

Lawrence Berkeley National Laboratory

Recent Work

Title

LINEAR DICHROISM OF LIGHT HARVESTING BACTERIOCHLOROPHYLL PROTEINS FROM RHODOPSEU-DOMONAS SPHAEROIDES IN STRETCHED POLYVINYL ALCOHOL FILMS

Permalink

<https://escholarship.org/uc/item/27t851x4>

Author

Bolt, John

Publication Date

1978-08-01

LINEAR DICHROISM OF LIGHT HARVESTING BACTERIOCHLOROPHYLL
PROTEINS FROM RHODOPSEUDOMONAS SPHAEROIDES IN
STRETCHED POLYVINYL ALCOHOL FILMS

John Bolt and Kenneth Sauer

August 1978

RECEIVED
LAWRENCE
BERKELEY LABORATORY

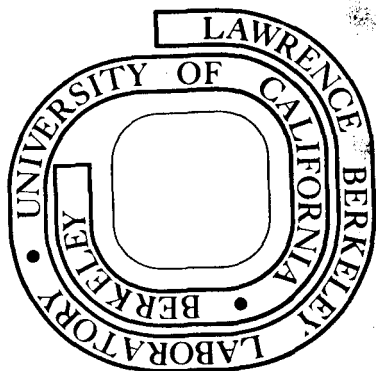
OCT 3 1978

LIBRARY AND
DOCUMENTS SECTION

Prepared for the U.S. Department of Energy
under Contract W-7405-ENG-48

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-7987
c2*

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.

LINEAR DICHROISM OF LIGHT HARVESTING BACTERIOCHLOROPHYLL
PROTEINS FROM RHODOPSEUDOMONAS SPHAEROIDES IN
STRETCHED POLYVINYL ALCOHOL FILMS

John Bolt and Kenneth Sauer

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

ABSTRACT

Light-harvesting bacteriochlorophyll-protein complexes from Rhodospseudomonas sphaeroides 2.4.1 and R-26 mutant are solubilized in SDS and imbedded in polyvinyl alcohol. Stretching induces orientation, and the linear dichroism of visible and near infrared absorption is analyzed. Based on a simple model, angles between the particle axis and the transition dipole moments are found. In the near infrared absorption band of the R-26 light-harvesting protein the dichroic ratio varies from 1.30 to 1.57. Using the absorption curves the band is resolved into two exciton components. In the visible absorption band the dichroic ratio has a constant value of 0.43 for the R-26 protein but varies with wavelength for the wild type light-harvesting protein. This variation is attributed to an additional bacteriochlorophyll not present in the R-26 protein.

INTRODUCTION

The light harvesting bacteriochlorophyll a and carotenoid of purple photosynthetic bacteria are found complexed with low molecular weight peptides of approximately 10,000 daltons [1-7]. With selective solubilization of the chromatophore membranes these complexes can be isolated. Sauer and Austin separated a complex from the carotenoidless mutant of Rhodospseudomonas sphaeroides, R-26, which contained two polypeptides and two bacteriochlorophylls [6]. In the presence of sodium dodecylsulfate (SDS) this complex is not further aggregated, and it absorbs in the near infrared with a single maximum at 853 nm. The circular dichroism, CD, spectrum shows a double Cotton effect, negative to long wavelengths, and centered on the 853 nm absorption band. A similar complex from the wild type Rps. sphaeroides contains an additional bacteriochlorophyll and a carotenoid and exhibits absorption maxima at 799 nm and 850 nm. Cogdell and Thornber classify this complex as B800-B850 [7]. The 800 nm absorption band of the wild type complex can be irreversibly and selectively bleached, leaving a complex with essentially the same absorption and CD properties as the complex isolated from the mutant R-26 [6]. Thus, we believe the complex from R-26 is an altered form of the wild type complex. This paper reports evidence to support this belief.

Recently Rafferty and Clayton reported the linear dichroism of the reaction center bacteriochlorophyll-protein complex in a stretched gelatin film [8]. We use a similar approach, inbedding the protein detergent particles in polyvinyl alcohol as the stretching matrix. The information gained is the orientation of the transition dipoles with respect to the stretching axis. Because bacteriochlorophyll has two well-separated electronic transitions, Q_x and Q_y , which are believed to be polarized in the monomer bacteriochlorophyll close to the bacteriochlorin symmetry axes [9], and because these pigment

protein complexes involve a small number of bacteriochlorophylls, they are suitable for determining the geometry of the pigment molecules from optical spectra.

MATERIALS AND METHODS

Bacteria strains R-26 and 2.4.1 of Rps. sphaeroides were grown as described by Sauer and Austin [6]. Chromatophores were prepared by the method of Clayton and Wang [10] and were purified on a 0.6/1.2 M discontinuous sucrose gradient. The light-harvesting complex (LH-wt) from wild type chromatophores was prepared according to published procedures [2, 11]. The protein complex was dialyzed against 0.01 M Tris HCl, pH = 7.6, henceforth called buffer, and stored at -20°C. The light-harvesting complex (LH-R26) from R-26 was prepared as follows: Chromatophores were adjusted to an absorbance of 0.5 cm^{-1} at 860 nm when diluted one hundred-fold. Dodecyl-dimethylamine oxide (DDAO) was added to a final concentration of 1% and the suspension was centrifuged over a 0.6/1.1 M discontinuous sucrose gradient at 90,000 g for three hours. The green material at the center sucrose interface was collected with a syringe and dialyzed overnight against buffer. The dialyzate was centrifuged one hour at 80,000 g. The pellet was resuspended in a minimum volume of buffer containing 1% SDS. The protein was then purified on a Sephadex G-150 column at 13°C. The material from 15 ml of chromatophores was applied to a column 29 x 2.5 cm and eluted with 0.4% SDS in buffer. Fractions with the ratio of absorbance at 855 nm to absorbance at 275 nm greater than 2.3 were pooled, dialyzed against buffer and stored at -20°C. Low molecular weight polyvinyl alcohol (PVA) from Touzart et Matignon was dissolved as a 35% solution in 50 mM Tris HCl to a final pH = 7.0. The dialyzed proteins were concentrated by centrifugation and resuspended in buffer containing 3.5 mM SDS to an absorbance at 853 nm of 25 cm^{-1} .

Due to the labile nature of the 800 nm band, 50 mM dithioerythritol (DTE) was included in the final solution of LH-wt protein. One half to 2.0 ml of the protein solution was mixed with 2.5 ml of the PVA solution at room temperature. After allowing the bubbles to rise overnight the solution was spread over a glass microscope slide, 25 x 76 mm, and dried in a nitrogen atmosphere at room temperature in the dark. The PVA film was peeled off the glass slide and hydrated at 100% humidity for one hour. A center portion was cut, clamped into a simple stretching device and stretched slowly to about half maximum. The film was rehydrated for 30 minutes before stretching to the final value. The extent of stretching is measured by stretch ratio, R_s , as defined by Land [12]. Stretch ratio is the axial ratio of the ellipse into which a hypothetical circle on the film is distorted.

Absorption measurements were performed using a Cary 14 recording spectrophotometer with a Hamamatsu R928 photomultiplier. Glan-Thompson prisms were mounted in sample and reference beams. The plane of the PVA film was mounted perpendicular to the beam with the stretch axis vertical. Absorption spectra with polarizations parallel and perpendicular to the stretch axis were measured by rotating the polarizing prisms for vertical and horizontal polarizations respectively. Baselines with vertical and horizontal polarizations were measured separately. Absorption of films made and stretched with no proteins present was coincident with the baseline after correcting for the baseline shift at 930 nm. The baseline shift was attributed to reflection and a small amount of light scattering. The plane of the PVA film was tilted by mounting dry films on a goniometer, with the rotating axis vertical and passing through the center of the beam.

RESULTS AND DISCUSSION

Figure 1 shows the absorption of the LH-R26 protein parallel and perpendicular to the stretch axis. In the Q_y region (820-920 nm) there is a distinct change in dichroic ratio across the band. The sigmoid shape of the ratio suggests two overlapping electronic transitions with different dichroic ratios. The midpoint of this sigmoid curve is 6 to 8 nm to the red of the wavelength of maximum absorbance, λ_{\max} . In the 850 nm absorption band of the LH-wt protein (Fig. 2) this behavior is also observed, suggesting similarity of the two complexes.

In the Q_x region of the LH-R26 protein (Fig. 1) the dichroic ratio is constant within the precision of the measurement. By contrast, the LH-wt protein has a distinct change across the Q_x absorption band. When DTE is omitted from the PVA film the 797 nm absorption band is bleached (not shown). This also decreases the dichroic ratio on the short wavelength side of the 590 nm absorption band but not on the long wavelength side. In solution, the selective bleaching at 800 nm is accompanied by a shift of the Q_x λ_{\max} from 589 nm to 592 nm. Thus, the increase in A_{\parallel} at shorter wavelengths in the Q_x region of the intact LH-wt protein is correlated with the presence of the 800 nm absorption band.

The dichroic ratio across the carotenoid absorption is constant from 440 nm to 520 nm.

Rafferty and Clayton reported orientation of reaction centers in unstretched gelatin films [8]. This was a result of the shrinkage in thickness by a factor of 30 on drying the gelatin film. The PVA films shrink to no less than one-fourth the original thickness. As a result, no dichroism was detected in unstretched, dried PVA films when A_{\parallel} and A_{\perp} were recorded with the sample tilted by 45 degrees.

To extract quantitative information from the linear dichroism presented in Figs. 1 and 2 it was necessary to establish a model for the orientation

phenomenon. This model must include the shape and extent of aggregation of the protein detergent particles and also the extent of orientation achieved by stretching. We will assume that orientation is uniaxial along the direction of stretch and that the particle contains a unique long axis. (However, an oblate ellipsoid model has not been eliminated.) Unfortunately, the aggregation state of the proteins cannot be directly determined in the PVA glass. The absorption and circular dichroism spectra are unaffected by incorporation into PVA film. In addition the films show no sign of particle aggregation and are optically clear. For these reasons we feel justified in treating the LH-R26 protein as individual units containing two polypeptides, SDS and two bacteriochlorophylls, and the LH-wt protein as a unit containing two polypeptides, SDS, three bacteriochlorophylls and one carotenoid [6].

According to the model of Fraser, the degree of orientation can be described by a fraction, f , of particles aligned perfectly with the stretch axis and a fraction, $1-f$, randomly oriented [13]. The dichroic ratio, D , can be expressed as a function of f and θ , the angle of the transition dipole to the particle axis:

$$D = \frac{A_{||}}{A_{\perp}} = \frac{f \cos^2 \theta + \frac{1}{3}(1-f)}{\frac{1}{2} f \sin^2 \theta + \frac{1}{3}(1-f)} \quad (1)$$

Other choices for plausible distribution functions can be related to equation (1) [4].

Two approaches were used to assess the orientation parameter f . The first is based on the restrictions inherent in equation (1). In Fig. 3, f is plotted as a function of θ and D . Typical values for the dichroic ratio are 1.55 (Q_y) and 0.4 (Q_x), which restrict f to values greater than 0.15 and 0.5 respectively. Since f must be the same for all dipoles within the same sample, we restrict it to values greater than 0.5. For dichroic ratios close to 1, the choice of f is not critical; but for increasingly

greater dichroism, the value of θ varies with f more significantly. Another approach is to plot the dichroic ratios as a function of amount of stretching. Figure 4 plots D versus R_s at various wavelengths for a sample of the LH-wt protein. With stretch ratio greater than 3 the dichroic ratios approach limiting values. This implies that a maximum value of f has been reached, but it does not imply that f equals 1. Table I lists values of θ based on observed dichroic ratios and using several possible values of the orientation parameter.

The apparent presence of two electronic transitions in the Q_y region is best interpreted in terms of oscillators with unequal strengths centered at different wavelengths. (Equally weighted oscillators at different wavelengths would result in a CD zero crossing and a dichroic ratio transition with midpoint centered at the peak of the absorption curve). Exciton coupling of two chromophores predicts splitting of the degenerate transitions into nondegenerate orthogonal transitions, designated plus and minus. Using the simple dipole model of the exciton coupling, the oscillator strengths (F_+ and F_-) of the exciton transitions should sum to twice the monomer oscillator strength [16]. The ratio F_+/F_- gives the angle, β , between the two monomer dipoles by equation (2):

$$\frac{F_+}{F_-} = \frac{1 + \cos\beta}{1 - \cos\beta} \quad (2)$$

The total Q_y absorption band is resolved into these two exciton absorption bands by writing expressions for A_{\parallel} and A_{\perp} including both exciton components, A_+ and A_- :

$$\begin{aligned} A_{\parallel} &= A_+ \cos^2 \theta_+ + A_- \cos^2 \theta_- \\ A_{\perp} &= \frac{1}{2} A_+ \sin^2 \theta_+ + \frac{1}{2} A_- \sin^2 \theta_- \end{aligned} \quad (3)$$

The assumption of perfect orientation, $f = 1$, does not affect the shape of the

resulting bands. θ_+ and θ_- are found from the limiting values of dichroic ratio, 1.30 and 1.57, in Fig. 1. Equations (3) can be solved simultaneously for A_+ and A_- at every wavelength:

$$A_+ = \frac{2A_{\perp} \cos^2 \theta_- - A_{\parallel} \sin^2 \theta_-}{\sin^2 \theta_+ \cos^2 \theta_- - \cos^2 \theta_+ \sin^2 \theta_-} \quad (4)$$

$$A_- = \frac{2A_{\perp} \cos^2 \theta_+ - A_{\parallel} \sin^2 \theta_+}{\sin^2 \theta_- \cos^2 \theta_+ - \cos^2 \theta_- \sin^2 \theta_+}$$

The resolution is plotted in Fig. 5. Taking the peak to peak separation as representative of the exciton splitting, then $\Delta\nu = 200 \text{ cm}^{-1}$. Using the relative areas under A_+ and A_- to estimate the oscillator strengths, then $F_+/F_- = 1.53$ and $\beta = 78^\circ$ from equation (2).

In the Q_x region of the LH-R26 protein no change in dichroic ratio across the band was observed. The CD in this band also shows a double Cotton effect, indicating exciton splitting of this transition. Therefore, the two exciton transition dipoles are tilted at the same angle to the particle axis. For the LH-wt protein, with one additional bacteriochlorophyll per molecule of complex, the change in linear dichroism across the band allows resolution into a band representing the two bacteriochlorophylls which are coupled and a band representing the third bacteriochlorophyll. The ratio of areas under the curves should be 2:1. The choice of limiting dichroic ratios is more difficult than for the Q_y band. We define A_{12x} and D_{12x} as the absorption and dichroic ratio due to the coupled bacteriochlorophylls; A_{3x} and D_{3x} are similarly defined for the third bacteriochlorophyll. On the long wavelength side $D_{12x} = 0.33$ is chosen in accord with the LH-R26 values. The A_{\parallel} and A_{\perp} are then fit to A_{12x} and A_{3x} using equation (4) for different values of D_{3x} until the area under A_{3x} is half

the area under A_{12x} . The fit is plotted in Fig. 6. D_{3x} is found to be 1.65. The λ_{\max} for A_{12x} is 593 nm in agreement with the λ_{\max} of the LH-R26 protein.

CONCLUSION

From these simple experiments we have been able to deduce information concerning the geometry of the transition dipoles in the light harvesting proteins. Figure 7 summarizes the data. The LH-R26 protein contains two bacteriochlorophylls; both Q_x and Q_y absorption transitions are coupled. The Q_y absorption band is resolved into its exciton components which are tilted at approximately 48 and 51 degrees to the particle axis, Fig. 7(a). In the plane containing the two Q_y exciton dipoles, the monomer dipoles can be found by using the simple point dipole theory for the exciton interaction; Fig. 7(b). The Q_x exciton transitions are tilted at 65 degrees to the particle axis but cannot be resolved. The LH-wt protein contains an additional bacteriochlorophyll and a carotenoid, Fig. 7(c). The carotenoid transition dipole is tilted at 58° to the particle axis. The Q_x and Q_y transitions of the third bacteriochlorophyll are not coupled to the other two bacteriochlorophylls; they are tilted by 48 and 51 degrees to the particle axis, such that the normal to the porphyrin plane is tilted by 67 degrees to that axis. More information is needed to obtain a complete picture of the geometry of the pigments. A detailed study of the fluorescence polarization will be used to extend this picture of bacteriochlorophyll arrangement.

ACKNOWLEDGEMENTS

We thank Dr. W. W. Parson for suggesting the method of orienting the proteins and Dr. Jacques Breton for many helpful discussions and encouragement.

This research was supported by the Division of Biomedical and Environmental Research of the U. S. Department of Energy.

REFERENCES

1. J.P. Thornber (1970) *Biochemistry* 9, 2688-2698.
2. R.K. Clayton and B.J. Clayton (1972) *Biochim. Biophys. Acta* 283, 492-504.
3. P.J. Fraker and S. Kaplan (1972) *J. Biol. Chem.* 247, 2732-2737.
4. A.A. Moskalenko and Y.E. Erokhin (1978) *FEBS Lett.* 87, 254-256.
5. R. Feick and G. Drews (1978) *Biochim. Biophys. Acta* 501, 499-513.
6. K. Sauer and L.A. Austin (1978) *Biochemistry* 17, 2011-2019.
7. R.J. Cogdell and J.P. Thornber (1978) Abstracts of the Ciba Foundation Symposium No. 61, "Chlorophyll Organization and Energy Transfer in Photosynthesis", 8.
8. C.H. Rafferty and R.K. Clayton (1978) *Biochim. Biophys. Acta* 502, 51-60.
9. K. Sauer (1975) in *Bioenergetics of Photosynthesis*, Govindjee, ed., Academic Press, N.Y., 115-181.
10. R.K. Clayton and R. Wang (1971) *Methods in Enzymology* 23, 696.
11. P. Heathcote and R.K. Clayton (1977) *Biochim. Biophys. Acta* 459, 506-515.
12. E.H. Land (1951) *J. Opt. Soc. Am.* 41, 957-963.
13. R.D.B. Fraser (1953) *J. Chem. Phys.* 21, 1511-1515.
14. R.D.B. Fraser (1958) *J. Chem. Phys.* 28, 1113-1115.
15. M. Beer (1956) *Proc. Roy. Soc. (London)* A236, 136-140.
16. E.A. Dratz, A.J. Schultz and K. Sauer (1967) *Brookhaven Symp. Biol.* 19, 303-318.

Table I. Angles of the Transition Dipoles to the Long Axis of
Light Harvesting Protines

The values are based on equation (1).

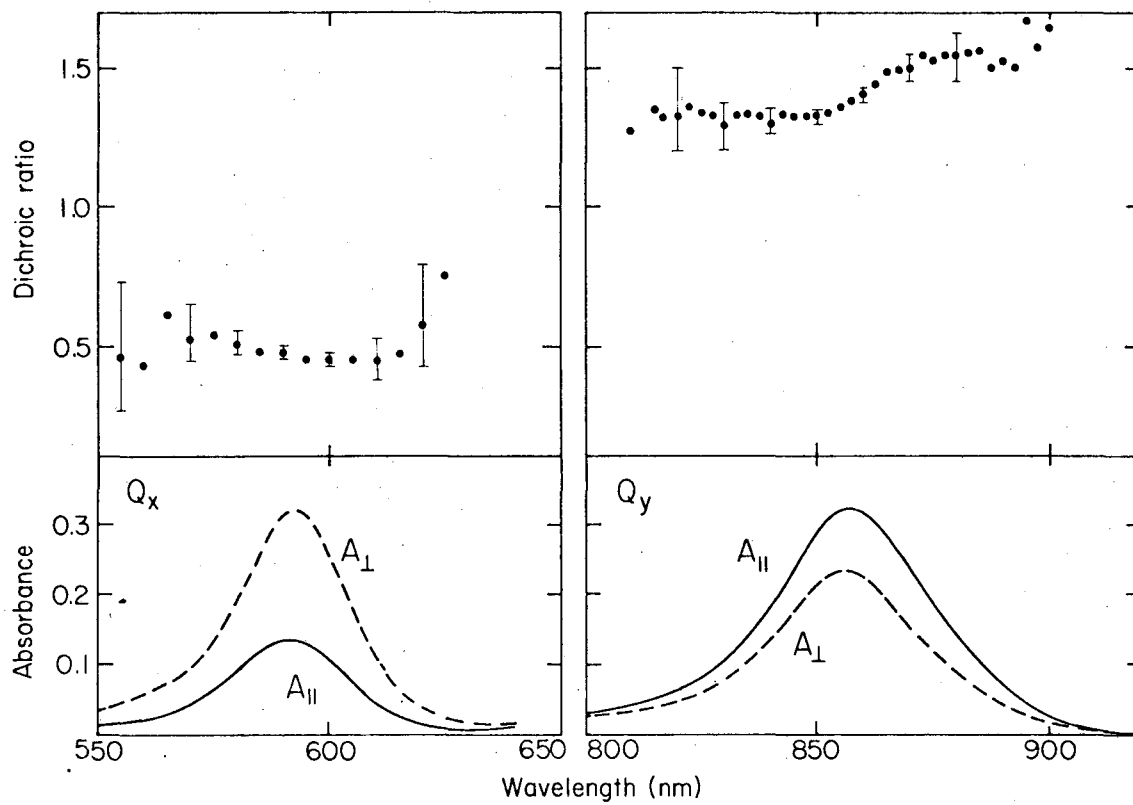
	Wavelength, nm (Dichroic ratio)	θ , degree		
		f=0.5	f=0.75	f=1.0
LH-R26	885 (1.57)	42.3	46.4	48.5
	835 (1.30)	47.6	49.9	51.1
	593 (0.43)	81.8	69.3	65.1
LH-wt.	885 (1.56)	42.5	46.5	48.5
	835, 800 (1.36)	46.4	49.1	50.5
	610 (0.38)	--	71.4	66.4
	575 (0.84)	59.5	57.8	57.1
	510 (0.78)	61.5	59.1	58.0
	A_{12x}^* (0.33)	--	73.8	67.9
	A_{3x}^* (1.65)	40.9	45.5	47.8

* See text for explanation of Q_x absorption bands A_{12x} and A_{3x} .

FIGURE CAPTIONS

- Fig. 1. Absorption parallel (A_{\parallel}) and perpendicular (A_{\perp}) to the stretch axis is shown for the LH-R26 protein in stretched polyvinyl alcohol film. In the Q_y region $R_s = 2.5$. A more concentrated sample was used for better precision in the Q_x region, and $R_s = 3.1$.
- Fig. 2 Absorption parallel and perpendicular to the stretch axis for LH-wt protein in stretched PVA. $R_s = 2.9$.
- Fig. 3. The dependence of θ , angle between particle axis and transition dipole, on the orientation parameter, f , for several values of the dichroic ratio, D . Values of D are labeled for each curve. Plotted from equation (1); adopted from M. Beer [15].
- Fig. 4 The dependence of dichroic ratio on stretching. The sample is LH-wt protein. A_{\parallel}/A_{\perp} is plotted versus stretch ratio at several wavelengths.
- Fig. 5. Resolution of the Q_y region of LH-R26 absorption. $2A_{\perp} + A_{\parallel}$ is the sum of experimental values from Fig. 1. A_+ and A_- are the exciton components. Resolution is based on equation (4) with $\theta_+ = 51.1^\circ$ ($D_+ = 1.30$) and $\theta_- = 48.5^\circ$ ($D_- = 1.57$).
- Fig. 6 Resolution of the Q_x region of LH-wt absorption. $2A_{\perp} + A_{\parallel}$ is from Fig. 2. A_{12x} is absorption attributed to the two bacteriochlorophylls absorbing at 850 nm. A_{3x} is absorption attributed to bacteriochlorophyll absorbing at 800 nm. Resolution is based on equation (4) with $\theta_{12x} = 67.8^\circ$ ($D_{12x} = 0.33$) and $\theta_{3x} = 47.8^\circ$ ($D_{3x} = 1.65$).

Fig. 7. Orientations of the transition dipoles are sketched with respect to the particle stretch axis, Z, using vector representation of the transition dipoles. (a) A_- and A_+ represent the two exciton components of the 853 nm band of the LH-R26 protein. They are orthogonal, and the normal to the plane containing them makes an angle of 43° with Z. The exciton components of the Q_x band make an angle of 65° with Z. (b) In the plane containing A_+ and A_- , the two monomer transition dipoles, A_1 and A_2 , are shown. (c) Additional components present in the LH-wt protein are shown. The square represents the plane of the third bacteriochlorophyll to which the 800 nm absorption band is attributed.



XBL 788-4099 A

Figure 1 Bolt and Sauer

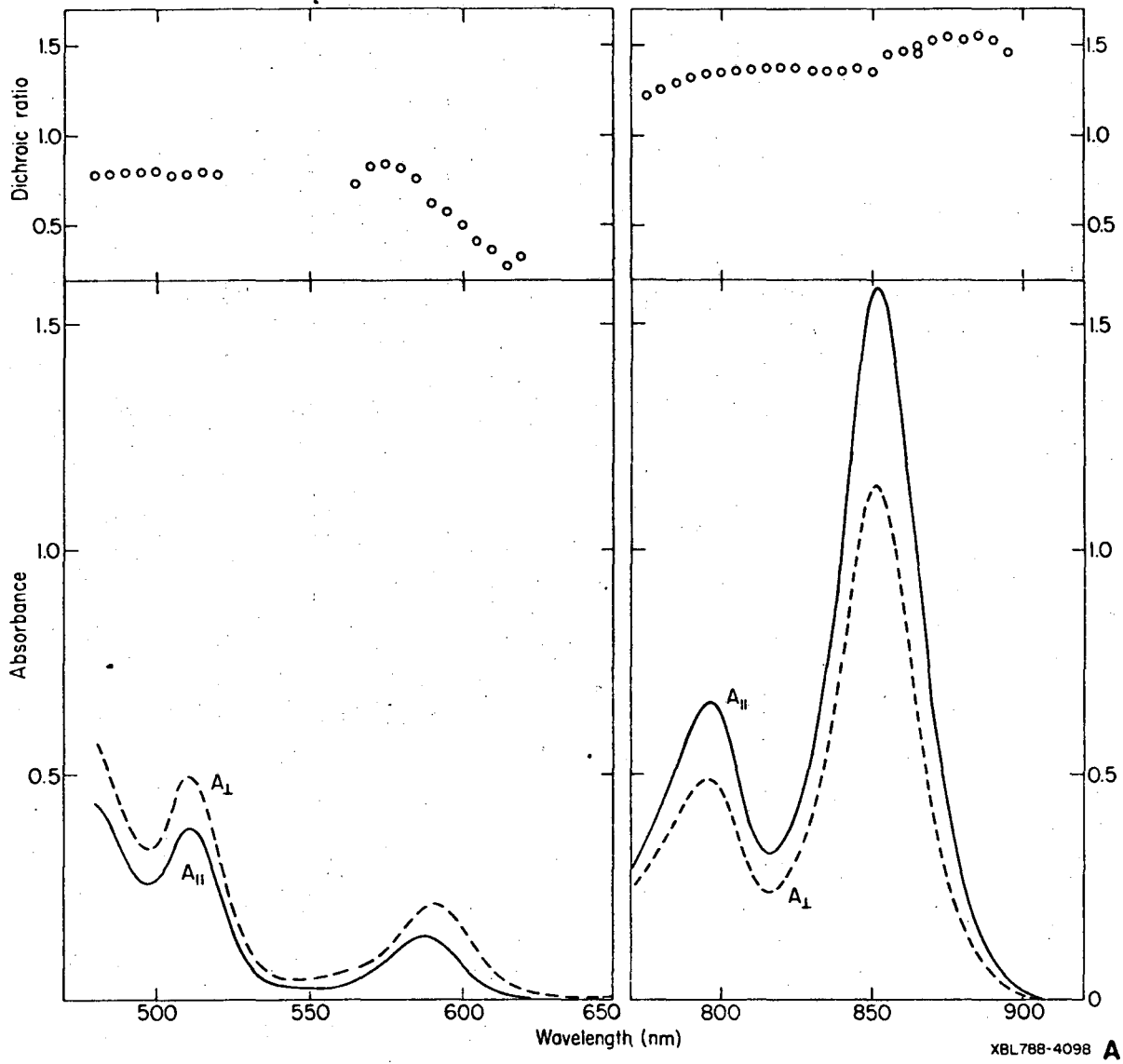
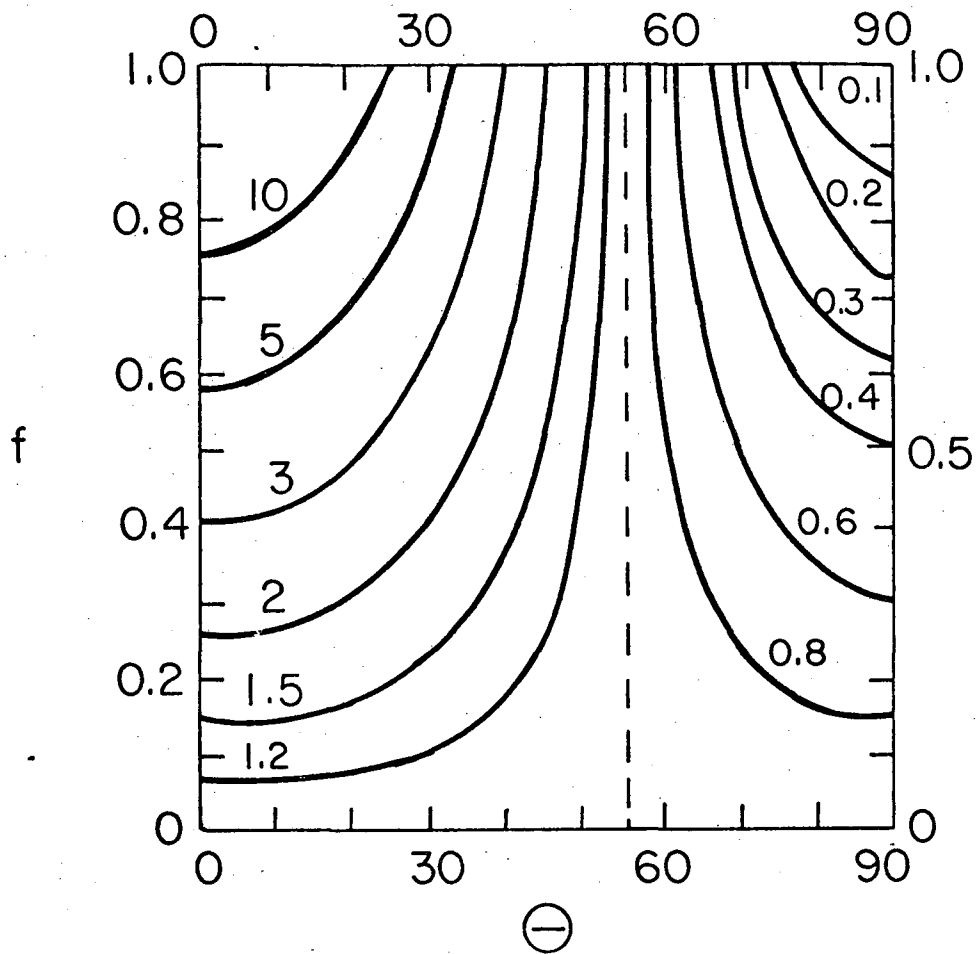
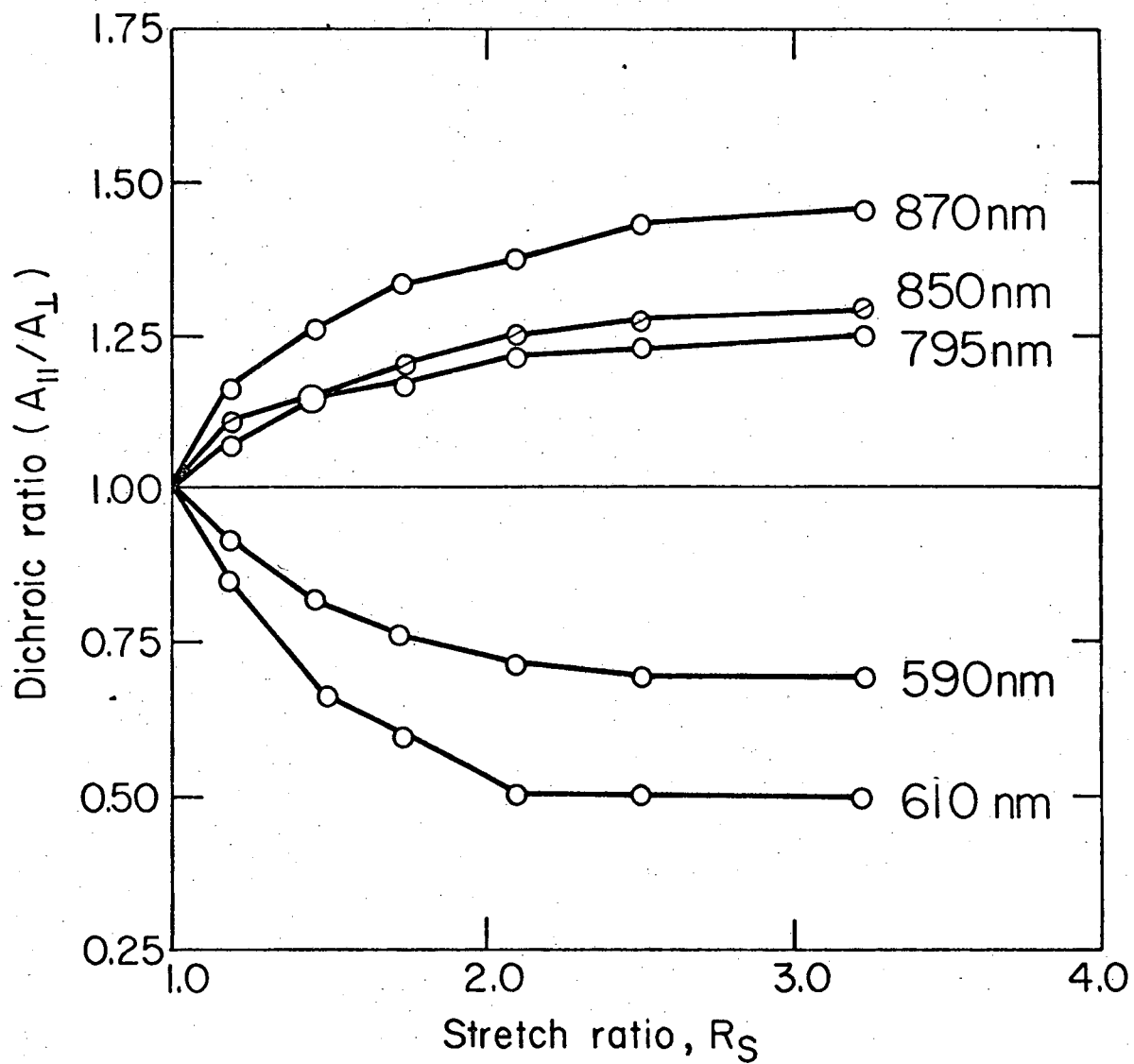


Figure 2 Bolt and Sauer



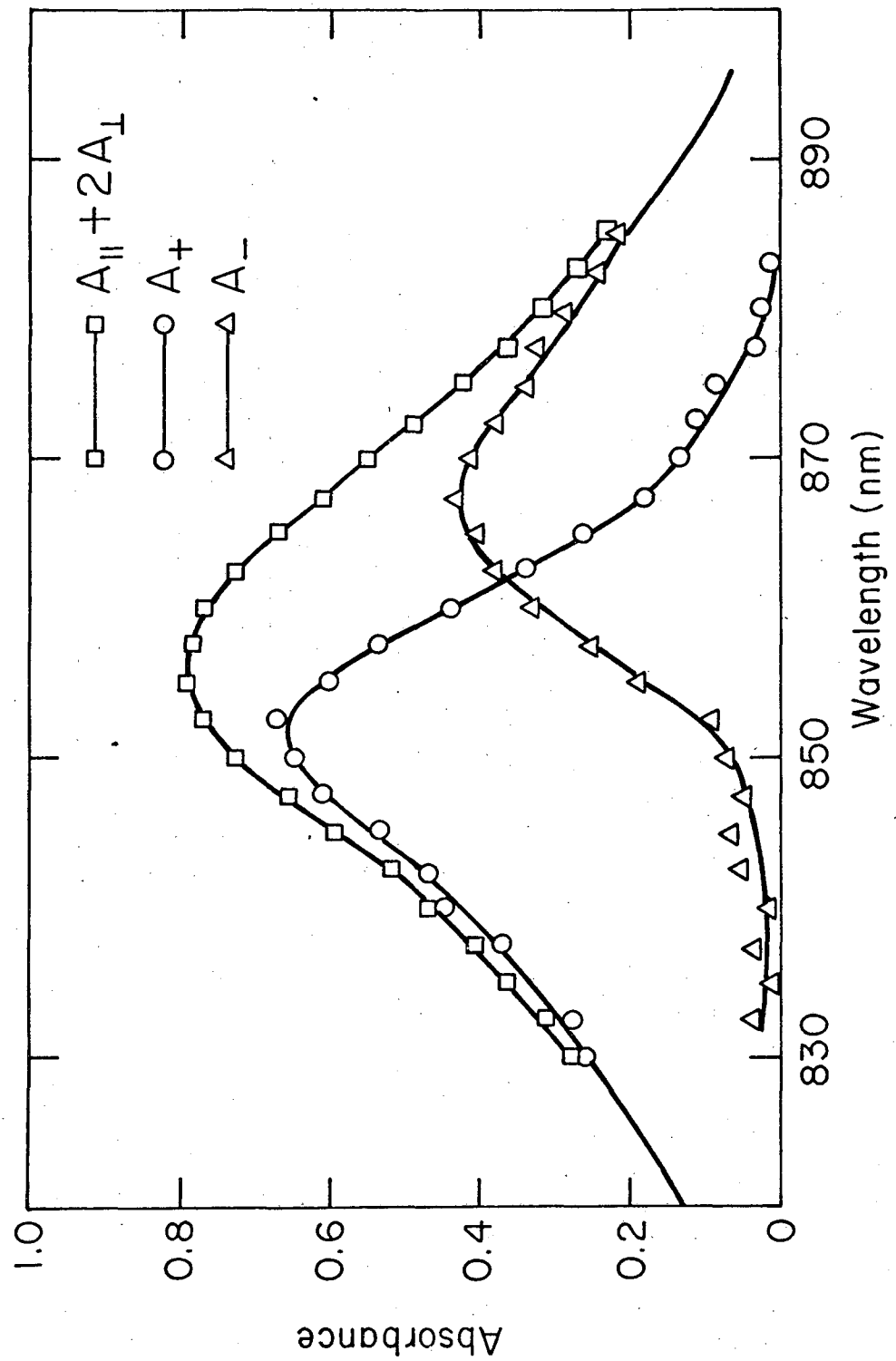
XBL 787-4093

Figure 3 Bolt and Sauer



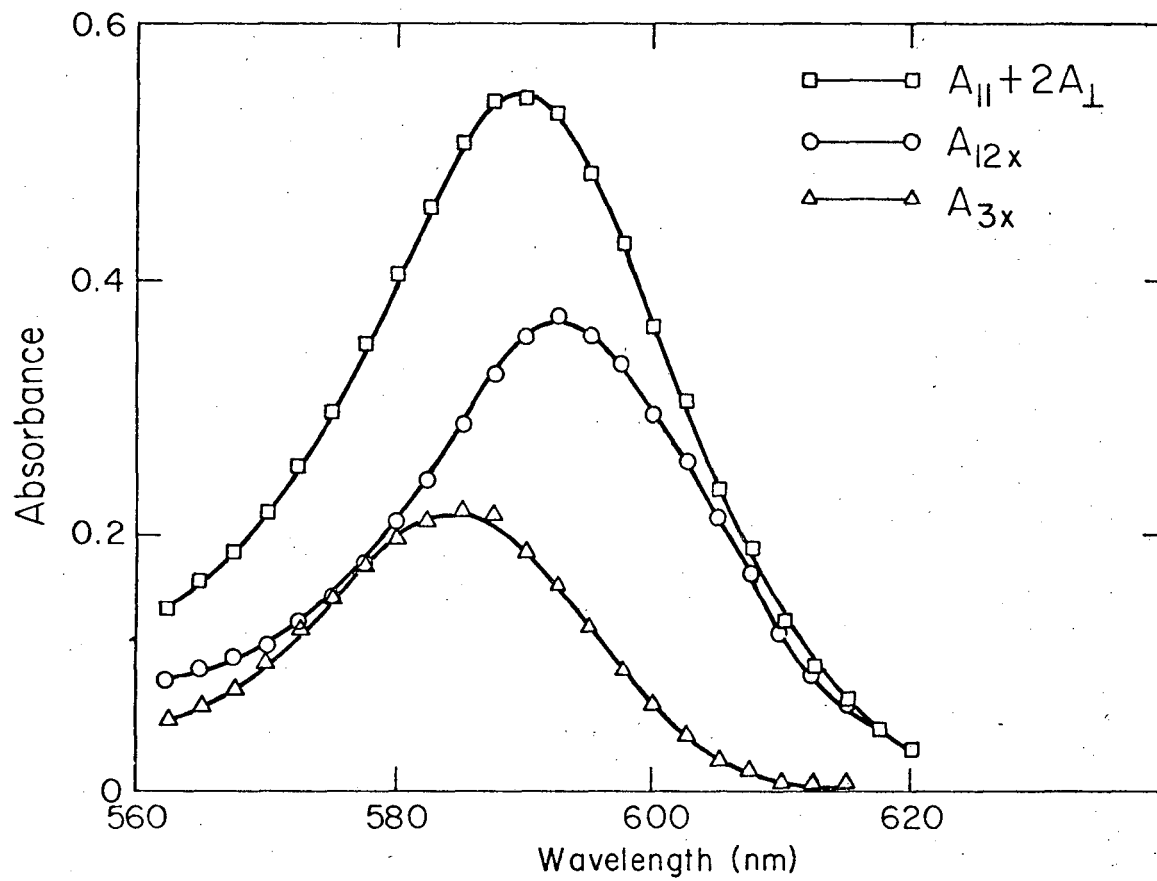
XBL 787-4092

Figure 4 Bolt and Sauer



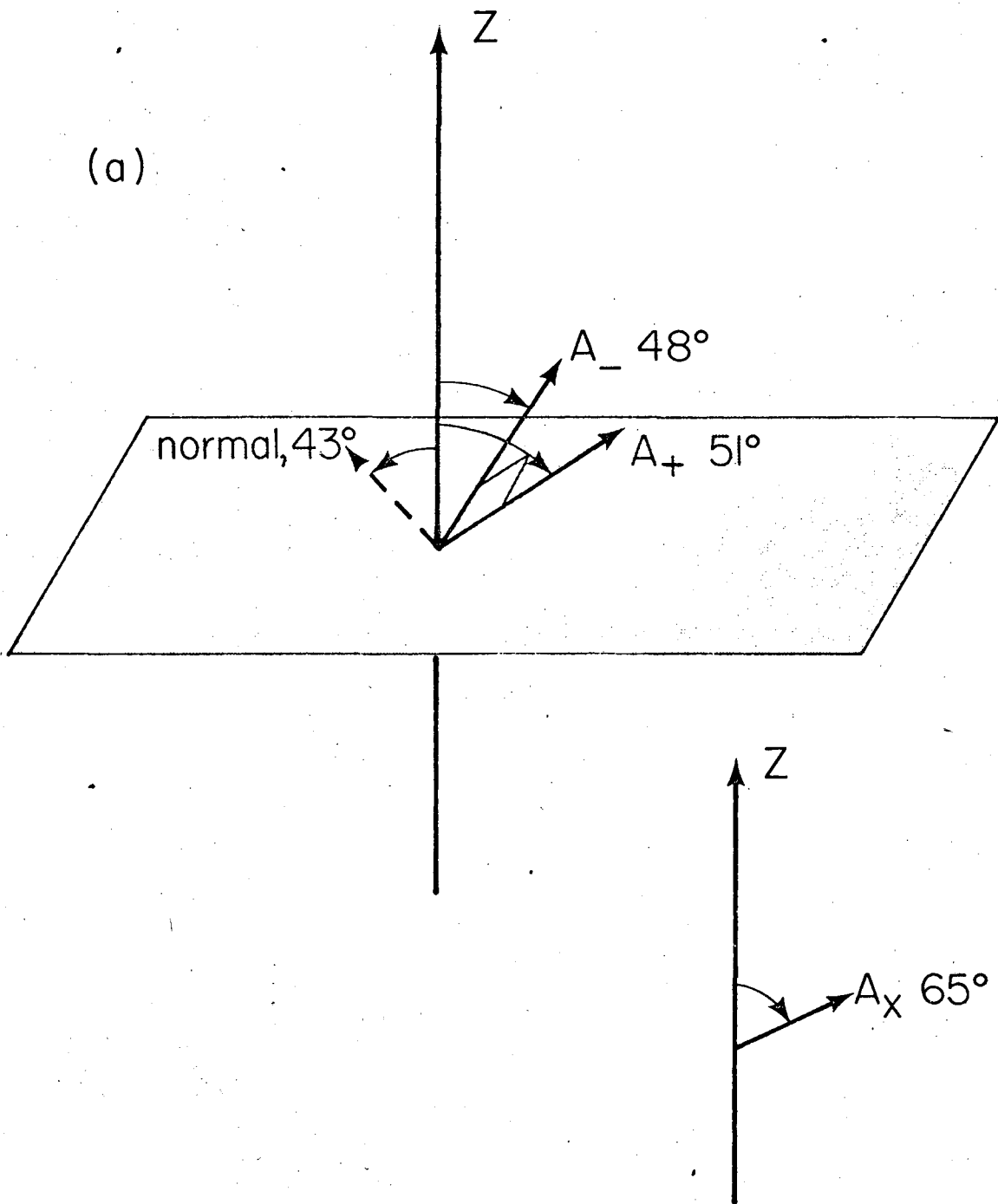
XBL 788 - 4094 A

Figure 5 Bolt and Sauer



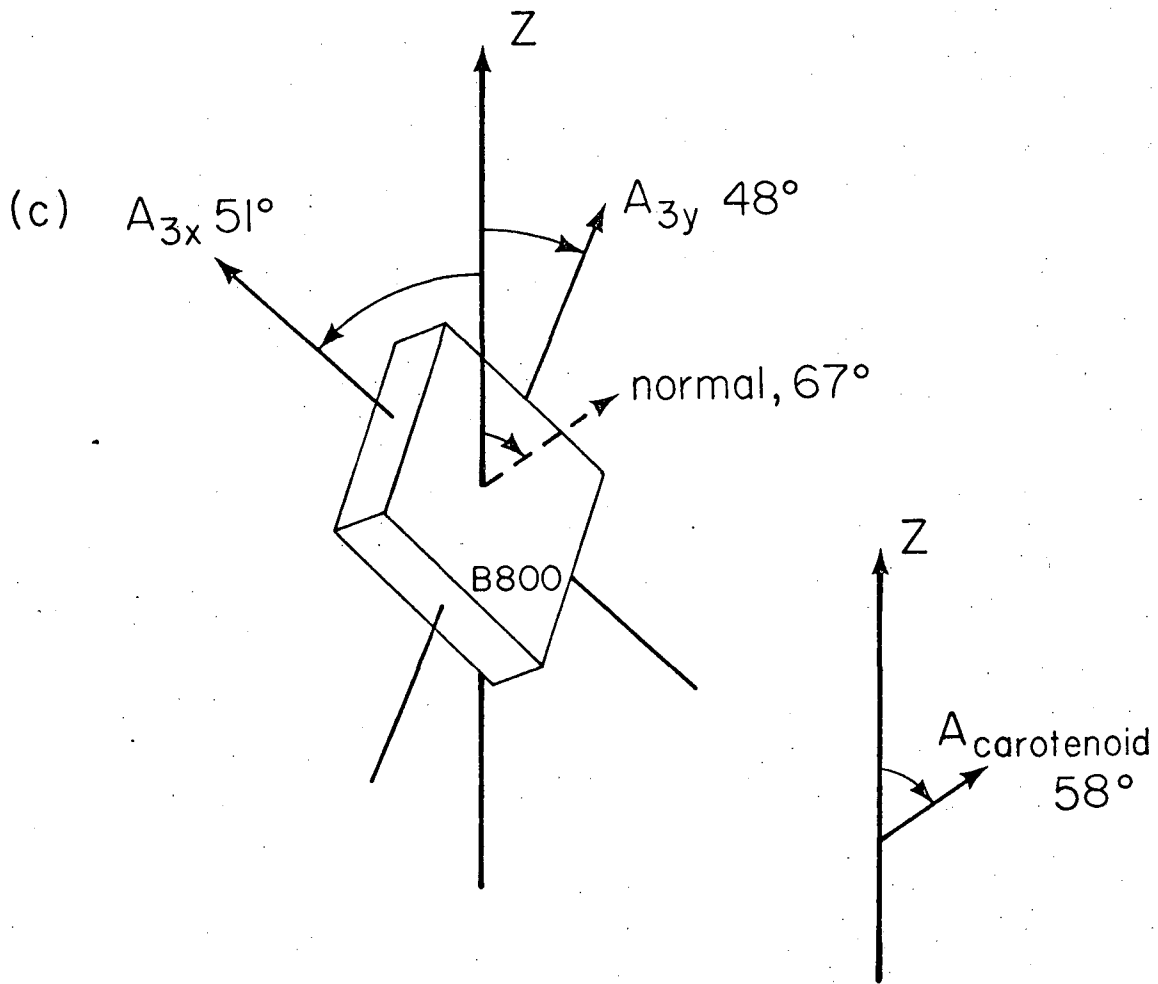
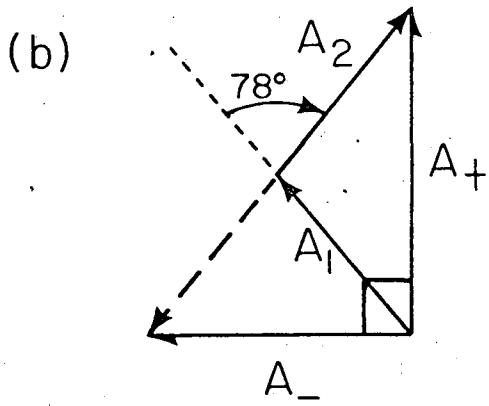
XBL 788-4097 A

Figure 6 Bolt and Sauer



XBL 788-4096

Figure 7 a Bolt and Sauer



XBL788-4095

Figure 7 b,c Bolt and Sauer

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.

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