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How Protein Transmembrane Segments Sense the Lipid Environment†

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ABSTRACT: Integral membrane proteins have central roles in a vast number of vital cellular processes. A structural feature that most membrane proteins have in common is the presence of one or more α-helices with which they traverse the lipid bilayer. Because of the interaction with the surrounding lipids, the organization of these transmembrane helices will be sensitive to lipid properties like lateral packing, hydrophobic thickness, and headgroup charge. The helices may adapt to the lipids in different ways, which in turn can influence the structure and function of the intact membrane protein. In this review, we will focus on how the lipid environment influences two specific properties of transmembrane segments: their lateral association and their tilt with respect to the bilayer normal.

Genome sequencing data have revealed that approximately one out of four proteins encoded by DNA is a membrane protein (*1*). These proteins play essential roles in many life processes, such as cell growth and division, uptake of food, communication between cells, and sensory perception. To understand the role of membrane proteins in health and disease, knowledge of the molecular mechanisms through which these proteins function is needed. This requires not only structural information about the protein itself but also information about how the lipid environment affects its structure and organization. While at this moment still relatively few crystal structures of membrane proteins are available, mostly due to the difficulty of handling these hydrophobic proteins, more and more general rules are being established that allow us to understand how proteins sense the lipid environment.

Membrane proteins come in a huge structural variety, but they have one property in common: they contain one or more hydrophobic regions with which they span the membrane, most often either as a single α-helix or as a bundle of α-helices. Many properties of membrane proteins are determined by interactions between these helices and the surrounding lipids, whereby the helices can act as sensors of the lipid environment. Indeed, changes in lipid composition may elicit a variety of responses, including changes in helix tilt, orientation, or conformation, or changes in helix−helix interactions. In addition, nonhelical regions and hingelike motions may occur (*2*) or be induced by the lipid environment. For membrane proteins, such changes in behavior of the transmembrane (TM)[1] segments may in turn lead to structural changes in the extramembranous parts or to formation or dissociation of oligomeric structures. Thus, the

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[1] Abbreviations: TM, transmembrane; PG, phosphatidylglycerol; MD, molecular dynamics; PC, phosphatidylcholine; WALP, tryptophan-flanked α-helical model peptide; KALP, lysine-flanked α-helical model peptide; NMR, nuclear magnetic resonance spectroscopy.

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interplay between membrane-spanning segments of proteins and their lipid environment is a key in obtaining a general mechanistic understanding of structural and functional properties of proteins in membranes.

This review discusses recent insights into lipid−protein interactions and how these can influence membrane proteins. In particular, we will focus on how the orientation of TM helices and their tendency to self-associate can be modulated by the surrounding lipid bilayer. First, we will briefly review the architecture of TM helices, as a central key for understanding their interactions with lipids.

Architecture of Transmembrane α-Helices

Membrane-spanning helices found in membrane protein can be divided into two regions: a hydrophobic TM segment, which interacts with the hydrophobic core of the bilayer, and the residues flanking the membrane-spanning segments that are located at the membrane−water interfacial region (*3*). The hydrophobic core of the lipid bilayer is ∼30 Å thick, while the membrane−water interfacial region makes up an ∼15 Å thick region on both sides of the membrane (*3*). As the length of an α-helix increases ∼1.5 Å per residue, membrane-spanning α-helices have to be ∼20 residues long or more to traverse the hydrophobic core of the lipid bilayers.

On the basis of known membrane protein structures, the hydrophobic segment of TM helices has been reported to be 20−25 residues long (*1*). These protein segments are highly enriched in hydrophobic residues like valine, leucine, alanine, isoleucine, and phenylalanine (*4*, *5*). Most of these residues seem to be randomly distributed along the TM segments, but valine and leucine are found more frequently in the center of the bilayer (*4*). In multispanning proteins, alanine has a preference for the interior of α-helical bundles while bulky residues tend to be on the "outer side" (*5*).

Although relatively rare, polar residues are also an important part of TM helices. Since such residues tend to be shielded from exposure to the hydrophobic acyl chains, they are likely to play a role in helix−helix association and hence in the folding of multispanning membrane proteins. The most frequently found polar residues in TM segments are threonine and serine (*4*). These residues appear to have no preference for either transmembrane or nontransmembrane segments (*5*). Their distribution in membrane proteins may be explained by their ability to form hydrogen bonds with adjacent carbonyl oxygens (*6*). Further, these relatively small side chains have been proposed to be associated with tight packing of helices (*7*).

Ionizable residues are found even less frequently in TM segments than polar residues. They may play important roles in membrane protein function, for example, in bacteriorhodopsin, where ionizable residues are involved in proton transfer (*8*).

Glycine is fairly common in TM α-helices, where it may facilitate closer packing of the helices (*5*, *7*). This is probably why glycine is found more frequently in the interior of multispanning proteins than in single helices (*5*, *7*). Proline is found only to a small extent within TM helices (*5*), where it seems to be preferentially localized to the center of the bilayer (*9*). It induces kinks or bends and thus may have an important role in membrane protein folding, as reported, for example, for bacteriorhodopsin (*10*). Proline also may form molecular hinges that function as conformational switches in the TM helices of channels and receptors (reviewed in ref *11*).

Compared to the hydrophobic acyl chain region of the bilayer, the interfacial region presents a chemically complex environment with its carbonyl groups, lipid headgroups, and water molecules. Analysis of known structures of membrane proteins, e.g., cytochrome *c* oxidase, has shown that the interfacial regions are enriched especially in the aromatic residues tryptophan and tyrosine and the hetero-aromatic residue histidine (*5*, *12*). What gives these residues an increased affinity for the interfacial region are mostly their hydrogen bond forming ability and their dipolar but still hydrophobic character (*13*−*16*). Indeed, phenylalanine, which is aromatic but hydrophobic and lacking hydrogen bond forming ability, is distributed preferentially to the hydrophobic TM segment (*5*, *12*).

For tryptophan side chains, Granseth et al. (*17*) found that residues below the interfacial region tend to stretch toward the interface while residues located farther out tend to direct their side chains toward the bilayer core. Furthermore, studies on synthetic, tryptophan-flanked model peptides (*16*) and on single-span membrane proteins that were biosynthetically incorporated into the ER (*18*) suggested that tryptophan side chains preferentially reside close to the carbonyl region in phospholipid bilayers and resist displacement either toward the aqueous phase or toward the membrane interior, yet tryptophans do not act as topological determinants, since no bias was observed for these residues to remain at the cis side of the membrane upon biosynthetic insertion (*19*). The preferential localization of tryptophan at the lipid−water interface and some other aspects of membrane protein architecture are illustrated in Figure 1A for the K^+ channel KcsA (*20*). Figure 1B shows the structure of a tryptophan-flanked synthetic TM peptide which has been used as a model for a membrane-spanning segment of intrinsic membrane proteins (*21*).

The charged residues lysine, arginine, aspartate, and glutamate are frequently found more outward at the flanks of TM helices (*5*). In contrast to tryptophans, these residues play an important role in determining the topology of membrane proteins and are distributed according to the "positive-inside" rule. That is, when newly synthesized membrane proteins insert into a membrane, the positively charged lysine and arginine residues preferentially will stay at the cis side, while the negatively charged aspartate and glutamate residues are found more frequently at the trans side of the membrane (*12*).

Nonpolar residues in TM segments tend to point toward the middle of the bilayer, while polar residues have a tendency to stretch toward the aqueous phase (*17*, *22*). In particular, the longer, positively charged side chains in fact may reach the interfacial region from a position deeper in the hydrophobic part of the bilayer by what is known as snorkelling. This allows more flexibility in the positioning of side chains at the interface and hence more freedom in the localization and dynamics of the helices in the bilayer (*23*).

From the examples given above, it is clear that the architecture of TM segments plays an important role in determining the structure and dynamics of membrane proteins in a lipid bilayer. In the next part, we will focus on the

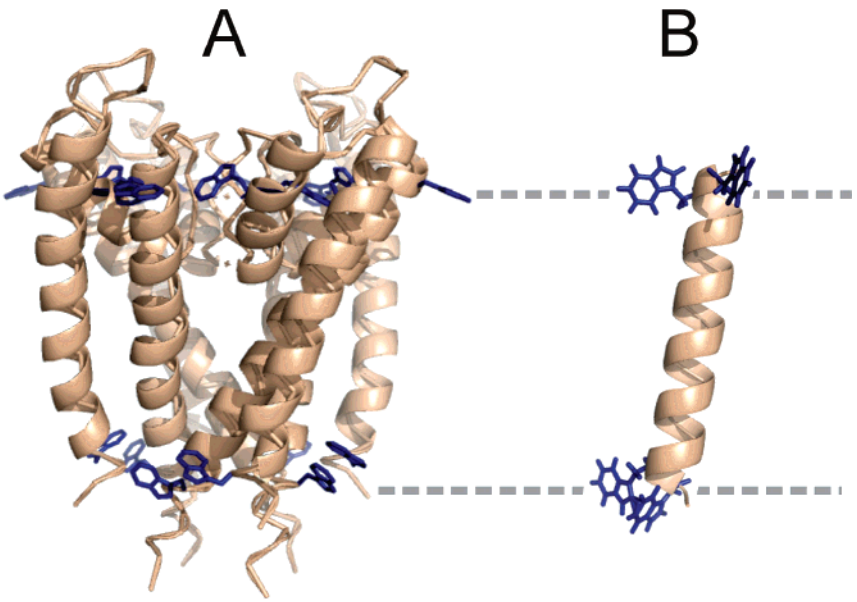

FIGURE 1: (A) Structure of the membrane-spanning parts of the potassium channel KcsA (*20*) (PDB entry 1BL8). This structure illustrates well the general architecture of transmembrane proteins with hydrophobic transmembrane helices in which the flanks are enriched with aromatic residues. (B) Model of a Trp-flanked transmembrane helix [HOC-AWW$(LA)_8$LWWA-NH_2] designed to mimic the membrane-spanning segment of natural membrane proteins. This figure was generated and rendered with PyMOL [DeLano, W. L. (2002) *The PyMOL Molecular Graphics System*, http//www.pymol.com].

importance of this architecture for self-association of membrane proteins and how this can be influenced by the lipid environment.

Lipid-Dependent Self-Assocation of Membrane Proteins

Interactions between TM α-helices are an important determinant for the structure of multispanning membrane proteins and for the assembly of membrane proteins into larger oligomeric complexes (*24*−*27*). In general, helices tend to associate when helix−helix interactions are more favorable than helix−lipid interactions. This can be accomplished in several ways, as schematically illustrated in Figure 2 and as discussed below.

First, many proteins contain helix−helix recognition motifs in their TM segments, which drive highly specific association, as schematically illustrated in Figure 2A. For such proteins, the helix−helix interaction will be much more favorable than helix−lipid interactions and the lipid environment will in general not significantly influence the oligomeric state. Several motifs that link TM helices together have been found (for reviews, see refs *28* and *29*), such as the well-characterized GXXXG pattern, which was first identified by Engelman and co-workers in glycophorin A (*30*). This motif appears to be a general one, since it also has been found in other membrane proteins (*31*, *32*). Another well-known motif is the leucine zipper (reviewed in ref *29*).

A simpler "motif" that can drive the association of TM helices is the presence of polar residues. Many proteins, for example, rhodopsin-like receptors, have well-conserved polar residues within the TM segments (*33*−*36*). Often such polar residues will be part of functional groups, but others may be there simply to facilitate protein assembly. In studies on model TM peptides, it was observed that the presence of asparagine, glutamine, aspartate, or glutamate residues strongly promotes oligomerization (*37*, *38*). Also, there are several antimicrobial peptides, e.g., alamethicin, that form helices of which one side is more hydrophilic and which

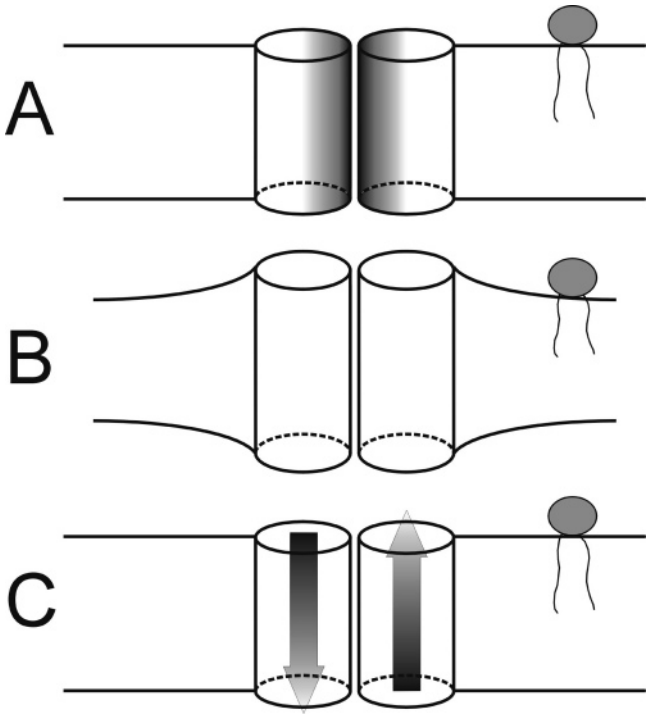

FIGURE 2: Schematic illustration of factors that can influence helix−helix interactions in membranes. (A) Specific recognition motifs or the presence of polar residues on one side of the helix promotes association of the helices. (B) Unfavorable interaction between helices and lipids is another factor that can promote helix self-association, as illustrated by the effect of positive mismatch between bilayer thickness and the hydrophobic length of the helices. (C) Favorable interactions between helix dipole moments may promote formation of antiparallel helices.

can spontaneously self-assemble in lipid bilayers to form transmembrane pores (*39*).

For several membrane proteins, helical fragments of the native proteins have been found to assemble by themselves to form functional units (*40*−*45*). For example, bacterior-

hodopsin can form a functional chromophore from fragments reconstituted in micelles or lipid bilayers (*40*−*42*). Thus, it is likely that there are many other interaction motifs in membrane proteins that have not yet been identified. Recently, Deber and co-workers suggested that one of the helices in a small multidrug resistance protein even has two interaction sides that promote self-assembly (*46*).

In the case in which TM segments do not contain any specific or aspecific motifs, helix−helix association may be driven simply by less favorable helix−lipid interactions. This would imply that in these cases the extent of helix−helix association is controlled by a delicate balance of forces, highly sensitive both to the properties of the lipid environment, such as lipid packing or bilayer thickness, and to the precise composition of the TM segments. In this view, unfavorable packing between the helices and surrounding lipids may be sufficient to drive oligomerization, for example, as a result of a mismatch between the hydrophobic thickness of the bilayer and the hydrophobic length of the protein TM segments, as illustrated in Figure 2B. Such mismatch-induced protein oligomerization was observed, for example, for bacteriorhodopsin in PC bilayers with different thicknesses (*47*). However, in this case, a rather extreme mismatch was required for oligomerization. For small, single-span peptides, the situation seems to be different. Using fluorescence methods, a relatively small mismatch between the hydrophobic length of lysine-flanked peptides and the bilayer thickness already was found to be sufficient to control helix−helix interactions (*48*, *49*). Similar observations were made with tryptophan-flanked peptides (*50*). It was suggested that in case of small, single-span peptides the lipids compensate less for the mismatch by stretching or disordering their acyl chains than in the case of proteins with a larger cross-sectional area (*51*, *52*). As a consequence, oligomerization may be promoted especially for small peptides or proteins.

The oligomeric state of proteins can also be influenced more directly by packing of the lipids. This is seen especially clearly in phases where the lipid chains are ordered. In lipid bilayers consisting of mixtures of liquid-crystalline phase regions with either gel phase or of liquid-ordered regions, α-helical TM peptides are expelled from the ordered phase (*53*−*55*). Also, cholesterol has been observed to promote helix−helix interactions of TM helices (*50*, *56*, *57*). Although in some cases this can be attributed to the chain ordering or bilayer thickening effect of cholesterol, in other cases it seems likely that self-association is due to the proteins being expelled from sterol-rich domains, as observed with the gammaM4 TM domain of the muscle nicotinic acetylcholine receptor (*57*). A special case is presented by the so-called striated domains, which are highly ordered linear aggregates of peptides and lipids that have been observed for tryptophan-flanked model peptides in gel phase bilayers (reviewed in ref *58*).

Lipid packing has also been modulated via addition of small alcohols, which partition at the lipid−water interface. This causes a looser packing of the bilayer interior, which may affect protein oligomerization. An example comes from recent studies of the potassium channel KcsA (*59*, *60*), where addition of short chain alcohols was seen to destabilize the KcsA tetramer in a membrane composition-dependent manner. The authors attributed these effects to changes in the lateral pressure profile across the lipid bilayer (reviewed in ref *61*). More recently, the short chain alcohol trifluoroethanol (TFE) was shown to destabilize a large number of oligomeric membrane protein complexes from *Escherichia coli* inner membranes (*62*).

Besides specific helix recognition motifs or poor packing between lipids and helices, also favorable dipole−dipole interactions may drive helix−helix association (Figure 2C). In a thermodynamic analysis of helix−helix interactions in PC bilayers, Yano and Matsuzaki studied the interactions between hydrophobic α-helical peptides consisting of alanine and leucine without interfacial anchoring residues (*63*). An increasing degree of dimerization was observed when the PC acyl chain length increased from 14 to 22 carbons, whereby the dimers preferentially adopted an antiparallel orientation. The peptides exhibited no increased level of oligomerization in thinner bilayers. Hence, the authors proposed that the major driving force for oligomerization in this study is not hydrophobic mismatch but rather the increasing strength of interactions between the helix macrodipoles as the partial charges are moving into a more hydrophobic environment (*63*). Consistent with this, in a study by Sparr and co-workers (*50*) on single-span tryptophan-flanked model peptides that were labeled with pyrene either on the N-terminus or on the C-terminus, fluorescence quenching was observed only when a mixture of both peptides was present, and it was concluded that antiparallel interactions are preferred. Computer modeling indicated that the antiparallel association is a result of favorable electrostatic interactions between the helix backbone dipoles in the antiparallel dimer (*50*). Thus, it can be concluded that favorable antiparallel packing of helices may contribute to the overall structure and stability of multispanning membrane proteins, while for proteins that form parallel dimers, specific interaction motifs may be required to overcome the less favorable helix−helix packing.

Interestingly, the role of hydrophobic mismatch in association of TM α-helices may be different when these helices contain motifs that promote specific assembly. With the M2 proton channel from influenza A virus (*56*), in contrast to the examples given above, oligomerization of the TM segments occurred most efficiently when the hydrophobic matching was optimal. A likely explanation is that the specific interactions between the helices require a certain TM orientation and that mismatch may influence this orientation. In the next section, the importance of the peptide composition and effects of lipid environment on the conformation and orientation of TM peptides will be discussed.

Orientation of Transmembrane Helices

An important determinant of membrane protein structure is the orientation of the TM α-helices in the bilayer. By changing the tilt or the direction of tilt (rotational angle), membrane proteins may switch, e.g., between active and inactive conformations. Such structural rearrangements can be large, as in the mechanosensitive channel MscL and the Ca^{2+}-ATPase from skeletal muscle sarcoplasmic reticulum (*64*, *65*), where the orientation of some of the TM helices changes completely with the functional state of the proteins. In other proteins, subtle fluctuations in tilt angle are sufficient. This is the case for rhodopsin and bacteriorhodopsin, which require only a small outward tilting of one α-helix,

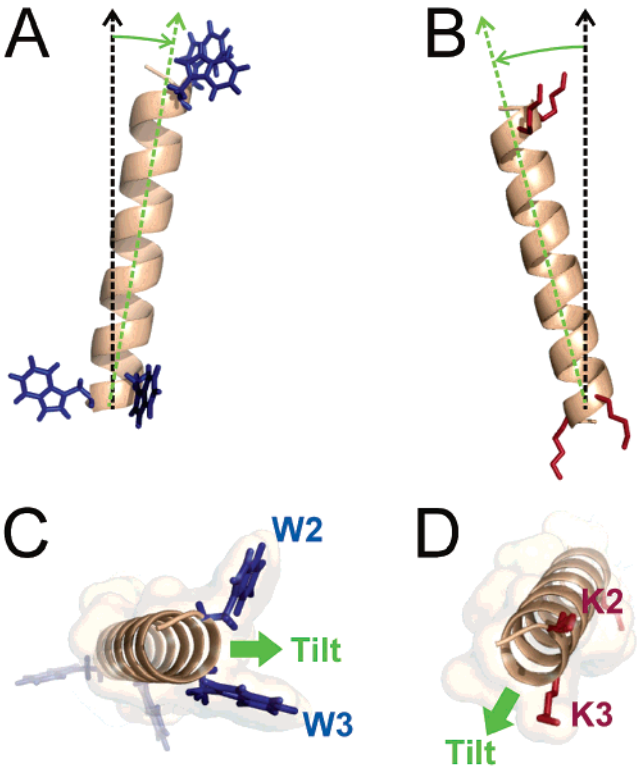

FIGURE 3: Flanking residues can influence the tilt and rotational angles of transmembrane α-helices. This figure illustrates the tilt and rotational angle of WALP23 (A and C) and KALP23 (B and D) in DMPC bilayers. The curved green arrows indicate the tilt angle in the plane of the picture (A and B). Rotational angles are represented by green arrows on helices viewed from the N-terminal side.

called the F-helix, to ensure their reprotonation as a so-called meta II or M2-state intermediate (*66*−*72*).

From known membrane protein structures, it has been concluded that on average the TM helices have a tilt angle of 22.0 ± 11.6° (*5*). What determines how an α-helix orients itself in a lipid bilayer? Again, this will strongly depend on the properties of both the helices and the lipids. A property of helices that is likely to be an important determinant of the orientation is its intrinsic propensity to tilt, which is determined by the nature of the different side chains and how they are distributed along the helix axis. For example, Harris et al. (*73*) described a class of peptides, known as "tilted peptides", that just on the basis of their amino acid sequence can be predicted to preferentially adopt a significantly tilted or oblique orientation in the membrane. These peptides that comprise, for example, viral fusion peptides are believed to play an important role in processes that involve modulation of the organization of the surrounding lipids, such as membrane fusion (*74*).

Another property that seems to be important for the orientation of TM helices is the nature of the flanking residues that anchor the termini to the lipid−water interface. This was, for example, suggested by studies on model peptides, which showed that lysine-flanked peptides tilt more easily than tryptophan-flanked peptides (*75*) (Figure 3A,B). Furthermore, in the mechanosensitive channel MscL (*76*), capping of TM helices with aromatic residues was observed to dramatically affect the function of the channel. Here, it was suggested that the strong anchoring of the aromatic residues to the interfacial region hinders the dramatic change in the tilting of the helices that is required to open the channels. For the KirBac1.1 potassium channel, it was suggested that the preference of the aromatic residues for the interfacial region is part of the driving force that cycles the protein between its open and closed conformation (*77*). Recently, it has also been suggested that the orientation of the amino-proximate TM domain of CREP-1 is determined by a tyrosine and an arginine residue, each on one end of the TM helix (*78*).

Besides the properties of the helices, also the lipid environment will be an important determinant for helix tilt. In particular, hydrophobic mismatch can be expected to be a driving force for tilt, much as it is for helix self-association (*21*). In this case, if the hydrophobic length of a helix is longer than the bilayer thickness, i.e., in situations of positive mismatch, the helix may tilt to reduce its effective length. That indeed proteins respond to changes in bilayer thickness by reorienting their TM helices is supported by many experimental observations. One example is simply the observation by Lee and co-workers in some proteins that tryptophans which flank the TM segments remain confined to the interfacial region, irrespective of bilayer thickness (*79*).

An almost perfect example of how bilayer thickness can influence the tilt angle of TM helices comes from a solid-state NMR study of the membrane protein Vpu from HIV-1 (*80*). Here the helical tilt angles were obtained from two-dimensional solid-state NMR measurements on oriented phospholipid bilayers containing Vpu protein with ^{15}N-labeled side chains. When the bilayer thickness was varied between 15.5 and 27 Å, the helical tilt angle responded by changing from 51° to 18° in the thinnest and the thickest bilayer, respectively, thereby fully compensating for the hydrophobic mismatch. Another example of mismatch-dependent reorientation of TM helices comes from studies by Hemminga and co-workers on the major coat protein M13 (*81*). By quantifying the Stokes shifts of fluorescent groups linked to cysteine residues at different positions along the helix, the authors calculated the tilt angle and rotational angle in PC bilayers of varying thicknesses. They found that the α-helix tilted with an angle of 36° with respect to the bilayer normal in di-14:1-PC bilayers. As the bilayer thickness was increased, the tilt angle decreased, reaching 18° in di-20:1-PC bilayers. Similar observations were made with the M2 protein from influenza A virus (*82*), a single-span α-helix that forms a tetramer in lipid bilayers. Also here, the helical tilt of the protein was found to decrease with an increase in bilayer thickness, as determined in PC bilayers using EPR on spin-labeled peptides.

A very systematic increase in helix tilt in response to hydrophobic mismatch was also observed for synthetic model peptides, consisting of a hydrophobic core of alternating leucines and alanines, flanked by either lysines or tryptophans in PC bilayers (*75*, *83*−*85*). However, for these so-called WALP and KALP peptides, the variations in bilayer thickness, as determined by geometric analysis of labeled alanines (*84*), seemed to lead to only modest adaptations in tilt angle as compared to what was observed with M13 and Vpu. The calculated tilt angles did not exceed 12° and were not large enough to compensate for the hydrophobic mismatch (*75*, *85*). In agreement with this, additional compensating mismatch responses were observed, including a small but systematic stretching of the acyl chains (*86*) increased extent

of helix−helix association. Thus, it seems that the tilt of peptides by itself is energetically unfavorable, possibly because tilting would disturb the packing of the surrounding lipids both at the interface and in the hydrophobic core. That lipid packing plays an important role is consistent with results from recent coarse grain simulations, where model proteins representing single-span helices were found to respond to hydrophobic mismatch by tilting, while systems with larger rigid proteins, which would disturb lipid packing more if they would tilt, responded by stretching of the lipid acyl chains (*87*). Interestingly, KALP peptides, which stretch the lipids less than WALP peptides, tilt slightly more (*16*, *75*). This suggests that the mode of anchoring to the interface can modulate the extent of tilt of helical TM segments as a response to positive mismatch. Consistent with this, it has been proposed for the transmembrane helix of synaptobrevin that the tilt angle is determined by the conserved WWKNLK sequence, which anchors the helix to the membrane−water interface at a specific angle (*88*).

The orientation of WALP and KALP peptides in lipid bilayers has also been studied by different MD approaches (*89*−*93*). In an extensive study with different length KALP peptides (*93*) in PC bilayers with varying thicknesses, a clear relationship between the degree of positive mismatch and helical tilt angle was observed, but the tilt angles were markedly larger than those experimentally determined by ^{2}H NMR. The reason for this is not clear at this moment. Also, recent simulations on WALP peptides, in which the lipid bilayer was represented by a "hydrophobic slab" instead of a full atom model, showed much larger tilt angles (*90*, *91*). However, since in these latter models the effect of tilting on the packing of the surrounding lipids cannot be taken into account, this may have led to overestimation of the tilt angles.

A likely reason for the relatively small tilt angles of WALP and KALP peptides is the rather symmetric distribution of side chains around the helix axis, which gives these peptides a low intrinsic propensity to tilt. It also has been suggested that self-association of peptides might lead to smaller tilt angles (*93*), which was observed in MD simulations of the nicotinic acetylcholine receptor gammaM4 TM helix, when comparing tilt angles with and without adjacent helices (*94*). Indeed, MD simulations showed that placement of two long KALP peptides close to one another in a bilayer leads to markedly reduced tilt angles (*93*). However, self-association is unlikely to be the explanation for the experimentally observed small tilt angles in the case of WALP and KALP peptides, since hardly any helix−helix association of WALP occurs at the peptide concentrations used in the tilt experiments (*50*), and also, in the MD simulations, spontaneous peptide aggregation was not observed (*93*).

Peptide composition and bilayer thickness are not the only determinants of the tilt angle of TM segments. The composition of the lipid headgroups and acyl chains can also be very important. For example, tilting as a consequence of positive mismatch may be promoted when anionic lipids are present and the helix has positively charged anchoring residues. In the case of M13 and Vpu, however, the tilt angles do not seem to be influenced by the presence of PG (*95*), indicating that for these proteins other factors are dominant in causing the relatively large tilt. As another example, since tilt will interfere with lipid packing, the packing properties of the lipids, both at the interface and at the hydrophobic core, will influence the energy cost of tilting. Thus, it is possible that helix tilt is facilitated in natural membranes, where a mixture of lipids is present to ensure optimal packing around tilted peptides.

Finally, besides the extent of tilt, also the direction of tilt, or the rotation angle, of TM segments may be determined by properties of both peptides and lipids. When the orientation of WALP and KALP peptides in PC bilayers was determined using deuterium NMR methods, it was found that the rotational angles are influenced by the anchoring residues (*75*) (Figure 3C,D) but that there is no clear dependence of the rotational angle on the degree of hydrophobic mismatch. For the M13 major coat protein, on the other hand, the rotational angle has been shown to vary systematically with bilayer thickness (*81*). The rotational angle of M13 was also affected by the presence of negatively charged lipids in the membrane. This is interesting since it indicates that specific charge−charge interactions may be important determinants for how helices orient in lipid bilayers. Unfortunately, compared to tilt angles, still relatively little information about rotational angles of helices in lipid bilayers is available.

CONCLUDING REMARKS

An increasing amount of evidence highlights the fact that the TM segments of proteins can adapt in different ways to the lipid environment into which they are embedded. It is also clear that even a minor change in, for example, the orientation of the TM segments in the lipid bilayer or their mode of association can have important structural and functional consequences for the intact membrane protein. How exactly a particular TM segment responds to the surrounding lipids will depend not only on its amino acid composition and the nature of the lipids with which it is in contact but also on its interaction with other TM helices. Bearing in mind the complex lipid composition and organization of biological membranes and the highly varied amino acid composition found in natural proteins, we conclude that a complete understanding of lipid−protein interactions is still far away.

Nevertheless, model system approaches that allow systematic characterization of properties of peptides and lipids have been promising. Such approaches have demonstrated, for example, the strength of anchoring interactions of tryptophan residues with the lipid−water interface and its possible implications for protein and lipid organization. It also has become clear how the extent of hydrophobic matching between TM helices and lipid bilayers can modulate both self-association and membrane orientation of TM segments. Importantly, applying principles obtained from model systems to biological systems appears to be valid. For example, studies on the mode of insertion of membrane proteins in the ER have yielded results that were fully consistent with those obtained in model systems (*96*, *97*). Thus, model system approaches should allow us to answer many different questions, like how the organization and assembly of proteins is modulated by kinks or bends in the TM segments or how it is affected by other interactions, e.g., charge−charge interactions in the interfacial region. Particularly intriguing in this respect is the recent discovery that the voltage sensor of some potassium channels, which contains several positively charged arginine residues, needs

the negatively charged phosphodiester of membrane lipids for functioning, as established by using model membranes with chemically synthesized lipid derivatives (*98*). Thus, the combination of chemical synthetic approaches with biophysical studies is a promising and powerful tool for establishing molecular details of membrane protein structure and function.

So far, most of the information about how TM segments of membrane proteins sense the lipid environment comes from studies on single helices. Nevertheless, it is clear that the situation is much more complex for larger membrane proteins, where interactions between TM segments also play a role. It can be expected that in particular solid-state NMR will be an important tool in investigating such proteins and how they are affected by the lipid environment. This is because NMR experiments combine the possibilities of obtaining structural details on an atomic level, of obtaining information on dynamics, of allowing systematic variation of the lipid environment, and of using isotopic labels that do not interfere with the behavior of the TM segments. However, in spite of important recent developments (for a review, see ref *99*), such experiments are still far from trivial, and understanding in detail how larger proteins are influenced by the lipid environment via their TM segments will remain an important future challenge.

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