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Review

Solid-state NMR approaches to measure topological equilibria and dynamics of membrane polypeptides

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ABSTRACT

Biological membranes are characterized by a high degree of dynamics. In order to understand the function of membrane proteins and even more of membrane-associated peptides, these motional aspects have to be taken into consideration. Solid-state NMR spectroscopy is a method of choice when characterizing topological equilibria, molecular motions, lateral and rotational diffusion as well as dynamic oligomerization equilibria within fluid phase lipid bilayers. Here we show and review examples where the ^{15}N chemical shift anisotropy, dipolar interactions and the deuterium quadrupolar splittings have been used to analyze motions of peptides such as peptaibols, antimicrobial sequences, Vpu, phospholamban or other channel domains. In particular, simulations of ^{15}N and ^{2}H-solid-state NMR spectra are shown of helical domains in uniaxially oriented membranes when rotation around the membrane normal or the helix long axis occurs.

Contents

The structures of several membrane proteins have been determined at high resolution [1,2] and these often are an important step to our detailed understanding of their functioning. Having available the structures of different conformational states, thereby tracing the structural changes in a stroboscopic manner, can be particularly helpful to gain insights into the molecular functioning. Examples are the many intermediates of the bacteriorhodopsin photocycle [3], or a number of structures of the skeletal muscle sarco-plasmic reticulum Ca-ATPase [4,5], as well as the homologous *Arabidopsis thaliana* auto-inhibited H^+-ATPase2 [6]. However, despite their high resolution, these structures are static, have in most cases been recorded at cryotemperatures and do not show the lipid bilayer. In contrast, the widely accepted fluid mosaic model suggests an inherently dynamic nature of the membrane with the proteins and lipids diffusing laterally and rotationally [7]. Therefore, additional information about the dynamic nature of the membranes and the proteins is key to fully understand the movements associated with their action. This is even more the case for membrane-associated peptides that are thoroughly immersed in the lipid environment and where interactions between peptides and lipids dominate [8].

Abbreviations: bR, bacteriorhodopsin; CD, circular dichroism; CP, cross polarization; CSA, chemical shift anisotropy; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; MAS, magic angle spinning; NMR, nuclear magnetic resonance; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol

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The dynamic nature of peptide-peptide interactions in a membrane has early on become apparent when the voltage-dependent channel formation by alamethicin has been investigated by single channel electrophysiological recordings. Alamethicin is a 20-amino acid hydrophobic peptide of fungal origin that is rich in Aib (α-amino*iso*butyric acid) and which exhibits antimicrobial activities (reviewed in [9–12]). This peptide is considered a paradigm for channel formation in biological membranes and has therefore been extensively studied by a variety of biophysical methods in the past. When added to lipid bilayers, well-defined patterns of successive increases in conductance levels are observed, each of a duration of a few milliseconds [13–15]. These step-wise changes resemble those seen in the presence of large voltage- or ligand-gated channel proteins (reviewed in [9–12]). The open alamethicin pore has been described by 'transmembrane helical bundles' or 'barrel staves' [10] which are composed of at least three [16] or four subunits [17]. As many as 20 different conductance states have been described [18] and it is believed that these correspond to differently sized bundles of transmembrane alamethicin helices [10]. Contrary to some of the previous models, recent molecular modeling calculations of the alamethicin channels suggest a less regular arrangement with a high degree of conformational variation of each subunit [19], an observation that is in agreement with recent experimental solid-state NMR data [20]. Conformational and/or topological heterogeneity has also been observed for phospholamban or sarcolipin [21,22]. Furthermore, a combination of solid-state NMR spectroscopy and molecular modeling has recently revealed interesting structural details on the GABA-TM2 domain [23].

Whereas alamethicin remains associated with the bilayer surface at low peptide-to-lipid ratios, pores of self-associated transmembrane helical bundles are formed above a threshold concentration [24]. A reorientation of the helix dipole and thereby an increase in the transmembrane population have also been suggested to form the basis of voltage gating [10,11]. In this context, the study of the in-plane to transmembrane equilibrium is of interest as it allows one to understand this regulatory event in a more quantitative manner [8].

NMR spectroscopy works in environments that preserve the character of solutions and/or of liquid crystalline membranes. It is therefore a method of choice to study molecular dynamics at high resolution, and the measurement of relaxation times and NOEs for spectral density mapping is a well-established method in solution NMR spectroscopy [25,26]. Due to inherent difficulties to obtain high-resolution NMR spectra in soft solids such as liquid crystalline membranes, these methods are less well established for biomolecules in such environments although first measurements of dynamics have been obtained from MAS NMR spectra of proteins in the solid state [27]. It is interesting to note that the solid-state NMR line widths observed for membrane samples and thereby the spectral resolution are inherently linked to the local dynamics of the protein [28–30].

1. NMR as a tool to study topological equilibria and molecular dynamics

Fig. 1 shows an example of the topological and conformational heterogeneity of a membrane-associated peptide. To monitor alamethicin alignment as a function of lipid composition, the peptide was reconstituted into uniaxially oriented membranes and the samples were investigated by proton-decoupled ^{15}N solid-state NMR spectroscopy. The ^{15}N chemical shift of ^{15}N labeled peptide bonds provides a direct indicator of the membrane alignment of helical polypeptides when these are reconstituted into bilayers that are typically oriented with the normal parallel to the magnetic field direction [31]. Whereas the chemical shift values of transmembrane peptides occur in the 200 ppm range, those of in-plane oriented helices resonate at frequencies<100 ppm [20,23,31–33]. Fig. 1A shows the ^{15}N chemical shift spectrum of alamethicin uniformly labeled with ^{15}N in oriented POPC, a simple mimetic of the zwitterionic membranes of eukaryotes, and Fig. 1B after reconstitution in POPE/POPG membranes, a model for bacterial membranes. Whereas the chemical shift values (between 195 and 230 ppm with a maximum at 208 ppm) indicate a transmembrane alignment of the peptide in POPC, an orientation parallel to the surface is adopted in POPE/POPG (chemical shifts between 68 and 125 ppm with a maximum at 95 ppm).

Fig. 1C and D show proton-decoupled ^{31}P solid-state NMR spectra of the same samples from which the ^{15}N solid-state NMR spectra were recorded (Fig. 1A, B). The ^{31}P chemical shift value is dependent on the alignment of the lipid head group relative to the magnetic field direction (B_o), the latter being collinear with the glass plate normal (n). For pure phosphatidylcholine membranes in their fluid state at the sample orientations represented in Fig. 1 ($B_o \parallel n$), values around 30 ppm are characteristic of lipids that align with their long axis parallel to B_o and resonances at −15 ppm are indicative of perpendicular alignments. The narrow ^{31}P NMR resonance in Fig. 1C therefore shows that the phospholipid membranes are well aligned with only a small contribution reaching into the −15 ppm region. The latter probably corresponds to the maximum intensity typically observed for a powder pattern line shape (i.e. a random alignment of non-oriented sample) [34].

The mixed POPE/POPG membrane exhibits two main intensities (Fig. 1D) corresponding to the differences in the ^{31}P chemical shift anisotropy of it two phospholipid components. Also in this case, a predominant alignment of the phospholipids parallel to B_o is observed albeit the distribution is less homogenous and the membranes exhibit higher mosaic spread. This observation agrees with previous data where amphipathic peptides that intercalate into the membrane interface in an in-plane fashion caused considerable membrane disordering [35–40].

The example shown in Fig. 1 illustrates that the peptide-lipid interactions are governed by sensitive equilibria although the acquisition time of many hours, even for a one-dimensional solid-state NMR spectrum, hampers the measurements of kinetic parameters characterizing these topological transitions. Previously, the membrane interactions of alamethicin were investigated in a variety of different environments by CD- [41,42] and solid-state NMR spectroscopies on oriented samples [43–45]. Using these techniques, transitions between the surface-associated state and transmembrane alignments of alamethicin and other members of the peptaibol family were characterized as a function of lipid composition, peptide-to-lipid ratio [24,41,42] and membrane hydrophobic thickness [20,45]. Lipid-dependent topologies, including barrel-stave pores, have also been observed pardaxin using solid-state NMR spectroscopy [35,46].

Topological equilibria have also been observed by solid-state NMR spectroscopy for a variety of other peptides and proteins including colicins [47,48], diphtheria toxin [49,50] and the C-terminal anchoring domain of the anti-apoptotic protein Bcl-x_L [51]. Furthermore, histidine-containing antimicrobial and transfection peptides have been designed in such a manner to change membrane orientation [52,53] and DNA association as a function of pH [54], an effect that has also been used to quantify the Gibbs free energies associated with the transfer of amino acid residues from the membrane interface to the hydrophobic interior [55,56].

Even though the equilibrium thermodynamics can be established and the differences in energies between topomers calculated [55,56], the question remains how fast the transfer between the in-plane and the transmembrane configuration occurs. This is a challenging task for systems where data acquisition is hampered by low sensitivity, but the investigation of histidine-containing amphipathic peptides has provided some indications [52,56,57]. Interestingly, at intermediate pH, the in-plane and transmembrane peptides exhibit well-separated ^{15}N resonances (Fig. 2) indicating that the exchange between the two alignments is slow on the 10^{-4} s time scale [52,56] although the orientational equilibrium is reversible within a few minutes of incubation [53].

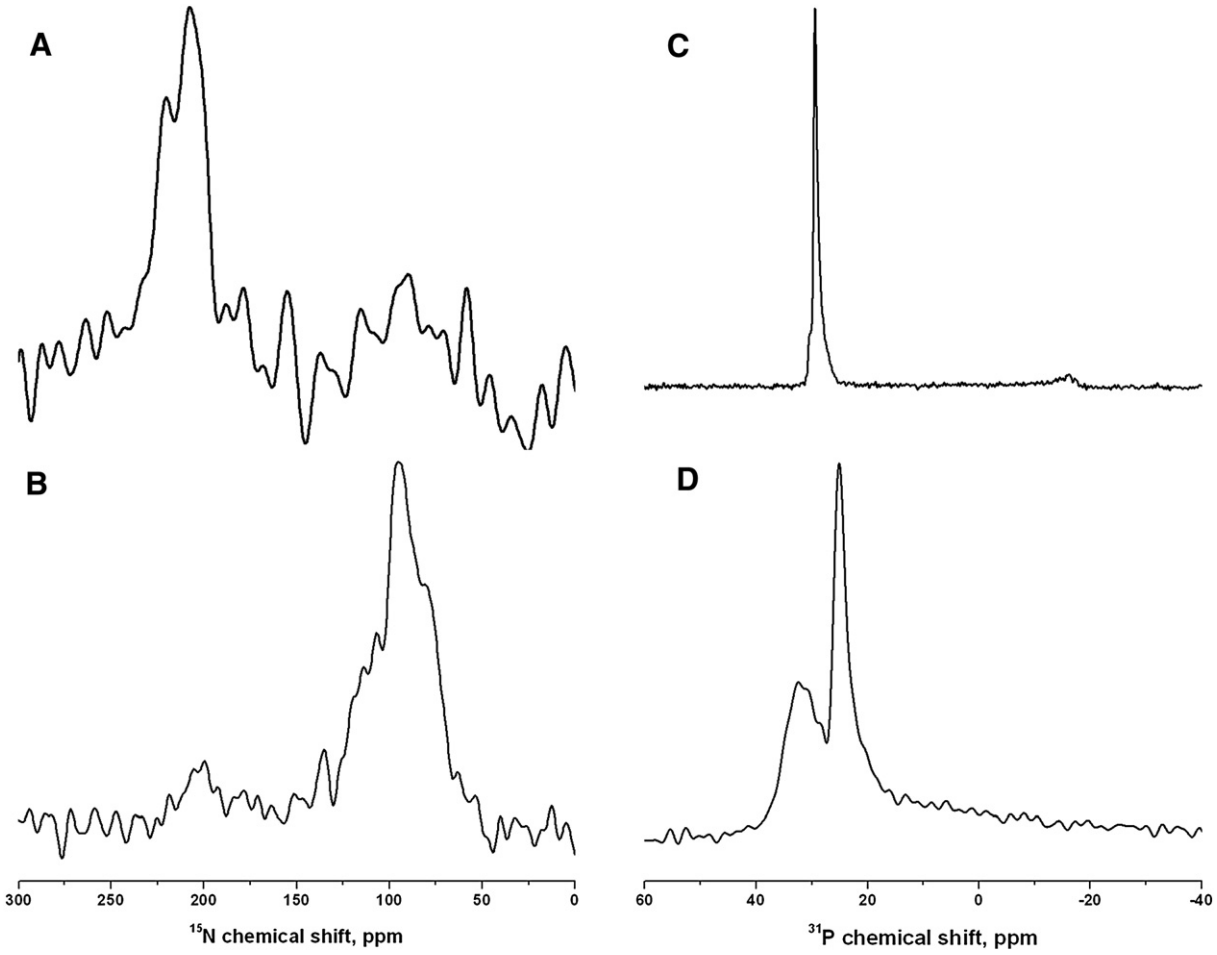

Fig. 1. Proton-decoupled oriented ^{15}N (A,B) or ^{31}P solid-state NMR spectra (C, D) of alamethicin in POPC membrane at a concentration of 1 mol% (A, C), or in POPE/POPG 3/1 bilayers at a concentration of 2 mol% (B, D). In the case of POPE/POPG membrane (B, D), pH 7.5 was set by addition of 1 mM Tris buffer solution and a temperature was maintained at 37 °C. For the sample of alamethicin in POPC, the temperature was maintained at 21 °C. The sample normal was aligned parallel to the external magnetic field. The effect of alamethicin on the order parameter of these membranes was published earlier [100].

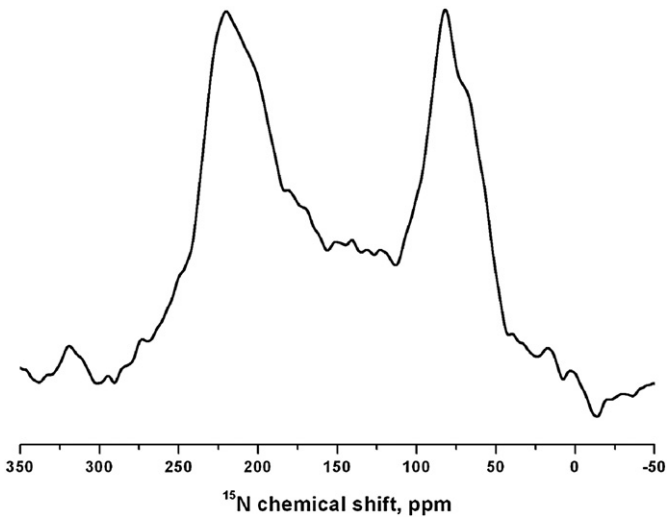

Fig. 2. Proton-decoupled ^{15}N solid-state NMR spectrum of LAH4 reconstituted in a membrane of POPC at a concentration of 1 mol% oriented with the normal parallel to the magnetic field direction. LAH4 is an amphipathic helical peptide, where the polar face consists of four histidines, with potent antimicrobial and DNA transfection characteristics [52,80]. The peptide changes from a transmembrane to an in-plane alignment in a pH-dependent manner [52] and the spectrum shows the situation at pH 6. The peptide was prepared labeled uniformly with ^{15}N according to [101]. A comparison of the effects of different peptide topologies on lipid order parameters is shown in [100].

The dynamics of membrane-associated polypeptides has also been explored by solid-state NMR investigations of the ^{1}H-^{1}H and ^{1}H-X dipolar couplings which are in the range of a few tens of kHz. At a given distance such as in covalent bonds, the reduction of the dipolar coupling is an indicator of motions faster than the spin interaction and has been explored to compare the dynamics of crystalline and membrane-bound colicin Ia [58]. Furthermore, by using this approach, it was shown that the biological activities of three tachyplesin antimicrobial peptides, when associated with lipid bilayers, correlated with the motional amplitudes observed within the peptide backbone, a finding that was interpreted with the different degree of aggregation of the β-sheet structures [59]. Interestingly, the dipolar interactions between nuclei have been exploited in various other ways, for example, by using different cross-polarization conditions on oriented samples to screen for the dynamical features of protein domains [60,61]. Furthermore, the orientation-dependent dipolar interactions are used to introduce a second dimension into static oriented solid-state NMR spectra [62,63], and by combining orientational information from dipolar interactions, chemical shift anisotropy and solution NMR data, highly refined structural models of membrane polypeptides can be obtained [39,64–66].

When bacteriorhodopsin (bR), labeled uniformly with ^{15}N has been investigated by static oriented CP solid-state NMR spectroscopy, the temperature dependence of the ^{15}N T_2 relaxation times is indicative of slow motions on the millisecond time scale [30]. On the other hand, the line broadening observed in ^{13}C CP-MAS spectra at

low temperatures indicates that the helices of bR in the purple membrane exhibit motions at frequencies of around 100 Hz [29,67]. The motional amplitudes and/or frequencies are increased after reconstitution of bR into DMPC membranes [30]. Motions of bR in the tens of microsecond correlation time regime have been found coupled to the phospholipids dynamics and these correlate with the activity of this proton pump [68]. In static oriented purple membrane samples, the slow motions result in considerable line broadening when the sample exhibits even a small degree of mosaic spread or conformational heterogeneity [30,69]. In this context, it should be noted that dark adapted bR is characterized by two distinct retinal conformers (e.g. [70,71]) which may contribute to the conformational heterogeneity of the protein backbone.

2. Methods to measure lateral diffusion in membranes

Another important aspect of membrane-polypeptide dynamics is the rotational and lateral diffusion in membranes. Commonly, optical techniques such as fluorescence recovery after photobleaching (FRAP, [72]) or fluorescence correlation spectroscopy (FCS) [73,74] are used to follow the diffusion along the membrane surface but these techniques require the labeling with bulky optical probes, which by themselves may have an effect on the diffusion and aggregation of the molecules. Therefore, non-invasive NMR methods have been developed for solutions and for membranes which make use of magnetic field gradients. As the strength of the magnetic field determines the speed of precession of the nuclear spin, magnetization phase differences develop during the duration of the magnetic field gradient. These can be used to encode the localization of the nuclei along the direction of the gradient. By applying a gradient of similar strength but with an opposite sign, the effect of the first gradient is cancelled provided that the spins have not changed their localization during the echo delays. Therefore, in pulse field gradient (pfg) NMR, the loss of signal after the second gradient pulse is a measure of diffusion [75]. As semi-solid samples such as membranes are characterized by considerable line broadening, the application of the technique is less obvious. However, by uniaxially orienting the membranes between glass plates and at alignments of the sample normal along the magic angle, rotational diffusion helps in obtaining well-resolved resonances [76,77].

Lateral diffusion experiments are widely applied to characterize the phase behavior of lipid bilayers of different compositions in particular in the presence of sterols [73,77]. Investigations of the formation of domains within lipid membranes (e.g. [78–80]), an effect known as lipid phase separation since the early days of lipidology, have become popular again after it has been speculated that 'rafts' may be important in biology. Although there are numerous indications for the biological significance of lipid phase separation within cellular membranes [81], the physical concept of microdomains is difficult to prove *in vivo* [82,83].

3. Rotational diffusion and aggregation in membranes

Whereas the rotational diffusion around the membrane normal of lipids and sterols (e.g. cholesterol) is characterized by correlation times in the ps–ns range and is of special interest for a microscopic description of the lipid phase behavior [84], the rotational motion of membrane peptides and proteins occurs in the μs–ms range. The latter time frame is accessible by solid-state NMR methods as it corresponds to chemical shift anisotropies (CSA) and deuterium quadrupolar interactions (in the range of 10–100 kHz). Optical techniques like polarized fluorescence photobleaching recovery (pFPR) can extent the measurement window into the ms range [85].

The coefficients describing rotational diffusion augment quickly with the size of the particle ($D_{rot} \propto 1 / R^2$) [86], therefore, the measurements are highly sensitive to the formation of multimers where aggregation results in reduced rotational correlation times. A number of channel-forming peptides have been shown to stably insert into the bilayers at transmembrane alignments (e.g. [20,87,88]). In these cases, channel opening is associated with conformational and topological changes and/or oligomer formation. Although from the oriented solid-state NMR spectra the structure and alignment of these channel peptides [20,87,88] have been calculated, the question often remains if the spectra represent the mono- or oligomeric forms of the channel [89]. We have therefore developed an approach where the ^{2}H solid-state NMR spectra of peptides labeled with methyl-deuterated alanines allow one to obtain information about the membrane rotational diffusion and thereby indirectly about the aggregation state in the membrane [90]. In contrast to many other techniques, such as e.g. fluorescence transfer energy measurements, the aggregation state can be tested by investigating the same samples that are also used for the structural analysis by merely tilting the oriented membranes by 90°. In this configuration, rotational diffusion around the membrane normal changes the molecular alignment relative to the magnetic field direction (B_o) [90]. As a consequence, the resulting NMR spectrum is a superposition of individual spectra each corresponding to a particular molecular alignment relative to B_o and a spherical powder pattern line shape is observed when rotational averaging is slow (Figs. 3B and 4B). However, in cases where rotational diffusion is fast, averaging of this powder pattern is observed (Figs. 3E and 4E) and this difference can be used to describe the rotational diffusion in a semiquantitative manner. Whereas the rotational diffusion of membrane peptides is 'frozen' in the gel-phase [90], it is sufficiently fast for averaging also below the phase transition temperature in the case of lipids and sterols [84].

Following this concept, it has been demonstrated that the ^{2}H quadrupolar line shape of the deuterated alanine methyl group is sensitive to differences in aggregation between transmembrane monomers and small oligomers (Fig. 4B, E) [90]. The technique shows, for example, that in a typical solid-state NMR sample the Influenza M2 transmembrane channel peptide occurs as a mixture between configurations exhibiting slow and fast rotational diffusion rates [89].

Notably, fast rotational diffusion also modifies the spectral appearance of non-oriented samples (e.g. Figs. 3C, F, I and 4C, F, I) and the resulting powder pattern line shapes can be used to obtain topological information about the peptides within lipid bilayers [31,91,92]. Furthermore, the dynamics in membranes has pronounced effects on the nuclear dipolar couplings and therefore the cross polarization efficiency and this has been used to qualitatively account for motions in membranes [59,93]. As the averaging of dipolar interactions depends on the alignment of the rotation axis relative to the magnetic field direction, cross-polarization efficiency drops when the dipolar couplings approach zero near the magic angle and results in distorted powder pattern line shapes [58,92,94]. Related effects also account for the difficulties of recording CP spectra of 20–30 mer in-plane aligned peptides reconstituted in oriented samples with the normal perpendicular to the magnetic field direction [90].

Finally, one needs to ask how such motions affect the analysis of oriented solid-state NMR spectra during structural studies. Figs. 3 and 4 show spectral simulations of a slightly tilted transmembrane helical peptide when reconstituted into oriented membranes at alignments of the sample normal (n) parallel (Figs. 3A, D, G and 4A, D, G) or perpendicular to the magnetic field direction (Figs. 3B, E, H and 4B, E, H) as well as of non-oriented samples (Figs. 3C, F, I and 4C, F, I). Notably, motions around the membrane normal do not affect the resonance position of the oriented line shape at sample alignments of the normal parallel B_o [31,95] (cf. Figs. 3A, D and 4A, D). Whereas in the static case, tilt angle information is directly obtained by comparing the resonance position with the static powder pattern (Figs. 3A and 4A), fast averaging of the chemical shift or quadrupolar tensor results in a symmetric powder pattern line shape where the low field shoulder corresponds to the $\delta_{||}$ tensor element (Figs. 3F and 4F). The $\delta_{||}$ element is oriented parallel to the main axis of motion which in the setting shown in Fig. 3D–F is collinear with the membrane normal

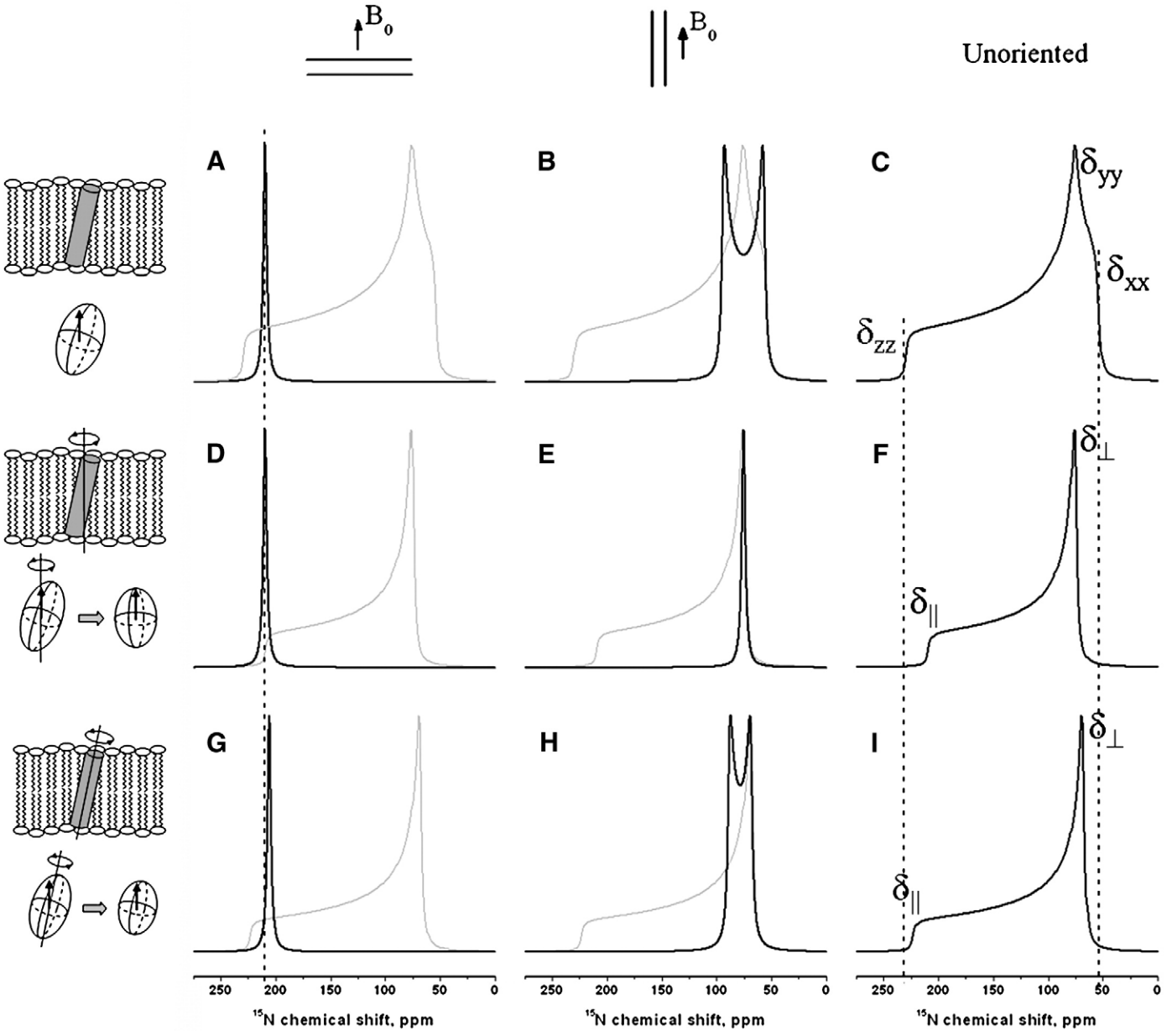

Fig. 3. Simulations of proton-decoupled ^{15}N solid-state NMR spectra of a 10° tilted transmembrane helical peptide, labeled at a single site with ^{15}N and reconstituted into membranes oriented with the bilayer normal parallel (A, D, G) or perpendicular to the external magnetic field (B, E, H). Panels C, F and I show spectra from non-oriented samples which are superimposed in grey onto the respective oriented spectra shown in the same row. The simulations account for rotation around the bilayer normal (D–F), or rotation around the helix axis (G–I) and are compared to the static case (A–C). The CSA tensor used for simulations is 54, 76 and 230 ppm with δ_{zz} covering an angle of 17° with the N–H bond ([31]). The ellipses shown to the left of the spectra illustrate the effects of averaging on the tensor where the vector represents the measured chemical shift value [31].

[31]. Therefore, by comparing the ^{15}N resonance frequency obtained from uniaxially oriented samples ($n \parallel B_o$) to the static tensor (or alternatively by comparing the averaged tensor to the static powder pattern), the tilt angle is obtained in the static case as well as in the case of rotational diffusion around the membrane normal (Fig. 3A, D). However, as discussed above, at settings with the membrane normal perpendicular to B_o, the oriented spectra are quite different when the cases of fast and slow rotational averaging around the membrane normal are compared to each other (Figs. 3B, E and 4B, E). By using this approach, averaging of the ^{15}N chemical shift anisotropy, and in some instances the alanine $^2H_3$ quadrupolar splitting, has been observed not only for transmembrane sequences such as Vpu, alamethicin or designed peptides but also for amphipathic helical peptides such as magainin or model sequences [45,89–92].

Furthermore, when the effects of motions around the helix axis are considered, the spectra shown in Figs. 3G–I and 4G–I are obtained. Here we only show simulations for the full averaging around the helix axis but motions that correspond to fluctuation movements rather than full rotation result in outcomes intermediate between the two extremes shown in Figs. 3A–C and 4A–C and Figs. 3G–I and 4G–I, respectively.

We suspect that large amplitude motions around the helix normal are most likely to occur for transmembrane peptides with a small hydrophobic moment (i.e. low amphipathity) and/or little peptide–peptide interactions [45,90,96–98]. It is likely that in the presence of motional averaging around the helix axis the peptide exhibits enough freedom to also rotate around the membrane normal, which would introduce spectral features of both regimes. In order to facilitate the analysis of such motions, we have performed simulations that reveal the resulting spectral modifications of oriented solid-state NMR spectra (Figs. 3 and 4).

When the effect of motional averaging around the helix axis is calculated for oriented samples ($n \parallel B_o$), only minor differences are

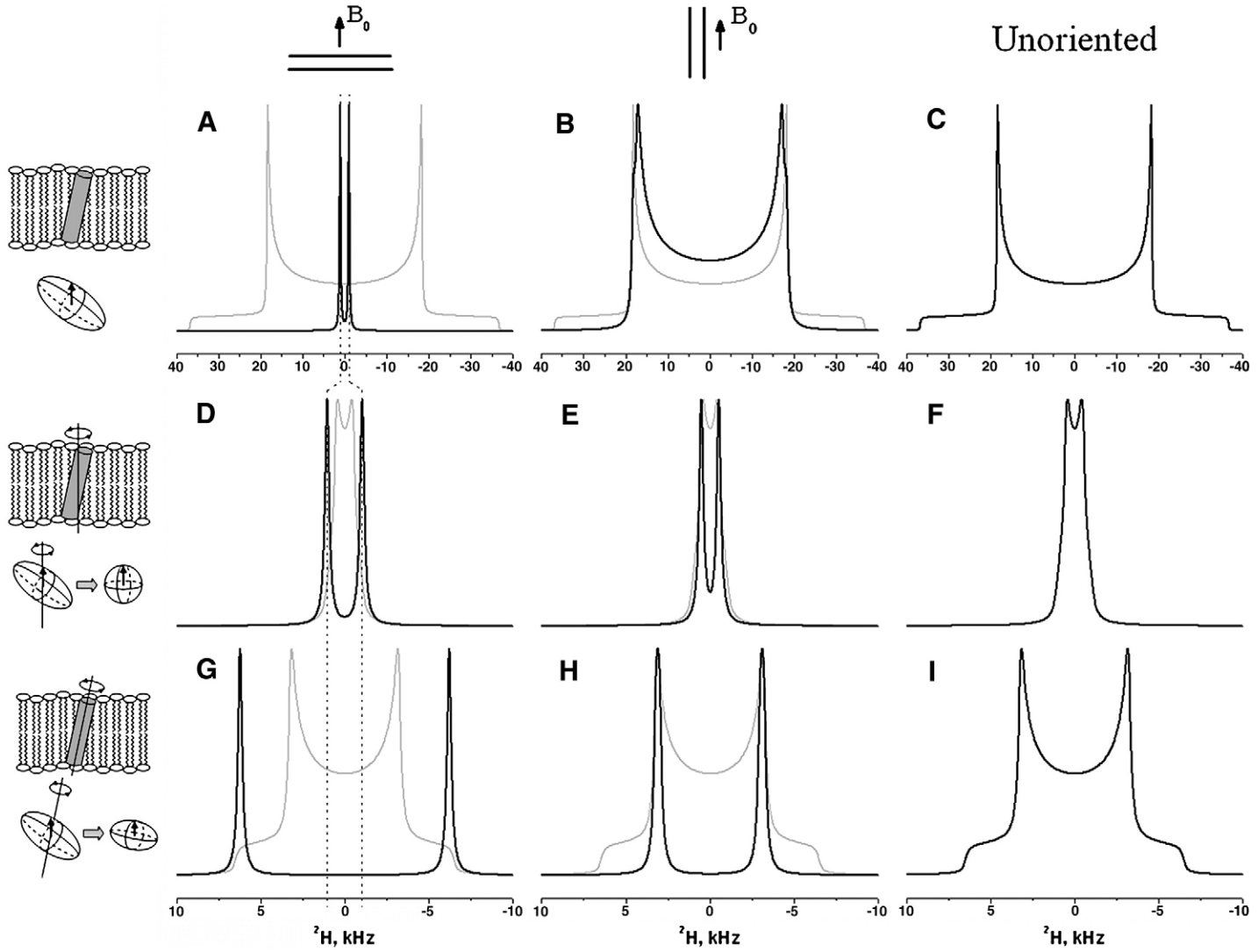

Fig. 4. Simulations of ^2H solid-state NMR spectra of a 10° tilted transmembrane helical peptide, $^2H_3$ labeled at a single alanyl methyl and reconstituted into membranes oriented with the bilayer normal parallel (A, D, G) or perpendicular to the external magnetic field (B, E, H). Panels C, F and I show spectra from non-oriented samples which are superimposed in grey onto the respective oriented spectra shown in the same row. The simulations account for rotation around the bilayer normal (D–F), or rotation around the helix axis (G–I) and are compared to the static case (A–C). The quadrupolar splitting used for simulations was 49 kHz aligned with the C_α–$C_\beta^2H_3$ bond taking into account three-site jump motions of the methyl group, librations and deviations from tetrahedral geometry [99]. Exact tensor orientations used for the simulations are $\varepsilon_{||} = 55°$ and $\varepsilon_\perp = -44°$, with $\varepsilon_{||}$ being the angle between the C_α–$C_\beta^2H_3$ bond and the peptide axis, and $\varepsilon_\perp$ the angle covered by the vector connecting the helix axis and C_α and the C_α–$C_\beta^2H_3$ bond projected onto a plane perpendicular to the helical axis. Please note that the quadrupolar splitting can be positive or negative and that a change of sign has occurred for panel G, the only splitting which is negative. The ellipses shown to the left of the spectra illustrate the effects of averaging on the tensor where the vector represents the measured quadrupolar splitting [31]. Please note the reduced scale in the case of motional averaging (D–I).

observed for the ^{15}N chemical shift positions (Fig. 3A, G) thereby reflecting the fact that the unique tensor element δ_{zz} of the amide chemical shift tensor (Fig. 3C; δ_{xx}, δ_{yy}, $\delta_{zz} = 54$ ppm, 76 ppm, 230 ppm) deviates by only a few degrees from the helix long axis [31].

In contrast, the C_α–$C^2_\beta H_3$ axis, which is also the alignment of the main tensor element of the deuterium quadrupole splitting in deutero-methyl-alanine, covers and angle of about 55° with the helix long axis [99] and a perfectly transmembrane oriented helix would result in a close to zero quadrupolar splitting. However, any movement around the helix long axis causes considerable changes in the spectral appearance of this site (cf. Fig. 4A–C with G–I). Molecular dynamics simulations indeed indicate that averaging over an ensemble of peptide orientations as it was observed for hydrophobic model peptides, results in pronounced modifications of the ^2H NMR spectra. Whereas on first view the quadrupolar splittings are suggestive of an apparently small tilt angle independent of lipid composition, i.e. even under conditions of hydrophobic mismatch, molecular dynamics simulations show considerably increased tilt angles albeit with a pronounced distribution of rotational pitch angles [97,98]. The ensemble of molecular alignments, taking into account the full distribution of tilt and rotational pitch angles observed during these simulations, allows one to back calculate the experimental deuterium NMR spectra [97,98] and the result agrees well with the tilt angle analysis from ^{15}N solid-state NMR spectra [96].

In conclusion, solid-state NMR spectroscopy works with peptides and proteins reconstituted with extended lipid bilayers in their fluid phase and has been used to investigate a number of dynamic aspects including topological equilibria, lateral and rotational diffusion as well as oligomerization. It thereby helps to establish a more complete picture of the events in fluid phase membranes than structural studies alone would provide.

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