The biological significance of lipid–protein interactions

2006 J. Phys.: Condens. Matter 18 S1281

(http://iopscience.iop.org/0953-8984/18/28/S11)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **18** (2006) S1281–S1291 doi:10.1088/0953-8984/18/28/S11

The biological significance of lipid–protein interactions

Heather E Findlay[1] **and Paula J Booth**

Department of Biochemistry, University Walk, University of Bristol, Bristol BS8 1TD, UK

E-mail: H.E.Findlay@bristol.ac.uk

Received 3 January 2006, in final form 24 April 2006
Published 28 June 2006
Online at stacks.iop.org/JPhysCM/18/S1281

Abstract

Biological membranes are complex environments, where membrane proteins are surrounded by a bilayer composed of many different types of lipid. The physical properties of the bilayer influence protein structure, folding and function, while specific interactions with lipid molecules can also contribute towards the biological activity of some membrane proteins. Improving understanding of these interactions has resulted in the development of synthetic lipid systems that allow the bilayer properties to be rationally manipulated *in vitro* to control protein behaviour.

1. Membrane organization

Biological membranes perform a number of roles crucial to survival. At a basic level they provide a permeability barrier to prevent proteins, ions and metabolites from leaking out of the cell and unwanted toxins leaking in. Additionally, they are involved in nutrient uptake and waste export, osmotic homoeostasis, cell-to-cell signalling and motility. They maintain electrochemical gradients and compartmentalize the cell. They have important roles in respiration and photosynthesis and support considerable enzymatic activity.

To accomplish these diverse tasks membranes have a correspondingly complex organization. A wide variety of lipids are present, with differing distribution between organisms and membranes within organisms. The inner membrane of Gram-negative bacteria contains a high percentage of phosphatidylethanolamine (PE), together with phosphatidylglycerol (PG) and cardiolipin. In contrast, Gram-positive bacterial cytoplasmic membranes are predominantly PG and diphosphatidylglycerol (DPG), with mainly saturated and branched acyl chains [1, 2]. Gram-negative bacteria are also surrounded by a second, outer membrane that displays an asymmetric lipid distribution. The inner leaflet is similar to that of the inner membrane, but the outer leaflet is composed of lipopolysaccharides (LPSs) [3]. Mammalian cells have cytoplasmic membranes that contain mostly phosphatidylcholine (PC), PE, phosphatidylserine

[1] Author to whom any correspondence should be addressed.

(PS), sphingomyelin (SM) and cholesterol [4]. In contrast, cholesterol is present only at much lower concentrations in the endoplasmic reticulum and mitochondrial membranes [5].

Membrane lipids can self-assemble into numerous different phases in aqueous solution, including micellar, lamellar, hexagonal and cubic phases (for reviews, see [6–12]). Type I lipids aggregate so that the polar/apolar interface curves towards the apolar region, and tend to form micelles. Type II lipids tend to curve towards the polar region. Most bio-lipids are type II, but can form either lamellar or non-lamellar phases, with the examples, respectively, of PC and PE being particularly well characterized [13]. However, the phase properties of biological membranes are far more complex, as a result of the broad mix of lipids, the monolayer asymmetry and the presence of embedded proteins.

Recently, there has been increasing evidence for the presence of lipid microdomains within the bilayer that have properties distinct from the surrounding lipid [14, 15]. These lipid rafts are proposed to be small, dynamic assemblies with high concentrations of cholesterol, sphingolipids and saturated phospholipids and create a local area of increased order and, potentially, bilayer thickness [5]. Certain proteins seem to be localized preferentially to raft lipid regions, and it has been suggested this may contribute towards the clustering of specific proteins for processes such as signal transduction. However, while these lipid domains can be clearly observed in model systems, the situation in native membranes is ambiguous. Limitations in techniques for the detection and isolation of rafts from cell membranes mean that their *in vivo* presence and biological relevance remain in question [16].

An elastic, fluid bilayer is vital for membrane integrity and function, and organisms such as *A. laidlawii* and *E. coli* have been shown to adapt their membrane lipid composition to maintain this in response to environmental challenges including temperature, pressure, pH and salinity [17]. Cells employ a variety of methods to maintain bilayer fluidity. The proportions of different polar head groups can be changed, and the degree of saturation, length or branching of the acyl chains can be altered [18, 19].

2. Membrane proteins

Anywhere from 20 to 80% of the membrane, by weight, is composed of protein. Indeed, a high proportion of the proteins in a cell are membrane proteins, with statistical analysis of genomic sequences predicting around 30% of the total protein content to be integral to the membrane [20]. The energy cost of transferring peptides into the non-polar environment of the bilayer is high, therefore only two structural motifs for crossing the membrane are permissible—α-helical bundles and β-barrels, where the complete internal hydrogen-bonding of the secondary structure reduces the energy cost [21]. There is, however, considerable variety within these two basic structural categories. Recently, an attempt has been made to classify membrane transport proteins on a rational basis [22]. Classes include channels and pores, electrochemical potential-driven transporters, primary active transporters, group translocators and transport electron carriers. In addition to the transporters found in the membrane, there are many enzymes and receptors, which are often of high pharmacological interest.

Despite the biological importance of membrane proteins, there have been relatively few 3D structures solved, with only around 90 distinct structures compared to the many thousands from soluble proteins (see http://www.mpibp-frankfurt.mpg.de/michel/public/memprotstruct.html). There are several difficulties inherent to working with membrane proteins, including generally poor over-expression in traditional heterologous systems [23], but a major problem is the complexity of the surrounding lipid bilayer of their native environment. Lipids are involved in specific interactions with membrane proteins, and also contribute to global bilayer properties, both of which can influence protein stability, folding and function.

3. Direct lipid–protein interactions with the bilayer

It is important for membrane proteins to form a good seal with the lipid bilayer to maintain structural integrity. The technique of spin-label electron paramagnetic resonance (EPR) has been useful in examining direct interactions at the lipid–protein interface. EPR provides information on the motion of labelled acyl chains within the bilayer, and can distinguish lipids whose mobility has been restricted due to interaction with the protein surface from those in the bulk lipid. The number of lipid molecules identified by this method as associated with the surface of a protein corresponds well with a calculated value of its hydrophobic surface area [24]. However, these interactions are rather transient, with off rate constants of around $1–2 \times 10^7\ s^{-1}$, for the lipid dissociating from the protein [25]. Although slower than the diffusion exchange rates of lipids in the fluid bilayer ($\sim 8 \times 10^7\ s^{-1}$) the difference is slight, suggesting these lipids are relatively loosely associated with the protein surface.

The loosely associated lipids described above are generally not found in crystal structures. The process of purification involves extensive exposure to detergent solutions, during which the protein is usually delipidated of all but the most tightly bound lipids. However, one structure that does contain a number of these surrounding lipids is that of the light-driven proton pump bacteriorhodopsin. Bacteriorhodopsin has seven transmembrane helices and forms a hexagonal array of trimers in the membrane. A 1.55 Å x-ray from crystals grown in lipid cubic phase was solved with 18 native purple membrane lipid molecules identified, covering approximately 80% of the hydrophobic surface area and forming a bilayer-like structure around the protein [26]. The acyl chains were aligned along distinct grooves on the protein surface. This complementarity on the lipid–protein interface is likely to be important for the effective integration of membrane proteins in the bilayer.

When lipids are structurally resolved, they tend to be situated in cavities or clefts in the protein structure, often between subunits of oligomeric complexes. In a 2 Å structure of the tetrameric bacterial potassium channel KcsA a single lipid was resolved, filling a groove at the interface of each adjacent monomer. The head group was too disordered to classify in the crystal structure, but subsequent biochemical analysis identified it as a PG molecule [27]. Similarly in bacteriorhodopsin a lipid molecule is found in several independent crystal structures wedged between neighbouring monomers [26, 28, 29]. The more common appearance of these types of lipids in structures in itself suggests tighter binding to the protein, and they are predicted to have specific roles in both the structure and function of membrane proteins.

Proteins themselves are also adapted to integrate stably into the membrane. There is a concentration of the polar aromatic residues Trp and Tyr on the exterior surface of membrane proteins at the lipid–water interface at the edge of the hydrophobic core area of the bilayer [30]. The indole ring of Trp partitions preferentially into this interface region and these amino acids have been suggested to act as an anchor for positioning the protein within the bilayer [31]. The 'aromatic belts' are observed in β-barrel as well as α-helical membrane proteins [32], though they are closer together, perhaps reflecting a thinner outer membrane structure compared to plasma membranes.

4. Global bilayer effects

The mix of lipids in biological membranes confers collective properties to the bilayer that can influence the proteins embedded within them. Membranes contain both lamellar and non-lamellar forming lipids. Lipids such as PC, PS, PG and SM are lamellar forming lipids that form fluid lamellar phases in physiological conditions, whereas PE and highly unsaturated

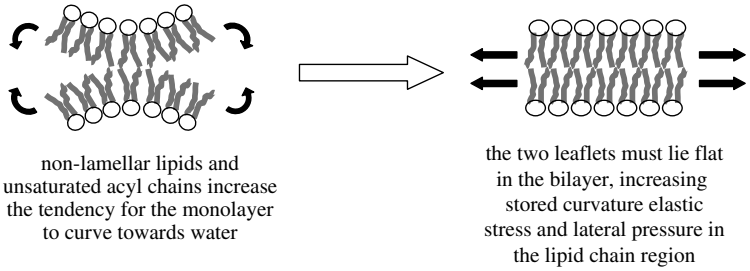

Figure 1. Curvature elastic stress of bilayers.

lipids form non-lamellar inverse hexagonal phases [8]. The addition of non-lamellar lipids to a membrane increases the propensity of each monolayer to curve towards the water. However, due to hydrophobic constraints, the two leaflets within a bilayer structure cannot separate, leading to an increase in curvature elastic stress. This is accompanied by an increase in lateral pressure in the central region of the membrane as a result of increased collisions between acyl chains, and potentially a corresponding decrease in pressure in the headgroup region of the bilayer [33] (figure 1). As the proportion of non-lamellar lipids is increased, eventually the stress will be released by a phase change to a bicontinuous cubic or hexagonal phase. Increasing the number of unsaturated lipid chains also increases the desire for monolayer curvature due to kinking of the chains, which in turn leads to an increase in stored curvature elastic stress. The alterations to lipid composition in different environmental conditions are predicted to be in response to sensing the curvature elastic stress and the requirement for a fluid bilayer [18, 34].

The hydrophobic thickness of a membrane can also influence protein structure and function. The energetic cost of burying polar groups within the bilayer or exposing non-polar groups to solvent requires that any hydrophobic mismatch between a protein hydrophobic surface area and bilayer thickness must be compensated for. There are several ways this can be achieved; for example, lipids can adapt to hydrophobic mismatch by stretching or disordering their acyl chains whilst proteins can aggregate or adjust the tilt of their helices in the membrane [35]. Mismatch has also been predicted to have a role in protein sorting. There are increasing levels of cholesterol present in membranes along the secretory pathway of eukaryotic cells, from endoplasmic reticulum, to the Golgi, to the plasma membrane, which could cause an increase in bilayer thickness. The average length of transmembrane domains of proteins that are sorted to the plasma membrane is five residues longer than that of proteins retained in the Golgi [5]. Increasing the length of transmembrane helices of Golgi proteins can cause them to be routed to the cell surface, whilst decreasing the length of helices of plasma membrane proteins can result in retention in the Golgi [36], and it has therefore been suggested that hydrophobic mismatch may be involved in preferential routing of plasma membrane proteins to the surface down a pathway of increasing bilayer thickness, possibly involving SM and cholesterol rich raft domains [15]. However, it has recently been shown that membrane proteins themselves may dictate the thickness of the surrounding bilayer to a greater extent than cholesterol content [37], indicating a more complicated role for hydrophobic mismatch in protein, and potentially, lipid sorting.

5. Protein stability

The lipid environment can have a profound effect on the stability of integral membrane proteins, both through general bilayer properties and specific binding. A 2.1 Å crystal structure of the

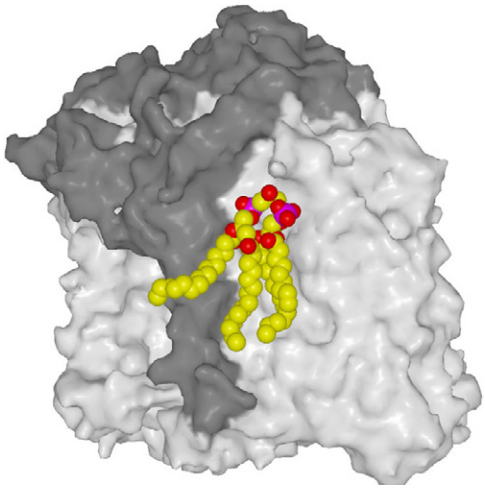

Figure 2. Surface representation of reaction centre with bound cardiolipin shown in spacefill (1QOV).

(This figure is in colour only in the electronic version)

purple bacterial reaction centre from *Rhodobacter sphaeroides* was shown to contain a single molecule of cardiolipin bound to the protein, in contact with all three subunits [38] (figure 2). The anionic headgroup of cardiolipin formed direct bonding interactions with basic residues on the protein, whilst the acyl chains lay along grooves on the surface. Mutagenesis of these basic residues prevented cardiolipin binding, as seen by x-ray crystallography, without otherwise appearing to affect protein expression or function [39]. However, the melting temperature of the protein was decreased by this mutation, suggesting a role for this cardiolipin molecule in reaction centre thermal stability.

Global bilayer forces also contribute towards protein stability. Bacteriorhodopsin reconstituted into the bilayers of the saturated lipid 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) denatured at a lower temperature than when the unsaturated 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was added, increasing the curvature elastic stress [40]. Reconstituted KcsA tetramer can be dissociated by 2,2,2-trifluoroethanol (TFE), by its partitioning into the headgroup region of the bilayer and consequently changing the lateral pressure profile. The denaturing concentration of TFE was found to be considerably higher for protein reconstituted into stressed bilayers containing 70% non-lamellar lipid 1,2-dioleoyl-*sn*-glycero-3-ethanolamine (DOPE) than DOPC only bilayers [41]. This result could be replicated with other small alcohols, where their ability to denature KscA was found to be correlated to their ability to alter lateral pressure within the bilayer [42]. Changes in curvature stress can also affect β-barrel proteins. Increasing the fraction of PE lipids in PC/PG bilayers was found to stabilize outer membrane porin OmpA against urea denaturation [3]. Increasing bilayer thickness was also found to increase OmpA stability [43].

6. Protein folding

There is increasing evidence for lipids playing an important role in membrane protein folding. The most widely studied example is that of bacteriorhodopsin. Denatured bacteriorhodopsin can be refolded from sodium dodecylsulfate (SDS) into lipid vesicles, with very high yields

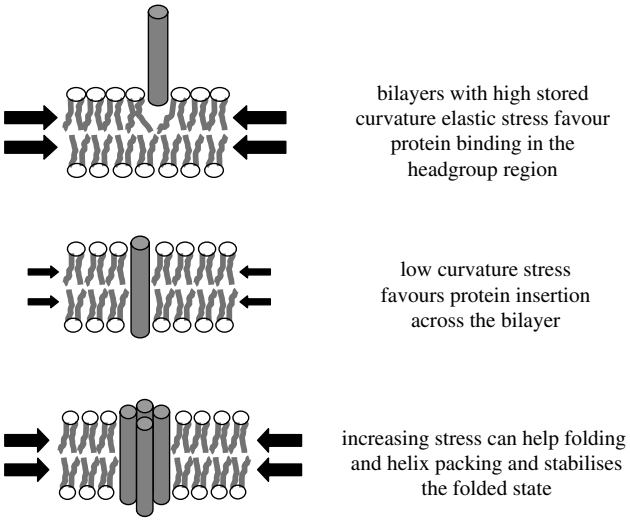

Figure 3. Different curvature stress may be optimal for different stages of membrane protein folding.

of regeneration [44]. It was found that the refolding yield could be adjusted in a predictable manner by changes to the bilayer curvature stress. The addition of non-lamellar lipid to 1,2-dipalmitoleolyl-*sn*-glycero-3-phosphocholine (DPoPC) vesicles increased the curvature stress and decreased recovery of functional protein, while correspondingly, relaxing the bilayer by the addition of saturated or single chain (lyso) lipids increased the folding yield [40]. Kinetics studies on the refolding process indicated that in stressed bilayers there was less total protein inserted into the bilayer, leading to less correctly folded protein. This is consistent with the increased activation energy for transmembrane helix insertion across the bilayer with increased PE content and therefore higher curvature stress [45]. Decreasing bilayer stress resulted in larger proportions of protein insertion, but could result in slower folding rates if the bilayer was relaxed too much.

These data have led to a model for the effect of bilayer properties on the folding and stability of membrane proteins, with different curvature stresses optimal for different stages of the folding process [46]. A stressed bilayer favours binding of the protein to the headgroup region, but a relaxed bilayer is necessary to allow full insertion across the membrane. However, a more stressed bilayer may be required for optimal folding rates and can increase stability of the folded state, probably as a result of increased lateral chain pressure (figure 3). The model can be related to standard protocols for reconstitution of membrane proteins *in vitro*. This involves pre-saturating the liposomes with detergent, which relaxes the bilayer and aids protein insertion. The detergent is subsequently removed by either dialysis or Biobeads, leading to a rise again of curvature stress, stabilizing the protein within the bilayer.

Specific roles for lipids in protein folding have also been observed *in vivo*. The *E. coli* lactose permease, LacY, is a 12 transmembrane helix proton/lactose symporter, that has a requirement for PE for correct folding [47]. When expressed in a PE-deficient strain of *E. coli*, with inducible PE synthesis, the first six helices of LacY displayed an inverted topology [48]. On the expression of PE post-assembly the correct topology was recovered, demonstrating that lipid composition, in addition to the protein primary sequence, can be a determinant of orientation in the membrane. A similar result has been seen with both the *E. coli* phenylalanine

permease PheP [49] and γ-aminobutyric acid (GABA) permease GabP [50] transporters, where the first two helices are reversibly orientated in a PE-dependent manner.

7. Protein function

The function of proteins is influenced by the lipid environment. Some respond directly to the curvature stress of the bilayer, such as the mechanosensitive channels and CTP:phosphocholine cytidylyltransferase (CCT). The large mechanosensitive ion channel from *E. coli*, MscL, is a homopentamer with each subunit contributing two transmembrane helices to form a channel with a high, non-selective conductance ($\sim$3 nS) [51]. MscL is activated in response to osmotic challenges to protect the cell from lysis. Purified protein is functional when reconstituted *in vitro*, suggesting that gating occurs as a result of changes in the bilayer itself [52]. In synthetic lipid systems, decreasing the curvature stress and lipid chain pressure by the addition of lysoPC lipids to one side of the bilayer was found to activate the MscL channels [53]. The presence of PE in the bilayer was found to counteract this, demonstrating that the effect is due to changes in bilayer pressures, not a specific effect of lysoPC. In contrast, changing the bilayer thickness by reconstituting into lipids with different chain lengths failed to induce activation of the channel. An alternative method of changing the bilayer pressure has been demonstrated for MscL. An azobenzene lipid mimic was included in the reconstitution, which has the property of reversible photoisomerization between a *cis* and *trans* conformation in the chain region [54]. The *trans* to *cis* isomerization increased the activity of the protein while the *cis* to *trans* decreased it, probably as a result of changes to the lateral pressure profile of the bilayer.

CCT is a rate-limiting enzyme in PC biosynthesis that is activated upon the binding of its amphipathic helical domain to lipid bilayers [55]. Activity of CCT is decreased in DMPC/DOPC vesicles compared to DOPC alone and increased in DOPC/DOPE vesicles, consistent with variations in curvature stress modulating function [56]. CCT is predicted to bind preferentially to stressed bilayers, where partitioning into the headgroup region allows a release of tension. Once bound, the enzyme is activated and PC is synthesized. An increase in the fraction of the lamellar lipid PC in the membrane results in a decrease in curvature stress, so that CCT binding is less favourable. This model demonstrates how a direct physical feedback of bilayer stress could be involved in membrane homeostasis, and how lipid composition can affect the function of peripheral as well as integral membrane proteins.

Lipid composition also modulates the activity of a range of other proteins, where physiological function is not directly related to membrane conditions. The photopigment rhodopsin is the major protein of rod outer segment cells, a seven transmembrane helical protein that activates the visual signal transduction cascade. To initiate the transduction, rhodopsin must undergo conformational changes to form an activated state. The proportion of protein in the activated form is influenced by the lipid environment. In native-like membrane conditions the activated state is favoured; however, this can be replicated using bilayers with high curvature stress containing non-bilayer and saturated lipids [57, 58]. Cholesterol also affects rhodopsin function. A large fraction of cholesterol in the membrane will increase the thermal stability of rhodopsin [59]; however, it also inhibits the formation of the activated state of the protein [58, 60]. For optimal function, the membrane must strike a balance between having enough cholesterol to stabilize the protein, without overly inhibiting its activity.

A balance must also be achieved for the best function of the peptide alamethicin. Alamethicin is a small 20 amino acid peptide that is capable of forming voltage-gated ion channels in lipid bilayers, with from four to 11 monomers forming helical bundles surrounding a central, conductive pore. Larger aggregations of peptide result in higher conductance states of the channel [61]. The binding free energy for monomeric alamethicin to vesicles increased linearly in proportion to increased curvature stress in the bilayer [62]. This results in reduced

partitioning of the peptide into stressed bilayers, and indeed increased peptide concentrations were required to obtain channels in planar bilayer systems of DOPE/DOPC compared to DOPC alone [63]. However, the probability of higher conductance states forming was found to increase with an increasing fraction of PE in the bilayer. Thus while low curvature stress favours initial peptide insertion, high curvature stress promotes monomer aggregation and correspondingly increased function.

Some lipids can have specific effects on protein function. The cytochrome bc_1 complex is inactivated by the delipidation of the protein [64]. Digestion of tightly bound lipids from bovine cytochrome bc_1 abolished function, but did not change the spectral properties of the complex. The addition of cardiolipin was necessary for the recovery of activity, suggesting a specific functional role for the lipid [65]. A 2.3 Å structure of yeast cytochrome bc_1 resolved several lipid molecules, including one of cardiolipin [66]. The cardiolipin headgroup is bound in close proximity to the site of quinione reduction, at the entrance to a potential proton-conducting pathway, and as such may be directly involved in proton uptake.

β-barrel function can also be modulated by lipids. The outer membrane protease OmpT folds to its native barrel structure *in vitro*, but is inactive in the absence of lipopolysaccharide [67]. Binding of LPS did not result in a large structural change, but kinetic analysis of OmpT and different LPS molecules demonstrated a minimum requirement of fully acylated lipid A for activity [68]. The mechanism for this interaction has not yet been determined.

8. Conclusions

Biological membranes are complex environments, which perform many functions fundamental to cell survival. This complexity is reflected in the large variety in lipid composition and number of membrane proteins. Lipid–protein interactions have been shown to influence the folding, the stability and the function of peptides and proteins, integral and peripheral membrane proteins, those with helical structures and those with barrel structures. These influences can be due to the binding of specific lipid molecules, stabilizing the protein structure within the bilayer, or acting as lipid cofactors, where they play an integral part in the function. Global bilayer properties, acting non-specifically, alter the environment surrounding proteins and consequently influence their structure and activity, whilst the incorporation of proteins into membranes can in turn influence the bilayer properties. Despite this complexity some general rules are becoming apparent. The membrane must be maintained at an optimal fluidity, usually just below the transition between lamellar and non-lamellar phases. Bilayers with low curvature stress favour protein insertion. Bilayers with high curvature stress improve stability and promote protein oligomerization.

When working with membrane proteins *in vitro* it is important to be aware of the potential influence of lipid–protein interactions. Samples may need to be supplemented with native lipids to meet specific requirements for function. Synthetic lipid systems are proving more effective at mimicking the native membrane environment than traditional detergent-based methods, creating bilayer conditions that can be simply and predictably manipulated with a view to improving stability or folding, and furthering the understanding of membrane protein reactions. These different methods for handling membrane proteins could potentially be useful for solving some of the expression problems inherent to working with them, and therefore help further the production of more 3D structures.

Acknowledgments

Heather Findlay is supported by the European Membrane Protein Consortium (E-MeP).

References

[1] O'Leary M W and Wilkinson S G 1988 Gram-positive bacteria *Microbial Lipids* ed C Ratledge (London: Academic) pp 117–201

[2] Wilkinson S 1988 Gram-negative bacteria *Microbial Lipids* ed C W Ratledge and S G Wilkinson (London: Academic) pp 299–488

[3] Tamm L K, Hong H and Liang B 2004 Folding and assembly of beta-barrel membrane proteins *Biochim. Biophys. Acta* **1666** 250–63

[4] Yorek M 1993 Biological distribution *Phospholipid Handbook* ed G Cevc (New York: Dekker)

[5] Bretscher M S and Munro S 1993 Cholesterol and the golgi apparatus *Science* **261** 1280–1

[6] Epand R 1997 *Lipid Polymorphism and Membrane Properties* (*Current Topics in Membranes* vol 44) ed D Fambrough and D Benos (New York: Academic)

[7] Hyde S, Andersson S, Larsson K, Blum Z, Landh T, Lidin S and Ninham B 1997 *The Language of Shape* (Amsterdam: Elsevier)

[8] Israelachvili J N 1991 *Intermolecular and Surface Forces* 2nd edn (London: Academic) p 450

[9] Larsson K 2000 Aqueous dispersions of cubic lipid–water phases *Curr. Opin. Colloid Interface Sci.* **5** 64–9

[10] Luzzati V 1968 X-ray diffraction studies of lipid–water systems *Biological Membranes* ed D Chapman (London: Academic) pp 71–123

[11] Luzzati V and Tardieu A 1974 Lipid phases—structure and structural transitions *Annu. Rev. Phys. Chem.* **25** 79–94

[12] Seddon J M 1990 Structure of the inverted hexagonal (HII) phase, and non-lamellar phase transitions of lipids *Biochim. Biophys. Acta* **1031** 1–69

[13] Seddon J M and Templer R 1995 Polymorphism of lipid–water systems *Structure and Dynamics of Membranes* ed R Lipowsky and E Sackmann (Amsterdam: Elsevier) pp 97–160

[14] Gandhavadi M, Allende D, Vidal A, Simon S A and McIntosh T J 2002 Structure, composition, and peptide binding properties of detergent soluble bilayers and detergent resistant rafts *Biophys. J.* **82** 1469–82

[15] Simons K and Toomre D 2000 Lipid rafts and signal transduction *Nat. Rev. Mol. Cell. Biol.* **1** 31–9

[16] London E 2005 How principles of domain formation in model membranes may explain ambiguities concerning lipid raft formation in cells *Biochim. Biophys. Acta* **1746** 203–20

[17] Hazel J R and Williams E E 1990 The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment *Prog. Lipid Res.* **29** 167–227

[18] Morein S, Andersson A, Rilfors L and Lindblom G 1996 Wild-type Escherichia coli cells regulate the membrane lipid composition in a 'window' between gel and non-lamellar structures *J. Biol. Chem.* **271** 6801–9

[19] Suutari M and Laakso S 1994 Microbial fatty acids and thermal adaptation *Crit. Rev. Microbiol.* **20** 285–328

[20] Wallin E and von Heijne G 1998 Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms *Protein Sci.* **7** 1029–38

[21] White S H and Wimley W C 1999 Membrane protein folding and stability: physical principles *Annu. Rev. Biophys. Biomol. Struct.* **28** 319–65

[22] Busch W and Saier M H Jr 2004 The IUBMB-endorsed transporter classification system *Mol. Biotechnol.* **27** 253–62

[23] Drew D, Froderberg L, Baars L and de Gier J W 2003 Assembly and overexpression of membrane proteins in Escherichia coli *Biochim. Biophys. Acta* **1610** 3–10

[24] Palsdottir H and Hunte C 2004 Lipids in membrane protein structures *Biochim. Biophys. Acta* **1666** 2–18

[25] Marsh D and Horvath L I 1998 Structure, dynamics and composition of the lipid–protein interface. Perspectives from spin-labelling *Biochim. Biophys. Acta* **1376** 267–96

[26] Luecke H, Schobert B, Richter H T, Cartailler J P and Lanyi J K 1999 Structure of bacteriorhodopsin at 1.55 A resolution *J. Mol. Biol.* **291** 899–911

[27] Valiyaveetil F I, Zhou Y and MacKinnon R 2002 Lipids in the structure, folding, and function of the KcsA K+ channel *Biochemistry* **41** 10771–7

[28] Belrhali H, Nollert P, Royant A, Menzel C, Rosenbusch J P, Landau E M and Pebay-Peyroula E 1999 Protein, lipid and water organization in bacteriorhodopsin crystals: a molecular view of the purple membrane at 1.9 A resolution *Struct. Fold Des.* **7** 909–17

[29] Cartailler J P and Luecke H 2003 X-ray crystallographic analysis of lipid–protein interactions in the bacteriorhodopsin purple membrane *Annu. Rev. Biophys. Biomol. Struct.* **32** 285–310

[30] Ulmschneider M B and Sansom M S 2001 Amino acid distributions in integral membrane protein structures *Biochim. Biophys. Acta* **1512** 1–14

[31] Braun P and von Heijne G 1999 The aromatic residues Trp and Phe have different effects on the positioning of a transmembrane helix in the microsomal membrane *Biochemistry* **38** 9778–82

[32] Schulz G E 2000 beta-Barrel membrane proteins *Curr. Opin. Struct. Biol.* **10** 443–7

[33] Cantor R S 1999 Lipid composition and the lateral pressure profile in bilayers *Biophys. J.* **76** 2625–39
[34] Gruner S M 1985 Intrinsic curvature hypothesis for biomembrane lipid composition: a role for nonbilayer lipids *Proc, Natl Acad. Sci. USA* **82** 3665–9
[35] Killian J A 1998 Hydrophobic mismatch between proteins and lipids in membranes *Biochim. Biophys. Acta* **1376** 401–15
[36] Munro S 1995 An investigation of the role of transmembrane domains in Golgi protein retention *Embo J.* **14** 4695–704
[37] Mitra K, Ubarretxena-Belandia I, Taguchi T, Warren G and Engelman D M 2004 Modulation of the bilayer thickness of exocytic pathway membranes by membrane proteins rather than cholesterol *Proc. Natl Acad. Sci. USA* **101** 4083–8
[38] McAuley K E, Fyfe P K, Ridge J P, Isaacs N W, Cogdell R J and Jones M R 1999 Structural details of an interaction between cardiolipin and an integral membrane protein *Proc. Natl Acad. Sci. USA* **96** 14706–11
[39] Fyfe P K, Isaacs N W, Cogdell R J and Jones M R 2004 Disruption of a specific molecular interaction with a bound lipid affects the thermal stability of the purple bacterial reaction centre *Biochim. Biophys. Acta* **1608** 11–22
[40] Allen S J, Curran A R, Templer R H, Meijberg W and Booth P J 2004 Controlling the folding efficiency of an integral membrane protein *J. Mol. Biol.* **342** 1293–304
[41] van den Brink-van der Laan E, Chupin V, Killian J A and de Kruijff B 2004 Stability of KcsA tetramer depends on membrane lateral pressure *Biochemistry* **43** 4240–50
[42] van den Brink-van der Laan E, Chupin V, Killian J A and de Kruijff B 2004 Small alcohols destabilize the KcsA tetramer via their effect on the membrane lateral pressure *Biochemistry* **43** 5937–42
[43] Hong H and Tamm L K 2004 Elastic coupling of integral membrane protein stability to lipid bilayer forces *Proc. Natl Acad. Sci. USA* **101** 4065–70
[44] Curran A R, Templer R H and Booth P J 1999 Modulation of folding and assembly of the membrane protein bacteriorhodopsin by intermolecular forces within the lipid bilayer *Biochemistry* **38** 9328–36
[45] Meijberg W and Booth P J 2002 The activation energy for insertion of transmembrane alpha-helices is dependent on membrane composition *J. Mol. Biol.* **319** 839–53
[46] Booth P J 2005 Sane in the membrane: designing systems to modulate membrane proteins *Curr. Opin. Struct. Biol.* **15** 435–40
[47] Bogdanov M and Dowhan W 1998 Phospholipid-assisted protein folding: phosphatidylethanolamine is required at a late step of the conformational maturation of the polytopic membrane protein lactose permease *Embo J.* **17** 5255–64
[48] Bogdanov M, Heacock P N and Dowhan W 2002 A polytopic membrane protein displays a reversible topology dependent on membrane lipid composition *Embo J.* **21** 2107–16
[49] Zhang W, Bogdanov M, Pi J, Pittard A J and Dowhan W 2003 Reversible topological organization within a polytopic membrane protein is governed by a change in membrane phospholipid composition *J. Biol. Chem.* **278** 50128–35
[50] Zhang W, Campbell H A, King S C and 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** 26032–8
[51] Sukharev S I, Martinac B, Arshavsky V Y and Kung C 1993 Two types of mechanosensitive channels in the Escherichia coli cell envelope: solubilization and functional reconstitution *Biophys. J.* **65** 177–83
[52] Sukharev S I, Blount P, Martinac B, Blattner F R and Kung C 1994 A large-conductance mechanosensitive channel in E. coli encoded by mscL alone *Nature* **368** 265–8
[53] Perozo E, Kloda A, Cortes D M and Martinac B 2002 Physical principles underlying the transduction of bilayer deformation forces during mechanosensitive channel gating *Nat. Struct. Biol.* **9** 696–703
[54] Folgering J H, Kuiper J M, de Vries A H, Engberts J B and Poolman B 2004 Lipid-mediated light activation of a mechanosensitive channel of large conductance *Langmuir* **20** 6985–7
[55] Kent C 1997 CTP: phosphocholine cytidylyltransferase *Biochim. Biophys. Acta* **1348** 79–90
[56] Attard G S, Templer R H, Smith W S, Hunt A N and Jackowski S 2000 Modulation of CTP:phosphocholine cytidylyltransferase by membrane curvature elastic stress *Proc. Natl Acad. Sci. USA* **97** 9032–6
[57] Brown M F 1994 Modulation of rhodopsin function by properties of the membrane bilayer *Chem. Phys. Lipids* **73** 159–80
[58] Litman B J and Mitchell D C 1996 A role for phospholipid polyunsaturation in modulating membrane protein function *Lipids* **31** S193–7
[59] Albert A D, Boesze-Battaglia K, Paw Z, Watts A and Epand R M 1996 Effect of cholesterol on rhodopsin stability in disk membranes *Biochim. Biophys. Acta* **1297** 77–82

[60] Albert A D and Boesze-Battaglia K 2005 The role of cholesterol in rod outer segment membranes *Prog. Lipid Res.* **44** 99–124
[61] Sansom M S 1993 Structure and function of channel-forming peptaibols *Q. Rev. Biophys.* **26** 365–421
[62] Lewis J R and Cafiso D S 1999 Correlation between the free energy of a channel-forming voltage-gated peptide and the spontaneous curvature of bilayer lipids *Biochemistry* **38** 5932–8
[63] Keller S L, Bezrukov S M, Gruner S M, Tate M W, Vodyanoy I and and Parsegian V A 1993 Probability of alamethicin conductance states varies with nonlamellar tendency of bilayer phospholipids *Biophys. J.* **65** 23–7
[64] Schagger H, Hagen T, Roth B, Brandt U, Link T A and von Jagow G 1990 Phospholipid specificity of bovine heart bc1 complex *Eur. J. Biochem.* **190** 123–30
[65] Gomez B, Jr and Robinson N C 1999 Phospholipase digestion of bound cardiolipin reversibly inactivates bovine cytochrome bc1 *Biochemistry* **38** 9031–8
[66] Lange C, Nett J H, Trumpower B L, and C and Hunte 2001 Specific roles of protein–phospholipid interactions in the yeast cytochrome bc1 complex structure *Embo J.* **20** 6591–600
[67] Kramer R A, Zandwijken D, Egmond M R and Dekker N 2000 In vitro folding, purification and characterization of Escherichia coli outer membrane protease ompT *Eur. J. Biochem.* **267** 885–93
[68] Kramer R A, Brandenburg K, Vandeputte-Rutten L, Werkhoven M, Gros P, Dekker N and Egmond M R 2002 Lipopolysaccharide regions involved in the activation of Escherichia coli outer membrane protease OmpT *Eur. J. Biochem.* **269** 1746–52