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CONDENSED-MATTER SPECTROSCOPY SPECTRAL METHODS FOR STUDY OF THE G-PROTEIN–COUPLED RECEPTOR RHODOPSIN. II. MAGNETIC RESONANCE METHODS

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Abstract

This article continues our review of spectroscopic studies of G-protein–coupled receptors. Magnetic resonance methods including electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) provide specific structural and dynamical data for the protein in conjunction with optical methods (vibrational, electronic spectroscopy) as discussed in the accompanying article. An additional advantage is the opportunity to explore the receptor proteins in the natural membrane lipid environment. Solid-state ²H and ¹³C NMR methods yield information about the both local structure and dynamics of the cofactor bound to the protein and its light induced changes. Complementary site-directed spin labeling studies monitor the structural alterations over larger distances and correspondingly longer time scales. A multi-scale reaction mechanism describes how local changes of the retinal cofactor unlock the receptor to initiate large-scale conformational changes of rhodopsin. Activation of the G-protein–coupled receptor involves an ensemble of conformational substates within the rhodopsin manifold that characterize the dynamically active receptor.

INTRODUCTION

NMR spectroscopy is considered to be one of the most informative methods for structural studies of biological molecules. However, its application to the study of G-protein–coupled receptors is complicated because they are membrane proteins with a large molar mass. Consequently, the NMR spectra are congested with multiple peaks due to homogeneous and inhomogeneous line broadening. For detergent solutions of G-protein–coupled receptors, the large molar mass results in slow molecular motion, with correspondingly short NMR relaxation times, yielding broadening and loss of resolution of multidimensional NMR spectra. In this context, NMR studies of membrane proteins can be subdivided into two types: the first uses ¹³C and ¹⁵N uniformly-labeled proteins and examines the complete protein; the second one is based on selective isotopic substitution, thereby allowing the structural investigation at a site-specific level. As one example, the first method has been applied to investigate the three-dimensional structure of the chemokine receptor CXCR1 [1]. On the other hand, solid-state NMR studies of rhodopsin are mainly focused on the second type of investigation involving the structural dynamics of the bound cofactor [2–10], which

we will discuss here in greater detail. In addition site-directed spin-labeling methods have been applied to rhodopsin to provide reliable quantitative information on the structural changes in rhodopsin during the activation process [11]. Lately, the accuracy of the distance measurements between the spin labels has been greatly improved due to the use of the double electron-electron resonance method (DEER) [12, 13]. By combining the global large-distance restraints from spin-label EPR with the shorter distances that detect the local structure from solid-state NMR, a more comprehensive picture can be obtained. Further angular restraints from solid-state NMR can provide additional valuable refinement. Such a combined magnetic resonance approach has much to contribute to our understanding of the structure and dynamics of G-protein-coupled receptors.

NUCLEAR MAGNETIC RESONANCE

The three-dimensional NMR structure of a G-protein-coupled receptor using uniformly ^{13}C and ^{15}N -labeled samples was recently described [1]. The authors investigated the chemokine receptor (CXCR1) for interleukin-8, which is the main mediator of the immune and inflammatory responses in humans in many disorders, including the growth of tumors. Three-dimensional NMR spectroscopy with detection of the ^{13}C carbon signal has allowed the acquisition of well-resolved spectra of the receptor without modifying its amino acid sequence, in a natural environment (phospholipid membranes) at physiological temperature and pH. In future work, one can assume that this method will be applied to determine the NMR structures of other G-protein-coupled receptors, as well as additional membrane proteins. The obtained CXCR1 structure should also facilitate molecular modeling of the receptor, in order to understand its interactions with low-molecular weight agonists and antagonists.

Using the second approach, Brown with co-authors have introduced site-directed ^2H -labeling of rhodopsin ligand (retinal) to study the structure and dynamics of the chromophore in various states of the receptor [8, 9]. Rhodopsin was regenerated with retinal containing selectively ^2H -labeled methyl groups at positions C18, C19, and C20 (in the literature sometimes a different notation is used: C5-Me, C9-Me, and C13-Me, respectively) [8–10, 14]. The receptor was recombined with phospholipid membranes, which were then aligned on ultrathin glass slides, and solid-state ^2H NMR spectra were measured (Fig. 1A). From ^2H NMR spectra of oriented samples, it is possible to calculate the orientation of the methyl groups relative to the principal long axis of rhodopsin. Theoretical calculations of ^2H NMR line shapes were performed using the following as fitting parameters: bond orientation of the $\text{C}-\text{C}^2\text{H}_3$ methyl group with respect to the long axis of rhodopsin (which is assumed to be parallel to the membrane normal), the width of the distribution of the local membrane normals with respect to the average membrane normal (director of the sample), the residual quadrupolar couplings of the deuterium nuclei (which are partially averaged due to rotation and reorientation of methyl groups), and the line broadening due to other interactions (dipolar, chemical shift anisotropy). A Gaussian distribution was used to characterize the deviation of the local membrane normal from the director due to imperfect membrane alignment on the glass slides.

The NMR structure of the retinal cofactor was calculated in the framework of the three-plane model (Fig. 1B,C), in which all the covalent bonds between carbon atoms (except C1–C16 and C1–C17) lie in three planes (A, B, and C), and only rotation around C6–C7 and C12–C13 bonds was allowed. Note that for the dark state, such a simplified model (Fig. 1B) failed to fit into the ligand-binding pocket of rhodopsin [9] without numerous steric clashes. Only the use of an extended model, where rotation of the polyene chain around the C11=C12 bond was not restricted (Fig. 1C), allowed us to dock the retinal into the binding pocket without steric clashes. Modeling of the retinal molecule using only the experimentally obtained methyl group orientations gives multiple possible conformations of the ligand. For example, with a fixed orientation of the B plane (Fig. 1B), a total of four orientations of the A plane are possible (which correspond to four possible C5=C6–C7=C8 torsion angles) for the same value of the bond angle between the C5-Me methyl group and the long axis of rhodopsin. The same is true for the orientation of plane C. Additional structural parameters are required to determine a unique configuration and orientation of the chromophore. Here, the distances between carbon atoms in the ligand molecule can be used, which are derived from the ^{13}C NMR rotational-resonance spectra [2, 3]. As a result of the analysis of the ^2H NMR spectra and the subsequent modeling, the structures of the retinal in dark state of rhodopsin [8, 9] (Fig. 1C) and metarhodopsin-I [9, 15] were determined. It turned out that the retinal molecule is essentially twisted in the dark state about its C11=C12 double bond in the direction of the photoisomerization (Fig. 1B). In the Meta-I state, the part of the polyene chain with a C13-methyl group rotates around the C11=C12 double bond towards the extracellular side of the membrane (Fig. 1B,C). As a result the polyene chain is straightened, and the β -ionone ring is displaced along the axis of the retinal in the direction of helix H5. In addition, a steric clash of the retinal with Trp265 may occur as a consequence of the polyene chain straightening, which is assumed to play an important role in the process of activation [9].

In the studies of de Groot et al. [2] (Fig. 2) and Watts et al. [3, 16] interatomic distances between pairs of selectively labeled ^{13}C carbons in retinal were measured using 1-D rotational resonance ^{13}C magic angle spinning (MAS) NMR spectroscopy, in the dark state and the Meta-I state of rhodopsin. In this method, additional fine structure or broadening of the ^{13}C NMR spectra is observed when the sample spinning frequency matches the difference of resonance frequencies of two ^{13}C nuclei. With appropriate conditions (favorable properties of the transverse relaxation and inhomogeneous line widths), the fine structure due to the rotational resonance effect can be resolved, where e.g. the resonance splitting ω_1 (Fig. 2) can be detected by taking the second derivative of the spectrum. Distance restraints obtained from the ^{13}C MAS NMR spectra, together with the orientational restraints for the ^2H -labeled methyl groups allow one to determine the structure of the ligand [9], similar to the case of solution NMR. In addition, Feng et al. have proposed a method for the direct determination of H–C–C–H torsion angle in retinal bound to rhodopsin using double-quantum heteronuclear local field NMR spectroscopy, where the ligand is ^{13}C -labeled at two adjacent positions (e.g. C10 and C11) in the polyene chain [4]. The method was used to determine H–C10–C11–H torsion angles of the chromophore in rhodopsin and metarhodopsin-I, giving values of $160 \pm 10^\circ$ and $180 \pm 25^\circ$, respectively [4]. The results are

consistent with models of the chromophore assuming a twisted conformation in the dark state and relaxed configuration after isomerization in the Meta-I state.

Correspondingly Kiihne et al. demonstrated a method of heteronuclear correlation NMR spectroscopy for the selective observation of intermolecular 3–7 Å contacts between the isotopically ^{13}C -labeled and unlabeled moieties of the large receptor proteins [5]. The method was applied to the unlabeled rhodopsin containing uniformly ^{13}C -labeled retinal. A number of $^1\text{H}_{\text{GPCR}}\text{--}^{13}\text{C}_{\text{ligand}}$ correlations of the carbon atoms of the ligand with amino acid residues were revealed and identified, for example, $^1\text{H}_{\text{GPCR}}\text{--}^{13}\text{C}_{\text{ligand}}$ correlation of C8 atom with an aromatic amino acid residue (identified as Trp 265) and many others were observed. The method can be useful for displaying contacts in the ligand-binding sites of membrane proteins, even for the small amount of unlabeled protein. Notably, such information can be crucial in structure-based drug design.

Using the method of ^{13}C two-dimensional dipolar-assisted rotational resonance (2D DARR), Smith et al. have evaluated the change of interatomic distances between the selectively ^{13}C -labeled retinal and a number of selectively ^{13}C -labeled amino acid residues in the ligand-binding pocket of the rhodopsin upon activation [6, 7, 17, 18]. This method allows determination of close contacts between the ^{13}C atoms in the protein. If the distance between the labeled atoms is 5 Å or less, the cross-peak in the 2D DARR ^{13}C NMR spectrum can be observed; at larger distances, the cross-peak vanishes. Thus, the changes in the position and configuration of the ligand in the binding pocket during the protein activation can be examined (Fig. 3). The main result of these studies is the displacement of the β -ionone ring of retinal in the direction of the helix 5 in the activation process. Initially, the possible value for the displacement of the ring was reported to be about 4–5 Å [6, 17]; in subsequent work, the value was reduced to 1.5 Å [7] as later confirmed by X-ray results [19–24]. However, rotation of the retinal around its long axis was not detected, in contrast to some X-ray structures. Consequently, the unambiguous structure and orientation of retinal in the active state remain to be established. It was also suggested [18] that displacement of the extracellular loop E2 connecting helix H4 and H5 away from the retinal binding site occurs upon activation (Fig. 3E). Because this displacement was not subsequently confirmed by X-ray data [23], it is assumed that it may be transitional.

Moreover, solid-state NMR relaxation is an extremely useful tool to study G-protein-coupled receptors, as it allows one to study molecular motions in the protein, as well as intramolecular interactions inaccessible to other methods. In Ref. [10, 14] the dynamics of methyl groups of the retinal cofactor in the dark, Meta-I, and Meta-II states of rhodopsin were investigated by ^2H NMR relaxation methods. Figure 4 shows the temperature dependences of ^2H NMR relaxation times of the Zeeman (T_{1Z}) and quadrupolar (T_{1Q}) orders for the selectively deuterated C5-, C9-, and C13-methyl groups of retinal. Solid-state ^2H NMR relaxation data can be interpreted either in the framework of our proposed generalized model-free (GMF) approach, that allows to calculate the spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ of the molecular fluctuations at the Larmor precession frequency of the nuclei ω_0 and at the double the Larmor frequency $2\omega_0$, respectively [14], or using models of the molecular motion. The theoretical T_{1Z} and T_{1Q} temperature dependences in Figure 4A were calculated for a model of the continuous rotational diffusion of the methyl groups, with

coefficients $D_{\parallel}$ (characterizing the rotation of the methyl group around the axis of symmetry) and $D_{\perp}$ (characterizing the reorientation of the methyl group relative to the average orientation). Alternatively a model of random rotational jumps between the minima of energy around the 3-fold symmetry axis with the jump rate constant k was considered. The activation energies for rotational motion of the methyl groups in various states of rhodopsin, obtained from temperature dependences of the relaxation times, were interpreted in terms of molecular interactions. Significant changes in the activation energies of rotational diffusion of the C9- and C13-methyl groups are observed by ^2H NMR relaxation in the transition from the dark state to the Meta-I and then to the Meta-II state [10, 14]. The differences are most likely due to ligand isomerization that leads to the changes of the interactions of methyl groups with the closest hydrogen atoms in the retinal. On the other hand, the activation energy of rotational diffusion of the C5-methyl group increases in Meta-I, and then decreases in the Meta-II state to a value smaller than in dark state. This could be the result of a more tightly packed environment of this methyl group in the pre-active state, due to the shift of the retinal β -ionone ring closer to helix 5, and interaction of the C5-methyl group with the side chain of the Glu122.

In addition, it is noteworthy that the NMR lineshape depends significantly on the molecular motions of the proteins. Information on the molecular dynamics can be obtained directly from the NMR spectra, in addition to the NMR relaxation times. For example, the rotation of the deuterated retinylidene methyl groups about their symmetry axes leads to a significant (by a factor of three) reduction of the residual quadrupolar coupling compared to an immobile C– ^2H bond, and to a corresponding narrowing of NMR lines. An additional reduction of the quadrupolar coupling constant by about 10 % compared to the spinning methyl group is observed in all three states (dark, Meta-I, and Meta-II) of rhodopsin, indicating a rapid reorientation of the methyl groups with respect to the average orientation within the range of $\pm 15^\circ$ [9]. Another example of the application of the NMR spectral lineshape to study molecular motions includes a combination of NMR spectroscopy with molecular dynamics (MD) simulations. Nygaard et.al [25] studied the structure and conformational dynamics of the β_2 -adrenergic receptor with ^{13}C -labeled methyl group of methionine using heteronuclear single-quantum coherence spectroscopy (HSQC). The MD simulations were used to provide a structural basis for interpretation of the NMR spectra from the view point of conformational transitions. The studies indicated conformational heterogeneity in both the agonist and inverse-agonist-bound receptor.

ELECTRON PARAMAGNETIC RESONANCE

The method of electron paramagnetic (spin) resonance (EPR) is based on the resonance absorption of electromagnetic radiation by atoms (or molecules) with unpaired electrons placed in a constant magnetic field [26]. The EPR method has been used to study photoinduced changes in the hydrophilic region of rhodopsin [27] and the interaction of rhodopsin with cytoplasmic proteins (transducin and arrestin) in the photoreceptor cell [28]. The application of site-directed spin labeling (SDSL) with cysteines used for spin labels [11, 29], showed displacement of the transmembrane helices of the protein in the activation process [11]. Six rhodopsin mutants each containing two reactive cysteines were prepared. Natural cysteines at positions 140, 316, 322 and 323 were replaced by serines. In each

mutant, the position of one cysteine (139 on helix 3) was kept constant, and the second one (on helix 6) was varied from 247 to 252. After photoactivation of rhodopsin, spectral narrowing of the EPR lines was observed for most samples, except 139–249 (where the changes were minor) and 139–250 (in this case the linewidth has increased). The analysis of the experimental results indicated changes in the interspin distances after activation that were interpreted as a rigid body rotation of the helix 6 with respect to helix 3, with the displacement of the cytoplasmic end of helix 6 away from the helix 3. This conclusion has been substantiated by recent X-ray data for activated rhodopsin [22–24].

The application of time-resolved EPR spectroscopy provides additional options for the study of the kinetics of molecular transformations in proteins. Knierim et al. [30], applying this technique together with flash photolysis measurements, established a temporal sequence of events in the activation process of rhodopsin. It was shown that the deprotonation of the Schiff base that binds the retinal to the protein, and the movement of the helix 6 are not synchronized events; the latter is an order of magnitude slower at 30 °C. On the other hand, the motion of the helix 6 and the proton uptake by Glu134 (a key event stabilizing the active state of the protein) occur at neutral pH within the same time frame. However, as indicated by pH-titration studies the proton uptake is a consequence rather than a cause of the helix motion.

Recently the method of double electron-electron resonance (DEER) [12, 13] has been applied by Hubbell et al. in studies of biological molecules. The method allows one to measure distances between pairs of spin labels up to 6 nm [13], which makes it possible to use label sites on the outer surface of the protein to minimize perturbation of the protein structure due to the spin label, or to the change of the configuration of the label itself when the protein is activated. As a result, it becomes possible to measure distances between two labels with an error of less than 0.1 nm. This method was first applied by Hubbell et al. [13] to study helical movements in rhodopsin during activation, and in particular, to establish the rotation of the cytoplasmic end of helix 6 away from the rest of the helical bundle (the analysis indicated displacement of 0.5 nm). This result was confirmed subsequently by X-ray crystallography of rhodopsin in the active state [22–24], providing an important validation of the SDSL technique.

ACTIVATION MECHANISM OF RHODOPSIN

Currently available data from applying biophysical and biochemical techniques have allowed one to draw important conclusions about the activation mechanism of visual rhodopsin. In the first article [31] we have discussed the reaction scheme obtained from UV-visible absorption and FTIR spectroscopy [32] which is also supported by the results of site-directed spin-labeling [30]. A more detailed picture is the following (Fig. 3E). It is proposed [9, 14] that after photoisomerization, the part of the retinal polyene chain near the Schiff base including the C13-Me methyl group rotates around long axis of the ligand. This leads to destabilization of the so-called ionic lock formed by the protonated Schiff base and its counterion (Glu 113), which stabilizes the receptor in an inactive dark conformation. The result is the deprotonation of the Schiff base. Additionally, X-ray analysis revealed the rearrangement of hydrogen bonds between Glu 122 on helix 3 and His 211 on helix 5, which

apparently is due to the displacement of β -ionone ring of the chromophore towards helix 5 after isomerization to all-*trans* retinal. The rearrangement of hydrogen bonding leads to the rotation of the cytoplasmic end of helix 5 in the direction of helix 6. The most significant restructuring of the hydrogen bonding network occurs, however, in the region of the second ionic lock stabilizing the dark state of the receptor that is formed by Glu134 and Arg135 on helix 3, and Glu247 and Thr251 on helix 6. As a result of the rearrangement of the hydrogen bonding network, the cytoplasmic end of the helix 6 moves away from the rest of the bundle of helices forming the binding site for transducin. The active conformation of rhodopsin is stabilized by a new network of hydrogen bonds between Glu247-Lys231-Thr251 and Tyr223-Arg135. Additionally, stabilization of the active Meta-II state occurs due to the protonation of Glu134. Currently, it remains to be established how structural changes in the ligand-binding pocket of rhodopsin lead to destabilization of the second ionic lock located at a considerable distance from retinal. One of the possible reasons is the approach of Tyr223 to Arg135 as a consequence of rotation of the cytoplasmic end of helix 5 towards helix 6. Alternatively, it can be a result of the increased amplitude of thermal fluctuations of the helices due to the rearrangement of hydrogen bonding network around retinal, and deprotonation of Schiff base after isomerization of the ligand, i.e. due to the presence of an ensemble of multiple active and inactive sub-states.

Clearly there are many questions still needing to be resolved to fully understand the mechanism of activation of rhodopsin as it occurs in a natural membrane lipid environment. One of the most important problems is whether the reorientation of the retinal along its axis occurs in rhodopsin binding pocket, and if it does, then when in the sequence of conformational changes of the protein does it occur? Does it happen in the Meta-I or in the Meta-II state? In this respect, the studies of modified rhodopsin (including modified retinals) and molecular modeling are particularly important, which on the one hand allow us to establish the contribution of specific molecular groups and interactions in the activation process, and on the other hand to show the kinetics of the transition from the inactive conformation of the receptor to the active state.

CONCLUSION

Spectral methods are indispensable tools to study the structure and dynamics of membrane proteins, including rhodopsin, whose activation mechanism remains incompletely understood. They allow one to study proteins in a natural membrane environment, thus providing information about the interactions of specific molecular groups in the temporal sequence of conformational changes. Even though the first X-ray structures for the active state of the receptor have only recently become available, many important details of the activation mechanism remain uncertain. For example, the structure of rhodopsin in the pre-active Meta-I state is currently unknown. Moreover, it is unclear how the conformational changes of the protein-bound ligand lead to the activating helical movements, or what is the role of equilibrium between the active Meta-II and inactive Meta-I states. Nevertheless, the last few years have witnessed striking improvements in experimental methods for the study of G-protein-coupled receptors. Especially the development of modern NMR techniques has enabled one to obtain the full structure of such proteins in a membrane environment [1]. Improved crystallization methods for X-ray analysis, development of numerical simulation

methods, and an integrated approach using a combination of methods suggests that important missing gaps in our knowledge of the activation mechanism of rhodopsin will be filled in the near future.

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Fig. 1.

(A) Temperature dependence of the experimental (solid lines) and theoretical (dotted line) solid-state ^2H NMR spectra of selectively ^2H -labeled C5-, C9-, and C13-methyl groups of the retinylidene chromophore bound to rhodopsin in the dark state [15]. Rhodopsin was recombined with POPC lipid membranes, which were then aligned on the glass slides. The central narrow peak observed at $T = -30^\circ\text{C}$ is due to the natural abundance of deuterium in water. (B) The carbon numbering scheme and retinal structures in dark state of rhodopsin obtained from ^2H NMR spectra for the simplified three-plane model [8]. (C) The extended (with rotation around the double bond C11=C12) three-plane model is adopted for analysis of the retinal conformation and orientation within the rhodopsin binding pocket [9, 15]. Figure is taken from Ref. [15].

Fig. 2.

Solid-state ^{13}C NMR spectra of the vinyl region obtained with cross-polarization and magic angle spinning for rhodopsin regenerated with retinal selectively ^{13}C -labeled at (A) positions C10 and C20, and (B) C11 and C20 [2]. The spectra were measured for the rotational resonance condition $n = 1$, with the rotation speed 11794 ± 3 kHz and 12592 ± 3 kHz, respectively at a resonance frequency of 100.6 MHz. Right panels show the second derivative of the signal for (C) 11-Z-[10,20- $^{13}\text{C}_2$]- and (D) 11-Z-[11,20- $^{13}\text{C}_2$]-retinylidene rhodopsin in the vinyl region. Figure is adapted from Ref. [2].

Fig. 3. Dipolar-assisted rotational-resonance (DARR) solid-state ^{13}C NMR reveals contacts of retinal $^{13}\text{C}16$ and $^{13}\text{C}17$ with phenylalanine, histidine, and methionine residues and changes in the structure of rhodopsin upon light activation. (A) Difference ^{13}C NMR spectrum of rhodopsin minus Meta-II showing the region of the $^{13}\text{C}16$ and $^{13}\text{C}17$ resonances of the retinal. (B) Section taken from the 2D DARR ^{13}C NMR spectra of rhodopsin containing ^{13}C -ring-labeled phenylalanine. The section passes through the diagonal of the ^{13}C resonances of the phenylalanine ring, showing only the area of the cross-peaks of the retinal $^{13}\text{C}16$ and $^{13}\text{C}17$ resonances. (C) Similar spectrum in the same region as in (B) in the Meta-II state. (D, E) Representative 2D ^{13}C DARR NMR spectra for the dark (black) and Meta-II (gray) states of rhodopsin ^{13}C -labeled at position C1 of (D) histidine and (E) methionine and regenerated with 11-Z-[16,17- $^{13}\text{C}_2$]-retinal. (F) Structure of rhodopsin in dark state (Protein Data Bank code 1U19). The most significant changes of the configuration of the ligand, the side chains of amino acid residues, and the helices of the protein are indicated by arrows. Figure is adapted from Ref. [7].

Fig. 4.

(A) The temperature dependences of the ^2H NMR relaxation times of Zeeman (T_{1Z} , filled symbols) and quadrupolar (T_{1Q} , empty symbols) order of selectively deuterated C5-, C9- and C13-methyl groups of retinal chromophore in the dark state. Theoretical dependences are shown in solid (T_{1Z}) and dashed (T_{1Q}) lines and were calculated for a model of continuous rotational diffusion with coefficients $D_{\parallel}$ and $D_{\perp}$ (for the case of $D_{\perp} = 0$), and for a model of random rotational jumps about the three-fold symmetry axis with jump rate k between the energy minima. (B) Schematic representation of rhodopsin with bound retinal in the dark state and rotational transformations characterized by the Euler angles $\Omega_{ij}(t)$ used in theoretical analysis of molecular mobility of deuterated methyl groups. The coordinate systems describe fluctuating orientations of the tensor of the quadrupolar interactions of the deuterium nuclei. Here PAS denotes the principal axis system of the tensor of the electric field gradient for the deuterium nucleus (z -axis is parallel to the C–D bond), M is the coordinate system associated with a methyl group (z -axis is parallel to the axis of symmetry and C–CD₃ bond), D is the coordinate system associated with the membrane (z -axis is parallel to the membrane normal $\mathbf{n}_0$), and finally L is laboratory coordinate system (z -axis is parallel to the magnetic field of the NMR spectrometer $\mathbf{B}_0$). Figure is adapted from Ref. [14].