

On the possible manifestation of harmonic-anharmonic dynamical transition in glassy media in electron paramagnetic resonance of nitroxide spin probes

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Continuous wave (cw) electron paramagnetic resonance (EPR) and echo-detected (ED) EPR were applied to study molecular motions of nitroxide spin probes in glassy glycerol and *o*-terphenyl. A linear decrease with increasing temperature of the total splitting in the cw EPR line shape was observed at low temperatures in both solvents. Above some temperature points the temperature dependencies become sharper. Within the model of molecular librations, this behavior is in qualitative and quantitative agreement with the numerical data on neutron scattering and Mössbauer absorption for molecular glasses and biomolecules, where temperature dependence of the mean-squared amplitude of the vibrational motion was obtained. In analogy with these data the departure from linear temperature dependence in cw EPR may be ascribed to the transition from harmonic to anharmonic motion (this transition is called dynamical transition). ED EPR spectra were found to change drastically above 195 K in glycerol and above 245 K in *o*-terphenyl, indicating the appearance of anisotropic transverse spin relaxation. This appearance may also be attributed to the dynamical transition as an estimation shows the anisotropic relaxation rates for harmonic and anharmonic librational motions and because these temperature points correspond well to those known from neutron scattering for these solvents. The low sensitivity of ED EPR to harmonic motion and its high sensitivity to the anharmonic one suggests that ED EPR may serve as a sensitive tool to detect dynamical transition in glasses and biomolecules. © 2006 American Institute of Physics. [DOI: 10.1063/1.2220571]

INTRODUCTION

Recently, much attention has been paid to a so-called dynamical transition in molecular glasses and biomolecules, which manifests itself in Mössbauer absorption of dissolved iron ions and in neutron scattering on protons of the matrix. Below the dynamical transition temperature, T_d , the mean-squared amplitude of motion $\langle r^2 \rangle$ linearly depends on temperature, which is ascribed to harmonic oscillations of the atoms. Above T_d the temperature dependence becomes much sharper and it is strongly nonlinear, which is interpreted as the onset of anharmonic motion. This transition was detected for molecular glasses,^{1–6} for proteins,^{7–16} for biomembranes,¹⁷ and other biomolecules.¹⁸ Dynamical transition is known to affect the physiological function of proteins.^{19,20} Typical $\langle r^2 \rangle$ values lie between 0.01 and 0.5 Å². Mössbauer absorption is sensitive to motion faster than 10⁷ s⁻¹, neutron scattering to motion faster than 10⁹ s⁻¹.

The possible manifestation of this phenomenon in continuous wave (cw) electron paramagnetic resonance (EPR) of nitroxide spin probes in glasses was discussed in Refs. 21 and 22 where the observed linear decrease with increasing temperature of the total splitting in the cw EPR line shape was ascribed to molecular harmonic librations. The mean-squared angular amplitude of libration $\langle \alpha^2 \rangle$ was estimated as ~0.01–0.1 rad². cw EPR line shape is sensitive to librational

motion faster than 10⁸ s⁻¹. The closeness of these numerical values with the above-mentioned analogous values for Mössbauer absorption and neutron scattering ($\langle r^2 \rangle$ taken in angstroms may be directly compared with $\langle \alpha^2 \rangle$ taken in radians, because the size of a librating molecule is around 1 Å) indeed supports this supposition.

Note that temperature dependence of cw EPR line shape in molecular glassy liquids may be also adequately explained within other models of molecular motion, such as isotropic or anisotropic Brownian diffusion, orientational jumps via fixed or arbitrary angles, and others.^{23–27} cw EPR spectra in glassy liquids, taken alone, are not critical to the motional model, because the line shape only slightly changes with temperature.²³

In this paper we explore the possible manifestation of harmonic-anharmonic transition in echo-detected (ED) EPR. ED EPR spectra are taken by measuring electron spin echo amplitude when scanning magnetic field and keeping constant the time separation τ between two echo-forming microwave pulses (so it is essentially a two-dimensional experiment). Comparing line shapes taken at different τ allows one to detect the field-dependent contribution to relaxation. As different field positions in the spectra are related with different nitroxide orientations relative to the external magnetic field, this contribution is also an orientation-dependent one (i.e., it presents anisotropic relaxation) and it is sensitive to orientational motion. ED EPR studies of nitroxide spin probes in molecular glasses^{22,28} and membranes^{29,30} have

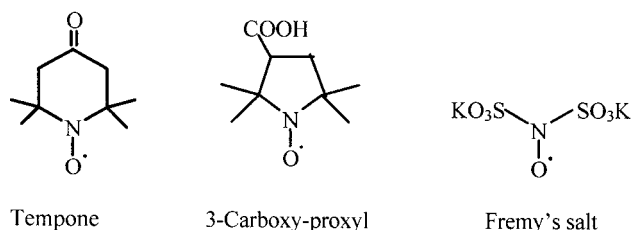
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shown that anisotropic relaxation may be quantitatively simulated within the model of fast orientational stochastic oscillations of the molecule (stochastic librations).

We study here nitroxides of several different types dissolved in two low-molecular glass formers, supercooled glycerol and *o*-terphenyl. Glycerol is a strongly polar media characterized by a net of hydrogen bonds while *o*-terphenyl is a nonpolar substance with van der Waals interactions between molecules. Both of these substances have been previously studied with neutron scattering in Refs. 2–4. Glycerol with added 6.5 wt % water and with dissolved $^{57}\text{Fe}^{2+}$ ions also was studied with Mössbauer absorption.¹

EXPERIMENT

Nitroxide spin probes used in this work were



Nitroxide Tempone (2,2,6,6-tetramethyl-4-piperidone *N*-oxide) was purchased (Sigma) and purified by recrystallization from hexane. 3-Carboxy-proxyl was synthesized by I.A. Grigoriev as described in Ref. 31, potassium peroxyamine disulfonate (Fremy's salt) was a gift from I.E. Sokolov. The solvents were glycerol and *o*-terphenyl (Aldrich). The nitroxide concentration was less than 10^{-3} M for all solutions. The sample of liquid *o*-terphenyl containing dissolved nitroxides was prepared above its melting point and then rapidly frozen. The transparency of the samples was checked before and after measurements, to prove that no crystallization had occurred.

Experiments were performed with a Bruker ESP 380E pulsed EPR spectrometer using either a standard Bruker rectangular cavity (cw EPR experiments) or a home-made rectangular cavity operating at H_{012} mode (ED EPR). For cw experiments, the operating parameters were chosen to avoid saturation and overmodulation. In pulsed experiments, the cavity was overcoupled to adjust the dead time to 120 ns. Two-pulse sequence, $\pi/2 - \tau - \pi - \tau - \text{echo}$, was employed. Duration of microwave pulses was 32 and 64 ns for $\pi/2$ and π pulses, respectively. Repetition frequency was 500 Hz, which allowed the signal to perfectly restore between applications of consequent pulse series.

A Bruker Variable Temperature Unit ER 4111VT was used with a Cu-constantan thermocouple placed in the sample tube to monitor the sample temperature. This setup provided temperature stabilization during the experiment within ± 1.5 K.

RESULTS AND DISCUSSION

The line shape of cw EPR spectra of nitroxides consists of three hfs components corresponding to three nitrogen spin projections and is determined by anisotropy of hfi and of g

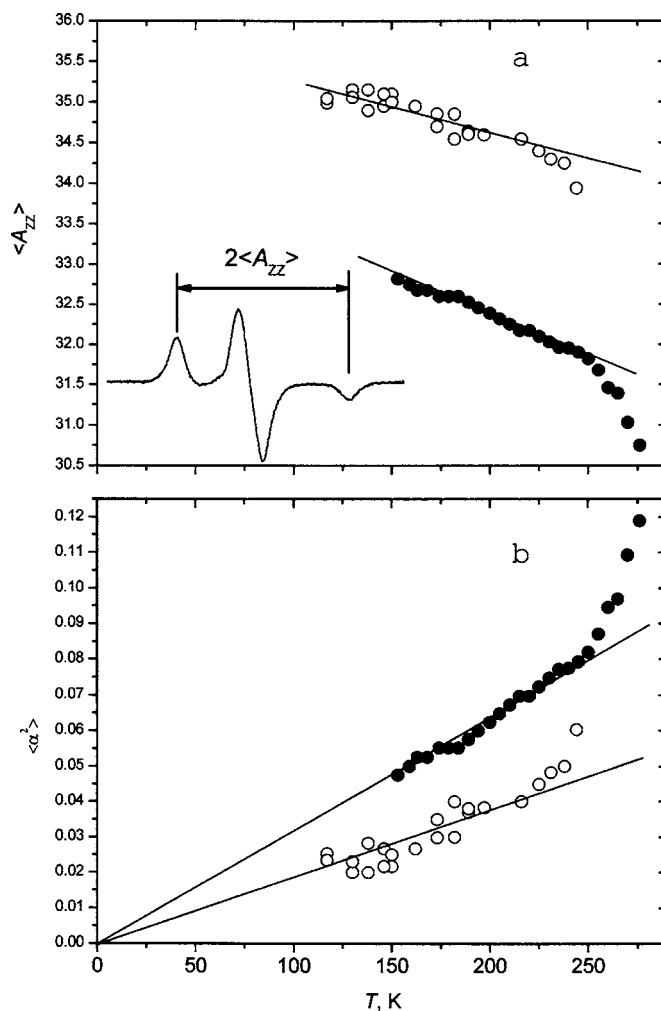


FIG. 1. (a) Temperature dependence of the splitting between two outer peaks (shown in the insert) for Tempone in glycerol (open circles) and in *o*-terphenyl (closed circles). The straight lines are drawn through low-temperature points. (b) The mean-squared amplitudes of angular motion, obtained from data (a) using Eq. (1).

factor. Figure 1(a) shows temperature dependence of the splitting between two outer peaks for Tempone in *o*-terphenyl and in glycerol. At low temperatures a noticeable linear decrease of the splitting with increasing temperature is observed. In *o*-terphenyl a sharp drop of the splitting is seen above 250 K. In glycerol an analogous drop, although less pronounced, is observed above 230 K.

Such linear decrease was discussed previously in Refs. 21 and 22 where it was assigned to harmonic librational motion. If librational motion is fast enough (the fast motion limit of the Redfield theory³²), the EPR line shape may be determined by the same equations as a solid-like EPR spectrum, with the principal hyperfine interaction (hfi) and g -factor values replaced by their motion-averaged counterparts.^{21,22} The splitting between the two outer peaks in Fig. 1 corresponds with good accuracy to the $2A_{ZZ}$ value where A_{ZZ} is the principal value of the hfi tensor (the molecular Z axis is perpendicular to the >NO plane). For simplicity we consider here the model of uniaxial librations with the motional axis lying in the >NO plane (for the motional axis parallel to the Z axis anisotropic relaxation is much

slower). If α denotes the deviation angle from the equilibrium and the potential well is symmetric, so that $\langle\alpha\rangle=0$, the motion-averaged $\langle A_{ZZ}\rangle$ value will be^{21,22}

$$\begin{aligned}\langle A_{ZZ}\rangle &= A_{ZZ}^0 - (A_{ZZ}^0 - A_{\perp}^0)\langle\sin^2\alpha\rangle \\ &\approx A_{ZZ}^0 - (A_{ZZ}^0 - A_{\perp}^0)\langle\alpha^2\rangle,\end{aligned}\quad (1)$$

where the superscript denotes data for a motionless molecule, A_{\perp} is the principal value for the perpendicular orientation (assuming axial symmetry of hfi tensor), and for small amplitude of motion $\langle\sin^2\alpha\rangle$ is replaced by $\langle\alpha^2\rangle$. We obtained A_{ZZ}^0 value by linear extrapolation of data in Fig. 1(a) to zero temperature (neglecting so the possible quantum effects in librations). As A_{\perp}^0 is much less than A_{ZZ}^0 , it was arbitrarily taken as 5 G. Temperature dependence of $\langle\alpha^2\rangle$ derived from data in Fig. 1(a) using Eq. (1) is shown in Fig. 1(b).

From Eq. (1) it follows that the linear temperature dependence of the hfi constants means that the mean-squared amplitude of libration motion $\langle\alpha^2\rangle$ is proportional to temperature. This behavior is typical of harmonic solids, because in the classical limit

$$\frac{I\Omega^2\langle\alpha^2\rangle}{2} = \frac{kT}{2},\quad (2)$$

where I is the moment of inertia, Ω is the oscillator frequency, k is the Boltzmann constant.

Equation (2) under some simple assumptions allows one to estimate Ω . Assuming the Tempone molecule to freely oscillate in the angular potential created by surrounding molecules, and taking $I \sim 5 \times 10^{-38}$ g cm² (for motion around the direction of the NO bond, estimated from the known molecular structure³³), from data of Fig. 1(b) we estimate $\Omega \sim 2 \times 10^{12}$ rad/s and $\Omega \sim 3 \times 10^{12}$ rad/s for Tempone in glycerol in *o*-terphenyl, respectively.

A departure from the linear dependence observed in Fig. 1 above 230 K in glycerol and above 250 K in *o*-terphenyl could be attributed to the onset of a large-amplitude anharmonic motion. The harmonic-anharmonic transition found previously from neutron scattering occurs for glycerol at ~ 200 K,² and for *o*-terphenyl at ~ 240 K.^{3,4} The agreement between these temperature points is good for *o*-terphenyl and worse for glycerol.

For 3-Carboxy-proxyl the analogous measurements showed the splitting increasing with temperature (data not given), which could be interpreted as a result of the temperature dependence of the polarity of the matrix that induces, because of the highly polar nature on the nitroxide itself, a temperature dependence of hfi.^{30,34} Also, carboxy group may rotate relative to the ring differently as a function of temperature, and results in different hfi. An analogous increase with temperature in a polar environment was reported previously.³⁰ Probably, experimental data for Tempone in glycerol also are influenced by temperature dependence of the polarity of the matrix. cw EPR data for nonpolar *o*-terphenyl glass in that interpretation are free from this concern.

ED EPR spectra for Tempone in glycerol and in *o*-terphenyl for different temperatures and τ values are given in Fig. 2. To exclude all isotropic relaxation processes that

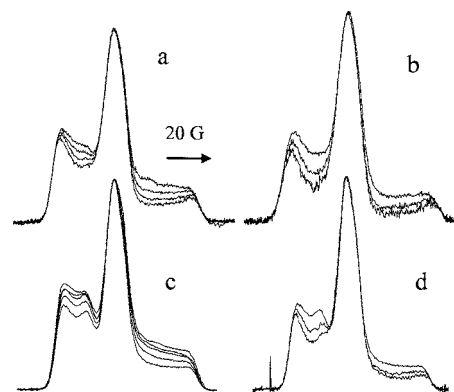


FIG. 2. ED EPR spectra for Tempone in glycerol (a), (b) and in *o*-terphenyl (c), (d), taken at different temperatures and at the same time separation τ (a), (c), and at the same temperature and at different τ (b), (d). Spectra are normalized to the same amplitude at the field position corresponding to the maximum of the spectrum. Temperatures are 181, 198, 205, and 211 K (a), 240, 250, 255, 260, and 265 K (c) (the intensities in the shoulders monotonically decrease with temperature increase), 216 K (b), and 260 K (d). $\tau=136, 256,$ and 400 ns (b), 200, 400, and 600 ns (d) (intensities in the shoulders monotonically decrease with τ increase), 176 ns (a), and 200 ns (c).

are expected to be field-independent, the spectra are normalized to the same value at the field position corresponding to the maximum amplitude. One can see remarkable temperature and τ dependencies in both cases, which imply the presence of anisotropic relaxation. The analogous data for 3-Carboxy-proxyl and for Fremy's salt in glycerol were reported previously.²² Note that dependence of amplitude on τ at any given field position is exponential with good accuracy,²² except for the very long delays. Below 180 K for glycerol and below 240 K for *o*-terphenyl we did not observe any noticeable temperature dependence of ED EPR line shape or its dependence on τ (data not given).

It was previously shown that ED EPR line shape may be precisely simulated employing the model of fast low-amplitude stochastic librations.²² For the whole set of data taken at different τ only a single fitting parameter was used in these simulations (spectral density of motion-induced deviation angle fluctuations). The good quantitative agreement between experiment and theory was considered as a strong argument in favor of the validity of the motional model.

To assess the intensity of motion at different temperatures, we use the relative intensity at some selected spectral position—see the inset in Fig. 3. Figure 3 present results for Tempone, Fremy's salt, and 3-Carboxy-proxyl in glycerol and for Tempone in *o*-terphenyl. One can see in Fig. 3 that the onset of ED EPR line shape temperature dependence starts near 195 K for glycerol and near 245 K for *o*-terphenyl. These temperatures are close to the above-cited temperatures T_d obtained by neutron scattering.

The sharp onset of spectral changes seen in ED EPR (Fig. 3) may be compared with the temperature behavior of cw EPR [Fig. 1(a)], where the noticeable changes are seen in a much wider temperature interval. The reason for this different behavior could be that harmonic motion has different impact on cw EPR and ED EPR line shapes. Fast harmonic motion averages A_{ZZ} and therefore produces, according to Eqs. (1) and (2), the noticeable temperature dependence of

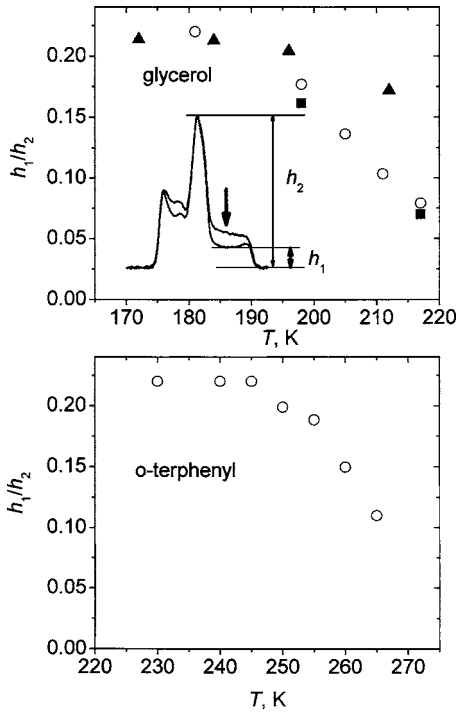


FIG. 3. The relative intensities of the lines in the ED EPR spectra taken at different temperatures for the field position indicated by arrow in the insert. Circles: Tempone, triangles: Fremy's salt, squares: 3-Carboxy-proxyl.

cw EPR line shape. In ED EPR the anisotropic relaxation rate for fast harmonic motion, R_{harm} , may be assessed as $\sim 10^{17} \langle \alpha^2 \rangle f / \Omega^2$ (Ref. 22), where f is the friction coefficient (taking into account that for nitroxides at X band the mean-squared amplitude of resonance frequency fluctuation $\langle \Delta \omega^2 \rangle \approx \gamma^2 (A_{ZZ} - A_{\perp})^2 \langle \alpha^2 \rangle \approx 10^{17} \langle \alpha^2 \rangle \text{ rad}^2 / \text{s}^2$). This value would be small for high frequency Ω . Indeed, for $\langle \alpha^2 \rangle \sim 0.1 \text{ rad}^2 / \text{s}^2$ and $\Omega \sim 3 \times 10^{12} \text{ rad/s}$ (see above), and if $f < \Omega$, we obtain $R_{\text{harm}} < 3 \times 10^3 \text{ s}^{-1}$. This rate is indeed small for typical τ values between 10^{-7} and 10^{-6} s .

For anharmonic motion relaxation rate R_{anharm} may be assessed in the following way. The equation for angular motion for free anharmonic oscillator is

$$I\ddot{\alpha} + I\Omega^2\alpha - I\Omega^2\frac{\alpha^2}{\alpha_0} = 0, \quad (3)$$

where α_0 is the parameter of anharmonicity. We seek the solution for the case of small anharmonicity, $\langle \alpha^2 \rangle \ll \alpha_0^2$, in the form of $\alpha = A \cos \Omega t + \delta$, where A is the amplitude and δ is an additive that is small as compared with A . (Note that $\langle \alpha^2 \rangle \approx A^2/2$). For δ we obtain from Eq. (3) the approximate equation:

$$\ddot{\delta} + \Omega^2\delta \approx \Omega^2\frac{A^2}{\alpha_0} \cos^2 \Omega t. \quad (4)$$

The solution of this equation includes as an additive a nonoscillating part, $\delta_0 = A^2/2\alpha_0$. This will result in a nonzero value of $\langle \alpha \rangle = \delta_0$. Under influence of thermal stochastic perturbations the A^2 value for a given oscillator will fluctuate (fluctuations of energy), producing fluctuations of δ_0 . In Redfield limit this will provide the relaxation rate

$$R_{\text{anharm}} \approx 10^{17} \delta_0^2 \tau_c = 10^{17} \frac{\langle \alpha^2 \rangle^2}{\alpha_0^2} \tau_c,$$

where τ_c is the correlation time for energy fluctuations. (The rigorous theory considering spin relaxation due to energy fluctuations in an oscillator may be found, e.g., in Ref. 35. Its results coincide in the case of classical motion with the result of this simple evaluation.) As this rate does not contain Ω in the denominator, anharmonic librational motion may produce spin relaxation fast enough to be detected in ED EPR. For example, for $\langle \alpha^2 \rangle \sim 0.1 \text{ rad}^2$, $\alpha_0 \sim 1 \text{ rad}$ and $\tau_c \sim 10^{-8} \text{ s}$ (the largest τ_c value when Redfield theory for a given $\langle \alpha^2 \rangle$ is still valid), we obtain $R_{\text{anharm}} \sim 10^7 \text{ rad/s}$ (cf. with R_{harm} value evaluated earlier).

Note that ED EPR line shape simulated for this model, except for the different meaning of spectral densities for the motion, must be exactly the same as that for the model of purely stochastic fast librational motion, which was employed in a number of previous studies.^{22,28-30}

Formally Eq. (1) is not valid for anharmonic motion, because of the nonzero $\langle \alpha \rangle$ value. However, for small anharmonicity, $\langle \alpha^2 \rangle \ll \alpha_0^2$, we have $\langle \alpha \rangle^2 \ll \langle \alpha^2 \rangle$, so the inaccuracy is small.

The observed sharp onset of manifestation of motion in Fig. 3 could imply that anharmonicity appears only above T_d . In other words, α_0 seems to be temperature-dependent and becomes apparently smaller above T_d .

CONCLUSIONS

Within the model of fast molecular librations, cw EPR data on spin probe nitroxides in molecular glasses provide temperature dependence of the mean-squared amplitude of molecular angular motion $\langle \alpha^2 \rangle$ [Fig. 1(b)], which very closely resembles the temperature dependence of the mean-squared amplitude of vibrational motion of atoms $\langle r^2 \rangle$ known from numerical neutron scattering and Mössbauer absorption studies.¹⁻²⁰ The observed sharp increase of $\langle r^2 \rangle$ temperature dependence above a certain temperature (dynamical transition temperature T_d) in these studies is ascribed to harmonic-anharmonic transition.

The librational model also quantitatively explains motional effects in ED EPR.^{22,28-30} In this work we found that motional effects in ED EPR appear at temperatures of 195 K for glycerol and 245 K for o-terphenyl (Fig. 3), which coincide with T_d values known for these glass formers from neutron scattering data.²⁻⁴ A simple qualitative explanation of this appearance may be given taking into account that anharmonicity results in a nonoscillating shift, which in turn may fluctuate for a given molecule because of energy fluctuations. This results in spectral diffusion which provides the appropriate relaxation pathway. This qualitative explanation is supported by the quantitative evaluation of the relaxation rate which employs rather reasonable parameters.

So, results of this work provide evidence that EPR of nitroxide spin probes is sensitive to harmonic-anharmonic dynamical transition in glassy media. However, for the rigorous justification of the suggested interpretation, further investigations are needed. At this stage, it is difficult to rule out

some other possibilities to explain the observed phenomena—like transition from fast librational motions to slower wobbling motions as the solvent softens. From this point of view, only the analogy with neutron scattering and Mössbauer absorption data, which are sensitive to fast motions only, now supports the suggested interpretation.

The applicability of ED EPR approach to study glassy states in complex molecular and biological systems has been previously demonstrated for living biological objects³⁶ and model phospholipids membranes.³⁰ In light of the results of the present work the previously observed transitions^{30,36} may be ascribed to dynamical transition.

ED EPR data may provide further insight into the phenomenon of dynamical transition. Neutron scattering and Mössbauer absorption are sensitive to atomic motion while ED EPR seems to reflect a motion of the molecule as a whole.³⁷ One may expect that such motion requires the rearrangement of the nearest surrounding. Therefore, the dynamical transition is most likely a cooperative phenomenon in which many molecules are involved. The cooperative nature of the transition is also supported by the fact that nitroxide probes of different structures indicate the onset of the transition at the same temperature (Fig. 3). Also, the energetic potential for librational motion probably is temperature-dependent, with increasing anharmonic contribution with increasing temperature.

ACKNOWLEDGMENTS

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