

# Lawrence Berkeley National Laboratory

## Recent Work

### Title

A NEW APPROACH TO THE THEORY OF LINEAR DICHROISM IN PARTIALLY ORDERED SYSTEMS: APPLICATION TO REACTION CENTERS AND WHOLE CELLS OF PHOTOSYN-THETIC BACTERIA

### Permalink

<https://escholarship.org/uc/item/3sg3h8r9>

### Author

Nairn, J.A.

### Publication Date

1979-08-01



# Lawrence Berkeley Laboratory

UNIVERSITY OF CALIFORNIA

## CHEMICAL BIODYNAMICS DIVISION

Submitted to Biophysical Journal

A NEW APPROACH TO THE THEORY OF LINEAR DICHROISM  
IN PARTIALLY ORDERED SYSTEMS: APPLICATION TO  
REACTION CENTERS AND WHOLE CELLS OF PHOTOSYNTHETIC  
BACTERIA

John A. Nairn, Richard Friesner, Harry A. Frank  
and Kenneth Sauer

August 1979

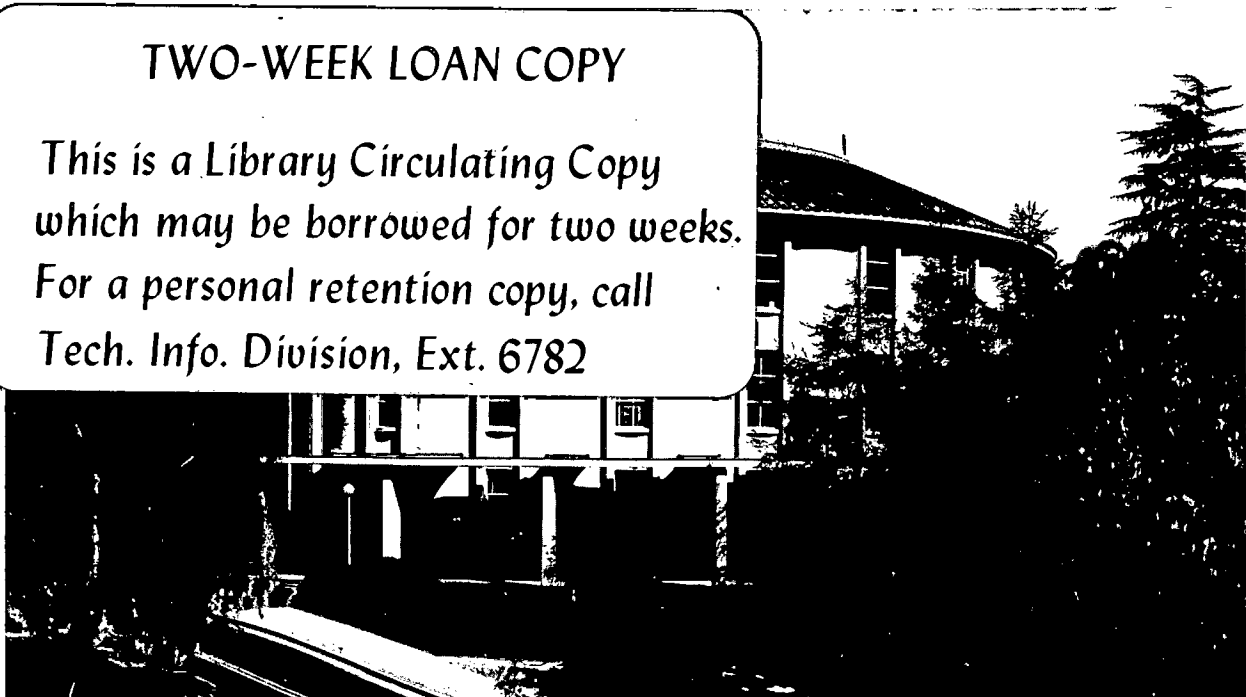
RECEIVED  
LAWRENCE  
BERKELEY LABORATORY

SEP 14 1979

LIBRARY AND  
DOCUMENTS SECTION

### TWO-WEEK LOAN COPY

*This is a Library Circulating Copy  
which may be borrowed for two weeks.  
For a personal retention copy, call  
Tech. Info. Division, Ext. 6782*



LBL-9655 e.1

## DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

A NEW APPROACH TO THE THEORY OF LINEAR DICHROISM  
IN PARTIALLY ORDERED SYSTEMS:  
APPLICATION TO REACTION CENTERS AND WHOLE CELLS  
OF PHOTOSYNTHETIC BACTERIA

John A. Nairn<sup>†</sup>, Richard Friesner,  
Harry A. Frank<sup>‡</sup>, and Kenneth Sauer

Department of Chemistry and  
Laboratory of Chemical Biodynamics  
Lawrence Berkeley Laboratory  
University of California  
Berkeley, CA 94720

<sup>†</sup>Supported by a University of California Regents Fellowship

<sup>‡</sup>Supported by a Postdoctoral Fellowship from the National  
Institutes of Health

## ABSTRACT

We have developed a new approach to the theory of linear dichroism in partially ordered systems. The description of the partially ordered ensemble uses a density of states function,  $D(\theta, \phi, \underline{\Delta})$ , which gives the probability that the direction of polarization for incident polarized light has spherical angles  $\theta$  and  $\phi$  in an axis system fixed with respect to the molecule;  $\underline{\Delta} = (\Delta_1, \Delta_2 \dots \Delta_n)$  is a set of parameters that describes the partial ordering. We derive new formulas for linear dichroism using the density of states function and then apply these formulas to the analysis of linear dichroism in reaction centers and whole cells of photosynthetic bacteria. One advantage of our approach is that the order parameter,  $\underline{\Delta}$ , provides a more complete description of the distribution function than the traditional order parameters used by other authors. Knowledge of  $\underline{\Delta}$  gives a good physical description of the partial ordering and allows one to calculate accurate limits for the range of possible orientations of the transition moments.

## INTRODUCTION

Linear dichroism refers to a dependence of the absorption of polarized light on the direction of polarization. This dependence arises because the molecular absorbance due to a transition moment  $\mu$  depends on the angle between  $\mu$  and the polarized field  $E$ . An analysis of linear dichroism data can, therefore, enable one to extract structural information on the orientation of the transition moment. For single crystals or perfectly ordered systems the theory is straightforward (1,2), but for partially ordered systems the theory is complex (2). We develop here a new approach to the theory of linear dichroism in partially ordered systems.

Most previous theories of linear dichroism in partially ordered systems have introduced an orientational distribution function  $P(\theta', \phi', \psi')$  which gives the probability that a molecule-fixed axis system has Euler angles  $\theta'$ ,  $\phi'$ , and  $\psi'$  with respect to the laboratory axis system (2) (see Fig. 1). After orientation averaging with the unknown function  $P(\theta', \phi', \psi')$  [or an expansion of  $P(\theta', \phi', \psi')$  (3,4), see Discussion] the linear dichroism formulas can be written in terms of order parameters which are integrals over some functions multiplied by  $P(\theta', \phi', \psi')$ . An example is the order parameter  $S$  defined when both the molecular reference frame and laboratory reference frame are axially symmetric; i.e. when  $P(\theta', \phi', \psi')$  depends only on  $\theta'$ . It is defined as

$$S = \frac{3\langle \cos^2 \theta' \rangle - 1}{2} \quad (1)$$

where  $\langle \cos^2 \theta' \rangle$  is an ensemble average of  $\cos^2 \theta'$  or

$$\langle \cos^2 \theta' \rangle = \int_0^{\pi/2} \cos^2 \theta' P(\theta') \sin \theta' d\theta' \quad (2)$$

Because the linear dichroism formulas depend only on order parameters like  $S$ , it is not possible to fully determine  $P(\theta', \phi', \psi')$  from a linear dichroism measurement; it is possible to obtain only the order parameters. If one can construct  $P(\theta', \phi', \psi')$  from a model for the system, however, it is possible to extract structural information in light of the specific model. This has been a major stumbling block for existing linear dichroism theories - no general method for constructing  $P(\theta', \phi', \psi')$  from a model has been described.

Our approach to linear dichroism will overcome this obstacle by using new techniques for describing partially ordered systems (5,6) which we have already applied to simulation of EPR spectra (7). These techniques involve a general method for calculating a density of states function  $D(\theta, \phi, \underline{\Delta})$  which gives the probability that the polarized field has spherical angles  $\theta$  and  $\phi$  in an axis system fixed with respect to the transition moment  $\underline{\mu}$  (see Fig. 2). Here  $\underline{\Delta}$  is a set of parameters  $(\Delta_1, \Delta_2, \Delta_3, \dots, \Delta_n)$  that describes the partial ordering. Note that  $D(\theta, \phi, \underline{\Delta})$  is a distribution function in a molecular axis system while  $P(\theta', \phi', \psi')$  is a distribution

function in the laboratory axis system. That is, we will be orientation averaging in a molecular axis system instead of a laboratory axis system. As will be shown later, this approach leads to simplification of the formulas in many instances.

The density of states function,  $D(\theta, \phi, \underline{\Delta})$  is calculated in the following manner: 1) From a model for the system, one determines a set of rotations which will rotate an axis system originally coincident with the laboratory axis system into an axis system which is a member of the ensemble of axis systems fixed with respect to the transition moment. 2) The rotations are assigned weighting functions giving the probability distribution in each rotation; e.g. a Gaussian weighted rotation about some axis will have the weighting function  $\exp(-\beta^2/\Delta^2)$ , where  $\Delta$  is the width of the Gaussian distribution. 3) Finally,  $D(\theta, \phi, \underline{\Delta})$  is obtained as an integral over the weighting function (see Appendix A and references 5 and 6 for more details). In effect, we have replaced order parameters that are integrals over  $P(\theta', \phi', \psi')$  with the parameters  $\Delta_1, \Delta_2 \dots \Delta_n$ . We believe that our parameters give a better physical description of the system.

The function  $D(\theta, \phi, \underline{\Delta})$  can be used to interpret many types of experiments on a system, and the results often allow one to specify  $\underline{\Delta}$  to within a small range (5-7). If all components of  $\underline{\Delta}$  can be measured, a linear dichroism experiment will yield the orientation of the transition moment in a molecular axis system. Unfortunately,  $\underline{\Delta}$  can rarely be



specified with certainty; our analysis then allows one to use the uncertainty in  $\Delta$  to place accurate limits on the orientation of the transition moment.

The next section describes our approach to the theory of linear dichroism based on the density of states formalism. The new approach is then applied to reaction centers and whole cells of photosynthetic bacteria. In the discussion, we compare our approach to others in the literature (1-4,8-13). We find that our approach is equivalent to others that use  $P(\theta',\phi',\psi')$ , but it has three distinct advantages. First, in comparison to parameters like  $S$ , the set of parameters  $\Delta_1, \Delta_2 \dots \Delta_n$  are more intuitively descriptive of the system, and they give a more complete definition of the distribution function. Second, it is straightforward to construct  $D(\theta, \phi, \Delta)$  from complex physical models and to interpret linear dichroism in light of these models. Third, by orientation averaging in a molecular axis system our approach is often much more efficient. For example, when neither the laboratory reference frame nor the molecular reference frame is axially symmetric,  $P(\theta', \phi', \psi')$  depends on all three angles, because it takes three Euler angles to specify the orientation of one axis system with respect to another. In contrast,  $D(\theta, \phi, \Delta)$  specifies the spherical angles of the applied field, which is a vectorial quantity. Because it takes only two angles to specify the orientation of a vector,  $D(\theta, \phi, \Delta)$  never requires more than two angles. We can, therefore, analyze complex models with fewer angular variables.

## THEORY

The dichroic ratio  $R$  of an absorption band is defined as the ratio of integrated absorption bands measured with light polarized parallel,  $E_{\parallel}$ , and perpendicular,  $E_{\perp}$ , to a given direction; i.e.

$$R = A_{\parallel} / A_{\perp} \quad (3)$$

where  $A_{\parallel}$  and  $A_{\perp}$  are the integrated absorbances. Most reported forms of linear dichroism can be related to  $R$ , as discussed in Appendix B. One notable exception is for experiments that directly measure  $A_{\parallel} - A_{\perp}$  (14-16) and normalize by dividing by  $A_r$ , which is the absorbance of the corresponding randomly oriented sample. We call this form the dichroic polarization, defined as

$$L = \frac{A_{\parallel} - A_{\perp}}{A_r} \quad (4)$$

When the laboratory reference frame is axially symmetric,  $L$  can be related to  $R$ , but in the general nonaxially symmetric case  $L$  cannot be related to  $R$ . Therefore, we also derive formulas for  $L$ .

Before continuing, let us formally define the two coordinate systems which we have already mentioned. The first coordinate system is the laboratory axis system (XYZ), and it is fixed in the laboratory reference frame. In the laboratory axis system  $E_{\parallel}$  and  $E_{\perp}$  are constant vectors. The second coordinate system is the molecular axis system (xyz), and it is fixed with respect to the transition moment whose linear dichroism

is being measured; that is, a unit vector  $\hat{\mu}$  in the direction of the transition moment  $\underline{\mu}$  is a constant vector

$$\hat{\mu} = (\mu_x, \mu_y, \mu_z) \quad (5)$$

in the molecular axis system. In general, the set of molecular axis systems defines a partially ordered ensemble. We have previously discussed a method for direct determination of the density of states functions  $D_{\parallel}(\theta, \phi, \Delta)$  and  $D_{\perp}(\theta, \phi, \Delta)$  (5,6). These functions give the probability that  $\underline{E}_{\parallel}$  and  $\underline{E}_{\perp}$  have spherical angles  $\theta$  and  $\phi$  in the molecular axis system; i.e. the probability that

$$\underline{E} = |\underline{E}|(\sin\theta \cos\phi, \sin\theta \sin\phi, \cos\theta) \quad (6)$$

Using the density of states formalism, we can now develop a new approach to the theory of linear dichroism.

We begin by calculating  $A_{\parallel}$ . The absorbance of a transition moment  $\underline{\mu}$  interacting with a polarized field  $\underline{E}$  is proportional to  $(\underline{\mu} \cdot \underline{E})^2$  or equivalently to  $\cos^2\beta$ , where  $\beta$  is the angle between  $\underline{\mu}$  and  $\underline{E}$ . For a partially ordered ensemble interacting with  $\underline{E}_{\parallel}$ , the absorbance is

$$A_{\parallel} = K \int_0^{\pi} d\theta \int_0^{2\pi} d\phi (\hat{\mu} \cdot \underline{E})^2 D_{\parallel}(\theta, \phi, \Delta) / \int_0^{\pi} d\theta \int_0^{2\pi} d\phi D_{\parallel}(\theta, \phi, \Delta) \quad (7)$$

where  $\hat{\mu}$  and  $\underline{E}$  are defined by Eq. (5) and Eq. (6), and  $K$  is an experimental constant which contains such parameters as extinction coefficient, concentration, and path length.

[Note that in Eq. (7) and throughout this paper we use unnormalized density of states functions; the denominator furnishes the required normalization]. Experimentally, the partial ordering is induced by exerting some type of force on the system, such as an alignment field or a mechanical stretch. Because the sign of the direction of these forces is arbitrary, the density of states has the following symmetry properties

$$(1) \quad D_{\parallel}(\theta, \phi, \underline{\Delta}) = D_{\parallel}(\theta, \phi + \pi, \underline{\Delta}) \quad (8)$$

and

$$(2) \quad D_{\parallel}(\theta, \phi, \underline{\Delta}) = D_{\parallel}(\pi - \theta, \phi, \underline{\Delta}) \quad (9a)$$

$$D_{\parallel}(\theta, \phi, \underline{\Delta}) = D_{\parallel}(\theta, \pi - \phi, \underline{\Delta}) \quad (9b)$$

That is  $D_{\parallel}(\theta, \phi, \underline{\Delta})$  is periodic with period  $\pi$  and is symmetric about  $\pi/2$ . Using only these symmetry properties, Eq. (7) reduces to

$$A_{\parallel} = K' \{ \mu_x^2 [T_{\parallel}(\underline{\Delta}) - F_{\parallel}(\underline{\Delta})] + \mu_y^2 F_{\parallel}(\underline{\Delta}) + \mu_z^2 [1 - T_{\parallel}(\underline{\Delta})] \} \quad (10)$$

where  $K'$  is a new constant,

$$T_{\parallel}(\underline{\Delta}) = \int_0^{\pi/2} d\theta \sin^2 \theta \int_0^{\pi/2} d\phi D_{\parallel}(\theta, \phi, \underline{\Delta}) / N_{\parallel}(\underline{\Delta}) \quad (11)$$

$$F_{\parallel}(\underline{\Delta}) = \int_0^{\pi/2} d\theta \sin^2 \theta \int_0^{\pi/2} d\phi \sin^2 \phi D_{\parallel}(\theta, \phi, \underline{\Delta}) / N_{\parallel}(\underline{\Delta}) \quad (12)$$

and

$$N_{\parallel}(\underline{\Delta}) = \int_0^{\pi/2} d\theta \int_0^{\pi/2} d\phi D_{\parallel}(\theta, \phi, \underline{\Delta}) \quad (13)$$

In a random sample,  $A_{\parallel} = A_r$ ,  $D_{\parallel}(\theta, \phi, \Delta) = \sin\theta$ ,  $T_{\parallel}(\Delta) = 2/3$ , and  $F_{\parallel}(\Delta) = 2/3$ . Using these facts, we find that  $K' = 3A_r$  and Eq. (10) for the ordered sample becomes

$$A_{\parallel} = 3A_r \{ \mu_x^2 [T_{\parallel}(\Delta) - F_{\parallel}(\Delta)] + \mu_y^2 F_{\parallel}(\Delta) + \mu_z^2 [1 - T_{\parallel}(\Delta)] \} \quad (14)$$

An analogous expression holds for  $A_{\perp}$ , where we define  $T_{\perp}(\Delta)$ ,  $F_{\perp}(\Delta)$ , and  $N_{\perp}(\Delta)$  using  $D_{\perp}(\theta, \phi, \Delta)$ . The dichroic ratio is thus given by

$$R = \frac{A_{\parallel}}{A_{\perp}} = \frac{\mu_x^2 [T_{\parallel}(\Delta) - F_{\parallel}(\Delta)] + \mu_y^2 F_{\parallel}(\Delta) + \mu_z^2 [1 - T_{\parallel}(\Delta)]}{\mu_x^2 [T_{\perp}(\Delta) - F_{\perp}(\Delta)] + \mu_y^2 F_{\perp}(\Delta) + \mu_z^2 [1 - T_{\perp}(\Delta)]} \quad (15)$$

and the dichroic polarization is given by

$$L = \frac{A_{\parallel} - A_{\perp}}{A_r} = 3 \{ \mu_x^2 [T_{\parallel}(\Delta) - T_{\perp}(\Delta) + F_{\perp}(\Delta) - F_{\parallel}(\Delta)] + \mu_y^2 [F_{\parallel}(\Delta) - F_{\perp}(\Delta)] + \mu_z^2 [T_{\perp}(\Delta) - T_{\parallel}(\Delta)] \} \quad (16)$$

Equations (15) and (16) take simpler forms when the density of states depends only on  $\theta$  and  $\Delta$ , which happens whenever the molecular reference frame is axially symmetric. Equations (11) and (12) become

$$T_{\parallel}(\Delta) = \frac{\int_0^{\pi/2} \sin^2 \theta D_{\parallel}(\theta, \Delta) d\theta}{\int_0^{\pi/2} D_{\parallel}(\theta, \Delta) d\theta} \quad (17)$$

and

$$F_{\parallel}(\Delta) = \frac{1}{2} T_{\parallel}(\Delta) \quad (18)$$

The dichroic ratio reduces to

$$R = \frac{T_{\parallel}(\Delta) + \mu_z^2 [2 - 3T_{\parallel}(\Delta)]}{T_{\perp}(\Delta) + \mu_z^2 [2 - 3T_{\perp}(\Delta)]} \quad (19)$$

If the laboratory reference frame is also axially symmetric and  $\tilde{E}_{\parallel}$  is along the symmetry axis,

$$A_r = \frac{1}{3}(A_{\parallel} + 2A_{\perp}) \quad (20)$$

When Eq. (20) holds, we can relate  $T_{\parallel}(\Delta)$  to  $T_{\perp}(\Delta)$ ; that relation is

$$T_{\perp}(\Delta) = 1 - \frac{1}{2}T_{\parallel}(\Delta) \quad (21)$$

Furthermore

$$L = \frac{3(A_{\parallel} - A_{\perp})}{A_{\parallel} + 2A_{\perp}} \quad (22)$$

L in this form is related to R, as shown in Appendix B. If  $\tilde{E}_{\parallel}$  is not along a laboratory symmetry axis, Eq. (20) is no longer valid, but we can still write

$$L = \frac{3}{2} \{ [T_{\perp}(\Delta) - T_{\parallel}(\Delta)] (3\mu_z^2 - 1) \} \quad (23)$$

We choose the z axis to be the axis of symmetry in the molecular axis system. The angle between the z axis and the transition moment  $\mu$  is (see Fig. 2)

$$\epsilon = \cos^{-1} \mu_z \quad (24)$$

From Eq. (19)

$$\epsilon = \cos^{-1} \left[ \frac{1}{3 + \frac{2(1-R)}{RT_{\perp}(\Delta) - T_{\parallel}(\Delta)}} \right]^{1/2} \quad (25)$$

We now consider some special cases of Eq. (19). One type of perfect ordering is where all molecular z axes line up with the laboratory Z axis. If linear dichroism is measured with  $\vec{E}_{\parallel} = (0,0,1)$  and  $\vec{E}_{\perp} = (1,0,0)$ , we have

$D_{\parallel}(\theta) = \delta(\theta)$ ,  $D_{\perp}(\theta) = \delta(\frac{\pi}{2} - \theta)$ ,  $T_{\parallel}(\Delta) = 0$ , and  $T_{\perp}(\Delta) = 1$  where  $\delta$  is a Dirac delta function.

Substitution into Eq. (19) gives the result first derived

by Fraser (8)

$$R = 2 \cot^2 \epsilon \quad (26)$$

The opposite extreme is a random sample, where  $D_{\parallel}(\theta) = D_{\perp}(\theta) = \sin \theta$  and  $T_{\parallel} = T_{\perp} = \frac{2}{3}$ .

The dichroic ratio is  $R = 1$ ; i.e. there is no linear dichroism.

In the general partially ordered case, a calculation of  $T_{\parallel}(\Delta)$  and  $T_{\perp}(\Delta)$  is sufficient to interpret the linear dichroism.

In the next section, we will apply these formulas to some examples. Most reports of dichroic ratios are ratios of the peak absorbances and not integrated absorbances. As long as the parallel and perpendicular lineshapes do not differ too much, the ratio of peak absorbances is a close approximation to the "true" dichroic ratio. We will therefore ignore this difficulty. Another difficulty arises from band overlap of several transitions. When band overlap occurs, it is difficult to measure the dichroic ratio by measuring peak absorbances. We will attempt to analyze only pure transitions and hence avoid this problem.

## RESULTS

The plan of attack for analyzing linear dichroism is the same for all systems. First, from a characterization of the absorption spectrum, one decides which bands are pure enough for an analysis. Second, from a consideration of the symmetry properties of the system, one calculates the parallel and perpendicular density of states,  $D_{\parallel}(\theta, \phi, \Delta)$  and  $D_{\perp}(\theta, \phi, \Delta)$ . Last, the formulas in the last section are used to extract all of the possible structural information. We begin by analyzing the linear dichroism for a common experimental situation.

## A. Gaussian Uniaxial Model

A common situation when the molecular reference frame is axially symmetric is that the symmetry axis tends to align along the direction of an applied force (e.g. magnetic field direction or stretch direction). If we take the applied force to be along the laboratory Z axis, then deviations from perfect order are manifested by a non-zero angle  $\beta$ , between the symmetry axis and the laboratory Z axis. A partially ordered ensemble will be described by a probability distribution in  $\beta$ ; in the Gaussian Uniaxial Model, we take the distribution to be a Gaussian of width

 $\Delta_G$ 

$$w_G = w(\beta) = \exp(-\beta^2 / \Delta_G^2) \quad (27)$$



This situation is illustrated in Fig. 3a; each symmetry axis that points along the cone of half angle  $\beta$  about the laboratory Z axis will have the same probability of occurrence.

We seek a set of  $n$  rotations  $R_1(\alpha_1), R_2(\alpha_2), \dots, R_n(\alpha_n)$  and a weighting function  $w(\alpha_1, \alpha_2, \dots, \alpha_n)$  that will generate the ensemble in the Gaussian Uniaxial Model. That is, a weighting function which describes the probability that the molecular axis system for a member of the ensemble is related to the laboratory axis system by  $n$  rotations of  $\alpha_1, \alpha_2, \dots, \alpha_n$  respectively. The ensemble in the Gaussian Uniaxial Model can be generated by the following three rotation scheme: a free rotation of  $\alpha$  about the laboratory Z axis (by free rotation, we mean the weighting function does not depend on  $\alpha$ ); a Gaussian weighted rotation of  $\beta$  about the laboratory Y axis; a free rotation of  $\gamma$  about the laboratory Z axis. The weighting function for these three rotations is given by Eq. (27). As discussed in references (5) and (6) (see Appendix A), the density of states is easy to calculate given  $w_G, \underline{E}_{\parallel}$  and  $\underline{E}_{\perp}$ . Typically,  $\underline{E}_{\parallel}$  is along the laboratory Z axis and  $\underline{E}_{\perp}$  is along the laboratory X axis (or any axis in the XY plane), which means  $D_{\parallel}(\theta, \Delta_G)$  and  $D_{\perp}(\theta, \Delta_G)$  are [in the notation of reference (6) - see Appendix A]

$$D_{\parallel}(\theta, \Delta_G) = D_{3,90}^{ZYZ}[\theta, w_G] \quad (28)$$

and

$$D_{\perp}(\theta, \Delta_G) = D_{1,0}^{ZYZ}[\theta, w_G] \quad (29)$$

$D_{\parallel}(\theta, \Delta_G), D_{\perp}(\theta, \Delta_G), T_{\parallel}(\Delta_G)$  and  $T_{\perp}(\Delta_G)$  for several values of  $\Delta_G$  are plotted in Fig. 4.

In Fig. 5, we plot the dichroic ratio,  $R$ , as calculated from Eq. (19), versus the angle between the symmetry axis of the particle and the transition moment. Note that perfect ordering,  $\Delta_G = 0.0$ , corresponds to  $R = 2\cot^2\epsilon$ , as shown in the Theory section. For perfect ordering,  $R$  can assume any

positive number; but as  $\Delta_G$  increases,  $R$  becomes bounded on both sides. This fact can be useful in determining an upper limit for  $\Delta_G$  in many instances. In Fig. 6, we plot  $\epsilon$  versus  $\Delta_G$  for various values of  $R$ . If  $R < 1$ ,  $\epsilon$  ranges from the perfect order value derived from Eq. (26) to  $90^\circ$  and, if  $R > 1$ ,  $\epsilon$  ranges from the perfect order value to  $0^\circ$ . Fig. 6 allows one again to infer limits on  $\Delta_G$  given a measurement of  $R$ , because  $\epsilon$  cannot fall outside the range  $0^\circ$  to  $90^\circ$ .

#### B. *Rhodospseudomonas sphaeroides* in Stretched Films

Two recent papers by Rafferty and Clayton (17,18) describe the linear dichroism spectra of reaction centers of *Rps. sphaeroides* in both stretched and unstretched films. The reaction center particles contain 4 bacteriochlorophyll a (BChl a) molecules and 2 bacteriopheophytin a (BPh a) molecules which all contribute to a complicated absorption spectrum (19). We choose to study the 860 nm transition because it is believed to be a pure transition of P860, which is a BChl a dimer that functions as the primary electron donor in *Rps. sphaeroides* (20).

We assume, as did Rafferty and Clayton (17,18), that the reaction center particles possess an axis of symmetry which tends to align with the stretch direction. As a first approximation, this assumption is isomorphic to the Gaussian Uniaxial Model; we take the width of the Gaussian distribution of particle orientations within their stretched film to be  $\Delta_S$ .

At 860 nm, Rafferty and Clayton (17,18) measure  $R = 2.28$  which means  $\epsilon < 43^\circ$ . Furthermore,  $\Delta_S \leq 1.0$  radian (see  $\Delta_G = 1.0$  curve in Fig. 5). We can narrow the limits on  $\Delta_S$  further by considering the value of  $R$  at a different wavelength. At 597 nm, they measure  $R = 0.48$ ; if we assume this to be a pure transition, we have a new limit:  $\Delta_S \leq 0.75$  radians. In reality, the 597 nm transition is not a pure transition, but this means that the 597 nm linear dichroism must contain at least one component whose  $R$  value is not greater than 0.48. Therefore, the limit of 0.75 radians on  $\Delta_S$  is an upper limit because it is based on the conservative assumption that the 597 nm transition is a pure transition. Rafferty and Clayton (17,18) did experiments on films that were stretched to different extents. At 860 nm in one such film, they determined  $R$  to be as high as 2.50. An  $R$  of 2.50 means that  $\epsilon$  must be less than  $42^\circ$ . Returning to the film where  $R = 2.28$ , we find that imposing the restriction of  $\epsilon \leq 42^\circ$  requires that  $\Delta_S$  must be greater than 0.30 radians. The final most conservative limits on  $\Delta_S$  are

$$0.30 \text{ radians} \leq \Delta_S \leq 0.75 \text{ radians} \quad (30)$$

From Eq. (25), the limits of  $\epsilon$  are

$$29^\circ \leq \epsilon \leq 42^\circ \quad (31)$$

Perhaps the Gaussian Uniaxial Model is an oversimplification, because it neglects the anisotropy of the unstretched film; i.e., it neglects the possibility that the particle symmetry axes lie in the plane of the unstretched film. We will therefore consider a more sophisticated model. Instead of giving equal weights to all symmetry axes that lie on the circle in Fig. 3a, we can use the model illustrated in Fig. 3b where all symmetry axes that lie on an ellipse have equal weights. Upon stretching, it is more likely that the tilt of the particle symmetry axis away from the laboratory Z axis is in the plane of the film than out of the plane of the film. Therefore, the ratio of the ellipse axes  $\Delta_e = b/a$  is less than 1. We call this model the Elliptical Gaussian Uniaxial Model; it can be generated by the same rotations as the Gaussian Uniaxial Model but the product of the weighting functions is now

$$w_{EG} = w(\beta, \gamma) = \exp(-\chi^2 / \Delta_S^2) \quad (32)$$

where

$$\chi = \tan^{-1} \left[ \tan \beta \left( \frac{\cos^2 \gamma}{\Delta_e^2} + \sin^2 \gamma \right)^{1/2} \right] \quad (33)$$

Despite the loss of axial symmetry in the laboratory reference frame,  $D_{\parallel}(\theta, \Delta_S, \Delta_e)$  and  $D_{\perp}(\theta, \Delta_S, \Delta_e)$  are still axially symmetric.  $T_{\parallel}(\Delta_S, \Delta_e)$  and  $T_{\perp}(\Delta_S, \Delta_e)$  for  $\Delta_e = 1.0$  (Gaussian Uniaxial Model) and  $\Delta_e = 0.2$  are plotted in Fig. 4c. We can pick a trial value for  $\Delta_e$  and analyze the linear dichroism data just as we did with the Gaussian Uniaxial Model. We find that the limits on  $\epsilon$  in equation (31) are independent of  $\Delta_e$ .

Rafferty and Clayton (17,18) calculated  $\epsilon$  to be  $40.8^\circ$  by assuming that an extrapolated value of  $R = 2.68$  at  $860$  nm corresponds to perfect order. This value falls within our limits but, if the extrapolation is invalid, the range of  $\epsilon$  in equation (31) provides a more realistic interpretation of their data. The question can be resolved by using  $D_{\parallel}(\theta, \Delta_S)$  and  $D_{\perp}(\theta, \Delta_S)$  to analyze other types of experiments and thereby pin down  $\Delta_S$ .

C. *Rhodospseudomonas sphaeroides* in Unstretched Films

Rafferty and Clayton (17,18) also did some linear dichroism experiments on unstretched film containing reaction center particles of *Rps. sphaeroides*. We use a model similar to Rafferty and Clayton's (17,18) which assumes that the reaction center particle symmetry axis lies close to the plane of the film. However, the film is not perfectly ordered; some of the symmetry axes are not in the plane of the film but are tilted out of the film by an angle  $\beta$ . The partial ordering is thus described by a probability distribution in  $\beta$  for which we use a Gaussian

$$w_{US} = w(\beta) = \exp(-\beta^2/\Delta_{US}^2) \quad (37)$$

where  $\Delta_{US}$  is the width of the Gaussian distribution.

The Gaussian Uniaxial Model is not appropriate here. But, using the coordinate system shown in Fig. 3b, this ensemble can be generated with the following three rotation scheme: a free rotation of  $\alpha$  about the laboratory Z axis;

a Gaussian weighted rotation of  $\beta$  about the laboratory Y axis; a free rotation of  $\gamma$  about the laboratory X axis. The product of the weighting functions for these rotations is given by Eq. (37).

To observe linear dichroism in unstretched films, the experiment must be done with a light propagation direction that is not normal to the plane of the film. Fig. 7. shows the geometry employed by Rafferty and Clayton (17,18). Within the boundaries of the film  $\underline{E}_{\parallel} = (0,0,1)$  and  $\underline{E}_{\perp} = (\sin\psi, \cos\psi, 0)$  where  $\psi = 37.3^\circ$ . In the notation of reference 6,

$$D_{\parallel}(\theta, \Delta_{US}) = D_{3,90}^{ZYX}[\theta, w_{US}] \quad (38)$$

and

$$D_{\perp}(\theta, \Delta_{US}) = D_{1,52.7}^{ZYX}[\theta, w_{US}] \quad (39)$$

$D_{\parallel}(\theta, \Delta_{US})$ ,  $D_{\perp}(\theta, \Delta_{US})$ ,  $T_{\parallel}(\Delta_{US})$ , and  $T_{\perp}(\Delta_{US})$  for several values of  $\Delta_{US}$  are plotted in Fig. 8.

From the range of  $\epsilon$  given in Eq. (32), we can determine a range for  $\Delta_{US}$ ; that is, a linear dichroism analysis will tell us how well the reaction center particles orient in unstretched films. Rafferty and Clayton (17,18) measured a dichroic ratio of  $R = 1.14$  at 860 nm. From Eq. (19), we find that the width of the Gaussian distribution in  $\beta$  is restricted to the range

$$0.45 \text{ radian} \leq \Delta_{US} \leq 0.90 \text{ radian} \quad (40)$$

In the next two sections, we consider some additional systems where experiments have been done that determine  $\Delta$  to within a small range.

D. *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris*  
in a Magnetic Field: The Density of States

Both *Rps. viridis* cells and *Rps. palustris* cells are cylindrical in shape (21), and they can be aligned in a magnetic field such that the long axis of the cylinder is perpendicular to the alignment field (22). Inside the cells are cylindrical membrane shells (21) containing bound chromophores. We choose the molecular axis system to have its z axis along the membrane normal. We choose a model which assumes that the chromophores are bound in a fixed relation to and distributed around the membrane normal, an assumption which is consistent with experiments. In this model, the molecular reference frame is axially symmetric. Furthermore, the angle  $\epsilon$  will be the angle between the membrane normal and the transition moment.

In this system, we cannot use the Gaussian Uniaxial Model as an approximation. The reason is that the z axis of the molecular axis system does not align preferentially along the magnetic field. It is the whole cells that are oriented by the magnetic field; the ensemble of molecular axis systems distributed throughout the membranes is oriented as a consequence. As a model, we assume that



the long axis of the cylinder representing the cell is perpendicular to the alignment field and that deviations from perfect order are due to deviations of the membranes from perfect cylinders. The angle between the actual membrane normal and the hypothetical perfect cylinder normal is assumed to have a Gaussian distribution with width  $\Delta_C$ . ( $\Delta_C = \Delta_{RV}$  for *Rps. viridis* and  $\Delta_P$  for *Rps. palustris*.) Calculation of  $D_{\parallel}(\theta, \Delta_C)$  and  $D_{\perp}(\theta, \Delta_C)$  is complicated by the morphology; briefly, using the coordinate system shown in Fig. 9 we found that a four rotation scheme ZXYZ (angles  $\alpha, \beta, \gamma$ , and  $\chi$ ) with weighting function  $w_C = \exp(-\beta^2/\Delta_C^2)$  will generate this ensemble (see reference 5 or 7 for details). The first two rotations locate the molecular axis system with respect to the cylindrical membranes, and the last two rotations locate the cylinder with respect to the laboratory axis system. Note that the last two free rotations require that the cylinder is perpendicular to the alignment field. The density of states for  $E_{\parallel}$  along the alignment field and  $E_{\perp}$  perpendicular to the alignment field are

$$D_{\parallel}(\theta, \Delta_C) = D_{3,90}^{ZXYZ}[\theta, w_C] \quad (41)$$

and

$$D_{\perp}(\theta, \Delta_C) = D_{2,0}^{ZXYZ}[\theta, w_C] \quad (42)$$

$T_{\parallel}(\Delta_C)$  and  $T_{\perp}(\Delta_C)$  are plotted in Fig. 10.

In reference 7, we used  $D_{\parallel}(\theta, \Delta_C)$  and  $D_{\perp}(\theta, \Delta_C)$  to simulate the EPR spectra of the triplet state of the primary electron donors in both *Rps. viridis* and *Rps. palustris*. We were able to calculate the orientation of the principal magnetic axes with respect to the membrane normal. As a byproduct of our calculation, we found that  $\Delta_{RV}$  and  $\Delta_P$  are restricted to small regions:

$$0.3 \text{ radian} \leq \Delta_{RV} \leq 0.5 \text{ radian} \quad (43)$$

and

$$0.4 \text{ radian} \leq \Delta_P \leq 0.6 \text{ radian} \quad (44)$$

We can now make use of this information for linear dichroism calculations on the same systems.

#### *Rhodospseudomonas viridis* Linear Dichroism

The long wavelength absorption maximum in whole cells of *Rps. viridis* is due to antenna BChl b molecules. Because these molecules do not all have the same orientation with respect to the membrane normal, the long wavelength absorption does not correspond to a single molecular species with a unique orientation. This problem can be circumvented by treating absorption changes induced by unpolarized light and measuring  $\Delta A_{\parallel}$  and  $\Delta A_{\perp}$  with light polarized parallel and perpendicular to the alignment field respectively. For light induced absorption changes, there is a pure transition at 970 nm due to the oxidation of the reaction center BChl b dimer P970,

which functions as the primary electron donor in *Rps. viridis* (24). Lastly, we note that the same formulas that apply to R and L in the Theory section also apply to  $\Delta R$  and  $\Delta L$  defined using  $\Delta A_{\parallel}$  and  $\Delta A_{\perp}$ .

Pailletin et al. (13) have measured the absorption change linear dichroism of magnetically aligned whole cells of *Rps. viridis* and found  $\Delta L = -0.42$  at 970 nm. From Eq. (23) and the limits

of  $\Delta_{RV}$  in Eq. (42),  $\Delta L$  cannot be less than  $-0.33$ , which results in a discrepancy with experiment. There are two possible explanations: 1) The EPR experiments of Frank et al. (7) where  $\Delta_{RV}$  was determined were done under experimental conditions different from those of the linear dichroism experiments which may affect  $\Delta_{RV}$ . 2) Perhaps the cell morphology is not adequately defined by our model.

The large negative value for  $\Delta L$  indicates that  $\epsilon$  is probably close to  $90^\circ$ . The ideal experiment to do next is magnetophotoselection (25) on P970; this experiment combined with the results in reference 7 would yield an independent value of  $\epsilon$ .

#### *Rhodospseudomonas palustris* Linear Dichroism

The only linear dichroism measurements reported for *Rps. palustris* have been in direct absorption (26,27). Although there are no isolated transitions due to a single molecular species with a fixed orientation with respect to the membrane normal, we will analyze the three absorption bands at 590 nm, 800 nm, and 870 nm and interpret the results as an

average orientation of the transition moments contributing to those peaks.

At 590 nm, 800 nm, and 870 nm, Breton measured dichroic ratios in magnetically aligned *Rps. palustris* of 0.59, 0.81 and 0.80 respectively (26). From  $T_{\parallel}(\Delta_P)$  and  $T_{\perp}(\Delta_P)$  in Fig. 10, Eq. (19) and the range for  $\Delta_P$ , we find the following limits on the average angles

$$0 < \langle \epsilon_{590} \rangle < 20^{\circ} \quad (45)$$

$$72^{\circ} < \langle \epsilon_{800} \rangle < 81^{\circ}$$

and

$$71^{\circ} < \langle \epsilon_{870} \rangle < 78^{\circ} \quad (47)$$

In the next section, we use these ranges to investigate the orientation of *Rps. palustris* in a flow method.

#### E. *Rhodopseudomonas palustris* in a Flow System.

Morita and Miyazaki (27) have measured the linear dichroism of *Rps. palustris* oriented by a velocity gradient created in a flow system. In a flow system, rod like particles such as *Rps. palustris* tend to orient such that the long axis of the rod is along a line of constant velocity of the flowing solvent (28). Because the flow system of Morita and Miyazaki (27) has a square cross section perpendicular to the flow direction, any tilt of the long axis away from the flow direction will move the long axis out of a line of constant velocity. We can therefore qualitatively analyze this flow system as a set of cells that tend to orient with the long axis of the cell collinear with the flow direction (see Fig. 11). We have not attempted a detailed

analysis of flow orientation; instead we introduce a Gaussian distribution for the angle between the long axis of the cell and the flow direction  $\chi$  with width  $\Delta_F$

$$w = (\chi) = \exp(-\chi^2/\Delta_F^2) \quad (48)$$

To generate the flow system ensemble, we begin with the same first two rotations that were used with magnetic field alignment. The reason is that these rotations orient the molecular axis system with respect to the hypothetical perfect cylinders which means they are properties of the cell and not of the alignment method. Note that with the models we are using,  $\Delta_P$  is unaffected by the alignment method. Three more rotations are necessary to orient the cylinder with respect to the laboratory axis system.

The three rotations are: a free rotation of  $\gamma$  about the laboratory Y axis; a rotation of  $\chi$  about the laboratory X axis weighted by the Gaussian function in Eqn. (48); and a free rotation of  $\xi$  about the laboratory Y axis. This rotation scheme contains five rotations (ZXYXY) which makes the density of states very difficult to evaluate. We note, however that the fifth rotation is superfluous for the parallel density of states because it is a rotation about  $E_{\parallel}$  (see Fig. 11). Thus

$$D_{\parallel}(\theta, \Delta_P, \Delta_F) = D_{3,0}^{\text{ZXYZ}}[\theta, w_F]$$

where

$$w_F(\Delta_P, \Delta_F) = \exp(-\beta^2/\Delta_P^2) \exp(-\chi^2/\Delta_F^2)$$

Because the laboratory reference frame is axially symmetric and  $E_{\parallel}$  is along the laboratory symmetry axis, we can calculate  $T_{\perp}(\Delta)$  from  $T_{\parallel}(\Delta)$  by use of equation (21). In this way we avoid the necessity of having to calculate  $D_{\perp}(\theta, \Delta_P, \Delta_F)$ .

Morita and Miyazaki (27) measured dichroic ratios of 0.54, 1.27 and 1.26 at 590 nm, 800 nm,

and 870 nm respectively in flow oriented *Rps. palustris*. We first set  $\Delta_p$  to its lower limit of 0.4 radians; this limit corresponds to the limiting values in Eqs. (45) - (47) of  $\langle \epsilon_{590} \rangle = 20^\circ$ ,  $\langle \epsilon_{800} \rangle = 72^\circ$ , and  $\langle \epsilon_{870} \rangle = 71^\circ$ . Working backwards with equation (19), we find that  $\Delta_F = 0.95$  radians is consistent with these angles. This calculation reveals two important facts. First, because  $\Delta_p = 0.4$  is a lower limit,  $\Delta_F = 0.95$  radians is an upper limit. Second, the fact that one  $\Delta_F$  value reproduces all three angles indicates that the models are self consistent. Analogously, from the  $\Delta_p = 0.6$  radian limit, we find a lower limit on  $\Delta_F$  of 0.90 radians. In summary, the width of the Gaussian distribution in  $\chi$ , which is a measure of the extent of orientation by flow method, is between 0.90 and 0.95 radians.

## DISCUSSION

We have used the density of states formalism to develop a new approach to the theory of linear dichroism. Although equivalent to theories that introduce the orientational distribution function  $P(\theta', \phi', \psi')$ , our approach has several distinct advantages. Those advantages will be outlined in this section.

In our approach, we begin by calculating a density of states function that depends on  $\underline{\Delta}$  and then derive linear dichroism formulas that also depend on  $\underline{\Delta}$ . Previous authors have ignored the problem of calculating a distribution function and have instead introduced parameters, like  $S$  into the linear dichroism formulas (1,2,13). Although the linear dichroism formulas in both cases are mathematically equivalent, knowledge of  $\underline{\Delta}$  determines the distribution function  $D(\theta, \phi, \underline{\Delta})$  while knowledge of  $S$  yields no such information. To see why this is the case, we will consider an experimental system where both the laboratory reference frame and the molecular reference frame are axially symmetric.

In this example, a mathematically elegant approach to the introduction of order parameters like  $S$  is to expand  $P(\theta', \phi', \psi')$  in a series of Wigner rotation matrix elements (3). Because of the axial symmetry the expansion reduces to an expansion in Legendre polynomials (3,4).

$$P(\theta', \phi', \psi') = P(\theta') = \sum_{\ell} \frac{2\ell+1}{2} \langle P_{\ell}(\cos\theta') \rangle P_{\ell}(\cos\theta') \quad (52)$$

where  $P_{\ell}(\cos\theta')$  and  $\langle P_{\ell}(\cos\theta') \rangle$  are the  $\ell$ th Legendre polynomial and its ensemble average, respectively.

$$P_{\ell}(\cos\theta') = \int_0^{\pi} P_{\ell}(\cos\theta') P(\theta') \sin\theta' d\theta' \quad (53)$$



Following McBrierty,

$$A(\psi) = A_r [\langle P_2(\cos\theta') \rangle P_2(\cos\theta') 3\cos^2\psi - 1] + 1 \quad (54)$$

where  $\psi$  is the angle between the polarized field and the symmetry axis of the laboratory. Because  $P_2(x) = (3x^2 - 1)/2$ , the parameter  $S$  is equal to  $\langle P_2(\cos\theta') \rangle$ . Eq. (54) gives a mathematical interpretation of the  $S$  parameter; it is the coefficient of  $P_2(\cos\theta')$  when  $P(\theta')$  is expanded in a series of Legendre polynomials. If the expansion is slowly convergent,  $S$  will reveal almost nothing about  $P(\theta')$ . In contrast, the density of states approach represents  $D(\theta, \underline{\Delta})$  with the few parameters in  $\underline{\Delta}$ . As shown in our examples,  $\underline{\Delta}$  can often be narrowed to a small range which thereby determines the distribution function to a small range. Stated another way, because  $D(\theta, \underline{\Delta}) = \sin\theta' P(\theta')$  for our axially symmetric example, knowledge of  $\underline{\Delta}$  determines all of the moments of the expansion in Eq. (52) by

$$\langle P_\ell(\cos\theta') \rangle = \int_0^\pi P_\ell(\cos\theta') D(\theta', \underline{\Delta}) d\theta' \quad (55)$$

The density of states formalism provides an explicit formalism for direct determination of  $D(\theta, \phi, \underline{\Delta})$  from an arbitrary model. Previous attempts by Fraser (8-11) and Beer (12) to interpret linear dichroism have relied on simple models. One such model considers a

sample to have a fraction  $f$  of the molecules perfectly ordered and the remaining fraction  $1-f$  randomly ordered.

The dichroic ratio is then given by

$$R = \frac{2f\cos^2\varepsilon + (2/3)(1-f)}{f\sin^2\varepsilon + (2/3)(1-f)} \quad (56)$$

Because this model is probably not realistic,  $f$  is a relatively meaningless parameter. In contrast, our  $\Delta$  parameter derived from more realistic models reveals more details about the system. Also, it is straightforward to extend our techniques to include more complicated models. For example, instead of using Gaussian weighting functions, a rotation could be weighted by a potential energy function

$$w(\beta) = \exp[-E(\beta, \Delta)/kt] \quad (57)$$

where  $E(\beta, \Delta)$  is energy as a function of  $\beta$ ,  $k$  is Boltzmann's constant, and  $T$  is temperature. In principle, several experiments on a system could be used to develop a detailed explanation of the partial ordering.

Finally, the fact that we average orientations in the molecular axis system instead of the laboratory axis system leads to simplification of the formulas in many instances. When both the laboratory reference frame and the molecular reference frame are axially symmetric,  $P(\theta')$  depends only on  $\theta'$  and  $D(\theta, \Delta)$  depends only on  $\theta$ . But, when axial

symmetry in the laboratory reference frame is lost,  $P(\theta', \phi')$  depends on two angles,  $\theta'$  and  $\phi'$ , which are the spherical angles of the molecular reference frame symmetry axis in the laboratory axis system. In contrast,  $D(\theta, \Delta)$  still depends only on  $\theta$  because it is a distribution function in the axially symmetric molecular reference frame. We can, therefore, consider complex models with nonaxially symmetric laboratory reference frames while still in the realm of axial symmetry. An example is the Elliptical Gaussian Uniaxial Model for which there is no symmetry axis in the laboratory reference frame; we were still able to use an axially symmetric analysis. When all axial symmetry is lost,  $P(\theta', \phi', \psi')$  depends on all three angles; but  $D(\theta, \phi, \Delta)$  never depends on more than two angles, because it takes only two angles to specify the orientation of the polarized field in the molecular axis system. In conclusion, by working in an axis system fixed with respect to the transition moment, we gain computational efficiency.

## APPENDIX A

In reference 6, we give some simple formulas and techniques for calculating density of states functions. A brief summary of that approach is given here. The notation for a density of states is

$$D_{v,\psi}^{RS}[\theta,\phi,w] \quad (A-1)$$

where RS is the rotation scheme (i.e., the number and order of rotations required to generate the ensemble of molecular axis systems),  $v$  is the type of field vector [ $v = 1$  for  $E = (\cos\psi, \sin\psi, 0)$ ,  $v=2$  for  $E = (\cos\psi, 0, \sin\psi)$ , and  $v=3$  for  $E = (0, \cos\psi, \sin\psi)$ ],  $\psi$  is the angle in the type of field vector indicated by  $v$ ,  $\theta$  and  $\phi$  are the spherical angles of the field vector in the molecular axis system, and  $w$  is the weighting function. Note that  $D_{v,\psi}^{RS}[\theta,\phi,w]$  is a function of the weighting function  $w$ ; that is,  $D_{v,\psi}^{RS}[\theta,\phi,w]$  is a functional. We denote this fact with square brackets.

When one is faced with a density of states calculation, the procedure is as follows: (1) determine RS, the number and order of rotations required to generate the ensemble, (2) determine  $W(\alpha\beta\gamma\dots)$ , the product of the weighting functions, and (3) determine  $v$  and  $\psi$  for the field vector. The formulas for rotation schemes with 3 or 4 rotations are given in reference (6).

## APPENDIX B

Linear dichroism is reported in a variety of forms. Here, we will relate several of those forms to  $R = A_{\parallel} / A_{\perp}$  or  $L = (A_{\parallel} - A_{\perp}) / A_r$  which we have used in this paper.

After separate measurements of  $A_{\parallel}$  and  $A_{\perp}$ , the following four definitions of linear dichroism are sometimes found

$$LD_1 = \frac{A_{\parallel} - A_{\perp}}{A_{\parallel} + A_{\perp}} = \frac{R-1}{R+1} \quad (B-1)$$

$$LD_2 = \frac{A_{\parallel} - A_{\perp}}{A_{\parallel} + 2A_{\perp}} = \frac{R-1}{R+2} \quad (B-2)$$

$$LD_3 = \frac{A_{\parallel} - A_{\perp}}{\frac{1}{2}(A_{\parallel} + A_{\perp})} = \frac{2(R-1)}{R+1} \quad (B-3)$$

$$LD_4 = \frac{A_{\parallel} - A_{\perp}}{\frac{1}{3}(A_{\parallel} + 2A_{\perp})} = \frac{3(R-1)}{R+2} \quad (B-4)$$

The dichroic ratio  $R$  is related to these four forms by

$$R = \frac{1+LD_1}{1-LD_1} \quad (B-5)$$

$$R = \frac{1+2LD_2}{1-LD_2} \quad (B-6)$$

$$R = \frac{2+LD_3}{2-LD_3} \quad (B-7)$$

$$R = \frac{3+2LD_4}{3-LD_4} \quad (B-8)$$

Alternatively,  $A_{\parallel} - A_{\perp}$  can be measured by lock-in techniques (14-16) and normalized by dividing by  $A_r$  or  $A_{\text{unpol}}$ , which is

the absorbance of the oriented sample using unpolarized light. We have already discussed normalization of  $A_{\parallel} - A_{\perp}$  by  $A_{\perp}$ , which gives  $L$ .  $A_{\text{unpol}}$  can be written in terms of  $A_{\parallel}$  and  $A_{\perp}$ . Following Zbindin, (2)

$$A_{\chi} = A_{\parallel} \cos^2 \chi + A_{\perp} \sin^2 \chi \quad (\text{B-9})$$

where  $A_{\chi}$  is absorbance due to a field  $E_{\chi}$  whose polarization makes an angle  $\chi$  with  $E_{\parallel}$ . Integrating over  $\chi$ , we find

$$A_{\text{unpol}} = \frac{1}{2}(A_{\parallel} + A_{\perp}) \quad (\text{B-10})$$

Therefore

$$\frac{A_{\parallel} - A_{\perp}}{A_{\text{unpol}}} = \frac{A_{\parallel} - A_{\perp}}{\frac{1}{2}(A_{\parallel} + A_{\perp})} = LD_3 \quad (\text{B-11})$$

and we can relate this form to  $R$  by Eq. (B-7).

**ACKNOWLEDGEMENTS**

This work was supported, in part, by the Biomedical and Environmental Research Division of the U.S. Department of Energy under contract No. 2-7405-ENG-48 and, in part, by National Science Foundation Grant PCM 76-5074.

## REFERENCES

1. Hofrichter, J., and Eaton, W.A. 1976. Linear dichroism of biological chromophores. *Ann. Rev. Biophys. Bioeng.* 5:511-560.
2. Zbinden, R. 1964. *Infrared Spectroscopy of High Polymers.* Academic Press, New York. pp. 166-233.
3. McBrierty, V.J. 1974. Use of rotation operators in the general description of polymer properties. *J. Chem. Phys.* 61:872-882.
4. Rothschild, K.J., and Clark, N.A. 1979. Polarized infrared spectroscopy of oriented purple membrane. *Biophys. J.* 25:473-487.
5. Friesner, R., Nairn, J.A., and Sauer, K. 1979. Direct calculation of the orientational distribution function of partially ordered ensembles from the EPR lineshape. *J. Chem. Phys.* in press.
6. Friesner, R., Nairn, J.A. and Sauer, K. 1979. A general theory of the spectroscopic properties of partially ordered ensembles. I. One Vector Problems. Submitted.
7. Frank, H.A., Friesner, R., Nairn, J.A., Dismukes, G.C., and Sauer, K. 1979. The orientation of the primary donor in bacterial photosynthesis. *Biochim. Biophys. Acta.* in press.
8. Fraser, R.D.B. 1953. The interpretation of infrared dichroism in fibrous protein structures. *J. Chem. Phys.* 21: 1511-1515.



9. Fraser, R.D.B. 1956. Interpretation of infrared dichroism in fibrous proteins - the  $2\mu$  region. J. Chem. Phys. 24:89-95.
10. Fraser, R.D.B. 1958. Interpretation of infrared dichroism in axially oriented polymers. J. Chem. Phys. 28:1113-1115.
11. Fraser, R.D.B. 1958. Determination of transition moment orientation in partially oriented polymers. J. Chem. Phys. 29:1428-1429.
12. Beer, M. 1956. Quantitative interpretation of infrared dichroism in partly oriented polymers. Proc. Roy. Soc. (London). A236:136-140.
13. Paillet, G., Vermeglio, A., and Breton, J. 1979. Orientation of reaction center and antenna chromophores in the photosynthetic membrane of *Rhodospseudomonas viridis*. Biochim. Biophys. Acta. 545:249-264.
14. Chabay, I., Hsu, E.C., and Holzwarth, G. 1972. Infrared circular dichroism measurement between 2000 and 5000  $\text{cm}^{-1}$ :  $\text{Pr}^{+3}$ -tartrate complexes. Chem. Phys. Lett. 15:211-214.
15. Gale, R., McCaffery, A.J., and Shatwell, R. 1972. Linear dichroism, an adjunct to polarised crystal spectroscopy. Chem. Phys. Lett. 17:416-418.
16. Stein, R.S. 1961. A procedure for the accurate measurement of infrared dichroism of oriented film. J. Appl. Polymer Sci. 5:96-99.

17. Rafferty, C.N., and Clayton, R.K. 1979. Linear dichroism and the orientation of reaction centers of *Rhodopseudomonas sphaeroides* in dried gelatin films. *Biochim. Biophys. Acta.* 545:106-121.
18. Rafferty, C.N., and Clayton, R.K. 1978. Properties of reaction centers of *Rhodopseudomonas sphaeroides* in dried gelatin films: linear dichroism and low temperature spectra. *Biochim. Biophys. Acta.* 502:51-60.
19. Straley, S.C., Parson, W.W., Mauzerall, D.C., and Clayton, R.K. 1973. Pigment content and molar extinction coefficients of photochemical reaction centers from *Rhodopseudomonas sphaeroides*. *Biochim. Biophys. Acta.* 305:597-609.
20. Parson, W.W., and Cogdell, R.J. 1975. The primary photochemical reaction of bacterial photosynthesis. *Biochim. Biophys. Acta.* 416:105-149.
21. (a) Giesbrecht, P., and Drews, G. 1966. Über die organisation und die makromolekulare architektur der thylakoide "lebender" bakterien. *Arch. Mikrobiol.* 54:297-330.  
(b) Tauschel, H.-D., and Drews, G. 1967. Thylakoid-morphogenese bei *Rhodopseudomonas palustris*. *Arch. Mikrobiol.* 59:381-404.
22. Geacintov, N.E., Van Nostrand, F., Becker, J.F. and Tinkel, J.B. 1972. Magnetic field induced orientation of photosynthetic systems. *Biochim. Biophys. Acta.* 267:65-79.

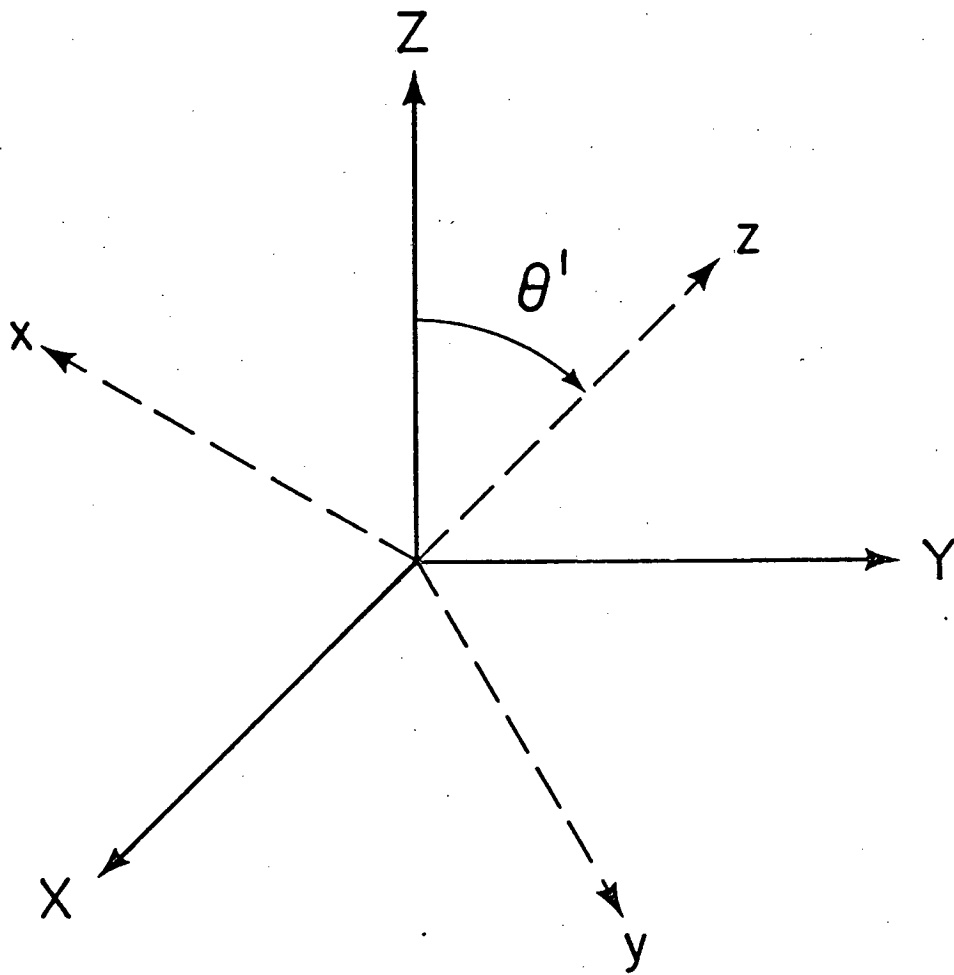
23. Breton, J., Michel-Villaz, M., and Paillotin, G. 1973. Orientation of pigments and structural proteins in the photosynthetic membrane of spinach chloroplasts: a linear dichroism study. *Biochim. Biophys. Acta.* 314:42-56.
24. Blankenship, R.E., and Parson, W.W. 1979. Kinetics and thermodynamics of electron transfer in bacterial reaction centers. In *Photosynthesis in Relation to Model Systems*. J. Barber, editor. Elsevier/North-Holland Biomedical Press, Amsterdam. pp. 71-114.
25. Frank, H.A., Bolt, J., Friesner, R., and Sauer, K. 1979. Magnetophotoselection of the triplet state of reaction centers from *Rhodopseudomonas sphaeroides* R-26. *Biochim. Biophys. Acta.* in press.
26. Breton, J. 1974. The state of chlorophyll and carotenoid *in vivo*, II - A linear dichroism study of pigment orientation in photosynthetic bacteria. *Biochem. Biophys. Res. Comm.* 59:1011-1017.
27. Morita, S., and Miyazaki, T. 1971. Dichroism of bacteriochlorophyll in cells of the photosynthetic bacterium *Rhodopseudomonas palustris*. *Biochim. Biophys. Acta.* 245:151-159.
28. Jeffery, G.B. 1923. The motion of ellipsoidal particles immersed in a viscous fluid. *Proc. Roy. Soc. (London)*. A102:161-179.

## FIGURE CAPTIONS

1. Relation between the laboratory axis system (Labeled X,Y, and Z) and the molecular axis system, (labeled x,y, and z).  $\theta'$  is one of the three Euler angles ( $\theta'$ ,  $\phi'$ , and  $\psi'$ ) that relate the laboratory axis system to the molecular axis system.
2. Arrangement of  $\underline{\mu}$  and  $\underline{E}$  in the molecular axis system.  $\theta$  and  $\phi$  are the spherical angles of  $\underline{E}$ .
3.
  - a) Schematic representation of the Gaussian Uniaxial Model.  $\beta$  is the half angle of the cone centered on the laboratory Z axis.
  - b) Schematic representation of the Elliptical Gaussian Uniaxial Model.  $\chi$  is the angle between the laboratory Z axis and the line in the YZ plane that points to the ellipse. a and b are the major and minor axes of the ellipse.
4.
  - a)  $D_{\parallel}(\theta, \Delta_G)$  for the Gaussian Uniaxial Model for several values of  $\Delta_G$  in radians.
  - b)  $D_{\perp}(\theta, \Delta_G)$  for the Gaussian Uniaxial Model for several values of  $\Delta_G$  in radians.
  - c)  $T_{\parallel}(\Delta_G)$  and  $T_{\perp}(\Delta_G)$  for the Gaussian Uniaxial Model ( $\Delta_e = 1.0$ ) and for the Elliptical Gaussian Uniaxial Model with  $\Delta_e = 0.2$ .

5. Dichroic ratio  $R$  versus angle  $\epsilon$  between the transition moment  $\underline{\mu}$  and the symmetry axis of the molecular reference frame. The plots are for the Gaussian Uniaxial Model with several values of  $\Delta_G$  in radians.
6. Angle  $\epsilon$  between the transition moment  $\underline{\mu}$  and the symmetry axis of the molecular reference frame versus  $\Delta_G$  in radians. The plots are for the Gaussian Uniaxial Model with several values of the dichroic ratio  $R$ .
7. Propagation of  $E_{\perp 1}$  through unstretched films as in the experimental set up of Rafferty and Clayton (17,18).
8.
  - a)  $D_{\parallel}(\theta, \Delta_{US})$  for unstretched films for several values of  $\Delta_{US}$  in radians.
  - b)  $D_{\perp 1}(\theta, \Delta_{US})$  for unstretched films for several values of  $\Delta_{US}$  in radians.
  - c)  $T_{\parallel}(\Delta_{US})$  and  $T_{\perp 1}(\Delta_{US})$  for unstretched films.
9. Definition of the angles and axis systems for *Rps. viridis* and *Rps. palustris* in a magnetic field. The alignment field  $\underline{H}_A$  is along the Z axis of the laboratory axis system (XYZ).  $E_{\parallel}$  is parallel to  $\underline{H}_A$ , and  $E_{\perp 1}$  is perpendicular to  $\underline{H}_A$ .  $\beta$  is the angle between the normal to the membrane  $\hat{n}$  ( $\hat{n}$  is also the z axis of the molecular axis system) and the hypothetical normal to a perfect cylinder  $\hat{N}$ .

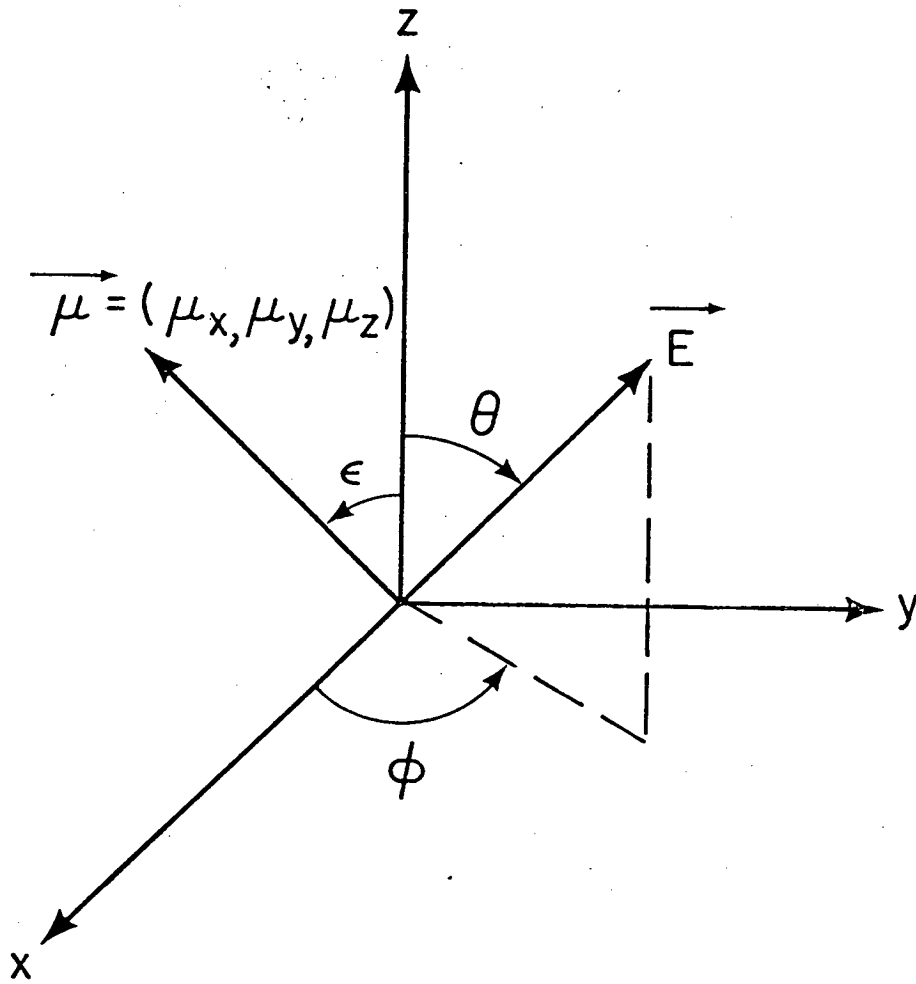
10.  $T_{\parallel}(\Delta_C)$  and  $T_{\perp}(\Delta_C)$  for *Rps. viridis* and *Rps. palustris* in a magnetic field.
11. Definition of the angles and axis systems for flow oriented *Rps. palustris*. The flow direction is along the Y axis of the laboratory axis system (XYZ).  $\underline{E}_{\parallel}$  is parallel to the flow direction and  $\underline{E}_{\perp}$  is perpendicular to the flow direction.  $\beta$  is the angle between the membrane normal  $\hat{n}$  ( $\hat{n}$  is also the z axis of the molecular axis system) and the hypothetical normal to a perfect cylinder  $\hat{N}$ .  $\chi$  is the angle between the flow direction and the long axis of the cylinder L.



XBL 796-4824

Nairn et. al.

Fig 1



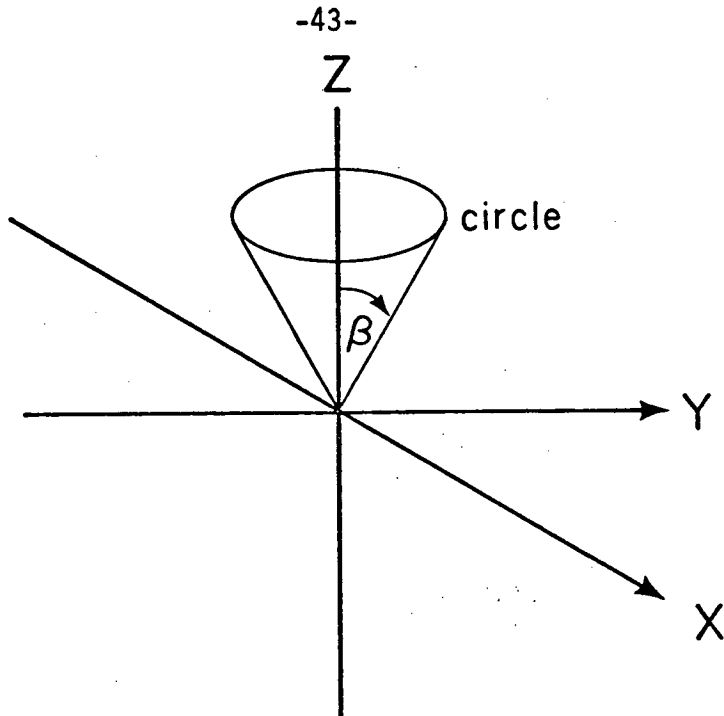
XBL796-4825

Naim et. al.

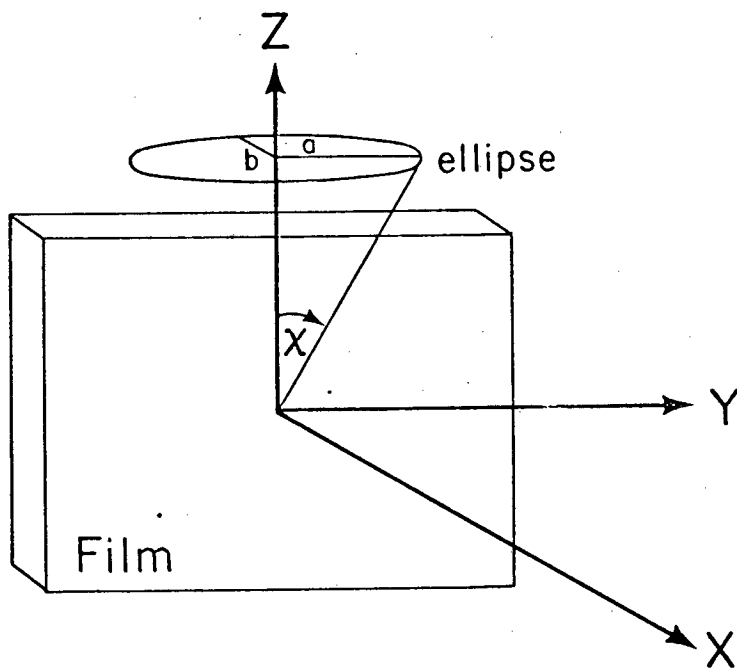
Fig 2



a)



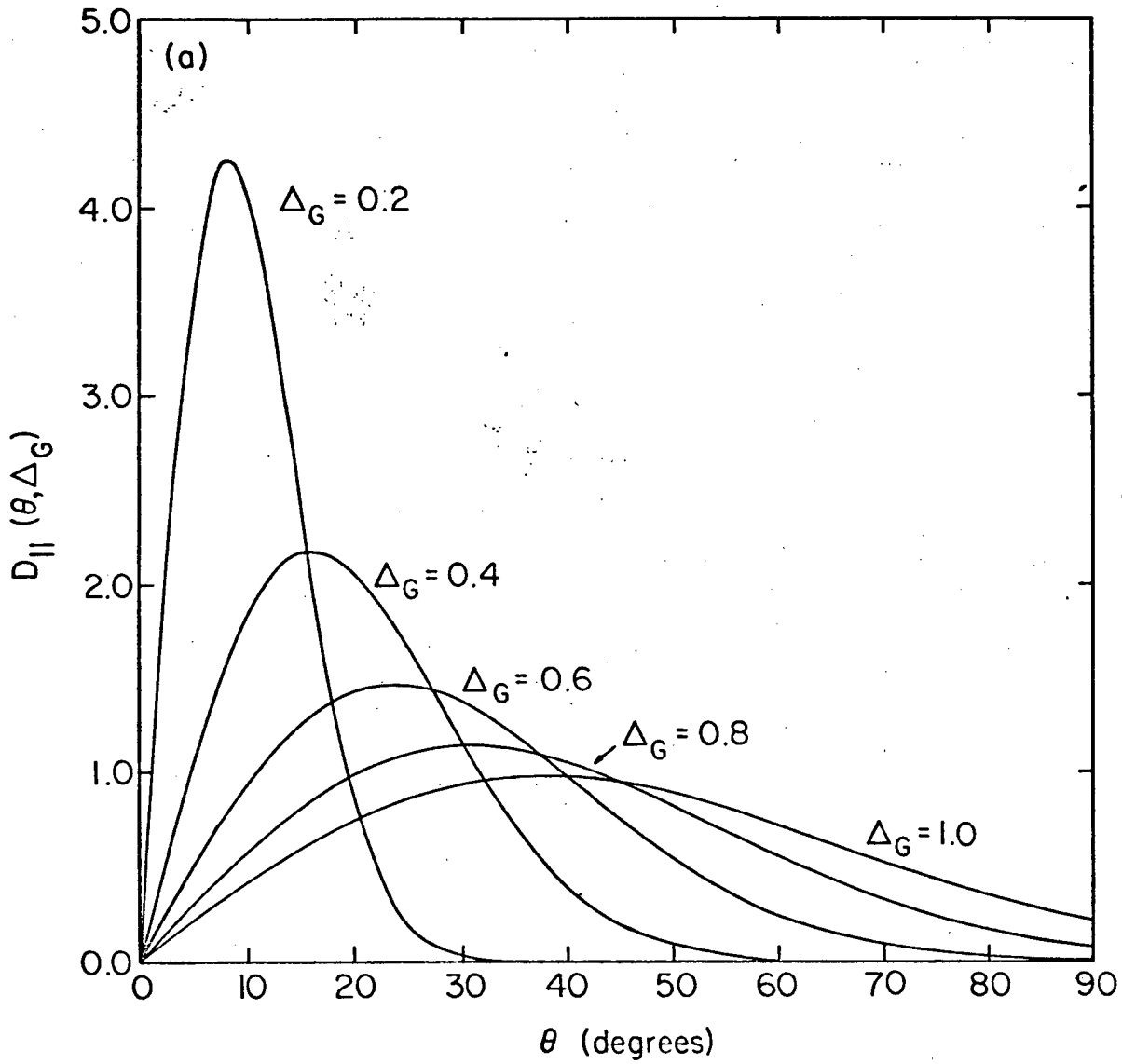
b)



XBL 796-4826

Nain et. al.

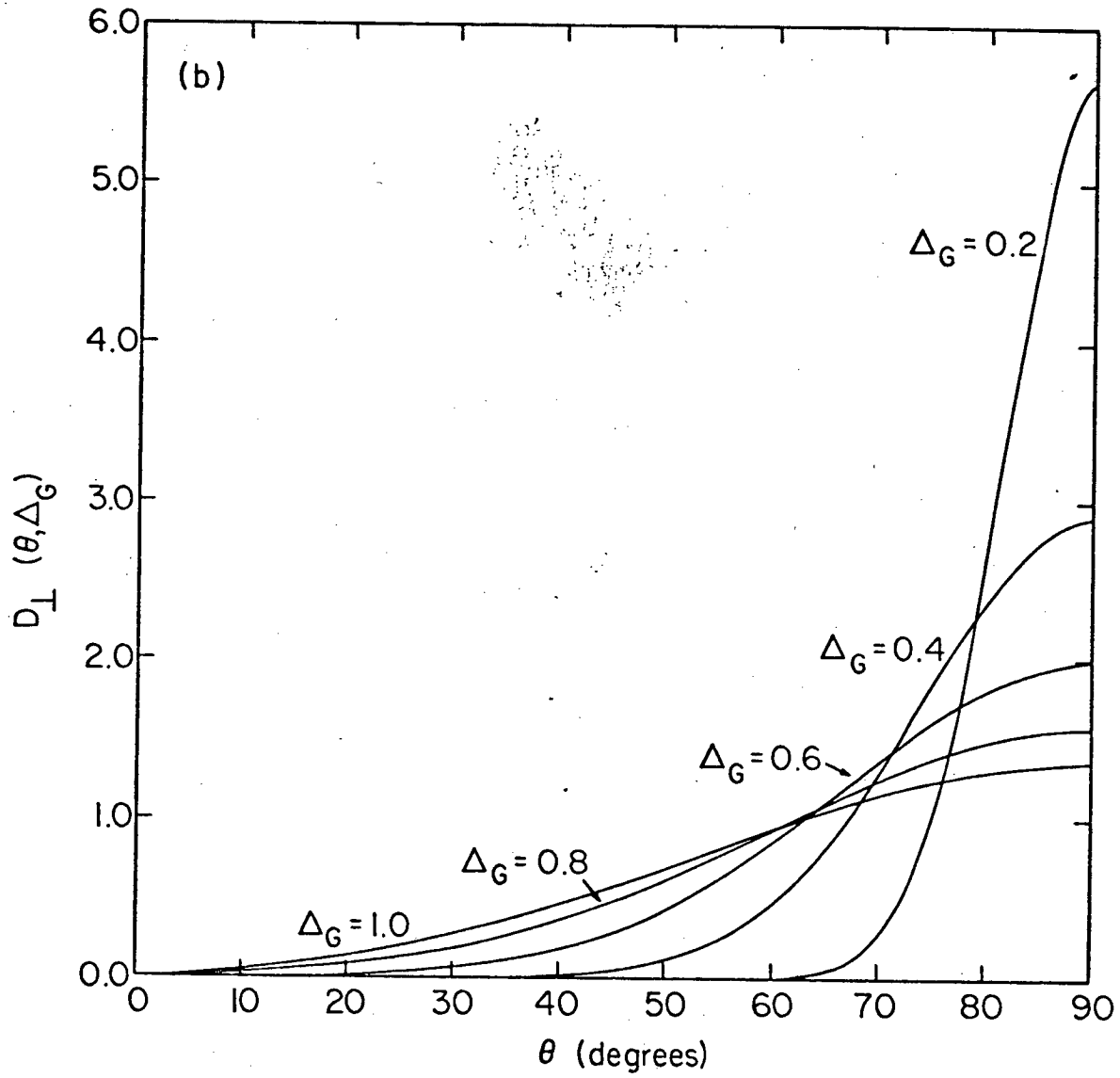
Fig 3



XBL798-4954

Naim et al.

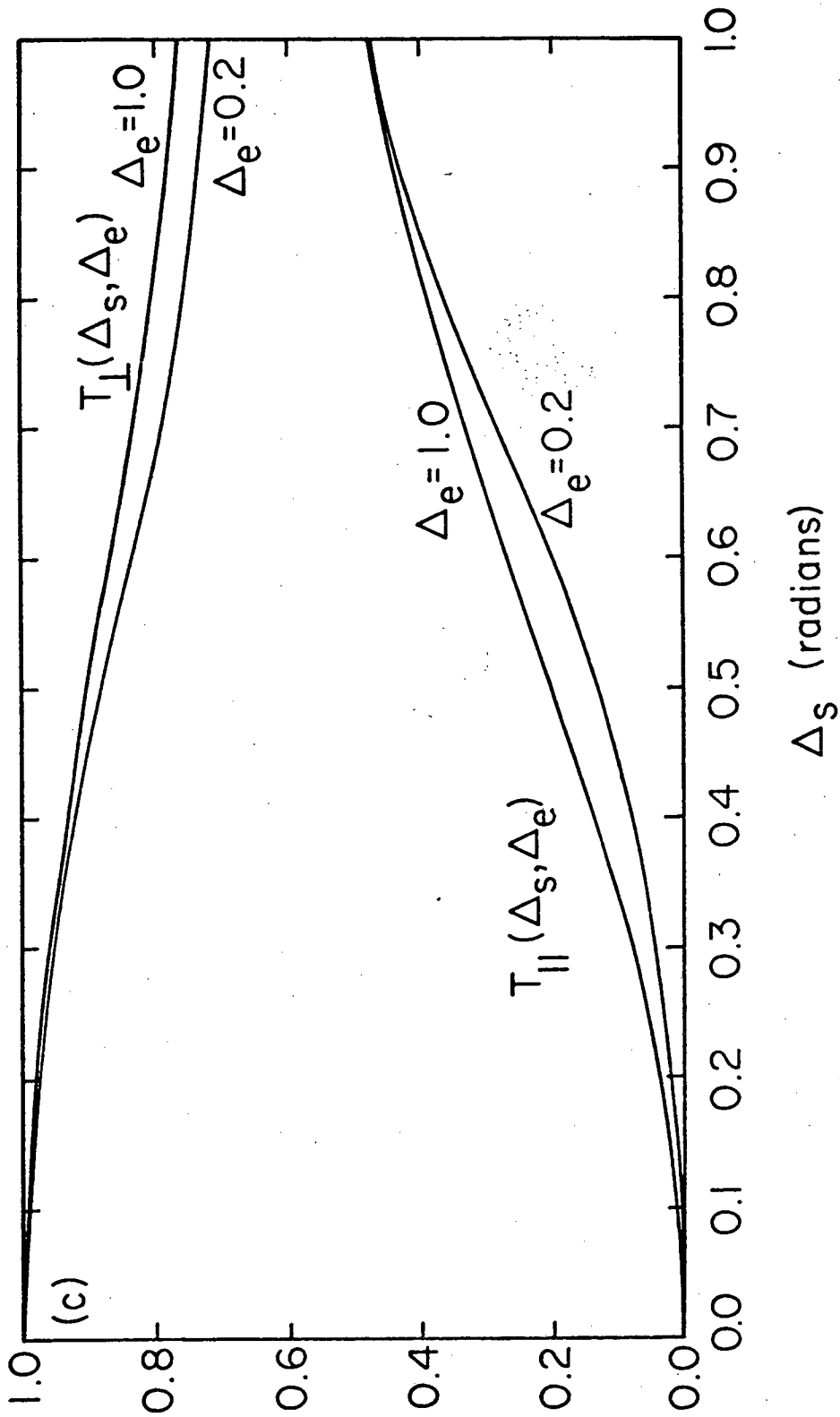
Fig 4a



XBL798-4955

Nain et. al.

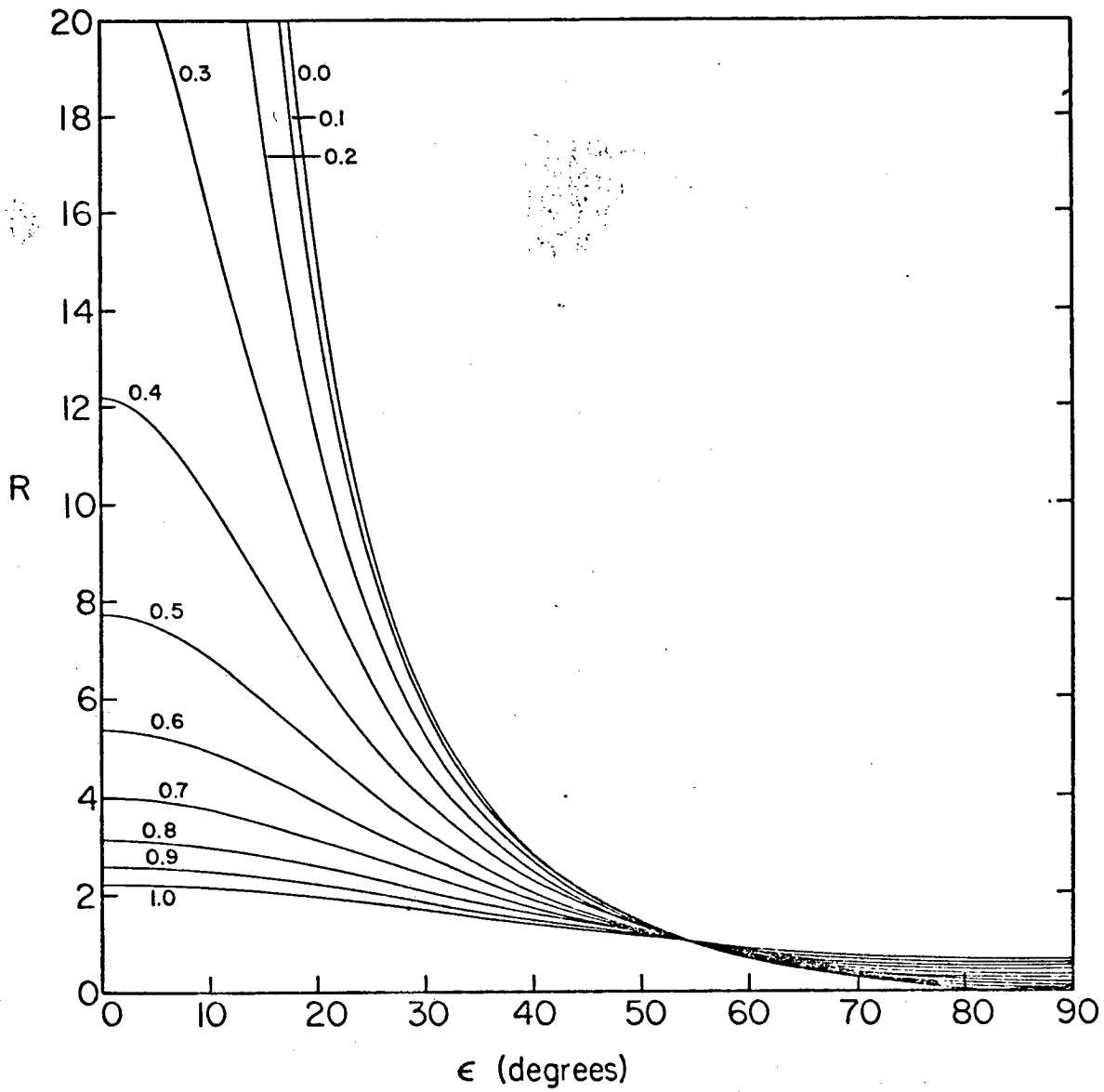
Fig 45



XBL798-4950

Naim et al.

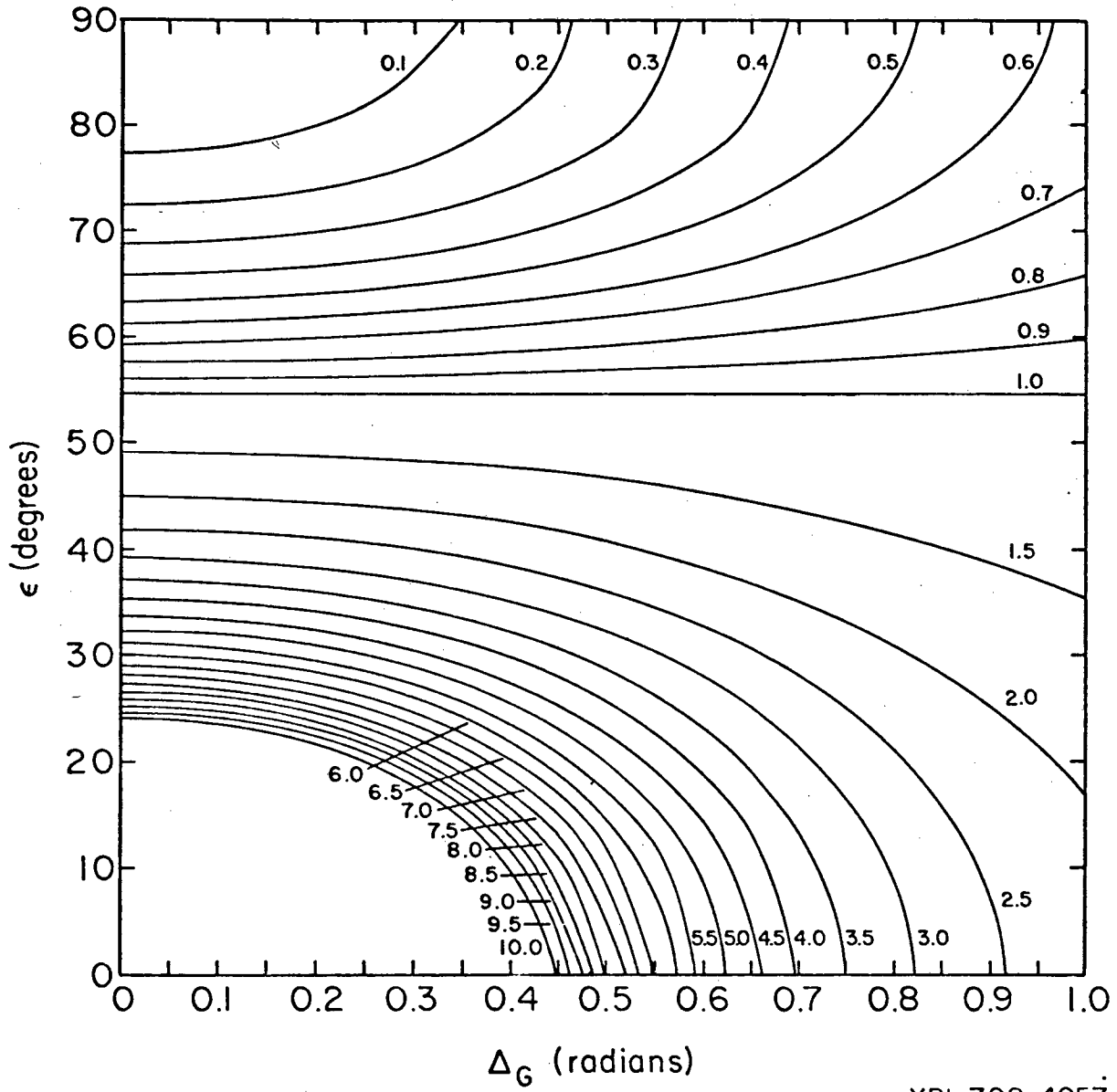
Fig 4c



XBL 798-4956

Naim et al.

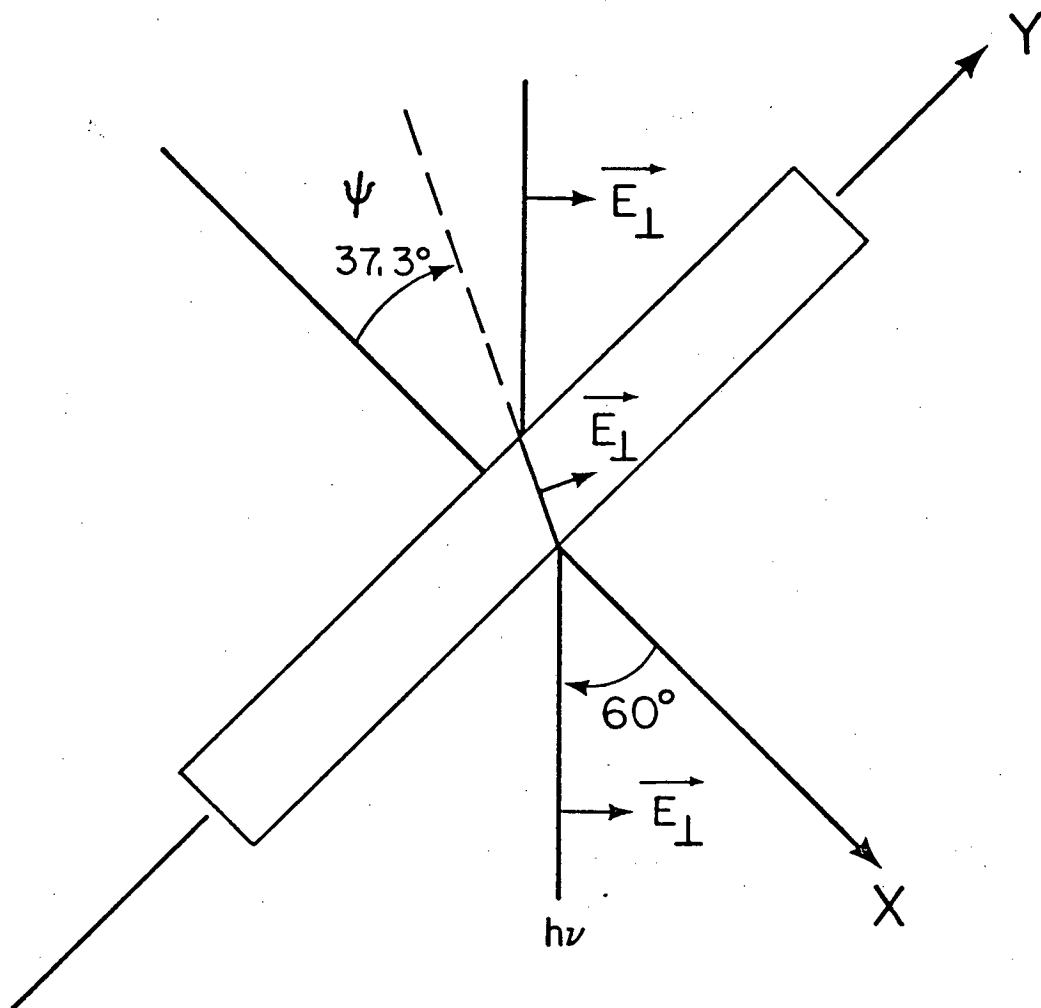
Fig 5



XBL 798-4957

Nairn et. al.

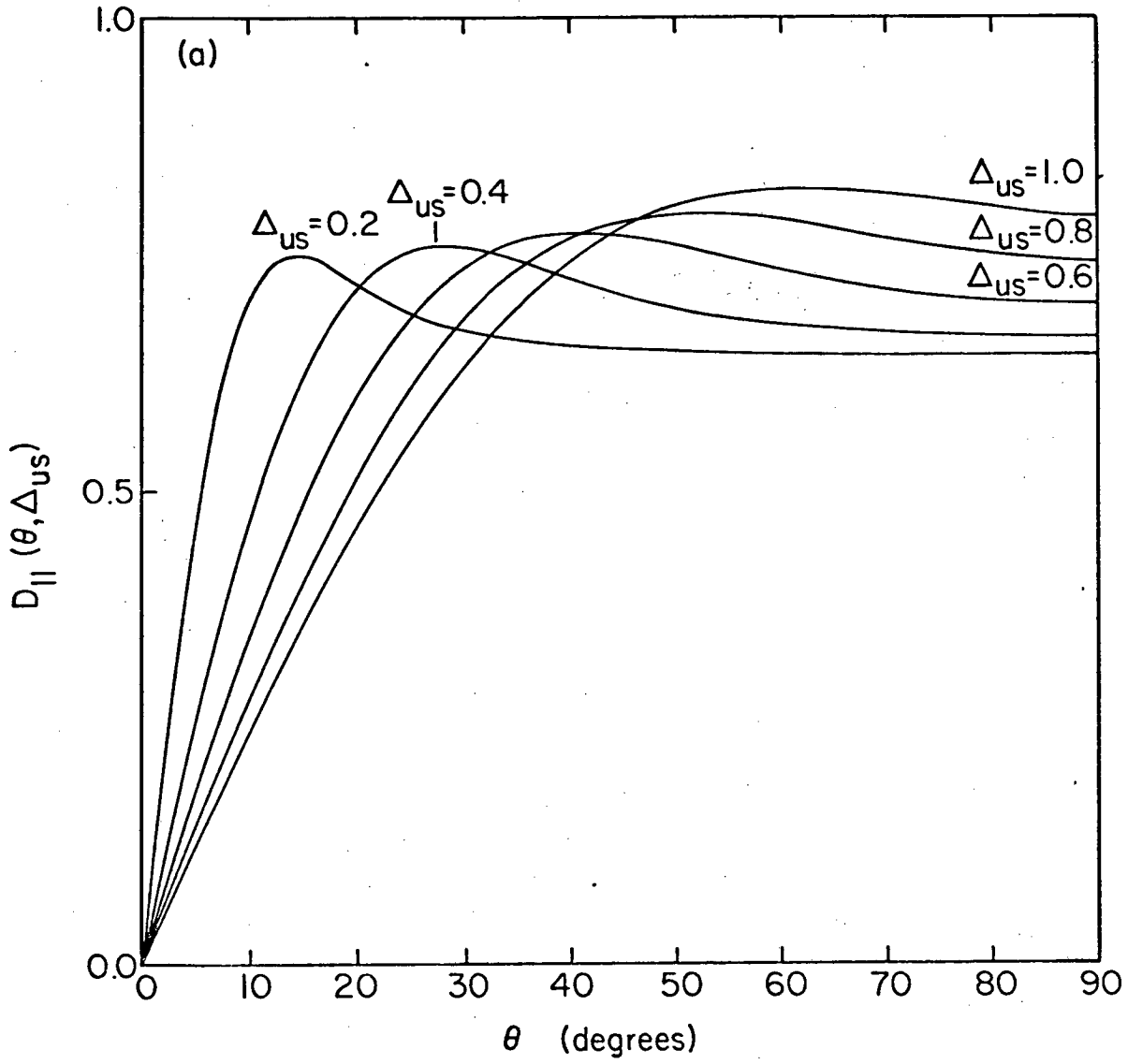
Fig. 6



XBL796-4827

Nairn et al.

Fig 7

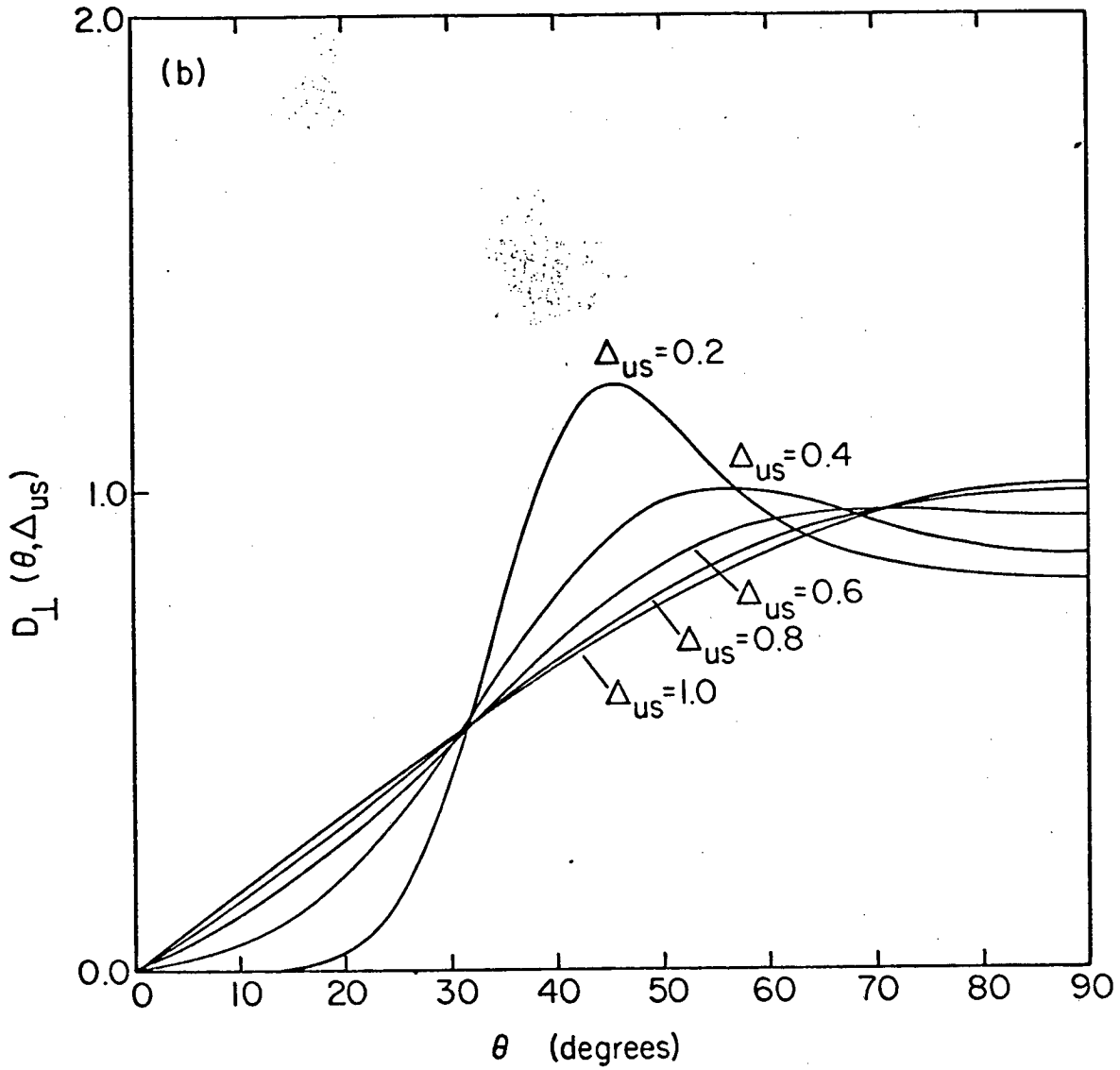


XBL798-4952

Nairn et. al.

Fig 8a

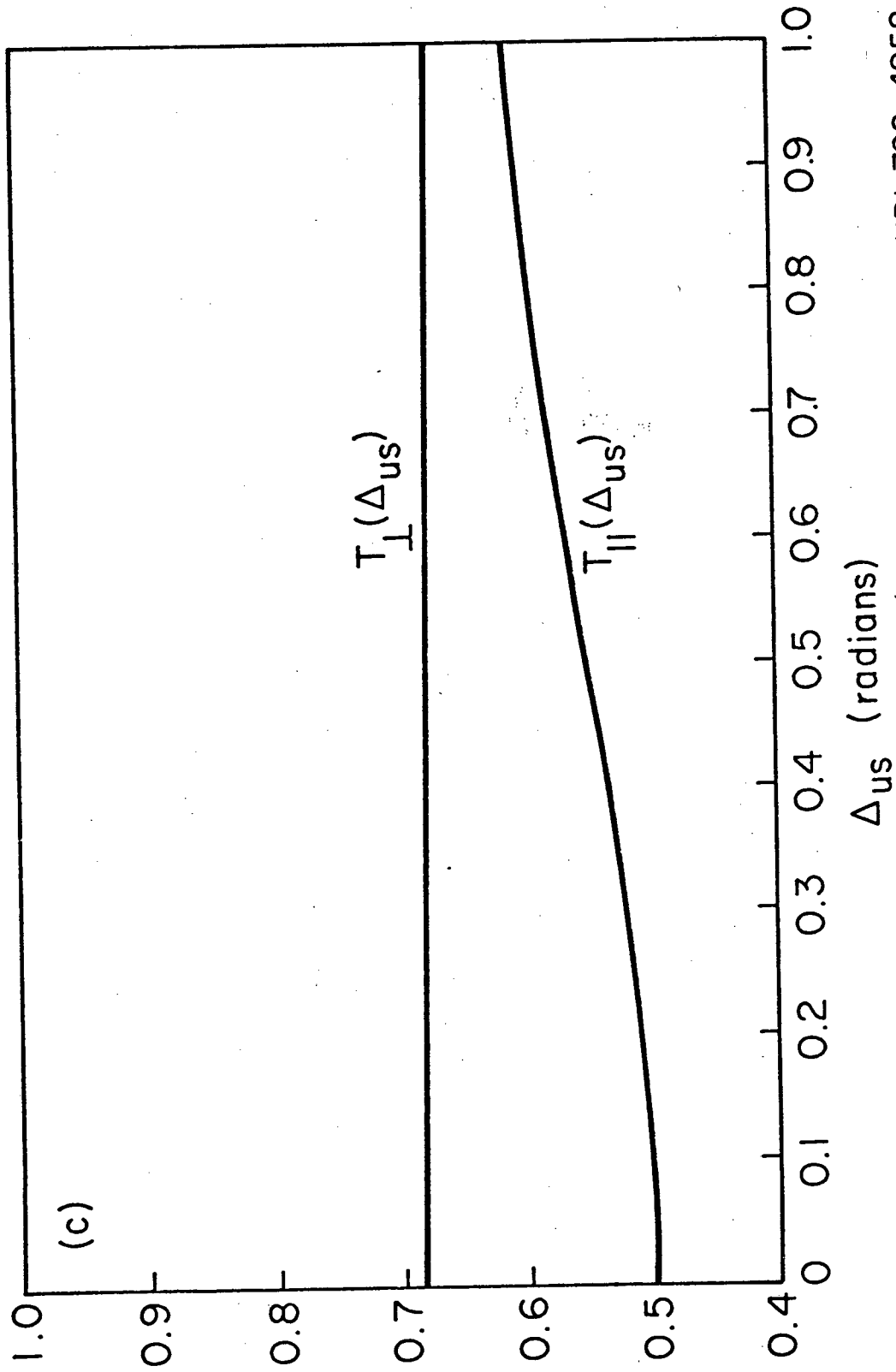




XBL 798-4953

Nairn et al.

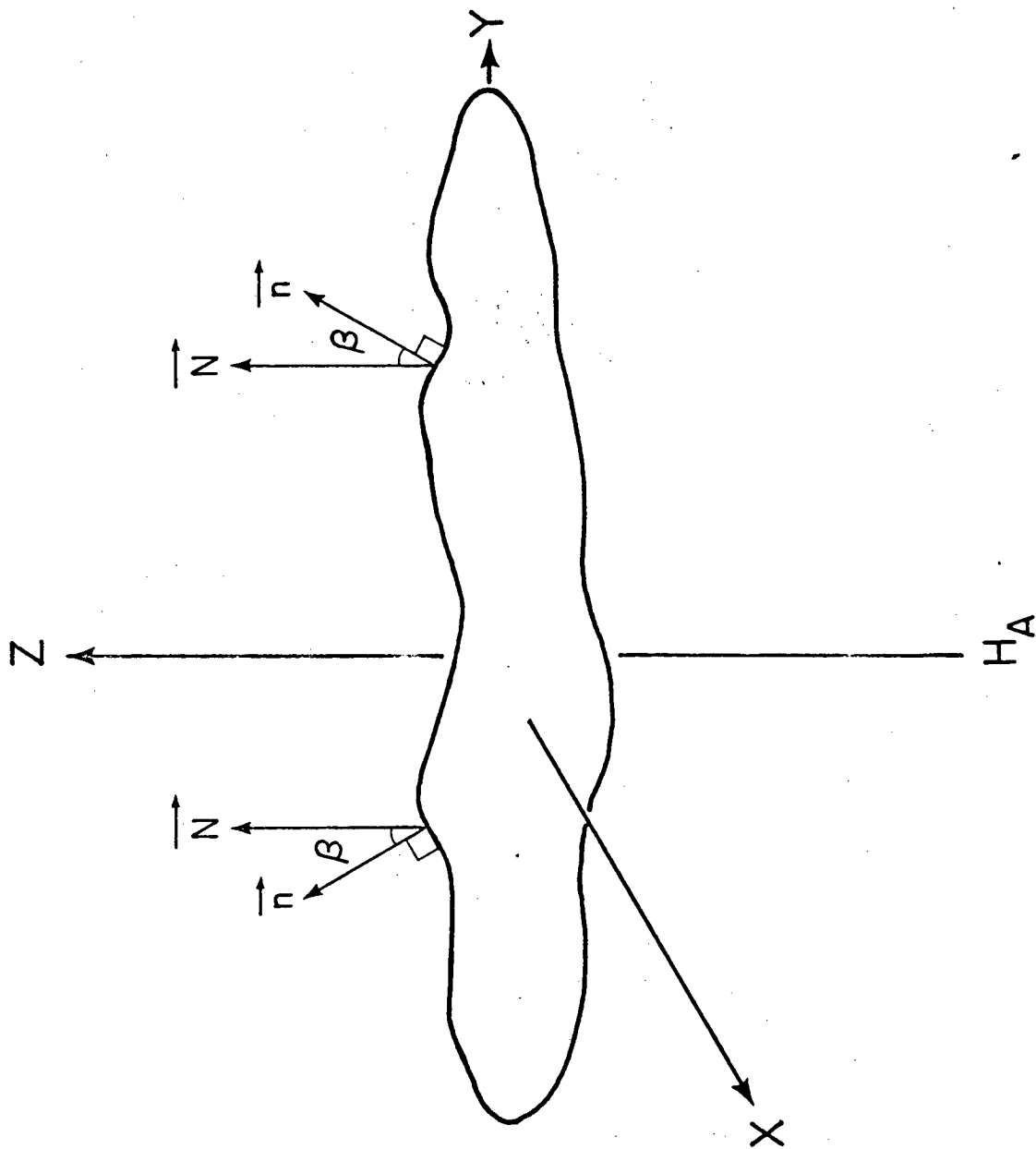
Fig 8b



XBL 798-4958

Nairn et al.

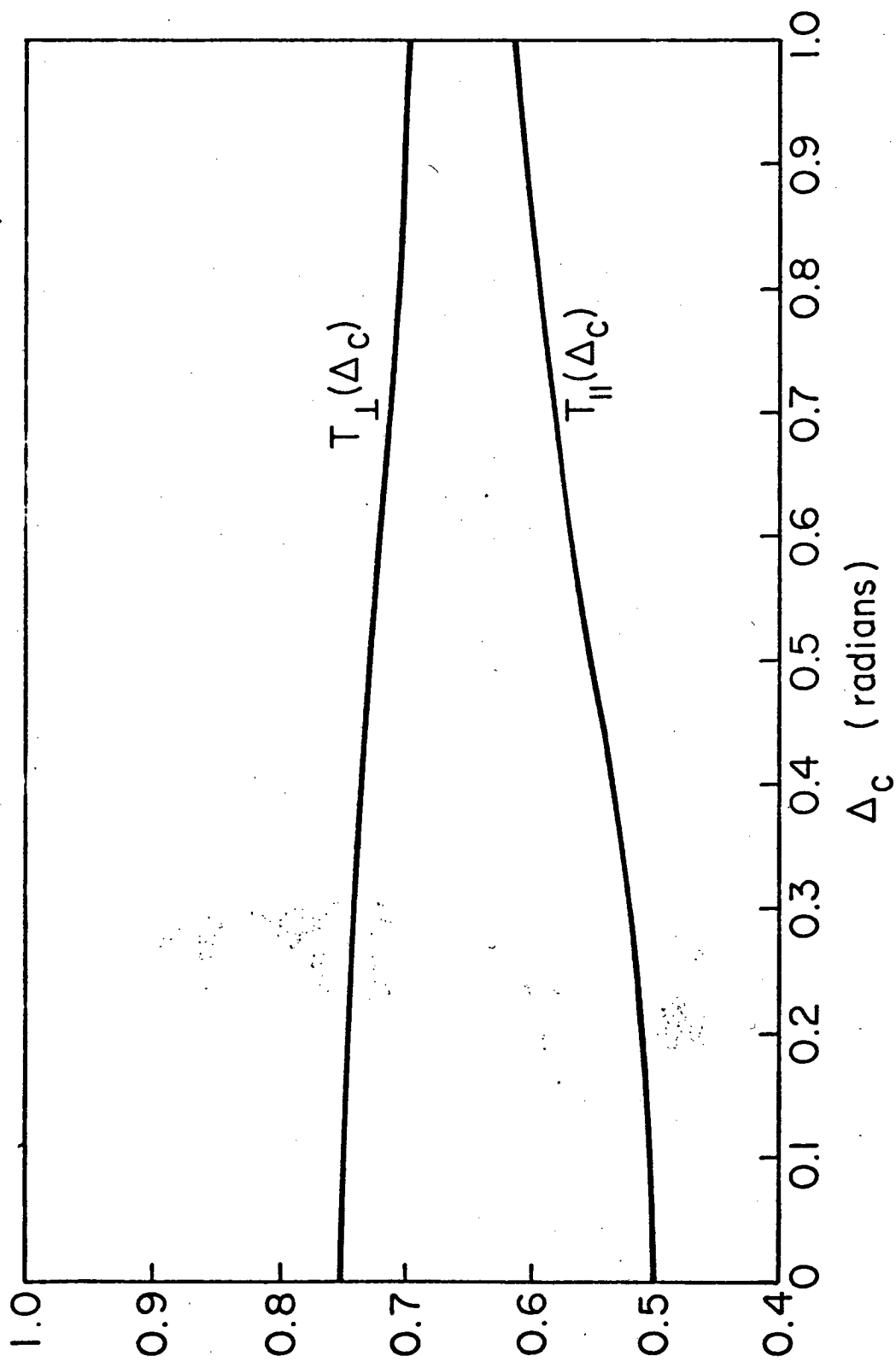
Fig 8c



XBL796-4828

Nairn et. al.

Fig 9

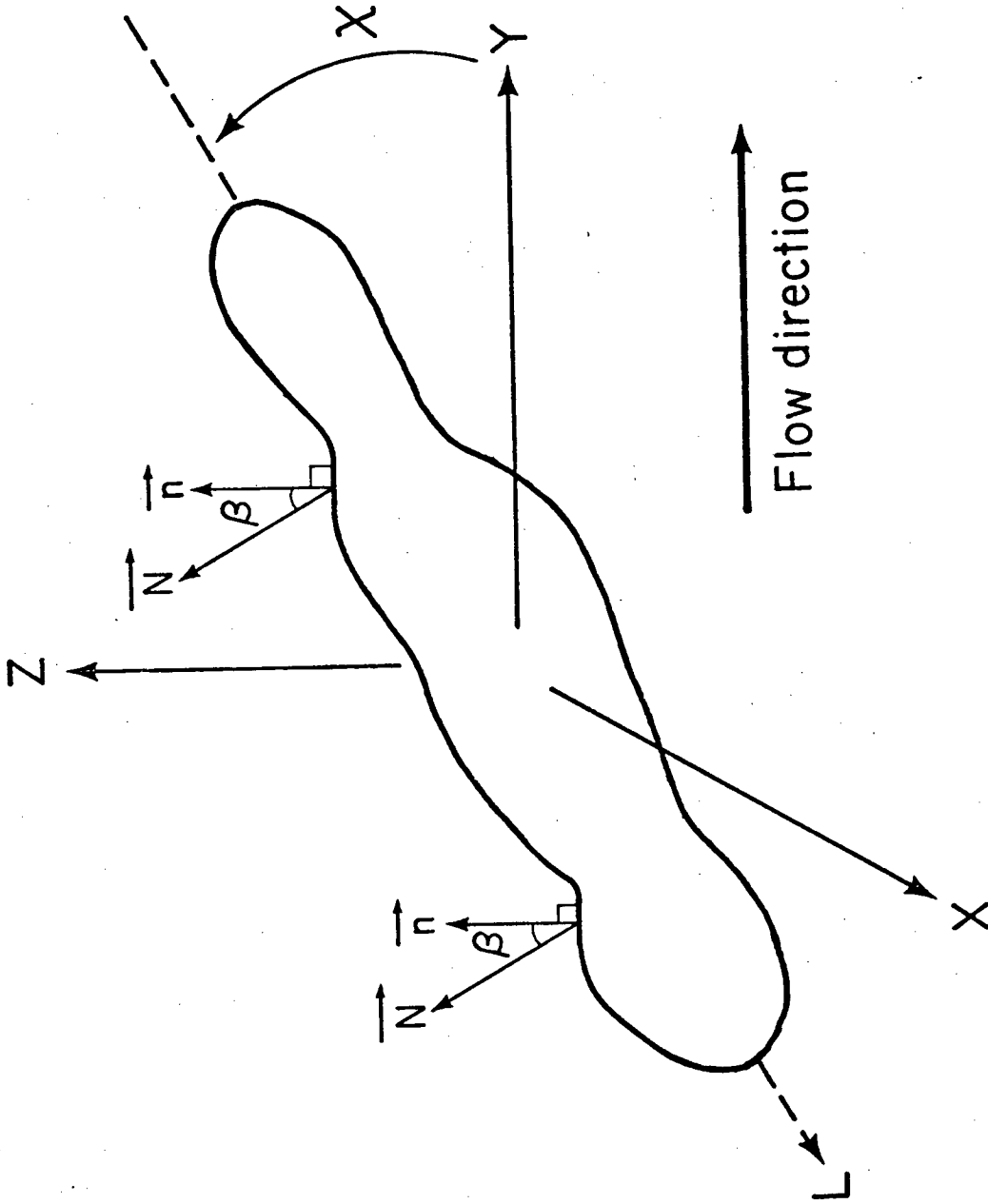


XBL798-4951

Nairn et al.

Fig 10

XBL 796-4829



Nairn et. al.

Fig 11

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

TECHNICAL INFORMATION DEPARTMENT  
LAWRENCE BERKELEY LABORATORY  
UNIVERSITY OF CALIFORNIA  
BERKELEY, CALIFORNIA 94720