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On the Interpretation of Fluorescence Anisotropy Decays from Probe Molecules in Lipid Vesicle Systems

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Measurements of fluorescence depolarization decays are widely used to obtain information about the molecular order and rotational dynamics of fluorescent probe molecules in membrane systems. This information is obtained by least-squares fits of the experimental data to the predictions of physical models for motion. Here we present a critical review of the ways and means of the data analysis and address the question how and why totally different models such as Brownian rotational diffusion and "wobble-in-cone" provide such convincing fits to the fluorescence anisotropy decay curves. We show that while these models are useful for investigating the general trends in the behavior of the probe molecules, they fail to describe the underlying motional processes. We propose to remedy this situation with a model in which the probe molecules undergo fast, though restricted local motions within a slowly rotating cage in the lipid bilayer structure. The cage may be envisaged as a free volume cavity between the lipid molecules, so that its position and orientation change with the internal conformational motions of the lipid chains. This approach may be considered to be a synthesis of the wobble-in-cone and Brownian rotational diffusion models. Importantly, this compound motion model appears to provide a consistent picture of fluorescent probe behavior in both oriented lipid bilayers and lipid vesicle systems.

KEY WORDS: Fluorescence anistropy decays; probe molecules; lipid vesicles.

REVIEW OF THE GENERAL PRINCIPLES OF PROBE MOLECULE TECHNIQUES

The study of the orientational order and rotational dynamics in membrane system is derived from a pragmatic wish to understand which physical processes are of prime importance for their biological function. While NMR spectroscopy yields the most direct information, it suffers from the distinct disadvantage that the investigations rely on the availability of selectively labeled molecules. Over the years it has proven to be experimentally more convenient to incorporate extraneous probe molecules in the membrane structure at the lowest possible concentration compatible with an acceptable signal/noise ratio. The focus is now on the behavior of these probe molecules, with the tacit assumption that they reflect the intrinsic behavior of the indigenous lipid molecules. While the use of probe molecules begs the question as to what exactly is being monitored, the quantitative interpretation of the results often turns out to be relatively straightforward. It has been argued that the information obtained from membrane systems in this way is compromised by structural perturbations caused by the incorporation of the probe molecules. Unfortunately, it has not been possible as yet to refute the latter criticism in a convincing way. At best, one can use different probe techniques with a common time window to avoid artifacts associated with probe-specific effects. Thus only

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effects common to all the experiments may be considered to be physically significant.

Fluorescence depolarization and electron spin resonance (ESR) techniques are particularly suitable for studying the molecular order and dynamics in membranes, since their intrinsic time window covers the range of rotational motions of the probe molecules [1,2]. It is important to bear in mind now that the behavior of the probe molecules is monitored indirectly, through the transition dipole moments in fluorescence depolarization experiments and the anisotropic hyperfine magnetic interactions in ESR experiments. Consequently, knowledge of the orientation of the transition dipoles or the hyperfine interaction tensor in the frame of the molecule must be known if the experimental signal is to be interpreted unequivocally in terms of the rotational motion and orientational order of the probe molecules themselves. Unfortunately, it is not always possible to obtain this molecular information from independent experiments.

The starting point in the description of the experimental observations, such as fluorescence anisotropy decay curves, is a model for the behavior of the probe in the membrane system. To this end, it is common to assume that each probe molecule experiences a local orienting potential imposed on it by the surrounding lipid molecules [3–5]. The orientational distribution function of the molecules relative to the normal to the bilayer surface is given simply by the Boltzmann distribution corresponding to this orienting potential. It is important to note that in anisotropic liquids, such as membranes, the *observed* dynamic effects, such as the depolarization of fluorescence, is determined by both the orienting potential and the thermal fluctuations in the system. This situation is in marked contrast to the case of isotropic liquids, where no orienting potential acts on the molecules [6]. While for isotropic liquids the motional information can be extracted from observations in a simple way, in membrane systems knowledge of the orienting potential is a prerequisite for the determination of the motional rates.

It has become accepted practice in the literature to discuss the orientational distribution of the probe molecules in terms of order parameters which can be determined experimentally [7]. The difficulty now is that the inverse step of reconstructing the distribution function from the limited number of order parameters is not a trivial task. Fluorescence depolarization measurements on cylindrically symmetric probe molecules, in principle, yield only the two order parameters $<P_2>$ and $<P_4>$. This provides us with very limited information about the behavior of the probe molecules in the membrane, as there is a large family of orientational distributions which yield the correct values for these known order parameters. Nevertheless, it is still possible to construct the broadest and smoothest distribution from the known order parameters in an objective way by invoking the maximum entropy method [7–11]. This stratagem is also useful in postulating reasonable forms for the orienting potential acting on the probe molecules, given the number and rank of order parameters which are accessible experimentally. We indeed make use of reasoning based on the MEM below to make informed guesses about the probable form of the orienting potential in membrane system.

The information on the rotational motions of the probe molecules is contained in the decay of the fluorescence depolarization following the application of a short light pulse [1,12,13]. The initial amplitude of the depolarization at time $t=0$ and the amplitude at long times after the pulse can be worked out theoretically in a model-independent way. Here we simply make use of the fact that the motion of the probe molecule is a stochastic process. These amplitudes can be expressed in terms of the order parameters. The time behavior of the decay process is determined by both the orienting potential and the rates of rotational motions. An analysis of this behavior in terms of the superposition of exponential decay components reveals the time scale on which motion takes place. However, it does not answer the question of the rates of motion. This information can be obtained only from an interpretation of the decays on the basis of a model describing the detailed behavior of the probe molecule in the membrane system [1,14,15].

The question which now needs to be faced is whether the particular experiment at hand can access sufficient physical information for the reliable characterization of the orienting potential. It has been established over the past years that the this is indeed the case in angle-dependent studies on oriented bilayer systems, on scanning either the polarization ratios or the ESR spectra as a function of the angle made relative to the normal to the bilayer surface [10,14–16]. Unfortunately, oriented bilayers are not always the system of choice and lipid vesicles afford a far more convenient experimental system. The difficulty with vesicle systems is that much information is lost because the experimental signals are an average over all the possible orientations of the bilayers relative to the polarization direction of the light or the applied magnetic field [17,18]. This is best illustrated in ESR, where many spectral features containing information are lost on going from oriented bilayers to vesicle or liposome configurations [19]. In fluorescence depolarization experiments all the model-independent in-

formation on the order parameter $<P_4>$ is lost, and only $<P_2>$ can be deduced from the long time plateau of the decays. For this reason, it has been stressed by a number of groups that great care must be used in the extraction of information from macroscopically isotropic, but microscopically ordered vesicle systems [17,18].

MODELS FOR ROTATIONAL MOTION AND THE GLOBAL TARGET ANALYSIS APPROACH

The common denominator to all the techniques providing information about rotational dynamics on a molecular scale is that the experimental data are analyzed using least-squares fits to the predictions of physical models for motion, the global target analysis approach. For this reason, it has become routine practice in the fluorescence field to apply either the "wobble-in-cone" model postulated by Kinosita *et al.* [20,21] or the Brownian rotational diffusion (BRD) model [1,3]. The only difference between these models is the form of the orienting potential acting on the molecules. The wobble-in-cone model assumes the simplest form of the potential, while the BRD model utilizes the MEM choice of the potential $U(\beta)$

$$U(\beta) = -kT\,[\lambda_2 P_2(\beta) + \lambda_4 P_4(\beta)] \qquad (1)$$

where P_L is the Legendre polynomial of rank L and β is the angle made between the long axis of the probe and the normal to the local membrane surface. This choice of the potential is based on the observation that only the order parameters $<P_2>$ and $<P_4>$ can, in principle, be obtained from the experiment in a model-independent way.

The discussion about the correct form of the orienting potential to be used in the global target analysis approach is unfortunately far too often concerned with statistical least-squares fitting criteria than with information content. For example, it has been cogently argued that the wobble-in-cone model is preferable to the BRD model, as it has fewer parameters for optimization to the experimental data [22]. While the wobble-in-cone model is undoubtedly conceptually simple, it imposes an impenetrable barrier on the motion of the probe molecules at some arbitrary orientation in the membrane structure. This constraint is difficult to reconcile with the generally accepted picture of the "fluid-mosaic" structure for a lipid bilayer or membrane system. Moreover, model-independent analysis of fluorescence depolarization experiments on oriented systems has revealed values for the order parameters $<P_4>$ which are impossible to accommodate in the framework of the wobble-in-cone model [23].

The BRD model itself is not above criticism, since the choice of the MEM potential implicitly assumes that the odd-rank order parameters such as $<P_1> = <\cos \beta>$ are identically zero. It is very much the question whether the potential shown in Eq. (1) is appropriate for probe molecules such as TMA-DPH, which are anchored to the bilayer interfaces and unlikely to tumble head-over-heels in the bilayer. Since each probe molecule is confined to one monolayer in the bilayer structure, we expect the value of $<P_1>$ to be significant. This term must now enter the expression for the MEM form of the orienting potential even though it cannot be determined in a model-independent way. It is important to note that the wobble-in-cone model does in fact yield nonzero values for the odd-rank order parameters, in contrast to the BRD potential, Eq. (1). The imponderable that will be left unanswered here is the question of the appropriate form for the orienting potential acting on the symmetric DPH molecules in bilayer systems.

ANALYSIS OF FLUORESCENCE ANISOTROPY DECAYS IN VESICLE SYSTEMS

The loss of information inherent in measurements on macroscopically isotropic vesicle system is manifested by ambiguities in the analysis of time-resolved fluorescence anisotropy decays obtained from DPH and TMA-DPH probe molecules [17,18,24,25]. Importantly it appears that both the BRD and the "wobble-in-cone" models are equally successful in describing fluorescence anisotropy experiments on a wide range of systems in the sense of satisfying the statistical criteria of least-squares fitting algorithms. Moreover, the BRD model yields a number of statistically equivalent solutions, each corresponding to a distinctly different orienting potential [24,25].

It has been demonstrated that the reason for the statistical equivalence of the two approaches lies in the fact that the fluorescence anisotropy decay consists of a sum of three independent correlation functions. While the different statistical solutions reproduce the same sum, they do so using markedly different individual contributions from the three decay components. Consequently, it is not possible to obtain unequivocal information about the orientational order and molecular dynamics from fluorescence anisotropy decays.

It is important to stress at this juncture that the problem sketched above is totally separate from the vexed question as to the whether the emission transition dipole

moment of DPH or TMA-DPH lies parallel to both the absorption transition moment and the long molecular axis.

The problems associated with the analysis of fluorescence anisotropy decays are perhaps best illustrated by our previous measurements on TMA-DPH embedded in vesicles of different lipid compositions [26]. A global target analysis of the anisotropy decay of TMA-DPH in vesicles of POPC using the BRD model with the potential shown in Eq. (1) (Fig. 1) shows that the fluorescence anisotropy decay curves consist of two very fast-decaying components and a single slow decay (Fig. 2). This behavior is apparent only in the BRD prescription for two particular forms of the potential. In the first case, the molecular orientational distribution function of the oblong probes is bimodal, with maxima for probes oriented with their axes parallel as well as perpendicular to the bilayer surface. This distribution, however, is difficult to rationalize in terms of the amphiphilic properties of the probes. For the second case, the probe molecules undertake a collective molecular tilt. The latter solution is at odds with the results of other physical techniques including ESR spectroscopy [27]. In both cases the BRD model achieves the combination of fast and slow anisotropy decay components by predicting probe distributions with two potential minima. Local probe motions within each minimum give rise to the fast anisotropy decay, whereas the slow components arise from transitions between them.

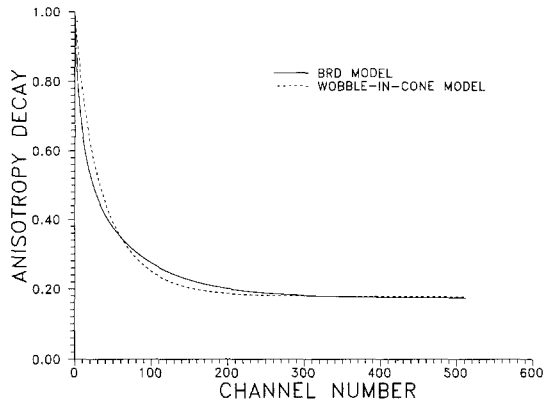

Fig. 1. The extracted fluorescence anisotropy decay from time-resolved measurements on TMA-DPH in vesicles of POPC using the BRD and "wobble-in-cone" models.

The same picture of two fast and one slow decay components is also revealed from fits of the same data to the wobble-in-cone model (Figs. 1 and 3). This behavior is inherent in the model, which confines the molecules to the upper-half of the bilayer with $\beta \leq \pi/2$. Consequently odd-rank order parameters, such as $<P_1>$, take on nonzero values and contribute to the decay rates. Interestingly, the decay components shown in Fig. 3 are indistinguishable from those obtained from the case of collective molecular tilt in the BRD model. Not surprisingly, similar values for the even-rank order parameters $<P_2>$ and $<P_4>$ are extracted from the wobble-in-cone analysis and the collective tilt solution of the BRD.

We now find that two models using three different orienting potentials nevertheless yield a common picture of fast and slow decay components for explaining the fluorescence anisotropy decay. We here argue that this

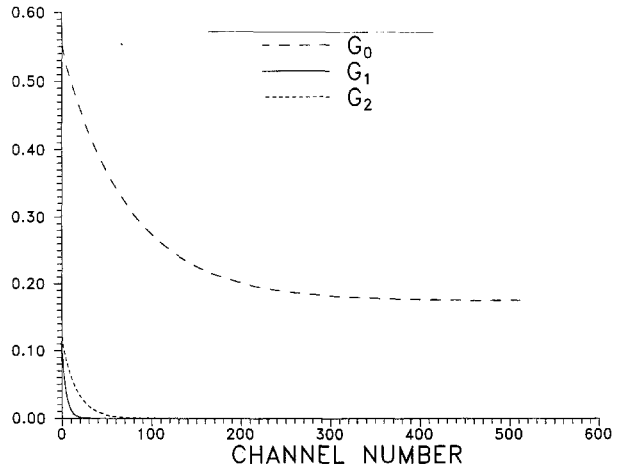

Fig. 2. The decay of the three correlation functions contributing to the fluorescence anisotropy decay shown in Fig. 1 according to the BRD model.

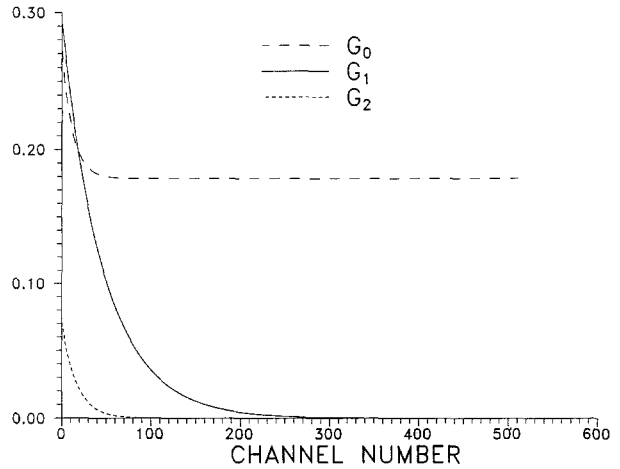

Fig. 3. The decay of the three correlation functions contributing to the fluorescence anisotropy decay shown in Fig. 1 according to the "wobble-in-cone" model.

time scale separation provides the key to understanding the physical nature of the rotational dynamics of probe molecules in membrane systems. A plausible and consistent description of the fluorescence anisotropy decay can be formulated on considering a model in which the probe molecules undergo fast, though restricted local motions within a slowly rotating "cage" in the lipid bilayer structure. This prescription has its roots in the slowly relaxing local structure model advanced by Freed and co-workers [28–30] for explaining ESR lineshapes of nitroxide probes embedded in nematic liquid crystals. The "cage" may be envisaged as a free volume cavity between the lipid molecules, so that its position and orientation change with the internal conformational motions of the lipid chains. The description of the dynamics of the probe molecules proposed here may be considered to be a synthesis of the wobble-in-cone and BRD models.

An important prediction of the compound motion model described in the next section is that the three components contributing to the fluorescence anisotropy decay have comparable time constants. This arises simply from the fact that the model mixes the fast and slow modes of motions in the depolarization process. The prediction is indeed borne out in time-resolved fluorescence depolarization experiments on oriented bilayer systems. The model proposed below does not overcome the intrinsic problem of the lack of information in vesicle systems, which is the root cause of the appearance of multiple equivalent statistical solutions in the least-squares fitting of fluorescence anisotropy decay curves. Nevertheless, it appears that it is often possible to discriminate between the solutions on simple physical grounds. The most important restrictive criterion is that the rotational motion of the probes in the cage is significantly faster than that of the cage in the bilayer. We have used this model to reanalyze our previous measurements of fluorescence anisotropy decays of TMA-DPH in vesicles of POPC. The results yield orientational distribution functions for the probes which are consistent with their amphiphilic nature.

It is an easy matter to fault the approach described here on the grounds that it contains five free model parameters instead of the two needed for the wobble-in-cone model or the three for the BRD model. However, measurements of fluorescence depolarization on oriented bilayer systems presented below show that the latter two models indeed provide an unsatisfactory explanation of the time scale separation.

We believe that in fact the wobble-in-cone and BRD models may be used in the interpretation of fluorescence anisotropy decays in investigations of general trends such as the variation of the order parameter $<P_2>$ and the motional rates on changing the temperature or chemical composition of the lipid. Detailed information on the orientational order and rotational motions of probe molecules in membrane systems can be obtained from the analysis of fluorescence depolarization measurements on oriented bilayer systems using the compound motion model approach.

THEORETICAL CONSIDERATIONS OF FLUORESCENCE ANISOTROPY DECAYS

Fluorescence Anisotropy

To capture the essence of the model we restrict the discussion to the probe molecule TMA-DPH. These molecules are commonly considered to behave as cylindrically symmetric objects with their absorption transition moments lying parallel to the long molecular axes. For sake of simplicity we also take the emission transition moment to be parallel to the absorption moment, even though this assumption is not borne out in experiments.

The fluorescence anisotropy $r(t)$ can be expressed as a sum of three correlation functions $G_k(t)$ [1,17,31]

$$r(t)/r(0) = [G_0(t) + 2G_1(t) + 2G_2(t)] \qquad (2)$$

where

$$G_k(t) = \langle D^2_{k0}(\Omega_{BM0}) D^{2*}_{k0}(\Omega_{BMt}) \rangle \qquad (3)$$

here D^L_{mn} are Wigner rotation matrix elements [32] and Ω_{BM0} and Ω_{BMt} denote, respectively, the orientation of the molecular axis at time $t = 0$ and t in the bilayer frame. For the sake of convenience, we consider here only the ratio $r(t)/r(0)$.

Time-Correlation Functions

The time-dependent intensities obtained from angle-resolved experiments on oriented samples can also be expressed in terms of the correlation functions as [10,11]

$$I_{HV}(t)/F(t) \propto 1 - S(2 - 3\sin^2\theta) + G_0(t)(1 - 3\sin^2\theta) - 3G_2(t)(1 - \sin^2\theta) \qquad (4)$$

$$I_{HH}(t)/F(t) \propto 1 - S(2 - 3\sin^2\phi - 3\sin^2\theta) + G_0(t)(1 - 3\sin^2\phi)(1 - 3\sin^2\theta) - 3G_1(t)\sin 2\phi \sin 2\theta + 3G_2(t)(1 - \sin^2\theta)(1 - \sin^2\phi) \qquad (5)$$

Here the first and second indices of the intensity denote the polarization of the excitation and emitted beam respectively, θ is the angle of incidence, and ϕ is the angle of emission measured relative to the normal to the bilayer surface. $S \equiv \langle P_2 \rangle$ is the order parameter of the transition dipole moments and is given by

$$S = \langle 3\cos^2\beta - 1 \rangle/2 \tag{6}$$

where β is the angle between the long molecular axis and the normal to the bilayer plane. The amplitudes of correlation functions at time $t=0$ can be expressed as linear combinations of the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ in a model-independent way. It is clear that the individual decay of each of the correlation functions $G_k(t)$ can be obtained simply by taking the appropriate linear combinations of the time-dependent intensities.

Inspection of Eqs. (4) and (5) shows, furthermore, that the fluorescence intensity decay $F(t)$ can be determined on excitation with horizontally polarized light with $\theta = \sin^{-1}(1/\sqrt{3})$ and $\phi = 0$. The fluorescence emission is observed through a polarizer with its axis set at 45° to the vertical.

The Compound Motion Model

We here treat the compound motions of the probe molecules to be a simple superposition of fast restricted motions within the cage and slow overall rotations of the cage itself. The theoretical framework for the evaluation of the correlation functions was formulated by Wallach [33] and subsequently extended to fluorescence and magnetic resonance spectroscopy by Szabo [31] and Freed and co-workers [28–30]. Only the salient points of the model are summarized below.

The rotational transformation bilayer frame ⧫ molecular frame is decomposed into the transformation bilayer ⧫ cage ⧫ molecule, so that

$$D^2_{k0}(\Omega_{BM}) = \sum_{m=-2}^{2} D^2_{km}(\Omega_{BC}) D^2_{m0}(\Omega_{CM}) \tag{7}$$

where Ω_{BC} and Ω_{CM} denote the orientation of the cage in the bilayer frame and the molecule in the cage frame, respectively. The assumption that the internal motion within the cage is independent of the motion of the cage itself implies that the correlation function $G_k(t)$ can now be expressed in terms of a linear combination of the products of correlation functions for motion of the probe within the cage, G^{CM}, and of the cage in the bilayer frame, G^{BC}:

$$G_k(t) = \sum_{m=-2}^{2} \langle D^2_{km}(\Omega_{BC0}) D^{2*}_{km}(\Omega_{BCt}) \rangle \langle D^2_{m0}(\Omega_{CM0}) D^{2*}_{m0}(\Omega_{CMt}) \rangle \tag{8}$$

$$= \sum_{m=-2}^{2} G^{BC}_{km}(t)\, G^{CM}_{m0}(t) \tag{9}$$

In deriving Eqs. (8)–(10) we have made use of the fact that the bilayer system possesses rotational symmetry about the normal to its plane. We note here that the decay of each of the three correlation functions $G_k(t)$, $k=0,1,2$, observed experimentally reflects the rates of both types of rotational motion.

The order parameters $\langle P_L \rangle^{BM}$ describing the orientational order of the probe molecules in each half of the bilayer are given by products of the order parameters of the molecule in the cage and of the cage within each monolayer:

$$\langle P_L \rangle^{BM} = \langle P_L \rangle^{CM} \langle P_L \rangle^{BC} \tag{10}$$

The broadest orientational distribution function in the bilayer compatible with these order parameters can be reconstructed using the MEM [7–11].

We assume here, for the sake of simplicity, that the cage can be taken to be a cone with half-angle β_0 and that the dynamics of the probe within it can be described in terms of a strong-collision model, with a jump time τ. The correlation functions G^{CM} can thus be expressed in a simple analytical form [1,31]. On the other hand, the motion of the cage in the bilayer frame is described in terms of the BRD model with an orienting potential of the form

$$U(\beta_{BC}) = -kT[\lambda_1 P_1(\beta_{BC}) + \lambda_2 P_2(\beta_{BC})] \tag{11}$$

This form of the potential, with $\lambda_1 \geq 0$, has been chosen as the simplest way of confining the cage to move within the upper half of the bilayer, $\beta_{BC} \leq \pi/2$.

It is important to note that this model predicts nonzero values for the odd-rank order parameters $\langle P_L \rangle^{BM}$. On the other hand, the odd-rank order parameters of the *total* bilayer system will be identically zero since the *macroscopic* orientational distribution function is now given by

$$F(\beta_{BM}) = N[\exp\{-U(\beta_{BM})/kT\} + \exp\{-U(\pi - \beta_{BM})/kT\}] \tag{12}$$

where N is a normalization constant and $U(\beta_{BM})$ is the effective orienting potential in each half of the bilayer.

RESULTS AND DISCUSSION

Angle- and time-resolved fluorescence depolarization experiments were carried out as described previ-

ously on oriented bilayers of POPC [26]. The individual decays of the correlation functions $G_k(t)$, $k=0,1,2$, were obtained by taking the appropriate linear combinations of intensity decays obtained at nine combinations of θ and ϕ, Eqs. (4) and (5). The decays of the three correlation functions using a channel width of 20 ps are shown in Fig. 4. The relative amplitudes of the correlation functions bear little resemblance to the corresponding amplitudes extracted from an analysis of the fluorescence anisotropy decays using the BRD and "wobble-in-cone" models (Figs. 2 and 3, respectively).

The long time plateau exhibited by the correlation function $G_0(t)$ corresponds to the value of S^2 obtained from both steady-state and time-resolved AFD experiments, Eqs. (4) and (5). The correlation functions $G_0(t)$ and $G_2(t)$ decay on a comparable time scale. In marked contrast, the correlation function $G_1(t)$ has additional very slow decay components. We show below that this is in fact a signature of the compound motion prescription.

We have so far not been able to obtain a satisfactory fit of the decay of correlation functions $G_k(t)$ shown in Fig. 4 using either the BRD or the "wobble-in-cone" model. The wobble-in-cone model fails to account for both the initial amplitudes of the correlation functions at time $t=0$ and the decay rates. The latter problem arises simply from the fact that the value of the order parameter $<P_4>$ associated with the cone angle needed to reproduce $<P_2>$ is far too low. The BRD model provides a better description of the data but fails to account for the behavior of the correlation function G_1(t) at long times.

The slow decay to zero of the correlation function $G_1(t)$, however, is consistent with the compound model motion. It arises from the mixing of the correlation functions of the motion of the cage in the bilayer and the probe molecules within the cage, Eq. (11). In general, correlation functions such as $G^{BC}{}_{11}(t)$ have very slow decay components with significant amplitudes. These can easily be confused experimentally with a long-time plateau. These long components are coupled into the experimentally observed correlation decays by virtue of the superposition of the two modes of motion. The same coupling also holds for the correlation function $G_2(t)$ but, in most cases, is too small to be observed. This finding thus provides strong experimental evidence in favor of the compound model description of the motion of probe molecules in bilayer systems.

The experimental fluorescence anisotropy decay curves from TMA-DPH molecules embedded in lipid vesicles of POPC reported by us previously [26] were reanalyzed using the compound motion model. The solutions of the least-squares fits were subjected to the usual statistical tests but were accepted only with the proviso that the motion of the probes within the cage was significantly faster than the motion of the cage in the bilayer. The latter acceptance test was crucial in discriminating between a number of statistically equivalent chi-square minima. The decays of the correlation functions $G_k(t)$ for TMA-DPH in vesicles of POPC obtained from the analysis are shown in Fig. 5. Their close resemblance to the corresponding correlation functions obtained directly from measurements on oriented POPC bilayers (Fig. 4) is a justification for the model. We find that the rotational diffusion rate for the TMA-DPH molecules in the cage is about 100 times faster than that for the cage in the bilayer structure. The orientational distribution function for TMA-DPH in vesicles of POPC is

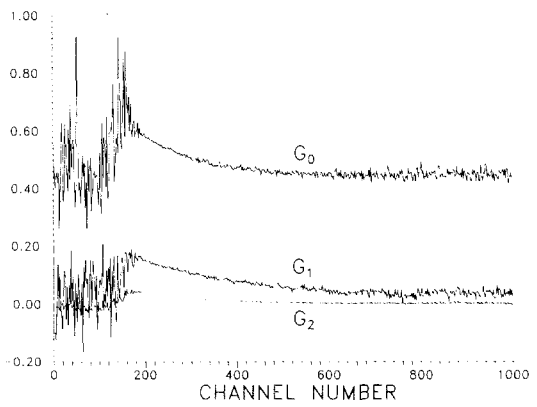

Fig. 4. The experimental decays of the three correlation functions contributing to the fluorescence anisotropy decay, obtained from angle-resolved measurements on oriented bilayers of POPC.

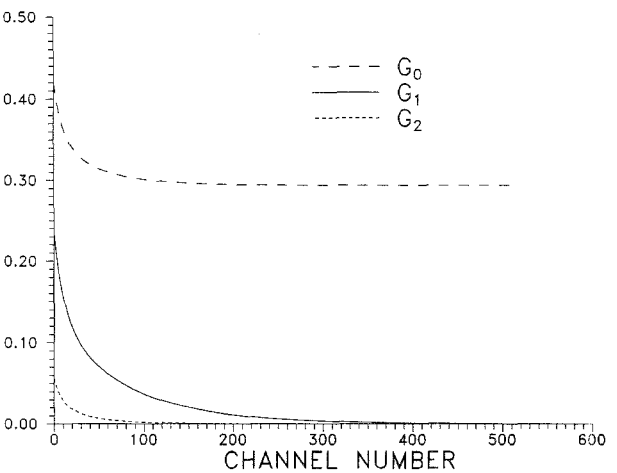

Fig. 5. The decay of the three correlation functions contributing to the fluorescence anisotropy decay from TMA-DPH in vesicles of POPC obtained from an analysis using the compound motion model.

shown in Fig. 6, together with the distributions obtained from the BRD and wobble-in-cone models. It can be seen that the compound motion model yields a unimodal distribution, with a negligible population of molecules lying with their axes parallel to the bilayer surface, $\beta = \pi/2$. We note that the distribution function of the TMA-DPH molecules for the bilayer as a whole can be obtained by reflecting the distributions shown in Fig. 6 for the wobble-in-cone and compound motion models about $\beta = \pi/2$.

CONCLUSIONS

We have shown here that the BRD model accounts for the fast and slow decay modes of the fluorescence anisotropy by predicting probe distributions with two maxima. The "wobble-in-cone" model achieves this time scale separation by virtue of the nonzero odd-rank order parameters. Although these models provide convincing fits to the fluorescence anisotropy decays, the extracted orientational distribution functions are difficult to reconcile with the physicochemical properties of the probes and the generally accepted picture of the "fluid-mosaic" structure of the lipid bilayer. A plausible and consistent picture of probe molecule dynamics in both oriented bilayers and vesicle systems can be obtained by implementing the compound motion model presented above. It is an easy matter to fault the approach described here on the grounds that it contains five free model parameters instead of the two needed for the wobble-in-cone model or the three for the BRD model. However, the measurements of the fluorescence depolarization on oriented bilayer systems show that the latter two models indeed provide an unsatisfactory explanation of the time scale separation.

We believe that, in fact, the wobble-in-cone and BRD models may be used in the interpretation of fluorescence anisotropy decays in investigations of general trends such as the variation of the order parameter $<P_2>$ and the motional rates on changing the temperature or chemical composition of the lipid. Detailed information about the orientational order and rotational motions of probe molecules in membrane systems can be obtained from the analysis of fluorescence depolarization measurements on oriented bilayer systems using the compound motion model approach.

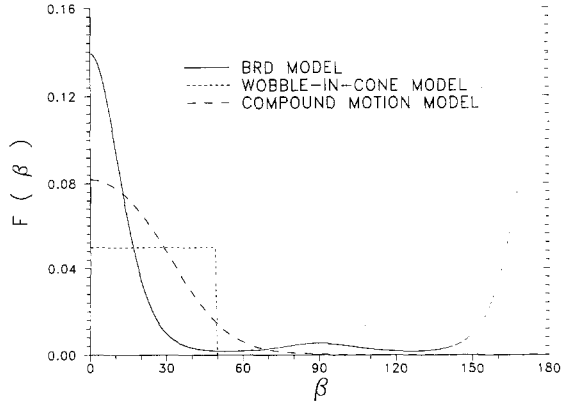

Fig. 6. The orientational distribution of TMA-DPH in vesicles of POPC obtained from the BRD, "wobble-in-cone", and compound motion models.

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