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DIRECT CALCULATION OF THE ORIENTATIONAL DISTRIBUTION
FUNCTION OF PARTIALLY ORDERED ENSEMBLES
FROM THE EPR LINESHAPE

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ABSTRACT

We have developed a method for the determination of the orientational distribution function of partially ordered systems from the EPR lineshape that is an alternative to the usual one of expansion in the elements of the Wigner rotation matrices. The method can be applied to distribution functions whose expansion in the rotation matrix elements are slowly convergent or where the EPR intensity cannot be computed analytically. It can also be used to extract geometrical information relating the principal magnetic axis system to the preferred direction of orientation. An application to a partially ordered ensemble of triplets observed in the photosynthetic bacterium *Rps. viridis* is given, and it is suggested that our technique may prove particularly valuable in analysis of biological systems.

I. INTRODUCTION

In a recent paper Hentschel *et al.*¹ presented a general method for determining the orientational distribution function of partially ordered ensembles of spin systems. The fundamental equation which is the starting point of such calculations can be written as

$$\bar{I}(H_0) = \iiint P(\alpha\beta\gamma) I(\alpha,\beta,\gamma,H_0) d\alpha d\beta d\gamma \quad (1)$$

where $P(\alpha\beta\gamma)$ is the probability that a member of the ensemble can be generated by an Euler rotation with angles α , β , and γ . $I(\alpha,\beta,\gamma,H_0)$ is the EPR intensity at field position H_0 for a member of the ensemble specified by $(\alpha\beta\gamma)$, and $\bar{I}(H_0)$ is the experimental intensity observed at H_0 .

Hentschel *et al.*,¹ following McBrierty², began by expanding the orientational distribution function $P(\alpha\beta\gamma)$ in terms of the elements of the Wigner rotation matrices.

$$P(\alpha\beta\gamma) = \sum_{\ell} \sum_m \sum_n P_{\ell mn} D_{mn}^{\ell}(\alpha,\beta,\gamma) \quad (2)$$

where

$$P_{\ell mn} = \frac{2\ell+1}{8\pi^2} \int_0^{2\pi} \int_0^{2\pi} \int_0^{2\pi} D_{mn}^{\ell}(\alpha,\beta,\gamma) P(\alpha\beta\gamma) d\alpha d\cos\beta d\gamma \quad (2a)$$

is the ℓmn th moment of the distribution function. When equation (2) is substituted into equation (1), the lineshape is expressed in terms of the moments $P_{\ell mn}$. By lineshape fitting with the moments $P_{\ell mn}$ as adjustable parameters, it is possible to determine these moments.

This method is advantageous for two reasons: [1] If the distribution function is of a suitable form, only a few terms in the expansion need be considered. For example, in an axially symmetric ensemble, P_{lmn} is zero if $m \neq 0$ or $n \neq 0$, and we can write

$$P(\alpha\beta\gamma) = \sum_l P_{l00} D_{00}^l(0, \beta, 0) \quad (3a)$$

$$= \sum_l P_{l00} P_l(\cos\beta) \quad (3b)$$

[2] If the EPR intensities $I(\alpha, \beta, \gamma, H_0)$, can be written (or approximated in closed form, the integral of Eq. (2) now consists of integrals over rotation matrix elements and some angular functions. These integrals can sometimes be evaluated analytically, allowing one to calculate subspectra corresponding to the various moments P_{lmn} .

In practice, one may encounter distribution functions which do not rapidly converge when expanded in rotation matrix elements. In such a case, the existence of many of the moments P_{lmn} would preclude a meaningful lineshape analysis. In addition, some EPR intensities have to be computed numerically for each orientation, which makes consideration [2] above irrelevant. In other words, it is not possible to calculate subspectra.

Another difficulty arises when the relation between the molecular axes undergoing partial ordering and the principle magnetic axes is not known, (e.g. the relation between a

partially oriented membrane and the principal magnetic axes of a membrane bound molecule). It is then best to pick trial distribution functions based on a model and attempt to fit the lineshape by treating the relation between axes as an adjustable parameter.

For these reasons, there is a need for a different, albeit equivalent, approach to determining distribution functions of partially ordered ensembles. Our approach is to reduce Eqn. (1) to a double integral over the magnetic variables θ and ϕ , specifying the orientation of the external magnetic field H_0 in the principal magnetic axis system. The form of $\bar{I}$ is then

$$\bar{I}(H_0) = \iint D(\theta, \phi) \cdot I(\theta, \phi, H_0) d\theta d\phi \quad (4)$$

where $I(\theta, \phi, H_0)$ is the EPR intensity measured at field position H_0 when the orientation of the external magnetic field is given by θ, ϕ . $D(\theta, \phi)$ is a density of states function, giving the probability that the external magnetic field has angular orientation between θ and $\theta+d\theta$, and ϕ and $\phi+d\phi$ in the principal magnetic axis system.

Rather than expanding $D(\theta, \phi)$ in a series of moments, we proceed by calculating the form of $D(\theta, \phi)$ directly from considerations of a physical model. In some cases, this

process is trivial, *e.g.* a Gaussian distribution about an axis of symmetry yields

$$D(\theta) = \exp[-\theta^2/\Delta^2] \quad (5)$$

where Δ is a disorder parameter. In the general case, expression of the distribution function in terms of θ and ϕ is more difficult.

We describe here a general method for calculating the density of states $D(\theta, \phi)$. The partially ordered ensemble is generated by set of distinct symmetry operations $R_1 \dots R_n$ with arbitrary weighting functions $g_1 \dots g_n$; the density of states $D(\theta, \phi)$ is obtained as integrals over the weighting functions which can be evaluated numerically. This method is appropriate for any functional form of the distribution function and for any type of magnetic Hamiltonian. It also explicitly provides for the determination of geometrical information (*e.g.* the relation between the molecular axes undergoing partial ordering and the magnetic axes) if this information is not known. Finally, the method is applied to the particular case of a polarized triplet signal observed in the photosynthetic bacterium *Rhodospseudomonas viridis*, whole cells of which have been aligned by magnetic field orientation.

II. THEORY

A. General Form of the Distribution Function

We first define a set of coordinate systems needed for the ensuing derivation. Our approach is essentially the same as that of Hentshel *et al.*¹ The only difference is that we do not define an axis system equivalent to their "laboratory system" which has the external magnetic field, H_0 , along its z axis. We instead define a "laboratory axis system" equivalent to their "sample system". In our "laboratory axis system", H_0 is a constant vector.

The three coordinate systems are then given as follows:

- 1) The laboratory axis system is a coordinate system with its z axis defined by the direction of the external forces that have produced orientation in the sample. In magnetic field alignment, the alignment field H_a , is along the z axis. The orientation of various members of the ensemble of spin systems is specified with respect to this fixed reference frame.
- 2) The principal magnetic axis system is the coordinate system in which the dipolar Hamiltonian $\mathcal{H} = \underline{S} \cdot \underline{D} \cdot \underline{S}$ is diagonal. It is related by a fixed set of Euler angles, α' , β' and γ' , to the intermediate axis system.
- 3) The intermediate axis system serves as a bridge between the laboratory frame and the magnetic axis system. One can think of the magnetic system as being enclosed in a cube; the unit vectors of the intermediate axis system ($\underline{x}'$, $\underline{y}'$, $\underline{z}'$) lie along 3 joined edges of the cube. The principal magnetic

axis system is fixed within the cube, so that an Euler rotation matrix $A(\alpha', \beta', \gamma')$ will bring the principal magnetic axis system into coincidence with the intermediate axes. This transformation is the same for every member of the ensemble.

The ensemble is generated as an array of these cubes in the laboratory axis system. We begin by placing the cube at the origin of the laboratory axis system such that the $\hat{x}', \hat{y}'$ and $\hat{z}'$ unit vectors are coincident with the $\hat{x}, \hat{y}$ and $\hat{z}$ unit vectors. Then the cube is rotated by n rotations ($R_1 \dots R_n$) in the laboratory frame into a member of the ensemble. Associated with each rotation is a distribution function $g_i(\alpha_i)$ giving the probability that the i th angular variable has the value α_i .

The task is to express the unit vectors of the rotated cube ($\hat{x}', \hat{y}', \hat{z}'$) in the laboratory coordinate system. This objective can be accomplished by applying the n rotations to $\hat{x}', \hat{y}'$ and $\hat{z}'$ which are originally equal to $(1,0,0)$, $(0,1,0)$ and $(0,0,1)$. Rotation of an arbitrary vector $\underline{u}(x,y,z)$ counterclockwise through an angle ϕ about an arbitrary axis is given by

$$\underline{u}'(x,y,z) = R^{-1}(\phi)\underline{u}(x,y,z) \quad (6)$$

where $\underline{u}'(x,y,z)$ is the rotated vector in the original coordinate system and $R(\phi)$ is a real orthogonal rotation matrix (See Appendix A). Thus, when the set of n rotations

is applied to $\hat{x}'$, $\hat{y}'$ and $\hat{z}'$, the result is

$$\hat{x}' = R_n^{-1}(\alpha_n) \dots R_1^{-1}(\alpha_1)(1,0,0) \quad (7a)$$

$$\hat{y}' = R_n^{-1}(\alpha_n) \dots R_1^{-1}(\alpha_1)(0,1,0) \quad (7b)$$

$$\hat{z}' = R_n^{-1}(\alpha_n) \dots R_1^{-1}(\alpha_1)(0,0,1) \quad (7c)$$

The external magnetic field in the intermediate axis system, H' , is now easily found to be

$$H' = (\hat{x}' \cdot H_o, \hat{y}' \cdot H_o, \hat{z}' \cdot H_o) \quad (8)$$

Rearrangement using equations (7a - 7c) yields

$$H' = [R_n^{-1}(\alpha_n) \dots R_1^{-1}(\alpha_1)]^T H_o \quad (9a)$$

$$= R_1(\alpha_1) \dots R_n(\alpha_n) H_o \quad (9b)$$

We denote the coordinates of H' by the following functions

$$H'_x = F_x(\alpha_1 \dots \alpha_n) \quad (10a)$$

$$H'_y = F_y(\alpha_1 \dots \alpha_n) \quad (10b)$$

$$H'_z = F_z(\alpha_1 \dots \alpha_n) \quad (10c)$$

The external magnetic field in the magnetic axis system, H'' , is then

$$H'' = A(\alpha', \beta', \gamma') \cdot H' \quad (11)$$

where α' , β' and γ' are constants, perhaps unknown.

Now we can write down the EPR intensity at magnetic field H_0 directly as an integral over the variables $\alpha_1 \dots \alpha_n$.

$$\begin{aligned} \bar{I}(H_0) = & \frac{1}{N} \int_{\alpha_1} \dots \int_{\alpha_n} I(H_x'', H_y'', H_z'') g_1(\alpha_1) \dots g_n(\alpha_n) \\ & \times d\alpha_1 \dots d\alpha_n \end{aligned} \quad (12)$$

where N is a normalization constant, and H_x'' , and H_y'' are functions of $\alpha_1 \dots \alpha_n$, α' , β' , and γ' .

The set of points $\{(\alpha_i)\}$ such that $H_x''^2 + H_y''^2 + H_z''^2 = H_0^2$ is a two dimensional manifold in $(n+1)$ space. We therefore transform to a new set of variables in which H_x'' , H_y'' , and H_z'' are functions of only two variables θ and ϕ . This transformation involves mapping the old manifold into a two dimensional manifold in three space.

This transformation can be accomplished by defining a new set of variables $v_1 \dots v_{n-2}$ to complement θ and ϕ ; θ and ϕ are defined to be the magnetic variables giving the location of H_0 in the principal magnetic axis system; $v_1 \dots v_{n-2}$ must be chosen to satisfy the formal requirements of the coordinate transformation. We thus perform the transformation

$$(H_0, \alpha_1, \dots \alpha_n) \rightarrow (H_0, \phi, v_1 \dots v_{n-2}, \theta) \quad (13)$$

under the constraints

$$H_x'' = \sin\theta \cos\phi \quad (14a)$$

$$H_y'' = \sin\theta \sin\phi \quad (14b)$$

$$H_z'' = \cos\theta \quad (14c)$$

and θ and ϕ are determined by the rotation angles $\alpha_1, \dots, \alpha_n$, α' , β' , and γ' . Application of the n-dimensional-change-of-variable theorem to equation (12) yields an integral in terms of the new variables

$$\begin{aligned} \bar{I}(H_0) = & \frac{1}{N} \int_{\theta} \int_{\phi} I[H''_x(\theta, \phi), H''_y(\theta, \phi), H''_z(\theta, \phi)] d\theta d\phi \\ & \times \int_{v_1} \dots \int_{v_{n-2}} \prod_{i=1}^n g_i[\alpha_i(\phi, v_1 \dots v_{n-2}, \theta)] \\ & \times \left| \frac{\partial \alpha_1 \dots \alpha_n}{\partial \phi, v_1 \dots v_{n-2}, \theta} \right| dv_1 \dots dv_{n-2} \quad (15) \end{aligned}$$

where $(\partial \alpha_1 \dots \alpha_n / \partial \phi, v_1 \dots v_{n-2}, \theta)$ is the Jacobian of the coordinate transformation.

By inspection, we can set

$$\begin{aligned} D(\theta, \phi) = & \frac{1}{N} \int_{v_1} \dots \int_{v_{n-2}} \prod_{i=1}^n g_i[\alpha_i(\phi, v_1 \dots v_{n-2}, \theta)] \\ & \times \left| \frac{\partial \alpha_1 \dots \alpha_n}{\partial \phi, v_1 \dots v_{n-2}, \theta} \right| dv_1 \dots dv_{n-2} \quad (16) \end{aligned}$$

Equation (16) is the key result of this paper. This equation expresses the distribution function of H_0 in any axis system that has a fixed relation to the intermediate axis system (e.g. Euler rotation angles α' , β' , and γ'). In particular, it gives the distribution of H_0 in the intermediate axis system when $\alpha' = \beta' = \gamma' = 0$. For use in equation (4), one specifically needs $D(\theta, \phi)$ for the distribution of H_0 in the principal magnetic axis system.

B. Generalized Coordinate Transformation

Our procedure will be to use equation (16) to calculate a distribution function in the intermediate axis system, $D'(\theta', \phi')$, where θ' and ϕ' specify the orientation of H_0 in the intermediate axis system, and then construct $D(\theta, \phi)$ for the principal magnetic axis system by use of the Euler rotation matrix $A(\alpha', \beta', \gamma')$. This approach is advantageous because one does not have to worry about the angles α' , β' , and γ' when calculating $D'(\theta', \phi')$. Also, careful choice of an intermediate axis system simplifies the calculations. In particular, one should choose an intermediate axis system such that the first rotation (α_1) is about the laboratory z axis. Then, from equation (9)

$$H' = \begin{pmatrix} \cos\alpha_1 & \sin\alpha_1 & 0 \\ -\sin\alpha_1 & \cos\alpha_1 & 0 \\ 0 & 0 & 1 \end{pmatrix} R_2(\alpha_2) \dots R_n(\alpha_n) H_0 \quad (17)$$

More explicitly

$$H'_x = \sin\theta' \cos\phi' = F_x^{n-1} \cos\alpha_1 + F_y^{n-1} \sin\alpha_1 \quad (18a)$$

$$H'_y = \sin\theta' \sin\phi' = -F_x^{n-1} \sin\alpha_1 + F_y^{n-1} \cos\alpha_1 \quad (18b)$$

$$H'_z = \cos\theta' = F_z^{n-1} = F_z(\alpha_2 \dots \alpha_n) \quad (18c)$$

where

$$R_2(\alpha_2) \dots R_n(\alpha_n) H_0 = (F_x^{n-1}, F_y^{n-1}, F_z^{n-1}) \quad (19)$$

and F_x^{n-1} , F_y^{n-1} , and F_z^{n-1} are functions of $\alpha_2 \dots \alpha_n$.

The following transformation is convenient.

$$\begin{aligned} \alpha_1 &= f_1(\phi', v_1 \dots v_{n-2}, \theta') \\ \alpha_2 &= v_1 \\ &\cdot \\ &\cdot \\ &\cdot \\ \alpha_{n-1} &= v_{n-2} \\ \alpha_n &= f_2(v_1 \dots v_{n-2}, \theta') \end{aligned} \quad (20)$$

where α_n is defined by solving the equation

$$\cos \theta' = F_z(\alpha_2 \dots \alpha_n) \quad (21)$$

for α_n and setting $\alpha_2 \dots \alpha_{n-1}$ equal to $v_1 \dots v_{n-2}$.

Clearly,

$$\frac{\partial \alpha_i}{\partial v_j} = \delta_{i,j+1} \quad 2 \leq i \leq n-1 \quad (22)$$

and

$$\frac{\partial \alpha_i}{\partial \phi'} = 0 \quad i \neq 1 \quad (23)$$

Then, from equations (18a) and (18b)

$$\frac{\partial \sin \theta' \cos \phi'}{\partial \phi'} = -\sin \theta' \sin \phi' = (-F_x^{n-1} \sin \alpha_1 + F_y^{n-1} \cos \alpha_1) \frac{\partial \alpha_1}{\partial \phi'} \quad (24)$$

$$-\sin\theta'\sin\phi' = \sin\theta'\sin\phi'\left(\frac{\partial\alpha_1}{\partial\phi'}\right) \quad (25)$$

or

$$\frac{\partial\alpha_1}{\partial\phi'} = -1 \quad (26)$$

$$\alpha_1 = -\phi' + f_3(v_1, \dots, v_{n-2}, \theta') \quad (27)$$

Using equations (22), (23), and (26), the Jacobian, J , simplifies to

$$|J| = \frac{\partial\alpha_n}{\partial\theta'} \quad (28)$$

which can be obtained from (18c) by differentiating both sides with respect to θ' ,

$$-\sin\theta' = \frac{\partial F_z}{\partial\alpha_n} \frac{\partial\alpha_n}{\partial\theta'} \quad (29)$$

or,

$$|J| = \frac{\sin\theta'}{\left| \frac{\partial F_z(\alpha_2 \dots \alpha_n)}{\partial\alpha_n} \right|} \quad (30)$$

with $\alpha_2 \dots \alpha_n$ replaced by their transformed variables $v_1 \dots v_{n-2}, f_2(v_1 \dots v_{n-2}, \theta')$. Substitution into equation (16) yields

$$\begin{aligned} \mathcal{D}'(\theta', \phi') = & \frac{\sin\theta'}{N'} \int_{v_1} \dots \int_{v_{n-2}} g_1[-\phi' + f_3(v_1 \dots v_{n-2}, \theta')] g_n[f_2(v_1 \dots v_{n-2}, \theta')] \\ & \times \frac{\prod_{i=1}^{n-2} g_i(v_i) dv_i}{\left| \frac{\partial F_z[v_1 \dots v_{n-2}, f_2(v_1 \dots v_{n-2}, \theta')]}{\partial\alpha_n} \right|} \end{aligned}$$

If $g_1(\alpha_1) = 1$, we have an axially symmetric distribution function [i.e. $\mathcal{D}'(\theta', \phi')$ is a function only of θ']. If we have a randomly oriented system, it can be shown that

$$\mathcal{D}'(\theta', \phi') = \text{constant} \times \sin\theta' \quad (32)$$

Clearly, one has some choice about which variables to set equal to the v_i 's, which to set equal to ϕ' and which to set equal to a function of $v_1 \dots v_{n-2}$, and θ' . Intelligent decisions in this regard can be used to simplify equation (31).

It is now necessary to transform the distribution of $H_{\underline{O}}$ in the intermediate axis system to one in the principal magnetic axis system. The Euler rotation matrix $A(\alpha', \beta', \gamma')$ in equation (11) establishes the transformation from θ' and ϕ' to θ and ϕ . Since $A(\alpha', \beta', \gamma')$ is a constant rotation matrix, $\sin\theta/\sin\theta'$ is the Jacobian for the transformation. Therefore

$$\mathcal{D}(\theta, \phi) = \frac{\sin\theta}{\sin\theta'} \mathcal{D}'(\theta', \phi')$$

where

$$\theta = \cos^{-1}(H_z''/H_O)$$

and

$$\phi = \tan^{-1}(H_x''/H_y'')$$

specify the orientation of H'' in the principal magnetic axis system. H_x'' , H_y'' , and H_z'' are found from equation (11). The angles α' , β' , and γ' , which establish the relation between the intermediate axis system and the principal magnetic axis system

can be treated as adjustable parameters if they are not known. In conclusion, in this section, we have outlined a prescription for computing $\mathcal{D}(\theta, \phi)$ for all values of θ and ϕ .

III. APPLICATION - ORIENTATION AVERAGING IN *Rps. viridis*.

A. Description of the Experimental System

Our experimental results on the triplet signals obtained from the photosynthetic bacterium *Rps. viridis* will be described in detail elsewhere (see reference 3). Here we calculate, using the method of section II, the distribution function $\mathcal{D}'(\theta', \phi')$ for intact bacterial cells aligned in a magnetic field.

The *Rps. viridis* cell can be represented as a prolate ellipsoid. We shall for the moment ignore end effects (they are indistinguishable from another sort of imperfection in ordering, as will be explained later), and consider the cell to be a perfect cylinder. Inside the cell are membranes rolled into concentric cylindrical sheets having a common axis with the long axis of the cell. Embedded in the membranes are reaction center particles, which contain the paramagnetic species that gives rise to the observed light induced triplet signal. We assume that the principal axis system of the triplet is fixed with respect to the reaction center particle.

Linear dichroism⁴ and photoselection⁵ experiments suggest that there is a unique axis of the reaction center particle which is normal to the membrane surface. Our ensemble of intermediate axis systems is then a set of reaction center particles, randomly distributed in the cylindrical membrane structure, each of which has the designated axis normal to the membrane at that point.

A 21 kg magnetic field orients the *Rps. viridis* so that the long axis of the cylinder tends to lie in the plane normal to the orienting field. One can then freeze the sample and perform EPR experiments with the external magnetic field either parallel to, or perpendicular to, the alignment field H_a .

B. Symmetry Operations in the *Rps. viridis* Ensemble

We choose the $\hat{z}'$ intermediate axis to be the unique axis of the reaction center particle. This choice fits the guideline of sec. II, because we assume that an unrestricted rotation of the particle around this axis is possible. This operation is then our first rotation, and we designate the angular variable as λ .

Two further symmetry operations are possible in the laboratory frame; rotation of the cylindrical cell about its long axis, and its rotation around the direction of the alignment field. We arrange the laboratory coordinate system so that the z axis coincides with the alignment field axis.

Then, the rotation about H_a corresponds to a rotation about the laboratory z axis; the magnitude of this rotation is specified by the angle, χ , that the long axis of the cell makes with the laboratory Y axis. Rotation about the cylinder axis is given by an angle μ , that a designated normal to the cylinder surface makes with H_a (see fig.1).

It remains only to take into account imperfections in ordering. We consider two sources of this,

- 1) Incomplete alignment of the cells in the magnetic field (i.e., tilt of the long axis of the cell from the plane normal to the alignment field)
- 2) Deviation of the membranes from perfect cylindrical shape. This could arise in two ways: (a) distortion of the entire cell from an ellipsoidal shape; (b) local imperfections in the membrane surface.

A careful examination of the geometrical considerations indicates that the effect of both of these factors can be described by one parameter, the angle that the membrane normal makes with the projection of the long axis of the cell on the plane perpendicular to the alignment field (see fig.1).

For the case of perfect ordering, this angle, ψ , is always 90° . Both incomplete alignment and membrane curvature reduce the angle in identical fashion.

We define the complement of ψ , $90^\circ - \psi$, to be the "wobble" angle ω . We further assume that deviations of ω from 0° are random fluctuations, and thus set the probability distribution in ω to be

$$g_\omega(\omega) = \cos\omega \exp[-\omega^2/\Delta^2] = \cos\omega h(\omega) \quad (33)$$

where Δ is the "disorder parameter" giving the standard deviation of ω , and $\cos\omega$ is the proper angular volume element, corresponding to the arc length swept out by a vector inclined at angle ω when the cell is rotated around its long axis.

We introduce the "wobble" into the density of states calculation as a rotation around the axis perpendicular to the long axis of the cell. A careful choice of the order of operations then allows us to represent every symmetry operation as one rotation in the laboratory coordinate system (λ about the z axis, ω about the x axis, μ about the y axis, and χ about the z axis). The final result for the external magnetic field in the intermediate axis system, $\underline{H}'$, is given by

$$\underline{H}' = [R_4^{-1}(\chi) R_3^{-1}(\mu) R_2^{-1}(\omega) R_1^{-1}(\lambda)]^T \underline{H}_O \quad (34a)$$

$$= R_1(\lambda) R_2(\omega) R_3(\mu) R_4(\chi) \cdot \underline{H}_O \quad (34b)$$

C. $\underline{H}_0$ Parallel to $\underline{H}_a$

We set $\underline{H}_0 = (0,0,1)$, i.e. along the alignment field direction. Then, the rotation $R(\chi)$ is unnecessary, since the projection of $(0,0,1)$ on the $\hat{x}'$, $\hat{y}'$, and $\hat{z}'$ axes is unaffected by a final rotation around the z axis. Following section IIb, we obtain

$$\alpha_1 = \lambda \rightarrow -\phi' + f_3(v_1, \theta') \quad (35a)$$

$$\alpha_2 = \omega \rightarrow v_1 \quad (35b)$$

$$\alpha_3 = \mu \rightarrow f_2(v_1, \theta') \quad (35c)$$

From (34) without $R(\chi)$,

$$F_z = \cos \theta' = \cos \mu \cos v_1 \quad (36a)$$

$$\text{or} \quad \cos \mu = \cos \theta' / \cos v_1 \quad (36b)$$

Evaluation of $\partial F_z / \partial \mu$ and substitution into equation (31) results in

$$\mathcal{D}'_{\parallel}(\theta') = \frac{\sin \theta'}{N'_{\parallel}} \cdot \int_{\cos v_1=1}^{\cos v_1=\cos \theta'} \frac{g_{\omega}(v_1)}{(1 - \frac{\cos^2 \theta'}{\cos^2 v_1})^{1/2}} \cdot \frac{1}{\cos v_1} dv_1 \quad (37)$$

Substituting for v_1 , we transform the above integral (see Appendix B) to

$$\mathcal{D}'_{\parallel}(\theta') = \frac{\sin \theta'}{2N'_{\parallel}} \cdot \int_0^{\pi} h(v_1) du \quad (38)$$

where $v_1 = \cos^{-1}[\frac{1}{2}(1 + \cos^2 \theta' + \cos u \sin^2 \theta')]^{1/2}$.

This integral has no singularities, and can be easily evaluated numerically. Fig. 2 plots $\mathcal{D}'_{\parallel}(\theta')$ vs. θ' for several values of the disorder parameter, Δ .

D. $\underline{H}_0$ Perpendicular to $\underline{H}_a$

We now set $\underline{H}_0 = (0,1,0)$ [a choice of $(1,0,0)$ would be equivalent). Proceeding as before, we obtain

$$\underline{H}' = R_1(\lambda) R_2(\omega) R_3(\mu) R_4(\chi) \cdot \begin{vmatrix} 0 \\ 1 \\ 0 \end{vmatrix} \quad (39)$$

This yields

$$\alpha_1 = \lambda \rightarrow -\phi' + f_3(v_1, v_2, \theta') \quad (40a)$$

$$\alpha_2 = \omega \rightarrow v_1 \quad (40b)$$

$$\alpha_3 = \chi \rightarrow v_2 \quad (40c)$$

$$\alpha_4 = \mu \rightarrow f_2(v_1, v_2, \theta') \quad (40d)$$

From equation (39)

$$F_z = \cos \theta' = \cos \mu \cos \omega \cos \chi + \sin \omega \sin \chi \quad (40e)$$

$$\text{or } \cos \mu = (\cos \theta' - \sin v_1 \sin v_2) / (\cos v_1 \cos v_2) \quad (40f)$$

Evaluation of $\partial F_z / \partial \mu$, substitution into equation (31), and evaluation of the v_2 integral yields (see Appendix C for details)

$$D_1'(\theta') = \frac{\sin \theta'}{N_1'} \cdot \int_0^{\pi/2} h(v_1) G(\theta', v_1) dv_1 \quad (41)$$

where

$$G(\theta', v_1) = 2K(k) \{ [1 + \sin(\theta' + v_1)] [1 + \sin(\theta' - v_1)] \}^{-1/2} \quad (42)$$

$$k = \left\{ [1 + 2(\sin(\theta' + v_1) + \sin(\theta' - v_1))] / \{ [1 + \sin(\theta' - v_1)][1 - \sin(\theta' + v_1)] \} \right\}^{1/2}$$

for $\sin(\theta' - v_1) + \sin(\theta' + v_1) \geq 0$.

$K(k)$ is the complete elliptic integral of the first kind. The integrand has a singularity at $\theta' + v_1 = \pi/2$ (here $k=1$). In spite of this singularity, the integral can still be evaluated numerically. Fig. (3) plots $\mathcal{D}'_1(\theta')$ vs. θ' for several values of Δ .

CONCLUSION

Our method of determining the orientational distribution function of a partially ordered system from the EPR lineshape is, in a sense, conceptually different from that of Hentschel *et al.*¹ This latter treatment makes no physical assumptions about the form of the distribution function other than that it can be conveniently expanded in the Wigner rotation matrix elements, and the effectiveness of their parametrization of the distribution function is thus dependent upon how rapidly the series of moments $P_{\ell mn}$ converges. Furthermore, no explicit prescription is given for extracting geometrical information (*i.e.* relation of the principal axis system to the preferred alignment direction), although presumably it would be possible to introduce parameters specifying these quantities.

Our method is based on constructing a distribution function which from the first takes into account the mathematical nature of the ordering in the system, and the symmetry properties of the ensemble. The choice of fitting parameters can thus be

tailored to one's physical intuition about the ordering of the system and not restricted to a rigidly prescribed expansion. In the problem described in section III, for example, the distribution function was fit to a single Gaussian parameter, Δ , whereas expansion in angular functions could require many terms (and thus require fitting many parameters) to insure convergence. We were also able to fit two geometrical parameters specifying the orientations of the normal to the membrane in the principal magnetic axis system of the triplet (see reference 3). The fit was relatively sharp when compared to experimental error, *i.e.* only a small set of values of the three above parameters gave an adequate fit of the theoretical spectra to the experimental EPR intensity measured parallel and perpendicular to the alignment field.

It was also possible to include in our calculation orientation-dependent intersystem crossing to the ground state singlet (see reference 3). This phenomenon makes a moment analysis very difficult, because analytical "subspectra" cannot be easily worked out for this case.

Our approach has particular appeal in applications to biological problems. In many simpler systems (*e.g.* crystals, liquid crystals) high degrees of ordering are common, and typical distribution functions are trigonometric terms with little random variation; here, expansion in Wigner functions

is straightforward and fruitful. Our method would probably yield equivalent results, but would be unlikely to produce a significant saving in computer time or gain in conceptual understanding.

Biological systems, however, are complex heterogeneous structures and often contain inhomogeneous ensembles which have small but significant statistical variations. Such variations blur the observation of orientation effects, making the oriented EPR spectra look more like a random spectrum and less like that of a single crystal.

The resulting smaller and less sharp effects make it difficult to extract meaningful quantitative information about such systems. It is in this regard that we hope that our formulation will prove valuable. We have shown that it is possible to take into account the random disorder that is characteristic of biological ensembles and still extract sharp values for the relatively invariant properties of the ensemble members; we have also obtained a good estimate of the degree of disorder present. These results³ were achieved with a modest amount of computer time, and from a conceptual viewpoint that is perhaps more intuitive and easily understandable than the Wigner rotation matrix formalism.

The method described here can be applied to calculation of other properties of partially ordered ensembles. For example, optical properties like linear dichroism can be determined if the distribution function can be modeled effectively.

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APPENDIX A:

The matrices that describe the rotation are defined as follows. $R_{\hat{u}}(\phi)$ is the rotation matrix for rotation counter-clockwise through an angle ϕ about an axis in the $\hat{u}$ direction. The matrix elements of $R_{\hat{u}}(\phi)$ are given by

$$[R_{\hat{u}}(\phi)]_{ij} = \cos(\angle x_i x'_j) \quad (A-1)$$

where $(\angle x_i x'_j)$ is the angle between the i th axis of the original coordinate system and the j th axis of the coordinate system obtained after the rotation.

APPENDIX B

Equation (38) is obtained from equation (32) by the following substitution:

$$\cos u = \frac{-1}{\sin^2 \theta'} (1 + \cos^2 \theta' - 2 \cos^2 v_1) \quad (\text{B-1})$$

The upper limit at $\cos v_1 = \cos \theta'$ is transformed to $\cos u = -1$, or $u = \pi$. The lower limit at $\cos v_1 = 1$ is transformed to $\cos u = +1$ or $u = 0$. From equation (B-1), one can readily obtain

$$dv_1 = \frac{\sin u \sin^2 \theta' du}{4 \sin v_1 \cos v_1} \quad (\text{B-2})$$

$$\cos^2 v_1 = \frac{1}{2} (1 + \cos^2 \theta' + \cos u \sin^2 \theta') \quad (\text{B-3})$$

and

$$\sin v_1 = \left[\frac{1}{2} \sin^2 \theta' (1 - \cos u) \right]^{1/2} \quad (\text{B-4})$$

Substitution into equation (37) yields

$$\begin{aligned} \mathcal{D}'_{\parallel}(\theta') &= \frac{\sin \theta'}{N'_{\parallel}} \int_0^{\pi} h(v_1) [\sin u \sin^2 \theta' du] / \{4 \left[\frac{1}{2} \sin^2 \theta' (1 - \cos u) \right]^{1/2} \\ &\times \left[\frac{1}{2} + \frac{\cos^2 \theta'}{2} + \frac{\sin^2 \theta'}{2} \cos u - \cos^2 \theta' \right]^{1/2} \} \quad (\text{B-5}) \end{aligned}$$

which simplifies to

$$\mathcal{D}_{\parallel}(\theta) = \frac{\sin \theta'}{2 N'_{\parallel}} \int_0^{\pi} h(v_1) du \quad (\text{B-6})$$

where

$$v_1 = \cos^{-1} \left\{ \left[\frac{1}{2} (1 + \cos^2 \theta' + \cos u \sin^2 \theta') \right]^{1/2} \right\}.$$

APPENDIX C

Using equations (31) and (40e), the perpendicular density of states can be written down

$$\rho'_1(\theta') = \frac{\sin\theta'}{N'_1} \int_{v_1} \int_{v_2} \frac{h(v_1)}{\cos v_1 \sin \mu \cos v_2} dv_1 dv_2 \quad (C-1)$$

Solving equation (40e) for $\sin \mu$ and substituting into equation (C-1) yields

$$\rho'_1(\theta') = \frac{\sin\theta'}{N'_1} \int_{v_1} \frac{h(v_1)}{\cos v_1} dv_1 \int_{v_2} \frac{dv_2}{\cos v_2} \times \{1 - [(\cos\theta' + \sin v_1 \sin v_2) / (\cos v_1 \cos v_2)]^2\}^{-1/2} \quad (C-2)$$

The denominator of the v_2 integral is quadratic in $\sin v_2$.

When expanded and factored, $\rho'_1(\theta')$ simplifies to

$$\rho'_1(\theta') = \frac{\sin\theta'}{N'_1} \int_{v_1} dv_1 h(v_1) \int_{v_2} dv_2 \{[\sin(\theta' - v_1) - \sin v_2][\sin(\theta' + v_1) + \sin v_2]\}^{-1/2} \quad (C-3)$$

The v_2 integration is performed over the region where the integrand is real; i.e. the $\sin v_2$ quadratic is greater than zero. This requirement means the v_2 integration is from $-(\theta' + v_1)$ to $(\theta' - v_1)$. Now, make the substitution $x = \sin v_2$ and equation (C-3) becomes

$$\rho'_1(\theta') = \frac{\sin\theta'}{N'_1} \int_{v_1} dv_1 h(v_1) \int_{-\sin(\theta' + v_1)}^{\sin(\theta' - v_1)} dx \{(x-1)(x+1)\}^{-1/2} \times [x - \sin(\theta' - v_1)][x + \sin(\theta' + v_1)]^{-1/2} \quad (C-4)$$

The x integral is an elliptic integral of the first kind where the limits of integration are two roots of the quartic in x . Using equation 254.00 in Boyd & Freidman⁵, the x integration can be performed. The end result for $\mathcal{D}'_1(\theta')$ is

$$\mathcal{D}'_1(\theta') = \frac{\sin \theta'}{N'_1} \int_0^{\pi/2} h(v_1) G(\theta', v_1) dv_1 \quad (C-5)$$

where $G(\theta', v_1)$ is given by equation (42) and the limits of the v_2 integration are by inspection 0 and $\pi/2$.

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FIGURE CAPTIONS

1. Axis systems and symmetry operations in the *Rps. viridis* ensemble. x, y , and z define the laboratory axis system; x', y' , and z' define the intermediate axis system; and x'', y'' , and z'' define the principal magnetic axis system. L is the long axis direction of the cell. P is the projection of L onto the xy plane. ψ is the angle between the membrane normal (z') and P . χ , μ , and λ are rotations about the indicated axes. H_A is the alignment field direction, and $H_{\parallel}$ and $H_{\perp}$ show the external magnetic field directions used in the EPR experiments. The insert displays a section of the cylindrical membrane surface.

2. Distribution function [$\mathcal{D}_{\parallel}(\theta')$] for H_0 parallel to H_a ;
 - a) Plot of $\mathcal{D}_{\parallel}(\theta')$ for several values of the disorder parameter Δ in radians. b) Plot of $\mathcal{D}_{\parallel}(\theta')/\sin\theta'$ versus θ' for several values of the disorder parameter Δ in radians. Note that $\mathcal{D}_{\parallel}(\theta')/\sin\theta'$ must approach a constant as Δ approaches ∞ ($\Delta = \infty$ corresponds to a random sample).

3. Distribution function [$\mathcal{D}_{\perp}(\theta')$] for H_0 perpendicular to H_a :
 - a) Plot of $\mathcal{D}_{\perp}(\theta')$ versus θ for several values of the disorder parameter Δ in radians. b) Plot of $\mathcal{D}_{\perp}(\theta')/\sin\theta'$ versus θ for several values of the disorder parameter Δ in radians. Note that $\mathcal{D}_{\perp}(\theta')/\sin\theta'$ must approach a constant as Δ approaches ∞ ($\Delta = \infty$ corresponds to a random sample).

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Fig. 1

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Fig 4 (insert)

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Fig 2a

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Fig. 26

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Fig. 39

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Fig. 3b

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