipid and the LacY transporter was predicted by MD simulations (Lensink et al., 2010). The PE headgroup forms salt-bridges to Asp68 and Lys69, which are part of a highly conserved motif in MFS transporters (Griffith et al., 1992). The interaction might have a role in coupling protein conformational fluctuations to fluctuations in the membrane (see main text). (The image was kindly provided by Dr Marc Lensink.)

2006). Van Klompenburg et al. (1997) determined that the presence of anionic lipids decreased the probability for positively charged loops to translocate to the other side of the membrane. As summarized by Dowhan and Bogdanov (2011), the protein net charge also plays a role in determining topology. Absence of PE (or PC) lead to a much higher negative surface charge density of the membrane, which increases the importance of the net-charge contribution to topological decisions relative to the positive-inside rule (Bogdanov et al., 2008). A rigorous quantitative explanation of these findings, including the question how whole transmembrane domains can reorient in the membrane, is currently yet to be determined.

5.3.2. LmrP

The MSF transporter LmrP is a proton-coupled secondary multidrug exporter of *Lactococcus lactis*. Like many other MFS transporters, it requires PE to undergo the conformational changes of its transport cycle (Gbaguidi et al., 2007). With PE, changes in the ionization states of solvent-exposed carboxylic residues are observed together with conformational changes and the mean pK_a value of all carboxylic residues was 6.5. In the absence of PE no transport and no conformational changes were detected while the mean pK_a value dropped to 4.5. The data were interpreted to mean that PE induced interactions between the membrane and the carboxylic residues so that these residues could participate in pH-induced conformational changes. Interaction with the lipid headgroup region reduced the exposure to solvent and therefore increased the pK_a. Hakizimana et al. (2008) showed that the interaction between carboxylic residue and lipid required the formation of a hydrogen bond because methyl-PE and dimethyl-PE resulted in the same activity as PE whereas PC (i.e. trimethyl-PE) abolished transport completely, similar to what was found for LacY (Chen and Wilson, 1984). A Asp68Cys mutant (located in the conserved GXXXD(R/K)XG motif (Griffith et al., 1992)) in PE liposomes showed no transport and the same structural characteristics as wildtype LmrP in PC liposomes. It is therefore likely that

Asp68 is directly involved in protein-lipid interaction, as also highlighted by the simulations of LacY in PE (Lensink et al., 2010).

5.3.3. GusB

The oligomeric glucuronide transporter GusB is stabilized in a pentameric state by natural *E. coli* lipids (about 80% PE) at room temperature, or by DMPC when stored above DMPC's phase transition temperature (about 29°C) (Ishii, 2010). Below the phase transition temperature it becomes unstable. These results suggest that the fluidity of the membrane can directly affect oligomeric states.

5.4. Branched Chain Amino Acid:Cation Symporter

The branched chain amino acid:cation symporter (LIVCS) family (TCDB #2.A.26) utilize the Na^+ or H^+ gradient to transport branched chain aliphatic amino acids. It has been observed that Leu transport by the proton driven Bca transporter of *Streptococcus cremoris* increased when increasing PE:PC ratio in proteoliposomes (Driessen et al., 1988). Glycolipids (native to *S. cremoris*) stimulated transport more than the non-native PE. Cardiolipin (CL) was not effective. Activity decreased with increasing degree of methylation from PE to PC. Both PE and PS activate Leucine transport, similar to the situation found in LacY and LmrP.

Further studies of the homologous transporter of *Lactococcus lactis* indicated that the carbon number of the acyl chains of PE and PC and thus the overall membrane thickness could modulate transport (in 't Veld et al., 1991), a result replicated for the Leu transporter of *Pseudomonas aeruginosa* (Uratani et al., 1987). The degree of acyl chain saturation only had a small effect on active Leu transport (in 't Veld et al., 1992).

5.5. Multidrug transporters

5.5.1. Small multidrug transporters

Drug export by EmrE (*E. coli*) and TBsmr (*Mycobacterium tuberculois*), members of the small multidrug resistance family (2.A.7.1) (Bay et al., 2008), is affected by bilayer composition (Charalambous et al., 2008). The membrane proteins were reconstituted into lipid vesicles of defined binary lipid mixtures, made from combinations of DOPC, DOPE, DOPG, and SoyPI. Proton-driven radiolabelled methyl viologen transport was measured as the initial transport rate as a function of the lipid mole fraction. DOPC hinders transport and decreases the initial rate to less than 10% of the value obtained with *E. coli* membrane lipids. Diluting the PC membrane to below a threshold of about 0.6 PC mole fraction by either DOPE or DOPG increases transport activity 4 to 20-fold. A native-like PE:PG or PE:PI membrane without any PC yields 25 to 70-fold faster initial rates than in pure DOPC. For instance, EmrE in 70%:30% DOPE:DOPG shows almost twice the initial transport rate than in *E. coli*-lipid vesicles.

5.5.2. P-glycoprotein

The ATP-powered P-glycoprotein (Pgp) transporter extrudes a diverse range of hydrophobic compounds. Efflux via Pgp is one of the major problems for drugs targeting cancer cells or the brain because tumor cells and the blood-brain barrier are enriched in Pgp. The interior cavity of the transporter is accessible from the cytosol and the inner leaflet of the membrane through portals (Aller et al., 2009). Hydrophobic drugs are believed to sequester into the membrane and to be taken up through the portals. Romsicki and Sharom (1999) showed that binding of drug substrates could be modulated up to a factor of 15 by the types of lipids in the membrane, their

acyl chain lengths, and the phase of the bilayer. Drug-transporter affinity increased from PE over PS to PC lipids and with decreasing acyl chain length (DPPC to DMPC). Affinity in the L_β gel phase was 2–4 times higher than in the liquid crystalline L_α phase. The change in affinity was positively correlated with the water:membrane partition coefficient of each tested drug and hence the most straightforward interpretation of the data is that the higher effective concentration of the drug in the bilayer lead to a higher observed affinity. In this case the properties of the membrane as a solvent for substrates indirectly affects Pgp-mediated transport.

5.5.3. HorA

The bacterial ABC multidrug transporter HorA of *Lactobacillus brevis* required PE for ATP-coupled substrate extrusion from liposomes (Gustot et al., 2010). When PE was replaced by PC, transport activity was abolished even though it hydrolyzed ATP at more than twice the rate than in PE. Spectroscopic studies showed that neither secondary structure nor insertion topology was affected. The structure of the transmembrane helix bundle of HorA in PC was tighter than in PE and the nucleotide-free Pgp and MsbA crystal structures. The average angle of α-helices with the membrane normal was reduced by 10°. In particular, Cys180 on helix TM4 was shown to be more exposed to the lipid phase in PC than in PE but it is an open question if specific headgroup-protein interactions or physical bilayer properties are driving the observed functional differences.

5.6. Betaine transporter BetP

The trimeric betaine transporter BetP of *Corynebacterium glutamicum* (Ressl et al., 2009), a member of the betaine-choline-carnitine family of transporters (TCDB #2.A.15), is activated by hyperosmotic and chill stress (such as a decrease in temperature to 10 to 15°C) and is stimulated by internal increase in K^+ concentration (Ozcan et al., 2007). Chill response was more difficult to elicit in cells grown at low temperatures, which had a markedly different fatty acid and phospholipid composition than cells grown at the optimum temperature of 30°C (Table 1). Because the chill response was independent from osmotic triggers it was concluded that chill activation of BetP is due to protein-lipid interactions. Recent crystal structures of BetP are likely to shed more light on the molecular details of regulation of this transporter my its membrane environment (Gärtner et al., 2011).

5.7. Mitochondrial ADP/ATP carrier

The mitochondrial ADP/ATP carrier (AAC), a member of the mitochondrial carrier family (TCDB #2.A.29), exchanges one ADP for ATP across the mitochondrial membrane. A homodimeric crystal structure of ACC appears to be mediated by two cardiolipin molecules inside the interface (Nury et al., 2005), which has been cited as an example of lipid-assisted dimerization (Ernst et al., 2010). Other lines of evidence, however, suggest that AACs function exclusively as monomers and that dimers formed during crystallization (Kunji and Crichton, 2010). Cardiolipin, which exists in high concentrations in the mitochondrial membrane (Table 1) had been shown to strongly influence ADP binding and the conformational transition required for transport (Beyer and Nuscher, 1996).

6. Conclusions

We presented a range of ion channels and transporters whose stability and function depends on the appropriate membrane environment or is at least modulated to varying degrees. The requirements range from general membrane properties such as fluidity, thickness, or surface charge to specific protein-lipid interactions where a lipid molecule acts like a ligand or co-factor. There is some evidence that zwitterionic PE lipids (e.g. for the MFS transporters) and the anionic PG (for bacterial ion channels) can interact with well-defined regions of the protein. The best example for chemical lipid-protein interactions is PIP_2, which has been shown to specifically regulate both channels and transporters. It differs from the other bilayer molecules discussed in that its primary biological purpose appears to be that of a signalling molecule although its contribution to the membrane surface charge is not negligible. The role of cholesterol was only mentioned briefly but it seems clear that it can affect membrane protein function both by modifying the physical properties of the bilayer and by specific interactions with proteins. In the systems discussed, the influence of lipids on transport properties could be related to changes in physical membrane properties such as fluidity, pressure profile, bilayer thickness and surface charge density, and to specific, chemical lipid-protein interactions.

Membrane proteins require an appropriate lipid environment for their structural integrity and in some cases for their correct insertion and possibly oligomeric state (Schneiter and Toulmay, 2007). From an organism's point of view, this is the strongest dependence on lipids because without the functional protein there would be no transport at all. Protein function is seen to be either regulated by lipids (e.g. transport can be enabled by the presence of a particular lipid species) or modulated (transport activity is shifted). The molecular basis of these effects has not been conclusively explained in many cases. Even though lipids are visible in many channel and transporter structures this does not necessarily imply a functional role beyond stabilisation of the transmembrane domain against polar solvent. The structural data on lipid-protein interactions for the aquaporins were included to show which level of detail is already possible to achieve but the functional implications remain to be elucidated.

The data we presented show clearly that many ion channels require specific properties for the surrounding annular lipids and those in direct non-annular binding sites in order to function properly. For transporters, the influence of the bilayer on function is not as well understood and there seem to be fewer clear-cut examples so far. We are only beginning to understand the molecular mechanisms at work, mainly thanks to the growing number of atomic resolution structures. It is, however, difficult to draw conclusions on lipid-protein interactions from structures crystallized outside their native membrane. Computer simulations of membrane proteins (Khalili-Araghi et al., 2009; Stansfeld and Sansom, 2011) have therefore become an important tool in the elucidation of protein-lipid interactions at the molecular level. In part this has become possible to the availability of force field parameters for lipids (Ulmschneider and Ulmschneider, 2009; Klauda et al., 2010; Poger and Mark, 2010; Piggot et al., 2011; Jämbeck and Lyubartsev, 2012; Piggot et al., 2012; Poger and Mark, 2012; Domański et al., 2010) that accurately describe the interaction between the membrane and the protein. Experiments in conjunction with simulations of proteins in realistic and complex membrane environments (Jo et al., 2009; Holdbrook et al., 2010; Piggot et al., 2011; Wennberg et al., 2012) will certainly be crucial to explain the interplay between the bilayer, the solvent, and the transport protein.

But how important are protein-lipid interactions? Many membrane proteins seem to be fairly tolerant against changes in membrane composition. It is often possible to functionally express prokaryotic or archaeal membrane proteins in *E. coli* or reconstitute them in liposomes with

simple lipid compositions very different from native membranes. Sanders and Mittendorf (2011) pointed out that tolerance of membrane protein function against varying membrane compositions might confer an evolutionary advantage and therefore a majority of transport proteins might only be weakly influenced by the surrounding lipids. On the other hand, cells go to great lengths to generate thousands of different lipids and constantly adjust their membrane composition—it seems unlikely that fundamental physiological processes such as transmembrane transport should not be regulated by such a complex lipid repertoire (van Meer et al., 2008).

References

Aharonovitz, O., Zaun, H. C., Balla, T., York, J. D., Orlowski, J., Grinstein, S., 2000. Intracellular pH regulation by Na^+/H^+ exchange requires phosphatidylinositol 4,5-bisphosphate. J Cell Biol 150 (1), 213–24.

Aller, S. G., Yu, J., Ward, A., Weng, Y., Chittaboina, S., Zhuo, R., Harrell, P. M., Trinh, Y. T., Zhang, Q., Urbatsch, I. L., Chang, G., 2009. Structure of p-glycoprotein reveals a molecular basis for poly-specific drug binding. Science 323 (5922), 1718–1722.

Andersen, O. S., Koeppe, II, R. E., 2007. Bilayer thickness and membrane protein function: An energetic perspective. Annu. Rev. Biophys. Biomol. Struct. 36, 107–130.

Aponte-Santamaría, C., Briones, R., Schenk, A. D., Walz, T., de Groot, B. L., 2012. Molecular driving forces defining lipid positions around aquaporin-0. Proc Natl Acad Sci U S A 109 (25), 9887–92.

Asteggiano, C., Berberián, G., Beaugé, L., 2001. Phosphatidyl inositol-4,5-bisphosphate bound to bovine cardiac Na^+/Ca^{2+} exchanger displays a MgATP regulation similar to that of the exchange fluxes. Eur J Biochem 268 (2), 437–42.

Baenziger, J. E., Corringer, P.-J., 2011. 3D structure and allosteric modulation of the transmembrane domain of pentameric ligand-gated ion channels. Neuropharmacology 60 (1), 116–125.

Baukrowitz, T., Fakler, B., 2000. K_{ATP} channels: linker between phospholipid metabolism and excitability. Biochem Pharmacol 60 (6), 735–40.

Bay, D. C., Rommens, K. L., Turner, R. J., 2008. Small multidrug resistance proteins: a multidrug transporter family that continues to grow. Biochim Biophys Acta 1778 (9), 1814–38.

Beyer, K., Nuscher, B., 1996. Specific cardiolipin binding interferes with labeling of sulfhydryl residues in the adenosine diphosphate/adenosine triphosphate carrier protein from beef heart mitochondria. Biochemistry 35 (49), 15784–90.

Bichet, D., Haass, F. A., Jan, L. Y., 2003. Merging functional studies with structures of inward-rectifier K^+ channels. Nat Rev Neurosci 4 (12), 957–67.

Bocquet, N., Nury, H., Baaden, M., Le Poupon, C., Changeux, J.-P., Delarue, M., Corringer, P.-J., 2009. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. Nature 457 (7225), 111–4.

Bogdanov, M., Dowhan, W., 1995. Phosphatidylethanolamine is required for *in vivo*function of the membrane-associated lactose permease of *Escherichia coli*. J Biol Chem 270 (2), 732–739.

Bogdanov, M., Heacock, P. N., Dowhan, W., 2002. A polytopic membrane protein displays a reversible topology dependent on membrane lipid composition. EMBO J 21 (9), 2107–16.

Bogdanov, M., Xie, J., Heacock, P., Dowhan, W., 2008. To flip or not to flip: lipid-protein charge interactions are a determinant of final membrane protein topology. J Cell Biol 182 (5), 925–35.

Bolivar, J. H., Smithers, N., East, J. M., Marsh, D., Lee, A. G., 2012. Multiple binding sites for fatty acids on the potassium channel KcsA. Biochemistry 51 (13), 2889–98.

Botelho, A. V., Huber, T., Sakmar, T. P., Brown, M. F., 2006. Curvature and hydrophobic forces drive oligomerization and modulate activity of rhodopsin in membranes. Biophysical Journal 91 (12), 4464–4477.

Braverman, N. E., Moser, A. B., 2012. Functions of plasmalogen lipids in health and disease. Biochim Biophys Acta 1822 (9), 1442–52.

Brohawn, S. G., del Mármol, J., MacKinnon, R., 2012. Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K^+ ion channel. Science 335 (6067), 436–41.

Brown, M. F., 2012. Curvature forces in membrane lipid-protein interactions. Biochemistry 51 (49), 9782–9795.

Cantor, R. S., 1999. Lipid composition and the lateral pressure profile in bilayers. Biophys J 76 (5), 2625–39.

Chang, A. B., Lin, R., Keith Studley, W., Tran, C. V., Saier, Jr, M. H., 2004. Phylogeny as a guide to structure and function of membrane transport proteins. Mol Membr Biol 21 (3), 171–81.

Charalambous, K., Miller, D., Curnow, P., Booth, P. J., 2008. Lipid bilayer composition influences small multidrug transporters. BMC Biochemistry 9 (31).

Chen, C. C., Wilson, T. H., 1984. The phospholipid requirement for activity of the lactose carrier of *Escherichia coli*. J Biol Chem 259 (16), 10150–8.

Cho, H., Lee, D., Lee, S. H., Ho, W.-K., 2005. Receptor-induced depletion of phosphatidylinositol 4,5-bisphosphate inhibits inwardly rectifying K^+ channels in a receptor-specific manner. Proc Natl Acad Sci USA 102 (12), 4643–8.

Clejan, S., Krulwich, T. A., Mondrus, K. R., Seto-Young, D., 1986. Membrane lipid composition of obligately and facultatively alkalophilic strains of *Bacillus* spp. J Bacteriol 168 (1), 334–40.

Cronan, J. E., 2003. Bacterial membrane lipids: where do we stand? Annu Rev Microbiol 57, 203–24.

Cronan, Jr, J. E., 1968. Phospholipid alterations during growth of *Escherichia coli*. J Bacteriol 95 (6), 2054–61.

Cybulski, L. E., de Mendoza, D., 2011. Bilayer hydrophobic thickness and integral membrane protein function. Curr Protein Pept Sci 12 (8), 760–6.

Dalton, K. A., East, J. M., Mall, S., Oliver, S., Starling, A. P., Lee, A. G., 1998. Interaction of phosphatidic acid and phosphatidylserine with the Ca2+-ATPase of sarcoplasmic reticulum and the mechanism of inhibition. Biochem J 329 (Pt 3), 637–46.

de Groot, B. L., Grubmüller, H., 2005. The dynamics and energetics of water permeation and proton exclusion in aquaporins. Current Opinion in Structural Biology 15 (2), 176–83.

Decher, N., Gonzalez, T., Streit, A. K., Sachse, F. B., Renigunta, V., Soom, M., Heinemann, S. H., Daut, J., Sanguinetti, M. C., 2008. Structural determinants of $Kv_{\beta}1.3$-induced channel inactivation: a hairpin modulated by PIP_2. EMBO J 27 (23), 3164–74.

Deol, S. S., Domene, C., Bond, P. J., Sansom, M. S. P., 2006. Anionic phospholipid interactions with the potassium channel KcsA: Simulation studies. Biophysical Journal 90 (3), 822–830.

Domański, J., Stansfeld, P., Sansom, M., Beckstein, O., 2010. Lipidbook: A public repository for force-field parameters used in membrane simulations. J Membr Biol 236 (3), 255–258.

Dowhan, W., Bogdanov, M., 2011. Lipid-protein interactions as determinants of membrane protein structure and function. Biochem Soc Trans 39, 767–774.

Driessen, A. J. M., Zheng, T., in 't Veld, G., Denkamp, J. A. F. O., Konings, W. N., 1988. Lipid requirement of the branched-chain amino-acid transport-system of *Streptococcus cremoris*. Biochemistry 27 (3), 865–872.

Enyedi, A., Flura, M., Sarkadi, B., Gardos, G., Carafoli, E., 1987. The maximal velocity and the calcium affinity of the red cell calcium pump may be regulated independently. J Biol Chem 262 (13), 6425–30.

Ernst, A. M., Contreras, F. X., Brügger, B., Wieland, F., 2010. Determinants of specificity at the protein-lipid interface in membranes. FEBS Lett 584 (9), 1713–20.

Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill, Jr, A. H., Murphy, R. C., Raetz, C. R. H., Russell, D. W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., van Meer, G., VanNieuwenhze, M. S., White, S. H., Witztum, J. L., Dennis, E. A., 2005. A comprehensive classification system for lipids. J Lipid Res 46 (5), 839–61.

Fahy, E., Subramaniam, S., Murphy, R. C., Nishijima, M., Raetz, C. R. H., Shimizu, T., Spener, F., van Meer, G., Wakelam, M. J. O., Dennis, E. A., 2009. Update of the LIPID MAPS comprehensive classification system for lipids. J Lipid Res 50 Suppl, S9–14.

Fairn, G. D., Grinstein, S., 2012. Precursor or charge supplier? Science 337 (6095), 653–654.

Fairn, G. D., Schieber, N. L., Ariotti, N., Murphy, S., Kuerschner, L., Webb, R. I., Grinstein, S., Parton, R. G., 2011. High-resolution mapping reveals topologically distinct cellular pools of phosphatidylserine. J Cell Biol 194 (2), 257–75.

Feller, S. E., Gawrisch, K., Woolf, T. B., 2003. Rhodopsin exhibits a preference for solvation by polyunsaturated docosohexaenoic acid. J Am Chem Soc 125 (15), 4434–4435.

Forrest, L. R., Krämer, R., Ziegler, C., 2011. The structural basis of secondary active transport mechanisms. Biochim Biophys Acta 1807 (2), 167–88.

Gamper, N., Shapiro, M. S., 2007. Regulation of ion transport proteins by membrane phosphoinositides. Nat Rev Neurosci 8 (12), 921–34.

Gärtner, R. M., Perez, C., Koshy, C., Ziegler, C., 2011. Role of bundle helices in a regulatory crosstalk in the trimeric betaine transporter BetP. J Mol Biol 414 (3), 327–36.

Gbaguidi, B., Hakizimana, P., Vandenbussche, G., Ruysschaert, J.-M., 2007. Conformational changes in a bacterial multidrug transporter are phosphatidylethanolamine-dependent. Cell Mol Life Sci 64 (12), 1571–82.

Gonen, T., Cheng, Y., Sliz, P., Hiroaki, Y., Fujiyoshi, Y., Harrison, S. C., Walz, T., 2005. Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. Nature 438 (7068), 633–8.

Griffith, J. K., Baker, M. E., Rouch, D. A., Page, M. G., Skurray, R. A., Paulsen, I. T., Chater, K. F., Baldwin, S. A., Henderson, P. J., 1992. Membrane transport proteins: implications of sequence comparisons. Curr Opin Cell Biol 4 (4), 684–95.

Grossfield, A., Feller, S. E., Pitman, M. C., 2006. A role for direct interactions in the modulation of rhodopsin by omega-3 polyunsaturated lipids. Proc Natl Acad Sci USA 103 (13), 4888–4893.

Gustot, A., Smriti, Ruysschaert, J.-M., Mchaourab, H., Govaerts, C., 2010. Lipid composition regulates the orientation of transmembrane helices in HorA, an ABC multidrug transporter. J Biol Chem 285 (19), 14144–51.

Hakizimana, P., Masureel, M., Gbaguidi, B., Ruysschaert, J.-M., Govaerts, C., 2008. Interactions between phosphatidylethanolamine headgroup and LmrP, a multidrug transporter. J Biol Chem 283 (14), 9369–9376.

Hamill, O. P., Martinac, B., 2001. Molecular basis of mechanotransduction in living cells. Physiol Rev 81 (2), 685–740.

Hansen, S. B., Tao, X., MacKinnon, R., 2011. Structural basis of PIP_2 activation of the classical inward rectifier K^+ channel Kir2.2. Nature 477 (7365), 495–8.

He, Z., Feng, S., Tong, Q., Hilgemann, D. W., Philipson, K. D., 2000. Interaction of PIP_2 with the XIP region of the cardiac Na/Ca exchanger. Am J Physiol Cell Physiol 278 (4), C661–6.

Hilgemann, D. W., Ball, R., 1996. Regulation of cardiac Na^+,Ca^{2+} exchange and K_{ATP} potassium channels by PIP_2. Science 273 (5277), 956–9.

Hilgemann, D. W., Feng, S., Nasuhoglu, C., 2001. The complex and intriguing lives of PIP_2 with ion channels and transporters. Sci STKE 2001 (111), re19.

Holdbrook, D. A., Leung, Y. M., Piggot, T. J., Marius, P., Williamson, P. T. F., Khalid, S., 2010. Stability and membrane orientation of the fukutin transmembrane domain: a combined multiscale molecular dynamics and circular dichroism study. Biochemistry 49 (51), 10796–802.

Hunte, C., Richers, S., 2008. Lipids and membrane protein structures. Curr Opin Struct Biol 18 (4), 406–411.

in 't Veld, G., Driessen, A. J. M., Denkamp, J. A. F. O., Konings, W. N., 1991. Hydrophobic membrane thickness and lipid protein interactions of the leucine transport-system of *Lactococcus lactis*. Biochim Biophys Acta 1065 (2), 203–212.

in 't Veld, G., Driessen, A. J. M., Konings, W. N., 1992. Effect of the unsaturation of phospholipid acyl chains on leucine transport of *Lactococcus lactis* and membrane permeability. Biochimica et Biophysica Acta (BBA) - Biomembranes 1108 (1), 31–39.

Ipsen, J. H., Mouritsen, O. G., Bloom, M., 1990. Relationships between lipid membrane area, hydrophobic thickness, and acyl-chain orientational order. the effects of cholesterol. Biophys J 57 (3), 405–12.

Ishii, N., 2010. Investigation on stability of transporter protein, glucuronide transporter from *Escherichia coli*. J Membr Biol 235 (2), 63–72.

Iwamoto, T., Nakamura, T. Y., Pan, Y., Uehara, A., Imanaga, I., Shigekawa, M., 1999. Unique topology of the internal repeats in the cardiac Na^+/Ca^{2+} exchanger. FEBS Lett 446 (2-3), 264–8.

Jämbeck, J. P. M., Lyubartsev, A. P., 2012. Derivation and systematic validation of a refined all-atom force field for phosphatidylcholine lipids. J Phys Chem B 116 (10), 3164–79.

Jensen, M. O., Tajkhorshid, E., Schulten, K., 2001. The mechanism of glycerol conduction in aquaglyceroporins. Structure 9 (11), 1083–93.

Jensen, M. Ø., Tajkhorshid, E., Schulten, K., 2003. Electrostatic tuning of permeation and selectivity in aquaporin water channels. Biophysical Journal 85 (5), 2884–99.

Jessen-Marshall, A. E., Paul, N. J., Brooker, R. J., 1995. The conserved motif, GXXX(D/E)(R/K)XG[X](R/K)(R/K), in hydrophilic loop 2/3 of the lactose permease. J Biol Chem 270 (27), 16251–7.

Jo, S., Lim, J. B., Klauda, J. B., Im, W., 2009. CHARMM-GUI membrane builder for mixed bilayers and its application to yeast membranes. Biophys. J. 97, 50–58.

John, S. A., Ribalet, B., Weiss, J. N., Philipson, K. D., Ottolia, M., 2011. Ca^{2+}-dependent structural rearrangements within Na^+-Ca^{2+} exchanger dimers. Proc Natl Acad Sci U S A 108 (4), 1699–704.

Kaback, H. R., Bibi, E., Roepe, P. D., 1990. β-galactoside transport in *E. coli*: a functional dissection of *lac* permease. Trends Biochem Sci 15 (8), 309–14.

Khalili-Araghi, F., Gumbart, J., Wen, P.-C., Sotomayor, M., Tajkhorshid, E., Schulten, K., 2009. Molecular dynamics simulations of membrane channels and transporters. Curr. Opin. Struct. Biol. 19 (2), 128–137.

Klauda, J. B., Venable, R. M., Freites, J. A., O'Connor, J. W., Tobias, D. J., Mondragon-Ramirez, C., Vorobyov, I., MacKerell, Jr, A. D., Pastor, R. W., 2010. Update of the CHARMM all-atom additive force field for lipids: validation on six lipid types. J Phys Chem B 114 (23), 7830–43.

Kobrinsky, E., Mirshahi, T., Zhang, H., Jin, T., Logothetis, D. E., 2000. Receptor-mediated hydrolysis of plasma membrane messenger PIP_2 leads to K^+-current desensitization. Nat Cell Biol 2 (8), 507–14.

Kruse, M., Hammond, G. R. V., Hille, B., 2012. Regulation of voltage-gated potassium channels by PI(4,5)P_2. J Gen Physiol 140 (2), 189–205.

Kunji, E. R. S., Crichton, P. G., 2010. Mitochondrial carriers function as monomers. Biochim Biophys Acta 1797 (6-7), 817–31.

Kurtz, I., Petrasek, D., Tatishchev, S., 2004. Molecular mechanisms of electrogenic sodium bicarbonate cotransport: structural and equilibrium thermodynamic considerations. J Membr Biol 197 (2), 77–90.

Law, C. J., Maloney, P. C., Wang, D.-N., 2008. Ins and outs of major facilitator superfamily antiporters. Annu Rev Microbiol 62, 289–305.

Lee, A. G., 2004. How lipids affect the activities of integral membrane proteins. Biochim Biophys Acta 1666 (1-2), 62–87.

Lee, A. G., 2011. Biological membranes: the importance of molecular detail. Trends in Biochemical Sciences 36 (9), 493–500.

Lensink, M. F., Govaerts, C., Ruysschaert, J.-M., 2010. Identification of specific lipid-binding sites in integral membrane

proteins. J Biol Chem 285 (14), 10519–26.
Liao, J., Li, H., Zeng, W., Sauer, D. B., Belmares, R., Jiang, Y., 2012. Structural insight into the ion-exchange mechanism of the sodium/calcium exchanger. Science 335 (6069), 686–690.
Linden, C. D., Wright, K. L., McConnell, H. M., Fox, C. F., 1973. Lateral phase separations in membrane lipids and the mechanism of sugar transport in *Escherichia coli*. Proc Natl Acad Sci U S A 70 (8), 2271–5.
Long, S. B., Tao, X., Campbell, E. B., Mackinnon, R., 2007. Atomic structure of a voltage-dependent K^+ channel in a lipid membrane-like environment. Nature 450 (7168), 376–382.
Majumdar, D., Bevensee, M. O., 2010. Na-coupled bicarbonate transporters of the solute carrier 4 family in the nervous system: function, localization, and relevance to neurologic function. Neuroscience 171 (4), 951–72.
Mangialavori, I. C., Corradi, G., Rinaldi, D. E., de la Fuente, M. C., Adamo, H. P., Rossi, J. P. F. C., 2012. Autoinhibition mechanism of the plasma membrane calcium pump isoforms 2 and 4 studied through lipid-protein interaction. Biochem J 443 (1), 125–31.
Marius, P., Alvis, S. J., East, J. M., Lee, A. G., 2005. The interfacial lipid binding site on the potassium channel KcsA is specific for anionic phospholipids. Biophysical Journal 89 (6), 4081–9.
Marius, P., Zagnoni, M., Sandison, M. E., East, J. M., Morgan, H., Lee, A. G., 2008. Binding of anionic lipids to at least three nonannular sites on the potassium channel KcsA is required for channel opening. Biophysical Journal 94 (5), 1689–98.
Marsh, D., 2008. Protein modulation of lipids, and vice-versa, in membranes. Biochim Biophys Acta 1778 (7-8), 1545–75.
Martinac, B., 2011. Bacterial mechanosensitive channels as a paradigm for mechanosensory transduction. Cell Physiol Biochem 28 (6), 1051–60.
Murata, K., Mitsuoka, K., Hirai, T., Walz, T., Agre, P., Heymann, J. B., Engel, A., Fujiyoshi, Y., 2000. Structural determinants of water permeation through aquaporin-1. Nature 407 (6804), 599–605.
Niggli, V., Adunyah, E. S., Carafoli, E., 1981. Acidic phospholipids, unsaturated fatty acids, and limited proteolysis mimic the effect of calmodulin on the purified erythrocyte Ca^{2+}-ATPase. J Biol Chem 256 (16), 8588–92.
Nishida, M., Cadene, M., Chait, B. T., MacKinnon, R., 2007. Crystal structure of a Kir3.1-prokaryotic Kir channel chimera. EMBO J 26 (17), 4005–15.
Nomura, T., Cranfield, C. G., Deplazes, E., Owen, D. M., Macmillan, A., Battle, A. R., Constantine, M., Sokabe, M., Martinac, B., 2012. Differential effects of lipids and lyso-lipids on the mechanosensitivity of the mechanosensitive channels mscl and mscs. Proc Natl Acad Sci U S A 109 (22), 8770–5.
Nury, H., Dahout-Gonzalez, C., Trézéguet, V., Lauquin, G., Brandolin, G., Pebay-Peyroula, E., 2005. Structural basis for lipid-mediated interactions between mitochondrial adp/atp carrier monomers. FEBS Lett 579 (27), 6031–6.
Nyholm, T. K. M., Özdirekcan, S., Killian, J. A., 2007. How protein transmembrane segments sense the lipid environment. Biochemistry 46 (6), 1457–65.
Obara, K., Miyashita, N., Xu, C., Toyoshima, I., Sugita, Y., Inesi, G., Toyoshima, C., 2005. Structural role of countertransport revealed in Ca^{2+} pump crystal structure in the absence of Ca^{2+}. Proc Natl Acad Sci U S A 102 (41), 14489–96.
Oliver, D., Lien, C.-C., Soom, M., Baukrowitz, T., Jonas, P., Fakler, B., 2004. Functional conversion between A-type and delayed rectifier K^+ channels by membrane lipids. Science 304 (5668), 265–70.
Opekarova, M., Tanner, W., 2003. Specific lipid requirements of membrane proteins — a putative bottleneck in heterologous expression. Biochimica et Biophysica Acta - Biomembranes 1610 (1), 11–22.
Ozcan, N., Ejsing, C. S., Shevchenko, A., Lipski, A., Morbach, S., Krämer, R., 2007. Osmolality, temperature, and membrane lipid composition modulate the activity of betaine transporter BetP in *Corynebacterium glutamicum*. J Bacteriol 189 (20), 7485–96.
Palmgren, M. G., Nissen, P., 2011. P-type ATPases. Annu Rev Biophys 40, 243–66.
Philipson, K. D., Bers, D. M., Nishimoto, A. Y., 1980. The role of phospholipids in the Ca^{2+} binding of isolated cardiac sarcolemma. J Mol Cell Cardiol 12 (11), 1159–73.
Phillips, R., Ursell, T., Wiggins, P., Sens, P., 2009. Emerging roles for lipids in shaping membrane-protein function. Nature 459 (7245), 379–85.
Piggot, T. J., Holdbrook, D. A., Khalid, S., 2011. Electroporation of the *E. coli* and *S. Aureus* membranes: Molecular dynamics simulations of complex bacterial membranes. The Journal of Physical Chemistry B 115 (45), 13381–13388.
Piggot, T. J., Pineiro, A., Khalid, S., 2012. Molecular dynamics simulations of phosphatidylcholine membranes: A comparative force field study. J Chem Theory Comput 8 (7168), 4593–4609.
Poger, D., Mark, A. E., 2010. On the validation of molecular dynamics simulations of saturated and cis-monounsaturated phosphatidylcholine lipid bilayers: A comparison with experiment. J Comp Theory Comput 6, 325–336.
Poger, D., Mark, A. E., 2012. Lipid bilayers: The effect of force field on ordering and dynamics. J Chem Theory Comput 8 (11), 4807–4817.
Powl, A. M., East, J. M., Lee, A. G., 2005. Heterogeneity in the binding of lipid molecules to the surface of a membrane protein: hot spots for anionic lipids on the mechanosensitive channel of large conductance MscL and effects on

conformation. Biochemistry 44 (15), 5873–83.
Powl, A. M., East, J. M., Lee, A. G., 2007. Different effects of lipid chain length on the two sides of a membrane and the lipid annulus of MscL. Biophysical Journal 93 (1), 113–22.
Powl, A. M., East, J. M., Lee, A. G., 2008. Importance of direct interactions with lipids for the function of the mechanosensitive channel MscL. Biochemistry 47 (46), 12175–84.
Radestock, S., Forrest, L. R., 2011. The alternating-access mechanism of MFS transporters arises from inverted-topology repeats. Journal of Molecular Biology 407 (5), 698–715.
Raetz, C. R., 1986. Molecular genetics of membrane phospholipid synthesis. Annu Rev Genet 20, 253–95.
Raetz, C. R. H., Reynolds, C. M., Trent, M. S., Bishop, R. E., 2007. Lipid A modification systems in gram-negative bacteria. Annu Rev Biochem 76, 295–329.
Rapedius, M., Fowler, P. W., Shang, L., Sansom, M. S. P., Tucker, S. J., Baukrowitz, T., 2007. H bonding at the helix-bundle crossing controls gating in Kir potassium channels. Neuron 55 (4), 602–614.
Reeves, J. P., Condrescu, M., 2008. Ionic regulation of the cardiac sodium-calcium exchanger. Channels (Austin) 2 (5), 322–8.
Ren, G., Reddy, V. S., Cheng, A., Melnyk, P., Mitra, A. K., 2001. Visualization of a water-selective pore by electron crystallography in vitreous ice. Proc Natl Acad Sci USA 98 (4), 1398–403.
Ren, X., Nicoll, D. A., Galang, G., Philipson, K. D., 2008. Intermolecular cross-linking of Na^+-Ca^{2+} exchanger proteins: evidence for dimer formation. Biochemistry 47 (22), 6081–7.
Ren, X., Nicoll, D. A., Xu, L., Qu, Z., Philipson, K. D., 2010. Transmembrane segment packing of the Na^+/Ca^{2+} exchanger investigated with chemical cross-linkers. Biochemistry 49 (39), 8585–91.
Ressl, S., Terwisscha van Scheltinga, A. C., Vonrhein, C., Ott, V., Ziegler, C., 2009. Molecular basis of transport and regulation in the Na^+/betaine symporter BetP. Nature 458 (7234), 47–52.
Rolfe, D. F., Brown, G. C., 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev 77 (3), 731–58.
Romero, M. F., Fulton, C. M., Boron, W. F., 2004. The SLC4 family of HCO_3^- - transporters. Pflügers Arch 447 (5), 495–509.
Romsicki, Y., Sharom, F. J., 1999. The membrane lipid environment modulates drug interactions with the P-glycoprotein multidrug transporter. Biochemistry 38 (21), 6887–96.
Roughan, P., Batt, R., 1969. The glycerolipid composition of leaves. Phytochemistry 8 (2), 363 – 369.
Saier, Jr, M. H., Tran, C. V., Barabote, R. D., 2006. TCDB: the transporter classification database for membrane transport protein analyses and information. Nucleic Acids Res 34 (Database issue), D181–6.
Saier, Jr, M. H., Yen, M. R., Noto, K., Tamang, D. G., Elkan, C., 2009. The transporter classification database: recent advances. Nucleic Acids Res 37 (Database issue), D274–8.
Sanders, C. R., Mittendorf, K. F., 2011. Tolerance to changes in membrane lipid composition as a selected trait of membrane proteins. Biochemistry 50 (37), 7858–67.
Schneiter, R., Toulmay, A., 2007. The role of lipids in the biogenesis of integral membrane proteins. Appl Microbiol Biotechnol 73 (6), 1224–32.
Seto-Young, D., Chen, C. C., Wilson, T. H., 1985. Effect of different phospholipids on the reconstitution of two functions of the lactose carrier of *Escherichia coli*. J Membr Biol 84 (3), 259–67.
Shirakabe, K., Priori, G., Yamada, H., Ando, H., Horita, S., Fujita, T., Fujimoto, I., Mizutani, A., Seki, G., Mikoshiba, K., 2006. IRBIT, an inositol 1,4,5-trisphosphate receptor-binding protein, specifically binds to and activates pancreas-type Na^+/HCO^{3-} cotransporter 1 (pNBC1). Proc Natl Acad Sci U S A 103 (25), 9542–7.
Sonoda, Y., Newstead, S., Hu, N.-J., Alguel, Y., Nji, E., Beis, K., Yashiro, S., Lee, C., Leung, J., Cameron, A. D., Byrne, B., Iwata, S., Drew, D., 2011. Benchmarking membrane protein detergent stability for improving throughput of high-resolution X-ray structures. Structure 19 (1), 17–25.
Soubias, O., Gawrisch, K., 2012. The role of the lipid matrix for structure and function of the GPCR rhodopsin. Biochimica et Biophysica Acta (BBA) - Biomembranes 1818 (2), 234–240.
Soubias, O., Jr, W. E. T., Hines, K. G., Mitchell, D. C., Gawrisch, K., 2010. Contribution of membrane elastic energy to rhodopsin function. Biophsyical Journal 99 (3), 817–824.
Squier, T. C., Bigelow, D. J., Thomas, D. D., 1988. Lipid fluidity directly modulates the overall protein rotational mobility of the Ca-ATPase in sarcoplasmic reticulum. J Biol Chem 263 (19), 9178–86.
Stansfeld, P. J., Hopkinson, R., Ashcroft, F. M., Sansom, M. S. P., 2009. PIP_2-binding site in Kir channels: Definition by multiscale biomolecular simulations. Biochemistry 48 (46), 10926–10933.
Stansfeld, P. J., Sansom, M. S. P., 2011. Molecular simulation approaches to membrane proteins. Structure 19 (11), 1562–1572.
Starling, A. P., Dalton, K. A., East, J. M., Oliver, S., Lee, A. G., 1996. Effects of phosphatidylethanolamines on the activity of the Ca2+-ATPase of sarcoplasmic reticulum. Biochem J 320 (Pt 1), 309–14.
Tajkhorshid, E., Aksimentiev, A., Balabin, I., Gao, M., Isralewitz, B., Phillips, J. C., Zhu, F., Schulten, K., 2003. Large scale simulation of protein mechanics and function. Advances in protein chemistry 66, 195–247.

Tajkhorshid, E., Nollert, P., Jensen, M. Ø., Miercke, L. J. W., O'Connell, J., Stroud, R. M., Schulten, K., 2002. Control of the selectivity of the aquaporin water channel family by global orientational tuning. Science 296 (5567), 525–30.

Taly, A., Corringer, P.-J., Guedin, D., Lestage, P., Changeux, J.-P., 2009. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. Nat Rev Drug Discov 8 (9), 733–50.

Tani, K., Mitsuma, T., Hiroaki, Y., Kamegawa, A., Nishikawa, K., Tanimura, Y., Fujiyoshi, Y., 2009. Mechanism of aquaporin-4's fast and highly selective water conduction and proton exclusion. J Membr Biol 389 (4), 694–706.

Thilo, L., Träuble, H., Overath, P., 1977. Mechanistic interpretation of the influence of lipid phase transitions on transport functions. Biochemistry 16 (7), 1283–90.

Tillman, T. S., Cascio, M., 2003. Effects of membrane lipids on ion channel structure and function. Cell Biochem Biophys 38 (2), 161–190.

Törnroth-Horsefield, S., Wang, Y., Hedfalk, K., Johanson, U., Karlsson, M., Tajkhorshid, E., Neutze, R., Kjellbom, P., 2006. Structural mechanism of plant aquaporin gating. Nature 439 (7077), 688–94.

Toyoshima, C., Inesi, G., 2004. Structural basis of ion pumping by Ca^{2+}-ATPase of the sarcoplasmic reticulum. Annu Rev Biochem 73, 269–92.

Tucker, S. J., Baukrowitz, T., 2008. How highly charged anionic lipids bind and regulate ion channels. J Gen Physiol 131 (5), 431–8.

Ulmschneider, J. P., Ulmschneider, M. B., 2009. United atom lipid parameters for combination with the optimized potentials for liquid simulations all-atom force field. J. Chem. Theo. Comp. 5 (7), 1803–1813.

Uratani, Y., Wakayama, N., Hoshino, T., 1987. Effect of lipid acyl chain length on activity of sodium-dependent leucine transport system in pseudomonas aeruginosa. J Biol Chem 262 (35), 16914–9.

van den Bogaart, G., Meyenberg, K., Risselada, H. J., Amin, H., Willig, K. I., Hubrich, B. E., Dier, M., Hell, S. W., Grubmüller, H., Diederichsen, U., Jahn, R., 2011. Membrane protein sequestering by ionic protein-lipid interactions. Nature 479 (7374), 552–5.

van der Does, C., Swaving, J., van Klompenburg, W., Driessen, A. J., 2000. Non-bilayer lipids stimulate the activity of the reconstituted bacterial protein translocase. J Biol Chem 275 (4), 2472–8.

van Klompenburg, W., Nilsson, I., von Heijne, G., de Kruijff, B., 1997. Anionic phospholipids are determinants of membrane protein topology. EMBO J 16 (14), 4261–6.

van Meer, G., Voelker, D. R., Feigenson, G. W., 2008. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 9 (2), 112–24.

Vemuri, R., Philipson, K. D., 1988. Phospholipid composition modulates the Na^{+}-Ca^{2+} exchange activity of cardiac sarcolemma in reconstituted vesicles. Biochim Biophys Acta 937 (2), 258–68.

Vemuri, R., Philipson, K. D., 1989. Influence of sterols and phospholipids on sarcolemmal and sarcoplasmic reticular cation transporters. J Biol Chem 264 (15), 8680–5.

Vitrac, H., Bogdanov, M., Heacock, P., Dowhan, W., 2011. Lipids and topological rules of membrane protein assembly: balance between long and short range lipid-protein interactions. J Biol Chem 286 (17), 15182–94.

von Heijne, G., 1986. The distribution of positively charged residues in bacterial inner membrane proteins correlates with the trans-membrane topology. EMBO J 5 (11), 3021–7.

von Heijne, G., 2006. Membrane-protein topology. Nat Rev Mol Cell Biol 7 (12), 909–18.

Wang, X. Y., Bogdanov, M., Dowhan, W., 2002. Topology of polytopic membrane protein subdomains is dictated by membrane phospholipid composition. EMBO J 21 (21), 5673–5681.

Wennberg, C. L., van der Spoel, D., Hub, J. S., 2012. Large influence of cholesterol on solute partitioning into lipid membranes. J Am Chem Soc 134 (11), 5351–61.

Weyand, S., Shimamura, T., Beckstein, O., Sansom, M. S. P., Iwata, S., Henderson, P. J. F., Cameron, A. D., 2011. The alternating access mechanism of transport as observed in the sodium-hydantoin transporter Mhp1. J. Synchrotron Rad. 18 (1), 20–23.

Wiggins, P., Phillips, R., 2005. Membrane-protein interactions in mechanosensitive channels. Biophys J 88 (2), 880–902.

Williamson, I. M., Alvis, S. J., East, J. M., Lee, A. G., 2002. Interactions of phospholipids with the potassium channel KcsA. Biophysical Journal 83 (4), 2026–38.

Wintermans, J. F. M., 1960. Concentrations of phosphatides and glycolipids in leaves and chloroplasts. Biochim Biophys Acta 44, 49–54.

Wu, J., McNicholas, C. M., Bevensee, M. O., 2009. Phosphatidylinositol 4,5-bisphosphate (pip2) stimulates the electrogenic na/hco3 cotransporter nbce1-a expressed in xenopus oocytes. Proc Natl Acad Sci U S A 106 (33), 14150–5.

Yaradanakul, A., Feng, S., Shen, C., Lariccia, V., Lin, M.-J., Yang, J., M, K. T., Dong, P., Yin, H. L., Albanesi, J. P., Hilgemann, D. W., 2007. Dual control of cardiac Na^{+}-Ca^{2+} exchange by PIP_2: electrophysiological analysis of direct and indirect mechanisms. J Physiol 582 (Pt 3), 991–1010.

Yorek, M. A., 1993. Biological distribution. In: Cevc, G. (Ed.), Phospholipids Handbook. Marcel Dekker, Inc., pp. 745–776.

Yu, F. X., Sun, H. Q., Janmey, P. A., Yin, H. L., 1992. Identification of a polyphosphoinositide-binding sequence in an actin monomer-binding domain of gelsolin. J Biol Chem 267 (21), 14616–21.

Zeidel, M. L., Nielsen, S., Smith, B. L., Ambudkar, S. V., Maunsbach, A. B., Agre, P., 1994. Ultrastructure, pharmacological inhibition, and transport selectivity of aquaporin channel-forming integral protein in proteoliposomes. Biochemistry 33 (6), 1606–1615.

Zhang, W., Bogdanov, M., Pi, J., Pittard, A. J., Dowhan, W., 2003. Reversible topological organization within a polytopic membrane protein is governed by a change in membrane phospholipid composition. J Biol Chem 278 (50), 50128–35.

Zhang, W., Campbell, H. A., King, S. C., Dowhan, W., 2005. Phospholipids as determinants of membrane protein topology. Phosphatidylethanolamine is required for the proper topological organization of the gamma-aminobutyric acid permease (GabP) of *Escherichia coli*. J Biol Chem 280 (28), 26032–26038.

Zhou, Y., Morais-Cabral, J. H., Kaufman, A., MacKinnon, R., 2001. Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 Å resolution. Nature 414 (6859), 43–8.

Zhu, F., Tajkhorshid, E., Schulten, K., 2001. Molecular dynamics study of aquaporin-1 water channel in a lipid bilayer. FEBS Lett 504 (3), 212–8.

Zhu, F., Tajkhorshid, E., Schulten, K., 2004. Theory and simulation of water permeation in aquaporin-1. Biophysical Journal 86 (1 Pt 1), 50–7.

Zinser, E., Sperka-Gottlieb, C. D., Fasch, E. V., Kohlwein, S. D., Paltauf, F., Daum, G., 1991. Phospholipid synthesis and lipid composition of subcellular membranes in the unicellular eukaryote *Saccharomyces cerevisiae*. J Bacteriol 173 (6), 2026–34.