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Peptides in lipid bilayers: the power of simple models

J Antoinette Killian and Thomas KM Nyholm

Interactions between proteins and lipids lie at the heart of virtually all membrane processes, but on a molecular level they are still poorly understood. Nowadays, simple model systems comprising designed transmembrane peptides in synthetic lipid bilayers are increasingly being recognized as powerful tools to uncover basic principles of protein–lipid interactions. Such model systems enable detailed analysis of how the properties of lipids influence the structure and dynamics of transmembrane helices, how these helices are anchored at the lipid–water interface, and how the length and composition of transmembrane segments influence the organization and dynamics of membrane lipids. In addition, well-characterized model systems have proven useful to refine computational approaches and to develop new techniques for studies of protein–lipid interactions.

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Introduction

Interactions between membrane proteins and lipids are essential to a huge variety of cellular processes, including transport, signalling and membrane biogenesis. However, little is understood of the role of protein–lipid interactions in these processes. This is because lipids and proteins can influence each other in so many different ways [1–3]. Lipids may affect the structure of membrane proteins by influencing their backbone conformation, the tilt and rotation angles of their transmembrane segments, or the orientation of their sidechains. They may influence the organization of membrane proteins by affecting their mode of self-association or change their localization by laterally directing them to specific membrane domains. *Vice versa*, membrane proteins may stretch or disorder the chains of surrounding lipids, promote transbilayer movement of lipids, influence their lateral organization, induce formation of different macroscopic phases, or promote processes such as membrane fusion or fission.

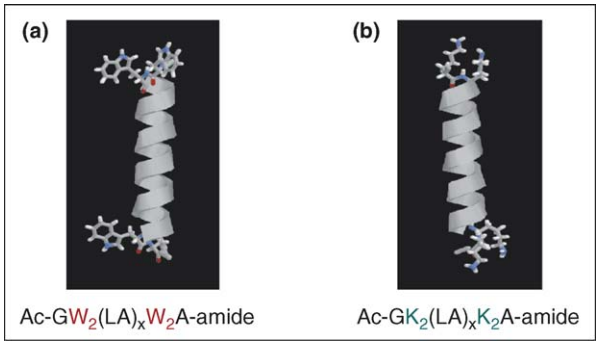
How to uncover the basic principles that govern the molecular consequences of protein–lipid interactions? Many researchers nowadays are using simple model systems comprising peptides that mimic transmembrane regions of proteins in synthetic lipid bilayers [3]. Examples of such model peptides are the so-called WALP and KALP peptides (Figure 1). They consist of a variable-length hydrophobic stretch of leucine and alanine residues, flanked, respectively, by tryptophan residues, which in natural membrane proteins are enriched at the lipid–water interface, and by lysine residues, which are generally located more outwards [4]. Both the design of such model peptides and the composition of the lipids can be systematically varied to answer specific questions. Below, recent progress will be discussed on how the use of simple peptide–lipid model systems has helped in understanding protein–lipid interactions.

How lipids influence membrane protein structure and organization

Model transmembrane peptides have been used in a variety of ways to investigate how the lipid environment can affect membrane proteins. General properties of lipids that can be sensed by membrane proteins include lipid packing, the lateral pressure profile across the membrane, intrinsic curvature of the lipids, bilayer thickness and electrostatic properties [1,2]. In particular, studies of the consequences of varying bilayer thickness have been useful as a tool to determine basic principles of protein–lipid interactions [3]. When the hydrophobic thickness of the bilayer is larger or smaller than the hydrophobic length of the transmembrane protein, this would lead to exposure of hydrophobic groups to a hydrophilic environment. As a consequence, proteins and lipids will tend to adapt their organization, as illustrated in Figure 2 for proteins in the case of positive mismatch, that is, when the transmembrane segments are relatively long. Studies of model systems have enabled these various consequences to be addressed in detail, hence gaining insight into properties of transmembrane helices such as the tendency to tilt, to oligomerize or to undergo conformational changes.

Many studies have indicated that transmembrane segments tilt as a response to positive mismatch, but the extent to which it occurs appears to vary considerably. Molecular dynamics studies [5,6,7•] and ^{15}N NMR experiments [8] on lysine-flanked model peptides suggested a considerable tilt, as did fluorescence studies of

Figure 1



Design of model peptides. Design of **(a)** WALP and **(b)** KALP peptides as models for transmembrane segments of intrinsic membrane proteins, as described in [3].

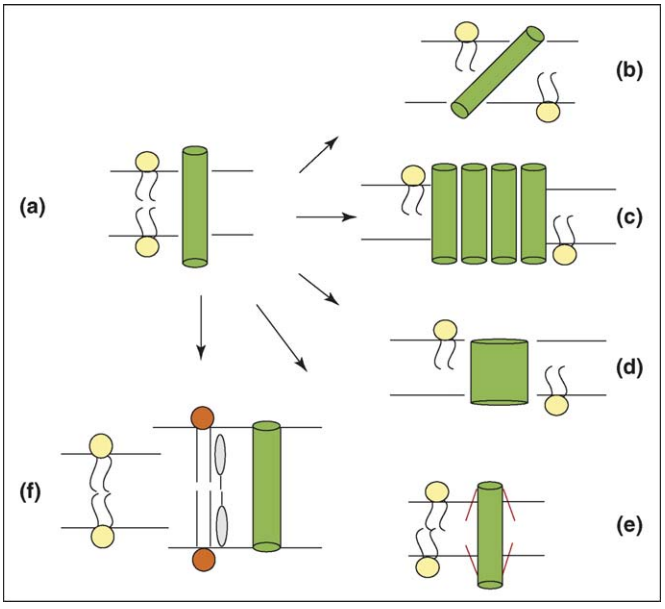
the natural single-span protein M13 [9]. For VpU, another single-span protein, tilt angles were found that were sufficiently large to fully compensate for mismatch [10•]. In contrast, ^{2}H NMR studies on WALP and KALP peptides showed only very small, albeit highly systematic, changes in tilt angle [11,12•]. Probable explanations for these apparent discrepancies include variations in experimental parameters, such as the level of hydration or the peptide concentration, and differences in peptide properties, such as its tendency to oligomerize or its intrinsic tendency to tilt, as determined by the distribution of sidechains around the helix axis. Also, lipid composition may be important, as lipid packing will be perturbed by tilting, both at the interface and in the hydrophobic region.

Recent computational studies by Sperotto and co-workers [13•] suggested that the extent of tilt is dependent on the cross-sectional diameter of the protein and that tilting occurs more easily for single-span peptides than for large proteins. However, in this study, large proteins were represented as rigid entities, whereas in natural multispan proteins the transmembrane segments may tilt individually and against one another. It is possible that multispan proteins in fact perturb lipid packing less and therefore tilt more easily than single helices. The same might hold for peptide aggregates.

Besides tilt, oligomerization has been reported as a response to mismatch. Using different fluorescently labelled model peptides, several groups unambiguously showed that the tendency to self-associate increases with either positive or negative mismatch [14,15,16•,17•], demonstrating that helix–helix association of membrane proteins can be promoted simply by less favourable helix–lipid interactions.

Studies of model peptides have enabled the identification of general properties of transmembrane segments that promote helix–helix association, such as the presence of

Figure 2



Possible consequences of hydrophobic mismatch for protein structure and organization. The green cylinder represents the hydrophobic part of a membrane protein. **(a)** Positive mismatch by itself would lead to exposure of hydrophobic groups to a hydrophilic environment at the interface. Possible adaptations are **(b)** tilting of transmembrane segments to reduce their effective length, **(c)** self-association, **(d)** changes in backbone conformation or **(e)** changes in the orientation of the sidechains. **(f)** In multicomponent systems, transmembrane segments that are too long might partition into thicker domains.

polar residues [18]. Such studies have also greatly contributed to understanding specific dimerization motifs, such as the GxxxG motif, as first identified by Engelman and co-workers [19]. Deber and colleagues [20•] even observed two recognition sites in one transmembrane helix, involved in folding and oligomerization of the protein.

Studies of model peptides have also provided insight into what specifies association in either parallel or antiparallel fashion. Sparr *et al.* [16•] showed by fluorescence measurements that WALP peptides tend to oligomerize as antiparallel dimers under conditions of mismatch. A similar observation was made by Yano and Matsuzaki [17•] for 'inert' model peptides without specific recognition motifs and without flanking residues. It was proposed that interhelical dipole–dipole interactions are responsible for the preferred antiparallel association. This was supported by modelling studies in which the dipoles of the backbone were reversed [16•].

Proteins can also respond to changes in the lipid environment by a conformational change of their backbone. Studies on model peptides in fluid phase bilayers with different thickness suggested that systematic adaptations of the helical pitch of peptides as a response to mismatch, if any, are at most very small [12•,21], although local distortions have been reported frequently [9,10•,11].

In natural membranes, cholesterol-enriched domains with different thickness are believed to occur [22] and proteins may be directed to such domains based on their hydrophobic length. Indeed, the length of transmembrane segments of monotopic membrane proteins is a major determinant of whether these proteins end up in the Golgi or in the cholesterol-rich plasma membrane [23]. However, matching alone is not sufficient, because several groups have reported that long model peptides do not partition into thicker, so-called liquid-ordered domains in model membrane systems [24–26].

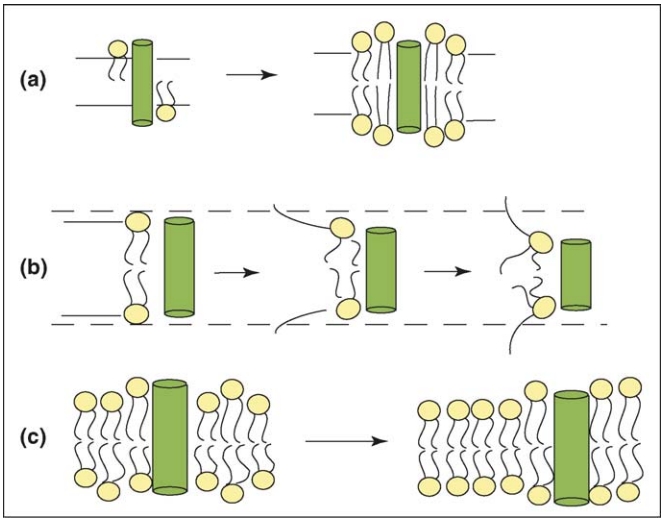
How membrane proteins influence lipid structure and organization

Just as lipids affect proteins, proteins also affect lipids in different ways, as illustrated in Figure 3. Again, much insight has been obtained from studies of the consequences of hydrophobic mismatch in model systems.

About 20 years ago, Mouritsen and Bloom [27] suggested in their 'mattress model' that lipids adapt to mismatch by stretching or disordering (Figure 3a), as indeed observed for model peptides by simulations [6,7•]. Also, experimental ^{2}H NMR studies on WALP peptides showed a systematic, albeit very small, response of lipid chain order to mismatch [28]. However, using X-ray diffraction of similar samples, Huang and co-workers [29] could not detect any bilayer thickness adaptation, possibly because the adaptations were too small. Interestingly, for the β-helical peptide gramicidin, which induced the largest chain ordering in the ^{2}H NMR experiments even though it was not the longest peptide studied [28], the X-ray measurements did detect an increase in bilayer thickness [29]. This may imply that proteins with larger cross-sectional areas cause more adaptation of the surrounding lipids.

For a variety of lipids, incorporation of short peptides was found to promote the formation of phases with negative

Figure 3



Possible mismatch-induced effects of proteins on lipids are **(a)** stretching of lipids under conditions of positive mismatch, **(b)** disordering of the lipid acyl chains and formation of non-lamellar structures under conditions of negative mismatch, and **(c)** sorting of lipids by recruitment of lipids with the best-matching length from mixtures of lipids.

curvature [30–32]. This can be understood in terms of an 'extreme' adaptation of the lipids by disordering their chains (Figure 3b). Similarly, incorporation of relatively long peptides may promote the formation of structures with positive curvature, although this has not been observed experimentally yet. It is even feasible that a combination of positive and negative mismatch, induced, for example, by a small transverse dislocation of transmembrane helices, may serve as a mechanism in nature to stabilize the formation of highly curved membranes in processes such as fusion or fission. So far, studies with model peptides on the role of transmembrane segments in membrane fusion have suggested that structural flexibility [33] and peptide length [32] are important for fusogenicity.

In biological membranes, mismatch also could play a role in lateral organization of the lipids, if proteins attract lipids with the best-matching hydrophobic length (Figure 3c). However, studies by different groups on model peptides in binary lipid mixtures in the fluid phase did not show a significant degree of such sorting [34•,35]. Also, in this case, it was suggested that the size or cross-sectional diameter of the protein may be an important factor [34•].

Finally, model peptides have contributed to understanding the process of transbilayer movement (flop) of lipids. De Kruijff and colleagues [36] showed that WALP and KALP peptides mediate flop of fluorescently labelled phospholipids without the need for dedicated flippases. Comparison with the flop efficiency of different natural membrane proteins suggested that translocation is mediated via the protein–lipid interface of relatively small proteins only. It was speculated that these proteins facilitate lipid translocation because they are more dynamic and engage in less stable protein–lipid interactions than larger membrane proteins.

Model peptides in studies of the interfacial anchoring properties of membrane proteins

Studies on lipid adaptations have also proven useful to obtain information on the anchoring behaviour of flanking residues at the lipid–water interface. For example, De Planque *et al.* [30] showed that the effective hydrophobic length of WALP and KALP peptides could be determined from the minimal lipid chain length required to induce formation of the inverted hexagonal (H_{II}) phase. Comparison of these effective lengths suggested that tryptophan residues anchor at the interface, whereas lysine preferentially positions its charged amino group close to the phosphates.

Mismatch studies in different lipid systems suggested that the length of lysine-flanked peptides can be effectively modulated by 'snorkelling', that is, stretching of the lysine sidechains with their positively charged amino group towards the interface [30,31,37]. Such studies also enabled estimation of the energy cost of snorkelling, which was found to be very low [38]. In general, however, very little is known about the orientations and possible adaptations of protein sidechains at the lipid–water interface.

Using conditions of both positive and negative mismatch, De Planque *et al.* [39] found that tryptophan residues resist displacement of their preferred interfacial localization towards both the hydrophobic part and the aqueous phase, and that interactions of tryptophans with the interface are strong enough to dominate effects of hydrophobic mismatch. This corresponds well with results from Braun and von Heijne [40] on the interfacial localization of biosynthetically inserted proteins in the endoplasmic reticulum, illustrating the validity of using model systems for understanding biological membranes.

Studies of both tryptophan- and lysine-flanked peptides showed that these peptides do not reorient fast about their helical axis, but that they do undergo fast reorientation about the bilayer normal [11,12•,41]. Özdirekcan *et al.* [12•] showed that the helices have a preferred direction of tilt and that interactions of the flanking residues with the lipid–water interface are important for determining this direction, rather than the composition of the transmembrane region. However, further studies on appropriate model peptides are required to establish the physical origin of the influence of flanking residues. In this respect, one promising recent development is the improved analysis of the orientation and motion of tryptophan sidechains at the interface using deuterium NMR methods [42].

Model peptides as a tool to explore new techniques and computational approaches

Well-characterized model peptides have proven valuable as tools to calibrate and explore new techniques in studies of lipid–protein interactions. Illustrative examples include studies by Huster and co-workers [43] using (labelled) WALP peptides to validate NMR relaxation measurements on protein sidechains in combination with lipid-attached spin probes as a tool to study membrane protein topology. Also, Nielsen *et al.* [44•] established a 'ruler' for determining the membrane depth of transmembrane or surface-bound regions of membrane proteins using electron paramagnetic resonance (EPR) methods. In addition, WALP peptides served as a convenient model to develop novel solid-state NMR approaches for structural studies of proteins in biomembranes [45,46].

Ganchev *et al.* [47••] used dynamic atomic force spectroscopy of cysteine-labelled WALP peptides to measure the forces required to remove single helices from ordered peptide-rich domains in lipid bilayers. Similar forces were

reported for peptides in unordered peptide-poor bilayers [47••] and for the removal of bacteriorhodopsin helices from the purple membrane [48], suggesting a common mechanism of membrane anchoring. In related studies on WALP peptides by Contera *et al.* [49], the force measurements were also simulated, providing more insight into the pulling process. Both studies suggested that the bilayer interface region plays an important role in stably anchoring proteins within membranes [47••,49].

Because, in general, they are small and well characterized, model peptides have become increasingly attractive for use in computational studies. Interesting examples are two recent studies on the mechanism of membrane insertion of hydrophobic peptides. Im and Brooks [50•], using an implicit membrane model, found that the peptides first become localized at the membrane–solvent interface, where they form helical structures. The central hydrophobic residues then insert into the membrane interior, after which a helical structure is formed throughout the peptide. However, simulations by Garcia and colleagues [51••], using an explicitly represented lipid bilayer, suggested a different sequence, in which the peptide first inserts spontaneously, followed by folding into an α helix with a transbilayer orientation. The authors suggested that transmembrane peptides may have different insertion and folding behaviour, depending on the exact properties of the peptides and lipids.

Molecular dynamics simulations can provide insights into properties of peptides and lipids that, in experimental studies, would require many separate experiments. An illustrative example is a recent study on different length KALP peptides by Kandasamy and Larson [7•], who showed that positive mismatch results predominantly in tilting and, to a lesser extent, in an increase in lipid order in the immediate vicinity of the peptide, whereas negative mismatch results in a combination of local bilayer bending and snorkelling of the flanking lysine residues. Except for the extent of tilt, these results are in excellent agreement with experimental studies.

Conclusions

The examples given above illustrate the power of model systems to analyze general principles of protein–lipid interactions. Optimal systems can now be designed to answer highly specific questions, such as whether the N or C terminus of a peptide is more dominant in determining the direction of tilt of transmembrane helices, how lipid sorting or the tendency of helices to tilt are affected by the size of the protein, how properties of the helix (e.g. regularity of the outer contour or the presence of helix-breaking residues) influence lipid flop or how peptide sidechains reorient at the lipid–water interface. Answers to such questions ultimately should enable us to predict the properties of proteins and lipids in any given membrane.

In the coming years, a cascade of new knowledge may be expected. This is because the model systems will be increasingly better characterized, in particular because the versatility of these systems should allow any discrepancies to be addressed and solved. This will make the systems increasingly attractive, leading to even better characterization and so forth. Thus, many new and exiting insights can be expected, of which it will be challenging in particular to find out how helix–helix interactions or the protein cross-sectional diameter influence helix–lipid interactions.

Once basic principles of peptide–lipid interactions have been established for model systems, it is important to determine whether the same holds for proteins in biological membranes. In this respect, one promising recent observation is that very similar rules apply to whether a peptide will integrate in a synthetic lipid bilayer as to whether an *in vitro* synthesized protein will integrate in the endoplasmic reticulum [52••].

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- •• of outstanding interest

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