ORIENTATIONAL ANISOTROPY OF NUCLEAR SPIN RELAXATION IN PHOSPHOLIPID MEMBRANES

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The observation that the spin–lattice relaxation (R_{1Z}) rates of pure phospholipid lamellar phases depend only weakly on their orientation in the liquid-crystalline state is explained. A relaxation model in which either segmental or molecular motions are described by anisotropic rotational diffusion in an ordering potential (M.F. Brown, J. Chem. Phys. 77 (1982) 1576) can account for the available ^{2}H R_{1Z} data to within experimental error. One possibility is that rotational isomerization breaks the symmetry of the static electric field gradient, leading to an asymmetric residual tensor which is further modulated by molecular motions.

The analysis of the spin–lattice relaxation rates (R_{1Z}) of lamellar phases of phospholipids [1–4] can provide an important experimental avenue towards understanding their molecular dynamics. One variable reflecting specific motional aspects is the dependence of R_{1Z} on the angle between the director axis (the normal to the lamellar surface) and the static magnetic field [4]. However, for pure phospholipids in the liquid-crystalline state, the anisotropy has remained something of an enigma in that only a weak or negligible angular dependence is found [1–3]. At present the anisotropy of R_{1Z} for phospholipid lamellar phases has not been satisfactorily explained.

In the case of randomly oriented, multilamellar dispersions of phospholipids in the L_α phase, powder type ^{2}H NMR spectra are obtained with little variation of R_{1Z} across the lineshape [2]. Although this result is consistent with a small angular dependence, Brown and Davis [2] have shown that orientational averaging of R_{1Z} can occur by translational diffusion of phospholipids about the curved surfaces of the lamellae. The influences of such translationally induced rotations can be largely circumvented by macroscopic alignment of bilayers on glass substrates. Brown [1] first reported that a significant angular anisotropy could not be detected in ^{2}H R_{1Z} measurements of oriented bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) with specifically deuterated acyl chains. This observation has now been substantiated by recent careful measurements of Jarrell, Siminovitch, and colleagues [3]. They have pointed out that the lack of a detectable anisotropy of R_{1Z} for liquid-crystalline phospholipids is inconsistent with a continuum model in which the relaxation is described by fluctuations of a local director; that is rotations of a single principal value of the coupling tensor about axes orthogonal to the average director [4]. It seems that specific molecular features need to be considered to explain the relaxation. One such model involves anisotropic rotational diffusion within the ordering potential of the membrane (cf. ref. [4]). Here the *rate* of the motions is described by the principal values of the rotational diffusion tensor, the *amplitude* by the order parameter for off-axial rotations, and the *angular anisotropy* is related to the principal values and orientation of the interaction tensor within the molecular frame [4]. The

present Letter shows that such a model can account for the angular dependence of the ^2H R_{1Z} values of phospholipid bilayers in the L_α phase to within experimental error.

The ^2H spin–lattice relaxation rate due to fluctuations in the nuclear quadrupolar interaction is given by [5,6]

$$R_{1Z}=\frac{3}{16}\left(\frac{e^2qQ}{\hbar}\right)^2 [J_1(\omega_0)+4J_2(2\omega_0)] \tag{1}$$

in which $e^2qQ/\hbar \equiv 2\pi\chi$, where χ is the quadrupole coupling constant, and ω_0 is the Larmor frequency. Unless stated otherwise all symbols have their usual meaning. In eq. (1) it is assumed that

$$J_M(\omega)=J_M^{(\delta)}(\omega)+J_M^{(\eta)}(\omega)+J_M^{(x)}(\omega)\,, \tag{2}$$

where $M=1$, 2 and ω $(=\omega_0, 2\omega_0)$ is a generic frequency variable. The symbol δ refers to the V_{zz} principal component of the electrostatic field gradient (EFG) tensor $\mathbf{V}$ $(\delta \equiv V_{zz}=eq)$, η is the asymmetry parameter $(\eta \equiv (V_{yy}-V_{xx})/V_{zz})$, and x denotes cross-terms involving the coupling parameters δ and η. For the case of isotropic motion, it can be shown that the latter vanish owing to the different transformation properties of the irreducible components of the EFG tensor under rotations, i.e. due to orthogonality of the Wigner rotation matrices on a unit sphere [7]. In terms of the reduced Wigner rotation matrix elements which transform the EFG tensor, expressed in a spherical basis, directly to the coordinate system of the static magnetic field B_0 (cf. ref. [6]), it follows that

$$J_M^{(\delta)}(\omega)=\langle d_{0M}^{(2)}[B(t)]^2\rangle j(\omega) \tag{3}$$

and

$$J_M^{(\eta)}(\omega)=\tfrac{1}{3}\eta^2\langle d_{2M}^{(2)}[B(t)]^2\rangle j(\omega)\,. \tag{4}$$

Here the angular brackets indicate time or ensemble averages, $B(t)$ is the time-dependent Euler angle between the V_{zz} and B_0 axes, and $d_{M'M}^{(2)}[B(t)]$ denotes elements of the reduced Wigner rotation matrix [7]. In isotropic media $\langle d_{0M}^{(2)}[B(t)]^2\rangle = \langle d_{2M}^{(2)}[B(t)]^2\rangle = \frac{1}{5}$ and the well-known result (p. 314 of ref. [5]) is obtained, where $j(\omega)=2\tau_c/(1+\omega^2\tau_c^2)$ and $\tau_c = 1/6D$ is the correlation time for isotropic reorientation with rotational diffusion constant D.

One can use the closure property of the group of rotations R_3 to decompose the matrix elements appearing in eqs. (2)–(4) into sums over various rotations, which are assumed to be statistically independent (cf. section III of ref. [4]). The problem of restricted motion, in which the irreducible EFG components are not averaged over all space, is somewhat more complicated than above, but can be simplified within certain approximations. For a model comprising anisotropic rotational diffusion in an orienting potential (cf. eqs. (2.5) and (3.5) of ref. [4]), one obtains that

$$J_M(\omega)=\sum_Q\sum_N |D_{0Q}^{(2)}(\Omega)-6^{-1/2}\eta[D_{-2Q}^{(2)}(\Omega)+D_{2Q}^{(2)}(\Omega)]|^2$$

$$\times\{\langle |D_{QN}^{(2)}[\Omega_0(t)]|^2\rangle - |\langle D_{QN}^{(2)}[\Omega_0(t)]\rangle|^2\delta_{Q0}\delta_{N0}\}|D_{NM}^{(2)}(\Omega_1')|^2 j_Q(\omega)\,. \tag{5}$$

The Euler angles $\Omega \equiv (\alpha, \beta, \gamma)$ refer to the fixed transformation [7] of the irreducible EFG components from their principal axis system to that of the diffusion tensor, $\Omega_0(t)$ to the time-dependent transformation to the director frame, and finally Ω_1' to transformation to the frame of the static magnetic field. Rotational symmetry about both the molecular and director axes is assumed, so that cross-terms involving rotation matrix elements with different values of the indices Q and N vanish; however cross-terms associated with the coupling parameters δ and η remain (vide supra). Neglecting the latter for simplicity and employing the properties of the rotation matrix elements [7] then leads to the following expression:

$$J_M(\omega)=\sum_Q\sum_N[d^{(2)}_{0Q}(\beta)^2+\tfrac{1}{3}\eta^2 d^{(2)}_{2Q}(\beta)^2]\{\langle d^{(2)}_{QN}[\beta_0(t)]^2\rangle-\langle d^{(2)}_{QN}[\beta_0(t)]\rangle^2\delta_{Q0}\delta_{N0}\}d^{(2)}_{NM}(\beta'_1)^2 j_Q(\omega)\,. \tag{6}$$

Eq. (6) extends previous results in closed form [4] to the case of a non-axially symmetric EFG tensor ($\eta\neq 0$). Here the Euler angle between the V_{zz} component of the EFG tensor and the diffusion tensor z-axis is denoted β, the time-dependent angle between the latter and the director axis (the lamellar normal) is $\beta_0(t)$, and β'_1 indicates the angle between the director and the static magnetic field. Eqs. (5) and (6) comprise three Lorenztians ($Q=0,\ \pm1,\ \pm2$), with spectral densities given by

$$j_Q(\omega)\equiv 2\tau_Q/(1+\omega^2\tau_Q^2)\,. \tag{7}$$

The correlation times τ_Q are related to the principal values $D_\parallel$ and $D_\perp$ of the rotational diffusion tensor in terms of

$$1/\tau_Q=6D_\perp+(D_\parallel-D_\perp)Q^2\,. \tag{8}$$

For simplicity, the diffusion tensor is presumed to be axially asymmetric, with an anisotropy parameter $\eta_D\equiv D_\parallel/D_\perp$, and a symmetric top approximation [4] is made (cf. p. 417 of ref. [8]).

The time dependence of the molecular motions in eq. (6) is contained entirely in the transformation from the principal axis system of the rotational diffusion tensor to the director. Owing to the cylindrical symmetry about the director axis, the term $\langle d^{(2)}_{00}[\beta_0(t)]\rangle^2$ is subtracted to account for the part of the interaction that is not modulated by the fluctuations [1,4,9,10]. The mean square values of the rotation matrix elements fall between the limits of isotropic motion and complete ordering, and are most conveniently evaluated in terms of their Clebsch–Gordan series expansions [7]. Since the bilayer is symmetric upon reflection through its midplane ($D_{\infty h}$ symmetry), only even ($j=0,\ 2,\ 4$) terms are included, in which case

$$d^{(2)}_{M'M}(\beta)^2=(-1)^{M-M'}\sum_{j\,\mathrm{even}}(2j+1)\begin{pmatrix}2 & 2 & j\\ M' & -M' & 0\end{pmatrix}\begin{pmatrix}2 & 2 & j\\ M & -M & 0\end{pmatrix}d^{(j)}_{00}(\beta)\,. \tag{9}$$

Here β is now a generic Euler angle [7], j the angular momentum, and

$$\begin{pmatrix}j_1 & j_2 & j_3\\ M_1 & M_2 & M_3\end{pmatrix} \tag{10}$$

denotes a Wigner 3-j symbol. Explicit formulae in terms of the order parameters $\langle P_2\rangle\equiv\langle d^{(2)}_{00}[\beta_0(t)]\rangle$ and $\langle P_4\rangle\equiv\langle d^{(4)}_{00}[\beta_0(t)]\rangle$ are given by eqs. (3.7) of ref. [4].

Some illustrative plots depicting the influences of three of the parameters of eqs. (1) and (6) are provided in fig. 1. Here we are interested primarily in R_{1Z} as a function of the angle β'_1 between the normal to the lamellar surface and the magnetic field direction. As can be seen, the behavior is fairly rich. Given that the EFG tensor is axially symmetric ($\eta=0$) and that $\beta=90°$, part (a) of fig. 1 shows normalized plots of the relaxation rate R_{1Z}, calculated using eqs. (1) and (6), versus β'_1 in which the diffusion tensor anisotropy parameter $\eta_D\equiv D_\parallel/D_\perp$ is varied. It is noteworthy that a reversal of the orientational anisotropy is found as η_D is increased; for $\eta_D=1$ it is observed that $R_{1Z}(0°)>R_{1Z}(54.7°)>R_{1Z}(90°)$, whereas the opposite is seen for $\eta_D=50$. Moreover, little or no anisotropy of R_{1Z} can occur as illustrated for $\eta_D=10$ in part (a) of fig. 1. Part (b) shows similar normalized plots of R_{1Z} as a function of β'_1 for an axially symmetric EFG tensor ($\eta=0$), in which $\eta_D=1$ and now the fixed angle β between the principal symmetry axes of the **V** and **D** tensors is varied. A reversal of the orientational anisotropy is also observed as β is varied from 0° to 90°, and a small angular dependence of R_{1Z} is possible as depicted for $\beta=45°$. The case of $\beta=0°$ corresponds to motion of a local unique axis, keeping all Wigner rotation matrix elements in the transformation ($N=0,\ \pm1,\ \pm2$), and is analogous to an order–director fluctuation model [3,4] in the limit of small angular displacements. For the case of a *non*-axially symmetric EFG tensor, part (c) of fig. 1 depicts normalized plots of R_{1Z} versus β'_1 in which $\eta_D=1$, and the angle β describing the orientation of the V_{zz} principal component is held constant ($\beta=0°$). The effect of increasing

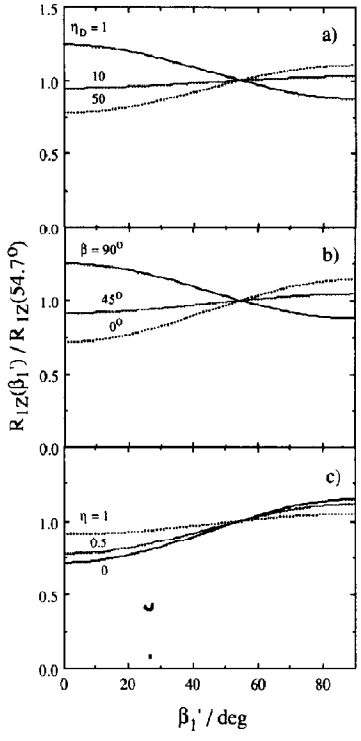

Fig. 1. Theoretical plots of the ^2H spin–lattice relaxation rate, R_{1Z}, as a function of the angle β_1' between the director axis and the main magnetic field. In each case, R_{1Z} has been normalized by dividing by the corresponding value at the magic angle (54.736°). In (a), it is assumed that the electrostatic field gradient (EFG) tensor **V** is axially symmetric ($\eta=0$) and that $\beta=90°$, where β is the angle between the EFG symmetry axis and the z-axis of the rotational diffusion tensor **D**. R_{1Z} is depicted as a function of the director orientation for a series of different values of η_D, the diffusion tensor anisotropy parameter. A significant dependence on orientation is found, which varies as a function of the anisotropy parameter η_D. In (b) an axially symmetric EFG tensor is assumed ($\eta=0$) with an isotropic diffusion tensor ($\eta_D=1$), and the angular dependence of R_{1Z} is illustrated for different values of β. The orientation dependence varies with the angle β between the z-axes of the **V** and **D** tensors. (c) shows the influence of the asymmetry parameter η of the EFG tensor for the case of $\eta_D=1$ and $\beta=0°$, i.e. the z-axes of the **V** and **D** tensors are coincident. The influence of increasing η is to reduce the magnitude of the orientational anisotropy. In each of the plots the additional parameters are held fixed at the following values: $\langle P_2\rangle=\langle P_4\rangle=0.4$; $\tau_0=1\times10^{-10}$ s; $\chi=170$ kHz; and $\nu_0=30.7$ MHz.

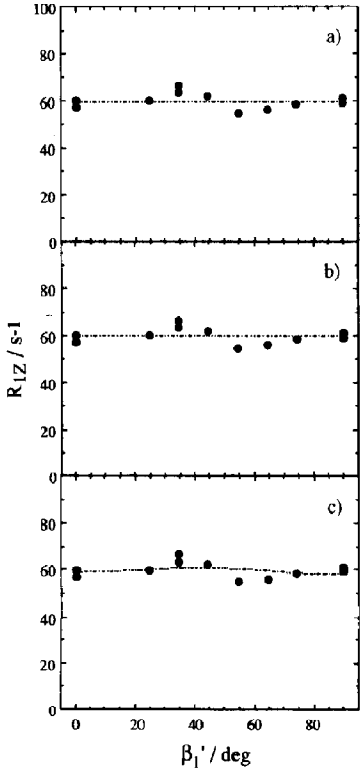

Fig. 2. Comparison of experimental and theoretical dependences of ^2H R_{1Z} values on the angle between the director axis and the main magnetic field. Three different fits are shown to experimental R_{1Z} data obtained at $\nu_0=30.7$ MHz for 2[4',4'-$^2H_2$]DMPC in the L_α phase at 30°C, i.e. deuterated specifically at the C-4 carbon of the *sn*-2 acyl chain [3]. In the first fit (a), a segmental picture is assumed, in which the static EFG symmetry axis is along the C-^2H bond direction ($\eta=0$; $\chi=170$ kHz) and the z-axis of the diffusion tensor is perpendicular to the C^2H_2 plane ($\beta=90°$). The fit assumes that the diffusion tensor is significantly anisotropic ($\eta_D=7$), with the following parameter values: $\langle P_2\rangle=\langle P_4\rangle=0.4$; and $\tau_0=3.9\times10^{-10}$ s. The other two fits correspond to a molecular picture, in which the static EFG tensor is reduced by segmental motions, and further modulated by molecular rotational diffusion. The second fit (b) assumes that the residual EFG tensor is approximately axially symmetric ($\eta=0$). In this case the R_{1Z} data are fit by a choice of the principal residual EFG symmetry axis relative to the diffusion tensor z-axis near the magic angle ($\beta=54.7°$), with the following parameters: $\eta_D=1$; $\langle P_2\rangle=\langle P_4\rangle=0.7$; and $\tau_0=1.4\times10^{-10}$ s. (For simplicity the static interaction constant of $\chi=170$ kHz is used in the fit; significance should not be ascribed to χ or τ_0.) The third fit (c) assumes that the residual EFG tensor is *non*-axially symmetric ($\eta\neq0$); that is the axial symmetry is broken by the segmental motions. Given that $\eta=1$ and $\chi=60$ kHz (cf. text), and assuming that one of the principal values of the residual EFG tensor is parallel to the long molecular axis ($\beta=0°$), a satisfactory fit to the data is obtained with the indicated parameters: $\eta_D=1$; $\langle P_2\rangle=\langle P_4\rangle=0.7$; and $\tau_0=1.9\times10^{-9}$ s. Thus the orientational anisotropy of R_{1Z} for liquid-crystalline phospholipid lamellar phases can be explained in at least three different ways.

the asymmetry parameter η is illustrated. As can be seen, when $\eta=0$ one has that $R_{1Z}(0°)<R_{1Z}(54.7°)<R_{1Z}(90°)$ as mentioned above. When η is increased, however, the magnitude of the anisotropy of R_{1Z} decreases, such that for $\eta=1$ rather little orientation dependence is observed.

In fig. 2 the data of Jarrell et al. [3] for liquid-crystalline 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), deuterated specifically at the C-4 segment of the *sn*-2 acyl chain, that is 2[4',4'-$^2H_2$]DMPC, are fit using eqs. (1) and (6). Here the predominant feature to be explained is the lack of a significant orientational anisotropy of R_{1Z} [3]. As noted above, models which consider only rotations of a single principal value of the coupling tensor about axes orthogonal to the average director, e.g. an order–director fluctuation model, predict a substantially greater orientation dependence [4]. Thus it appears that the analysis should be enriched by inclusion of axial rotations [4], and possibly by allowing the principal values and orientation of the EFG tensor to vary within the molecular frame. Due to the number of parameters a unique fit is unlikely, so that the data must be explained with values consistent with present knowledge of phospholipid lamellar phases. The theoretical fits in fig. 2 represent two different views of the motions giving rise to the relaxation; each is described by eq. (6).

First, it is assumed that the relaxation is due to *segmental motions*, in which the EFG is along the C–^{2}H bond direction. The relaxation then involves modulation of the *static* EFG tensor, e.g. by trans–gauche isomerizations, librational motions, and so forth. It follows that the static interaction constant χ is equal to about 170 kHz [11–13] and the EFG tensor is axially symmetric to a high degree of approximation. For simplicity, the effective segmental motions are modeled by small-step rotational diffusion with an axially symmetric diffusion tensor [4]. The orientation of the V_{zz} principal static EFG component, relative to the principal symmetry axis of the diffusion tensor, is described by the Euler angle β. It is also assumed that the residual EFG tensor remaining upon averaging by the segmental motions has an axis of threefold or greater symmetry, which may need further investigation (vide infra). Part (a) of fig. 2 shows that the R_{1Z} data of Jarrell et al. [3] can indeed be fit by such a model. To account for the lack of an orientation dependence, it is sufficient to assume that the z-axis of the diffusion tensor is perpendicular to the C^2H_2 plane ($\beta=90°$), and that the rotational diffusion tensor is significantly anisotropic, i.e. with different rates for axial and off-axial motions of the acyl chain segments ($\eta_D\approx5$–10). The magnitudes of the R_{1Z} values correspond to a correlation time τ_0 of about 4×10^{-10} s; cf. refs. [14–16].

A second alternative is to assume that R_{1Z} is due to *molecular motions*, such as wobbling or tilting of phospholipids within the bilayer, which are superimposed on more rapid internal motions of the chains; separation of time scales [4] is assumed. The faster segmental motions then alter the *static* EFG tensor through reduction of its principal values and/or a change of its orientation within the molecular frame. It is the *residual* EFG tensor preaveraged by segmental motions that is now modulated by the molecular fluctuations [4]. In this case eq. (6) corresponds to whole molecule rotational diffusion relative to the director axis. A complication is that the fixed orientation of the residual EFG tensor within the molecular frame is unknown. If it is assumed to be axially symmetric (which is reasonable for $\eta<0.5$), then a choice of the orientation of the principal residual EFG symmetry axis near the magic angle relative to the diffusion tensor ($\beta=54.7°$) suffices to explain the angular dependence of R_{1Z} (part (b) of fig. 2). It is also possible that the axial symmetry of the *static* EFG tensor is broken by segmental motions, yielding a *residual* EFG tensor which is *non*-axially symmetic ($\eta>0$). The anisotropy of R_{1Z} can then be explained simply by the coupling parameters δ and η representing the different principal residual EFG values fixed within the frame of the diffusion tensor. Here the elegant work of Huang, Griffin, and coworkers is noteworthy [17,18]. They have shown that rotational isomerization of the polymethylene chains of lipid bilayers can average the principal values of the static EFG tensor to principal residual components that depart significantly from axial symmetry. For a residual EFG tensor which is totally axially asymmetric, $\eta=1$ and the static interaction constant χ is reduced to $\langle\chi\rangle=\frac{3}{8}\chi=63.75$ kHz for two-site jumps [17,18]. It is plausible for illustrative purposes to assume that the residual EFG and diffusion tensors are diagonal within the same principal axis system, and that the coupling parameters δ and η are related to the principal residual EFG components parallel and perpendicular to the long molecular axis, respectively [17].

The observation of axially symmetric ^2H NMR spectra for liquid-crystalline phospholipid bilayers [11,12] moreover suggests that effective axial diffusion of the chains occurs in addition to their rotational isomerization. Then, the reduction of the *maximum* splitting from $\Delta\nu_Q = \frac{3}{4}\chi = 127.5$ kHz to values on the order of 50–60 kHz [11,12] for $\beta_l' = 0°$ may represent additional modes of rotational isomerization [11,17,18] and/or wobbling of the chain segments, which can be collective or noncollective in nature [4]. Part (c) of fig. 2 shows that the angular dependence [3] of the ^2H R_{1Z} values of liquid-crystalline 2[4',4'-$^2H_2$]DMPC can be fit using eq. (6) by assuming that $\eta = 1$, with an interaction constant of 60 kHz and an order parameter for the off-axial motion of $\langle P_2 \rangle \approx 0.7$. It should be remarked that it is sufficient to explain the *shape* of the R_{1Z} anisotropy by assuming that $\eta = 1$, whereas to fit the *magnitude* of R_{1Z} requires a sufficiently large residual EFG tensor. The angular anisotropy [3] and magnitude [10] of R_{1Z} can also vary as a function of chain segment position due to differences in the orientation and principal values of the residual EFG tensor within the molecular frame.

The above conceptual paradigm can also be employed to predict new results that can be tested experimentally. One such predictive test involves systems in which the orientation and principal values of the EFG tensor modulated by the motion are more confidently known than for the flexible acyl chains of liquid-crystalline phospholipids. Here R_{1Z} studies of mixtures of phospholipids with specifically deuterated cholesterol in the lamellar, liquid-crystalline state are of interest [19]. Owing to the rigidity of the sterol frame, it is likely that the static EFG tensor along the C–^2H bond axis is directly modulated by molecular motions [4]. The ability to predict the R_{1Z} angular anisotropy [19] for different C–^2H orientations relative to the cholesterol frame with a consistent set of dynamic parameters constitutes an important test of motional theories (not shown).

Finally, one should note that the angular dependent ^2H R_{1Z} studies [3] were conducted at a single Larmor frequency of 30.7 MHz, and that the above analysis does not consider other possible contributions to the relaxation [4]. There is evidence that a broad distribution of motions exists in liquid-crystalline phospholipid lamellar phases [14,15,20–25]. Given that the relaxation described by eq. (6) reflects molecular motions, then contributions from faster segmental fluctuations could be present, in addition to slower motions of a more collective nature. Previous treatments have considered explicitly the influences of local segmental motions [1,10] and collective chain fluctuations [4,15,26,27]. The present work suggests that molecular features [4,10,15] may also need to be included to explain current R_{1Z} data in the megahertz range.

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