The interaction of helical polypeptides with biological model membranes*

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Proton-decoupled solid-state ^{15}N NMR spectroscopy was used to investigate helical peptides reconstituted into oriented phospholipid bilayers. Hydrophobic channel peptides such as the *N*-terminal region of Vpu of human immunodeficiency virus (HIV-1) adopt transmembrane orientations, whereas amphipathic peptide antibiotics are oriented parallel to the bilayer surface. The alignment of helical peptides in lipid membranes was analysed in some detail using model peptides. In particular, peptides with pH-dependent topology and a series of peptides that allow one to study the contributions of specific interactions were designed. The energies of transfer of several amino acids from the in-plane to transmembrane localisation were determined. In addition, the alignment of peptides and phospholipids under conditions of hydrophobic mismatch have been investigated in considerable detail.

Key words: solid-state NMR, oriented bilayer, transmembrane polypeptide, amphipathic peptide, membrane equilibria, hydrophobicity scale, interface, Vpu protein of HIV-1, alameticin, LAH_4.

When interacting with biological membranes, hydrophobic or amphipathic helical peptides exhibit a multitude of properties, including antibiotic, fusogenic, and channel activities.[1,2] Elucidation of the structure and alignment of membrane-associated helices is essential for our understanding of the functional mechanisms of these peptides, which are often characterized by helical domains. This can be a difficult task, as these peptides exhibit highly dynamic properties and the detailed conformation and membrane alignment might vary with experimental conditions. Thus, it is often more difficult to find a structure—property relationships than one would have expected in view of the small size of these polypeptides.[3,4]

First, it has been suggested that membrane permeability and, hence, the antibiotic properties of linear peptide antibiotics are due to the formation of transmembrane helical bundles.[1] However, subsequent structural studies have shown that in membrane-like environment these peptides are α-helical and are oriented parallel to the bilayer surface.[1] However, it cannot be ruled out that a minor peptide population in a high-energy configuration is responsible for the activity. In this case, detection of the active states against an abundant background of inactive low-energy configurations using structural techniques would be difficult if not impossible.[5] Furthermore, the situation is complicated by the fact that the distribution between active and inactive configurations can be shifted upon variation of the experimental conditions.[4] These include changes in lipid composition or the temperature as well as the application of transmembrane electric fields.

Alternatively, quantitative estimation of the peptide—lipid interactions and thus prediction of the resulting peptide topologies would allow one to assess the contributions of different models. For this purpose, it is important to reveal the role of interactions that determine the structures and topologies of α-helical polypeptides in the membrane environment.[6—8]

The theoretical estimates of the interaction contributions made by in-plane and transmembrane peptides make it possible to substantiate the suggested models of peptide—membrane interactions.[5] The membrane association of amphipathic and hydrophobic polypeptides is governed by numerous equilibria of the type (Fig. 1)

Water-dispersed aggregates $\Leftrightarrow$

$\Leftrightarrow$ solution in an aqueous buffer $\Leftrightarrow$

$\Leftrightarrow$ membrane surface associated $\Leftrightarrow$

$\Leftrightarrow$ IP $\Leftrightarrow$ TM_1 $\Leftrightarrow$ TM_2 $\Leftrightarrow$ ... $\Leftrightarrow$ TM_n,

where IP stands for in-plane and TM for transmembrane peptide oligomers of *n* subunits.[4] In our laboratory, we have approached this problem by experimentally testing

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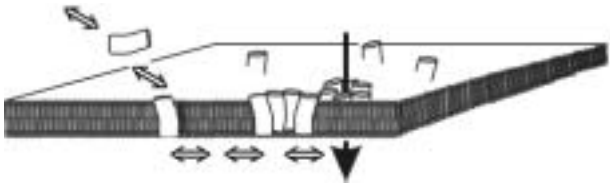

Fig. 1. Model for the equilibria in the peptide—bilayer association. The following sequence of events is schematically shown (from left to right): helical peptide soluble in an aqueous buffer ⟺ membrane surface associated peptide ⟺ IP ⟺ ⟺ TM_1 ⟺ TM_2 ⟺ ...

the alignment of designed model peptides whose amino acid composition has been modified in a systematic manner. In the next step, the results obtained were compared with the structural data, including the orientation and dynamics, of naturally occurring peptides. This review presents the results of our studies of the IP ⟺ TM equilibria for a variety of natural or model peptides using solid-state NMR spectroscopy in combination with other biophysical techniques.

Structural and topological analyses of polypeptides reconstituted into lipid bilayers have been performed using solid-state NMR, CD, and ATR-FT IR spectroscopies. In particular, solid-state NMR has proven to be a valuable method for the investigation of membrane-associated proteins and peptides.[9—13] The technique offers the distinct advantage, as it allows investigation of membrane polypeptides in their natural bilayer environments.

The nuclear interactions with the magnetic field are inherently anisotropic and, therefore, dependent on the orientation and conformation of the molecule with respect to the magnetic field direction.[13—17] In solution, fast molecular tumbling results in isotropic averaging of the nuclear interactions. However, the re-orientational correlation times of molecules associated with extended phospholipid bilayers are in general too long to efficiently average the ^{1}H, ^{13}C, ^{15}N, and ^{31}P chemical shifts or ^{2}H quadrupole interactions. In these cases, the anisotropic properties of these interactions are reflected in the NMR line shape of membrane-bound peptides or lipids.

The anisotropic chemical shift is mathematically described by second rank tensors, which are expressed in the principle axis system by three diagonal components, σ_{11}, σ_{22}, and σ_{33}. The conversion of this coordinate system into the laboratory frame (related to the direction of the magnetic field) is achieved by using Euler transformations around the angles Θ and Φ. As a result, the ZZ component of the chemical shift tensor, *i.e.,* that coinciding with the magnetic field direction, corresponds to the measured NMR chemical shift. This parameter is given by the equation

$$\sigma_{ZZ} = \sigma_{11}\sin^2\Theta\cos^2\Phi + \sigma_{22}\sin^2\Theta\sin^2\Phi + \sigma_{33}\cos^2\Theta.$$

We have investigated membrane-associated peptides labeled with ^{15}N either selectively, or specifically, or uniformly using proton-decoupled solid-state ^{15}N NMR spectroscopy. The static ^{15}N NMR chemical shift tensor of the amide bond is characterized by the σ_{22} and σ_{11} elements of the main tensor whose values are about 85 ppm and 65 ppm, respectively. The σ_{33} component is approximately 230 ppm.[18—22] In α-helical peptides, the NH vector and the σ_{33} component cover an angle of ~18° and are both oriented along the helix long axis to within a few degrees (Fig. 2, *a*). Due to the unique size of σ_{33} and its orientation almost parallel to the helix axis, the ^{15}N chemical shift provides direct information on approximate orientation of the helix within oriented phospholipid bilayers. When the sample is uniaxially oriented

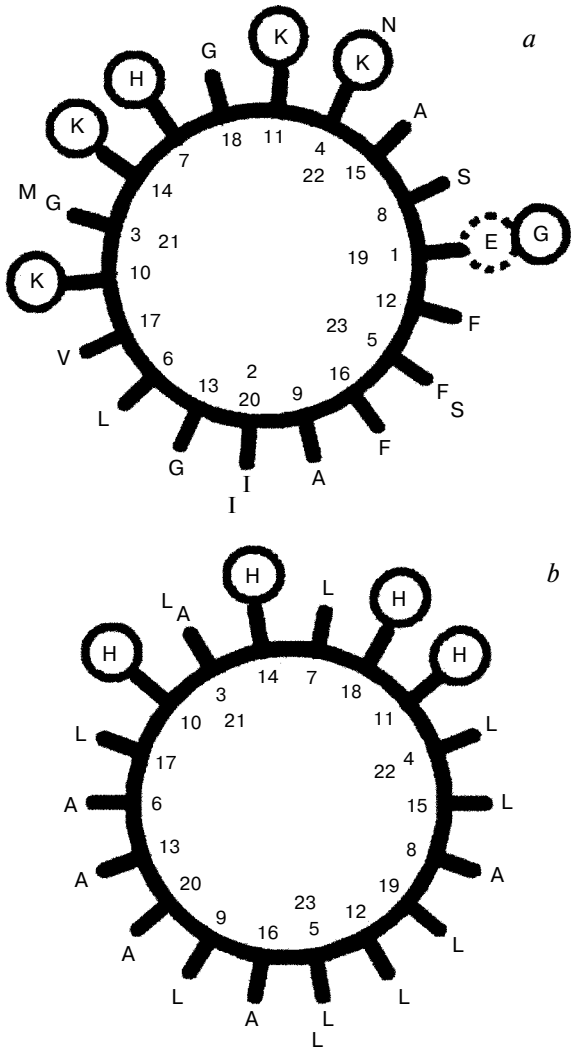

Fig 2 View down the helix axis of the peptide (the Edmundson helical wheel); magainin 2 (*a*) and LAH_4 (*b*). Both peptides exhibit a clear separation of charged (circled) and hydrophobic amino acids located along the opposite faces of the helix. These peptides were described in detail previously.[1,34,35]

with the membrane surface normal parallel to the magnetic field direction, transmembrane α-helical peptides exhibit ^{15}N chemical shifts greater than 200 ppm.[13] Conversely, the signals in the σ_{11}—σ_{22} range (*i.e.,* < 100 ppm) are indicative of orientations parallel to the membrane surface.

Proton-decoupled solid-state ^{15}N NMR spectroscopy was used to demonstrate that hydrophobic sequences, including the channel-forming M2 domain of influenza A,[23,24] the C-terminus of the Bcl-x_L peptide,* the peptide antibiotic alamethicin,[25,26] or the $\beta^{6.3}$-helix of gramicidin A have transmembrane orientation.[9] Studies of the Vpu viral protein of HIV-1 show an approximately 20° tilt angle of the N-terminal hydrophobic helix (with respect to the membrane surface), a nearly 90° in-plane alignment of the amphipathic helix close to residue 45, and the lack of efficient membrane interactions for residues 51—81.[27—29] The orientation of the amphipathic helix domain 32—51 does not depend on whether or not serines 52 and 56 are phosphorylated.[28] Other amphipathic α-helices, including a variety of antibiotic peptides, orient preferentially perpendicular to the membrane normal.[1,30]

Lysines are abundant in most amphipathic helices that exhibit antibiotic activities and membrane permeability. Thus magainins carry 4 or 5 positive charges over a total length of 26 residues (see Fig. 2, *a*). Despite their high charge density, lysine-containing helices have been suggested to form macroscopic structures in the active state, including transmembrane helix orientations.[31—33] This model stimulated us to investigaste the topological equilibria of lysine-containing model peptides in more detail.[34] The results indicate that hydrophobic model peptides with one lysine residue acquire stable transmembrane orientations. In the case of our model peptides, the hydrophobic interactions compensate efficiently the large overall hydrophilic energy contributions associated with this basic amino acid (pK ~ 10.5). However, when three or more lysine residues are located on the hydrophilic face of an amphipathic helix, stable in-plane orientations are obtained. These model peptides represent best the cecropin or magainin family of antibiotic peptides where four or more positively charged amino acids constitute the hydrophilic face of amphipathic α–helices.[1] Interestingly, when two lysines are placed in the central core of the otherwise hydrophobic sequence, an equilibrium between in-plane and transmembrane oriented peptides is observed.[34] This indicates that in the transition from the in-plane to the transmembrane configuration, the contributions of various interactions largely compensate each other. These experimental results obtained by solid-state NMR spectroscopy are in good agreement with molecular modeling calculations in which the alignment of monomeric peptides is tested in a low-dielectric slab flanked by interfaces where the dielectric constant changes in a sigmoidal manner within 4.5 Å.[34]

In the next step, the lysines were replaced by histidine residues. In water, histidine has p$K \approx 6$; therefore, histidine residues are readily protonated at pH values close to neutral. The pK values of histidine in the range of 5—6 were also measured for peptide sequences in the presence of dodecyl phosphocholine detergent micelles.[35] The peptide LAH_4 (KKALLALALHHLAHLALHLALALKKA) was synthesized in such a way that four histidine residues were incorporated in a sequence formed by leucine and alanine residues.[35] All histidine residues are located on one face of the helical structure as shown in Fig. 2, *b*. Therefore, on the one hand, this peptide exhibits clear-cut amphipathic properties. On the other hand, the hydrophobic core region consists of 21 amino acids and, therefore, it is able to span the lipid bilayer. Two lysines located at the N- and C-termini act as membrane anchors and help to solubilize the peptide. Proton-decoupled solid-state ^{15}N NMR measurements of LAH_4 reconstituted into oriented 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayers indicate that at low pH values, the peptide is aligned parallel to the membrane surface. This is in line with the pronounced amphipathic character of the peptide.[35] Conversely, at neutral pH, these peptides acquire transmembrane orientations.

In antibiotic tests, pH-independent inhibition of the growth of *E. coli* cells is observed at about 10 μM concentrations of the lysine-rich peptide antibiotic magainin 2. In a similar experiment at pH 5.5, LAH_4 proved to be about 1—2 orders of magnitude more active[36] and was found to have the in-plane orientation.[35] Conversely, at pH 7.4, the peptide LAH_4 occurs in the transmembrane orientation and demonstrates a much lower lytic activity.[35,36] This result indicates that in-plane oriented peptides are more powerful in antibiotic assays. Therefore, models based on the detergent-like properties of amphipathic peptides have been developed.[1,2,30,33]

Recently, it has been shown that the peptide LAH_4 acts as a potent DNA transfectant into eukaryotic cells.[37] This peptide is as powerful as the commercially available polyethylenimine,[38] but is considerably less toxic to the cells when compared to this polymer. Systematic analysis of several derivatives and investigations into the action of a number of pharmaceuticals indicate that they are inserted *via* the endosomes. Furthermore, only the peptides that acquire in-plane detergent-like orientation upon moderate acidification exhibit full transfection activity.

In order to evaluate the hydrophobicity of alanine, the insertion behavior of the peptide $K_3A_{18}K_3$ was studied.[39] This peptide has been designed for testing its membrane insertion properties. On the one hand, the available hydrophobicity tables predicted a trasmembrane orientation.[6,40,41] On the other hand, the considerable hydro-

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philic contribution of the peptide backbone is expected to reduce the hydrophobicity of this amino acid in peptides to an extent sufficient for the preferred association with the aqueous phase.[8,42] Indeed, studies of alanine-based peptides indicate efficient membrane insertion of the peptide $(SK)_2A_6WA_3(KS)_3$ and the lack of such interactions for $A_{27}YK_6$.[43,44] The proton-decoupled solid-state ^{15}N NMR spectroscopy performed in our laboratory indicates that for the peptide $K_3A_{18}K_3$, both in-plane and transmembrane helices are present in 1 : 1 ratio.[39] These experiments faced more difficulties than anticipated, as the fraction of transmembrane helices tends to decrease with time. The highest transmembrane contribution of $K_3A_{18}K_3$ observed in a series of NMR spectra is approximately 70%. Thus, the energy of transfer of one alanine from the in-plane to transmembrane orientation is close to ≥ -0.2 kJ mol^{-1} when averaged over 18 alanine residues within an α-helical secondary peptide structure. This result indicates that alanine, when considered in the context of membrane-associated helical peptides, occurs equally probably at the water—membrane interface or in transmembrane helical domains. Thus, this amino acid can serve as a reference point when the membrane localization of amino acids is considered. The equilibrium between in-plane and transmembrane orientations can be easily shifted by replacement of the alanine residues by leucines.[39,44]

The hydrophobicity value (ΔG^h) for alanine was taken from transfer experiments of $K_3A_{18}K_3$ (-0.2 kJ mol^{-1}), while the value for leucine was calculated using the relative hydrophobicity of alanine and leucine (see below). A comparison of these values with the values for the transfer of amino acid from water into oil determined previously[6] suggests that the hydrophilic contributions from the polypeptide backbone are ~6—7 kJ mol^{-1}, which is in good agreement with earlier estimates.[8,42]

Phospholipid bilayers are soft structures able to adapt to changes in the peptide form and surface. The question arises of how membranes can adapt to peptides with hydrophobic domains shorter or longer than the thickness of the hydrophobic bilayer. A previous theoretical work has predicted that the mismatch energy would increase in a parabolic manner when the difference between the hydrophobic thickness of the pure lipid membrane and the hydrophobic length of the peptide is considered.[45] However, only a limited set of experimental data for testing these predictions has been available so far.[46] Therefore, we studied the interactions between peptides and lipid membranes as a function of hydrophobic mismatch.[47] A series of alanine-leucine or polyleucine peptides flanked by lysine residues on both ends was synthesized. The hydrophobic core of these peptides encomasses 10 to 30 residues. Our results indicate that over a wide range of hydrophobic mismatch, these peptides acquire transmembrane orientations.[47] Only in those cases where the peptides are longer by more than 14 Å or shorter by at least 3 Å than the lipid bilayer thickness, were substantial deviations from the transmembrane orientation observed. Evidently, this asymmetric behavior indicates that some factors other than compression/expansion of the bilayer also contribute.

In this study, six different peptides were systematically combined with eight different phosphatidylcholine membranes. Analysis of this extensive data set shows that peptides that are too short do not disturb the order in phospholipid head groups. However, according to proton-decoupled solid-state ^{31}P NMR spectra, peptides that are longer than the tolerance for transmembrane orientation are characterized by a low degree of ordering. This means that strong interactions between long hydrophobic sequences and the phospholipid bilayer induce pronounced rearrangements in the phospholipid bilayer.

In addition, a regular decrease in the ^{15}N chemical shifts is observed as the calculated hydrophobic mismatch increases within the 14 Å tolerance.[47] This means that peptide adjustment to the bilayer thickness takes place *via* an increase in the helical tilt angle. This adjustment mechanism is inapplicable for peptides that are too short to span the hydrophobic thickness of the bilayer, providing an explanation for the pronounced asymmetry in the tolerance of hydrophobic mismatch. However, a quantitative analysis shows that the measured increase in the tilt angle is insufficient to fully compensate for the calculated hydrophobic mismatch.[47] Therefore, most probably, a combination of adjustment mechanisms is involved, in particular, the increase/decrease in the lipid order parameters (concomitant with changes in bilayer thickness) and conformational changes of the peptides.[46,48]

Our studies using model peptides show that sequences much shorter than the bilayer hydrophobic thickness are aligned predominantly parallel to the membrane surface.[47] Similarly, reconstitution of zervamicin II, a noncharged 16-membered peptide antibiotic from fungi, into oriented POPC membranes results predominantly in in-plane orientations.[26] A decrease in the hydrophobic thickness of the bilayer in the case of 1,2-dicapryl-*sn*-glycero-3-phosphocholine (di-C10:0-PC) results in transmembrane orientation of zervamicin II. However, in the case of alamethicin, composed of 20 amino acids, transmembrane orientation predominates under all conditions tested.[25,26] The difference between the total lengths of zervamicin II and alamethicin is only four amino acids, which corresponds to a ≤6 Å length of the α-helix. Therefore, it is surprising that the thickness of the phosphatidylcholine bilayers has to decrease by more than 10 Å (on passing from from POPC to di-C10:0-PC) in order to transfer zervamicin II into a stable transmembrane configuration.[26] Evidently, other factors such as conformational details of the membrane environment or the length of the terminal anchoring sequences also play a role.[49] In

addition, the hydrophobic moment of zervamicin II is higher than that of alamethicin,[50] thereby favoring orientations along the membrane interface.

Finally, we and other researchers have determined the energy contributions that play the major role in the in-plane to transmembrane (IP → TM) transition of helical peptides, namely, the pH-dependent energy of discharge of the amino acid side chain (ΔG^{d}), the changes in the hydrophobicity (ΔG^{h}) and the polarity (ΔG^{p}), the interactions determining the hydrophobic mismatch (ΔG^{m}) (see, for example, Refs 45—47), and energy changes caused by interactions with transmembrane electrostatic fields (ΔG^{ψ}). This results in the following overall change in the Gibbs free energy:

$$\Delta G = \Delta G^{h} + \Delta G^{d} + \Delta G^{p} + \Delta G^{m} + \Delta G^{\psi} + \Delta G^{\#},$$

where the last term ($\Delta G^{\#}$) includes all other contributions. For example, the changes in lipid—lipid interactions such as lipophobic,[51] van der Waals, and lipid head group interactions can be taken into account separately ($\Delta G^{l} + \Delta G^{W} + \Delta G^{hh}$), provided that they have not been already included in ΔG^{m}.

The discharge energy is derived from the corresponding chemical potentials and is calculated from the equation

$$\Delta G^{d} = n_i \cdot RT \cdot \ln r + 2.3RT \sum_i (pK_i - pH),$$

where r is the ratio of the charged to noncharged state of a functional group acceptable for membrane insertion (typically, this ratio is taken to be 99), n is the number of potential charge carriers i, and RT have their usual meanings.[35] If $pK - pH = 3$, ΔG^{d} amounts to 25—30 kJ mol^{-1}, which is considerably smaller than the corresponding Born energy needed to transfer a charge from high to low dielectric medium (the estimated value is 50—200 kJ mol^{-1}).[52]

In the case of LAH_4, this model predicts that in an acidic medium, hydrophilic interactions would exceed the pH-independent hydrophobic contributions of two leucines, one alanine, and the histidines, all being located on the same face. Slow neutralization of the solution results in a decrease in ΔG^{d} in a linear manner with a decrease in pH. As a result, at pH 7, interactions favorable for membrane insertion predominate and transmembrane orientation is attained.

According to previous studies, the energy contributions related to changes in the lipid packing during peptide reorientation ($\Delta G^{\#}$) are small.[51] Thus, our results indicate that the gain in hydrophobic energy in the IP → TM transitions is sufficient to compensate for the polar contributions of the uncharged histidine side chains. The IP → TM transition of LAH_4 is reversible; its midpoint was determined by proton-decoupled solid-state ^{15}N NMR and by ATR-FT IR spectroscopies at pH 6.1.[35,53]

Other histidine-containing peptides whose membrane insertion entails a regular variation of hydrophobic contributions have also been designed.[54] Thus replacement of leucine residues by alanine residues shifts the transition pH to higher values.[55] The introduction of other, more hydrophilic residues such as glycines or tyrosines excludes the IP to TM transition. Interestingly, in these experiments, tryptophan residues have also exhibited a pronounced tendency to reside at the membrane interface.[54] This more recent work reports a more elaborate model for calculating the IP → TM transition curves as a function of pH; the differences between transition pH were used to determine the relative hydrophobicities.[54]

In the case of α-helical peptides and in a lipid bilayer, the membrane insertion of leucine is about 5—6 kJ mol^{-1} more favorable than that of alanine. This difference is of the same order of magnitude when compared with previously obtained hydrophobicity values for these amino acids.[40] These studies deal with transitions of amino acids or small peptides from water into a hydrophobic environment[8] or from water to the membrane interface, respectively. These data also indicate that during the transfer of alanine or leucine into a hydrophobic environment, about 30% of the Gibbs free energy change is associated with the first step of transfer from water to the membrane interface.[8] The dielectric properties change rapidly within the interfacial region of the membrane;[8,56] therefore, the transfer energy should depend appreciably on the conformational details and the depth of interfacial penetration of the peptide.

The studies presented in this paper have helped to identify the interactions whose contributions are important during the IP → TM transitions. The knowledge of these interactions is important not only for the quantitative evaluation of functional models of peptide activity.[1] The study of model peptides[47] has also helped in the rational design of experimental conditions in which the alignment of naturally occurring peptides shows the lowest mosaic spread.[26] Well-ordered samples are important for a number of structural techniques including oriented solid-state NMR spectroscopy.[9,24]

Further research is required to improve our understanding of peptide—membrane interactions. The available improved hydrophobicity scales would allow researchers to improve considerably the membrane—protein prediction algorithms and thus our knowledge on the organization of bilayer-associated proteins. In addition, the effects of transmembrane electric fields and peptide oligomerization on polypeptide structure and topology need to be investigated.[4] Therefore, structural studies and methodological developments should include these interactions in quantitative analysis.[55,57]

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References

1. B. Bechinger, *Biochim. Biophys. Acta*, 1999, **1462**, 157.
2. Y. Shai, *Biochim. Biophys. Acta*, 1999, **1462**, 55.
3. M. S. P. Sansom, *Current Opinion in Colloid and Interface Science*, 1998, **3**, 518.
4. B. Bechinger, *Phys. Chem. Chem. Phys.*, 2000, **2**, 4569.
4. B. Bechinger, *Curr. Opin. Chem. Biol.*, 2000, **4**, 639.
5. B. Bechinger, *J. Membrane Biol.*, 1997, **156**, 197.
6. D. M. Engelman, T. A. Steitz, and A. Goldman, *Ann. Rev. Biophys. Biophys. Chem.*, 1986, **15**, 321.
7. G. Montich, S. Scarlata, S. McLaughlin, R. Lehrmann, and J. Seelig, *J. Biochim. Biophys. Acta*, 1993, **1146**, 17.
8. S. H. White and W. C. Wimley, *Annu. Rev. Biophys. Biomol. Struct.*, 1999, **28**, 319.
9. T. A. Cross, *Methods Enzymol.*, 1997, **289**, 672.
10. R. G. Griffin, *Nature Structural Biology, NMR Supplement*, 1998, **5**, 508.
11. J. H. Davis and M. Auger, *Progress in NMR Spectroscopy*, 1999, **35**, 1.
12. A. Watts, *Curr. Opin. Biotech.*, 1999, **10**, 48.
13. B. Bechinger and C. Sizun, *Concepts in Magnetic Resonance*, 2003, **18A**, 130.
14. U. Haeberlen, *High Resolution NMR in Solids, Selective Averaging*, Academic Press, New York, 1976.
15. R. G. Griffin, *Methods Enzymol.*, 1981, **72**, 108.
16. M. Mehring, *Principles of High Resolution NMR in Solids*, Springer, Berlin, 1983.
17. T. A. Cross and J. R. Quine, *Concepts in Magnetic Resonance*, 2000, **12**, 55.
18. C. J. Hartzell, M. Whitfield, T. G. Oas, and G. P. Drobny, *J. Am. Chem. Soc.*, 1987, **109**, 5966.
19. T. G. Oas, C. J. Hartzell, F. W. Dahlquist, and G. P. Drobny, *J. Am. Chem. Soc.*, 1987, **109**, 5962.
20. N. D. Lazo, W. Hu, and T. A. Cross, *J. Magn. Res.*, 1995, **107**, 43.
21. D. K. Lee, R. J. Wittebort, and A. Ramamoorthy, *J. Am. Chem. Soc.*, 1998, **120**, 8868.
22. D. K. Lee, Y. Wei, and A. Ramamoorthy, *J. Phys. Chem., B*, 2001, **105**, 4752.
23. F. A. Kovacs and T. A. Cross, *Biophys. J.*, 1997, **73**, 2511.
24. B. Bechinger, R. Kinder, M. Helmle, T. B. Vogt, U. Harzer, and S. Schinzel, *Biopolymers*, 1999a, **51**, 174.
25. C. L. North, M. Barranger-Mathys, and D. S. Cafiso, *Biophys. J.*, 1995, **69**, 2392.
26. B. Bechinger, D. A. Skladnev, A. Ogrel, X. Li, N. V. Swischewa, T. V. Ovchinnikova, J. D. J. O´Neil, and J. Raap, *Biochemistry*, 2001, **40**, 9428.
27. V. Wray, R. Kinder, T. Federau, P. Henklein, B. Bechinger, and U. Schubert, *Biochemistry*, 1999, **38**, 5272.
28. P. Henklein, R. Kinder, U. Schubert, and B. Bechinger, *FEBS Lett.*, 2000, **482**, 220.
29. B. Bechinger and P. Henklein, in *Viral Membrane Proteins: Structure, Function, Drug Design (in Preparation)*, Ed. W. Fischer, *Solid-state NMR Investigations of Vpu Structural Domains in Oriented Phospholipid Bilayers: Interactions and Alignment*, 2004.
30. B. Bechinger, *Critic. Rev. Plant Sci.*, 2004, **23**, 271.
31. A. de Waal, A. V. Gomes, A. Mensink, J. A. Grootegoed, and H. V. Westerhoff, *FEBS Lett.*, 1991, **293**, 219.
32. S. J. Ludtke, K. He, W. T. Heller, T. A. Harroun, L. Yang, and H. W. Huang, *Biochemistry*, 1996, **35**, 13723.
33. K. Matsuzaki, *Biochim. Biophys. Acta*,1998, **1376**, 391.
34. T. C. B. Vogt, P. Ducarme, S. Schinzel, R. Brasseur, and B. Bechinger, *Biophys. J.*, 2000, **79**, 2644.
35. B. Bechinger, *J. Mol. Biol.*, 1996, **263**, 768.
36. T. C. B. Vogt and B. Bechinger, *J. Biol. Chem.*, 1999, **274**, 29115.
37. A. Kichler, C. Leborgne, J. März, O. Danos, and B. Bechinger, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 1564.
38. A. Kichler, C. Leborgne, E. Coeytaux, and O. Danos, *J. Gene Medicine*, 2001, **3**, 135.
39. B. Bechinger, *Biophys. J.*, 2001, **82**, 2251.
40. G. von Heijne, *Eur. J. Biochem.*, 1981, **120**, 275.
41. J. Kyte and R. F. Doolittle, *J. Mol. Biol.*, 1982, **157**, 105.
42. N. Ben-Tal, A. Ben-Shaul, A. Nicholls, and B. Honig, *Biophys. J.*, 1996, **70**, 1803.
43. C. M. Deber and S. C. Li, *Biopolymers*, 1995, **37** , 295.
44. L. A. Chung and T. E. Thompson, *Biochemistry*, 1996, **35**, 11343.
45. O. G. Mouritsen and M. Bloom, *Biophys. J.*, 1984, **46**, 141.
46. J. A. Killian, *Biochim. Biophys. Acta*, 1998, **1376**, 401.
47. U. Harzer and B. Bechinger, *Biochemistry*, 2000, **39**, 13106.
48. M. R. de Planque, J. W. Boots, D. T. Rijkers, R. M. Liskamp, D. V. Greathouse, and J. A. Killian, *Biochemistry*, 2002, **41**, 8396.
49. M. R. R. de Planque, J. A. W. Kruijtzer, R. M. J. Liskamp, D. Marsh, D. V. Greathouse, R. E. Koeppe, B. De Kruijff, and J. A. Killian, *J. Biol. Chem.*, 1999, **274**, 20839.
50. M. S. P. Sansom, *Prog. Biophys. Molec. Biol.*, 1991, **55**, 139.
51. F. Jähnig, *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 3691.
52. J. N. Israelachvilli, S. Marcelja, and R. G. Horn, *Q. Rev. Biophys.*, 1980, **13**, 121.
53. B. Bechinger, J. M. Ruysschaert, and E. Goormaghtigh, *Biophys. J.*, 1999b, **76**, 552.
54. C. Aisenbrey, *PhD Thesis*, University Louis Pasteur, Strasbourg, 2003.
55. R. Kinder, *PhD*, Technical University, Munich, 1999.
56. G. Buldt, H. U. Gally, A. Seelig, J. Seelig, and G. Zaccai, *Nature*, 1978, **271**, 182.
57. A. Le Saux, J. M. Ruysschaert, and E. Goormaghtigh, *Biophys. J.*, 2001, **80**, 324.

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