has the holohedry $m\perp 2\perp 2$. (Veysseyre *et al.*, 1991). The subgroups of this holohedry are the following:

$$2\perp 2;\quad 2/m;\quad \bar{1}_4, \bar{1}, \bar{1};\quad \bar{1}\perp 2;\quad m\perp\bar{1}_4;$$
$$\bar{\bar{1}};\quad 2;\quad \bar{1}_4;\quad m;\quad \bar{1};\quad 1.$$

All these PSGs are DIPSGs of $E^5$. Therefore this family is a DI crystal family.

(2) The eleven DI crystal families of $E^5$.
In space $E^5$, eleven crystal families are DI families. In Table 5, we give their names, together with the WPV symbols of their holohedries and of their PSGs.

## Concluding remarks

The study of the different possibilities for the entries of the modulation vectors to be either rational or not enables us to define the DIPSOs, then the DIPSGs and the DI crystal families.

de Wolff, Janssen & Janner (1981) published a list of Bravais classes of the crystal families of $E^3$ necessary for the study of incommensurate phases of internal dimensions equal to 1, 2 or 3 and they proposed a notation for these Bravais classes.

In a previous paper, we established a connection between the two approaches and the two notations for the mono-incommensurate structures (Grebille, Weigel, Veysseyre & Phan 1990).

In a forthcoming paper, the same work will be developed for the di-incommensurate structures and some physical examples studied.

## References

GREBILLE, D., WEIGEL, D., VEYSSEYRE, R. & PHAN, T. (1990). *Acta Cryst.* A**46**, 234-240.
PHAN, T. (1989). Thesis, Paris, France.
PLESKEN, W. (1981). *Match*, no. 10, pp. 97-119.
VEYSSEYRE, R., PHAN, T. & WEIGEL, D. (1991). *Acta Cryst.* A**47**, 233-238.
WEIGEL, D., PHAN, T. & VEYSSEYRE, R. (1990). *Acta Cryst.* A**46**, 463-466.
WOLFF, P. M. DE, JANSSEN, T. & JANNER, A. (1981). *Acta Cryst.* A**37**, 625-636.

*Acta Cryst.* (1991). A**47**, 553-559

# Phase Determination for Membrane Diffraction by Anomalous Dispersion

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## Abstract

The phase problem of membrane diffraction is usually solved by the swelling method; however, this method does not always resolve the phases unambiguously. An alternative method of phase determination using anomalous dispersion is illustrated by the multiple-wavelength diffraction of membranes containing gramicidin ion channels. The anomalously scattering atoms are thallium ions bound to the channel. The result determines the location of the ion-binding sites in the gramicidin channel and the electron-density profile of the membrane. The applicability and limitation of the anomalous-dispersion method are discussed.

## Introduction

Membrane scattering has been used to determine the structures of membranes and to reveal structural properties of molecules embedded in membranes, such as cholesterol (*e.g.* Franks & Lieb, 1979), rhodopsin (*e.g.* Yeager, 1975) and ion channels (*e.g.* Olah, Huang, Liu & Wu, 1991). When membranes are in the smectic liquid-crystalline form, the resolution of membrane diffraction is usually limited to a few ångströms. Nevertheless, if heavy atoms in the system are bound to a few well defined sites, it is

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possible to determine their locations accurately using the low-resolution data. In view of the fact that structural data for membrane molecules are extremely difficult to obtain, membrane diffraction is indeed a valuable tool for membrane studies.

The standard way of solving the phase problem for membrane diffraction is the swelling method. Diffraction by a membrane sample is recorded at varying degrees of hydration. The phases are then determined by assuming that structure factors plotted in reciprocal space form a single smooth curve as the lamellar spacing changes with hydration. This assumption is justified if the bilayer structure is unchanging or changing slightly and continuously with hydration (Torbet & Wilkins, 1976). However, the swelling method does not always resolve the phases unambiguously. For example it does not work well if the range of swelling (*i.e.* the variation of the lamellar spacing) by hydration is small or if the bilayer structure changes significantly with hydration. It is therefore desirable to develop an alternative method of phase determination. In this paper we will discuss an application of the anomalous-dispersion technique to membrane diffraction. In particular, we will solve the phase problem of the diffraction from a membrane containing the gramicidin channel, which is a prototypical ion pore in biological membranes. The anomalously scattering atoms are Tl ions bound to the channel. The result determines the location of the ion-binding sites in the gramicidin channel.

The principle of this technique was recently reviewed by Karle (1989). The atomic scattering factor of an anomalously scattering atom may be written as

$$f = f^n + f'_\lambda + if''_\lambda, \tag{1}$$

where $f^n$ is the nonanomalous scattering factor measured at a wavelength much shorter than any of the absorption-edge wavelengths of the atom, and $f'_\lambda$ and $f''_\lambda$ are the real and imaginary parts of the dispersion correction; the wavelength ($\lambda$) dependence of the anomalous dispersion is denoted by the subscript. Correspondingly, the structure factor of anomalously scattering atoms is written as

$$F_{\lambda\mathbf{h}} = F^n_{\mathbf{h}} + F^a_{\lambda\mathbf{h}}, \tag{2}$$

where $\mathbf{h}$ is the vector of the Miller indices and

$$F^n_{\mathbf{h}} = \sum_{j=1}^{N} f^n_{j\mathbf{h}} \exp(2\pi i \mathbf{h} \cdot \mathbf{r}_j), \tag{3}$$

$$F^a_{\lambda\mathbf{h}} = \sum_{j=1}^{N} (f'_{\lambda j} + if''_{\lambda j}) \exp(2\pi i \mathbf{h} \cdot \mathbf{r}_j). \tag{4}$$

$\mathbf{r}_j$ is the position of the $j$th atom. The dispersion corrections are treated as constants of $\mathbf{h}$ and, for simplicity, written as

$$f'_{\lambda j} + if''_{\lambda j} = f^a_{\lambda j} \exp(i\delta_{\lambda j}). \tag{5}$$

We also write

$$F^n_{\mathbf{h}} = |F^n_{\mathbf{h}}| \exp(i\varphi_{\mathbf{h}}). \tag{6}$$

Suppose that there is only one type of anomalous atom in the crystal. Let $F^n_{1,\mathbf{h}}$ represent the structure factor of nonanomalous atoms and $F^n_{2,\mathbf{h}}$ the nonanomalous structure factor of the anomalously scattering attoms. It is straightforward to show that (Karle, 1989)

$$\begin{aligned}|F_{\lambda\mathbf{h}}|^2 = {} & |F^n_{1,\mathbf{h}}|^2 + [1 + Q(Q + 2\cos\delta_{2,\lambda})]|F^n_{2,\mathbf{h}}|^2 \\ & + 2(1 + Q\cos\delta_{2,\lambda})|F^n_{1,\mathbf{h}}||F^n_{2,\mathbf{h}}|\cos(\varphi_{1,\mathbf{h}} - \varphi_{2,\mathbf{h}}) \\ & + 2Q\sin\delta_{2,\lambda}|F^n_{1,\mathbf{h}}||F^n_{2,\mathbf{h}}|\sin(\varphi_{1,\mathbf{h}} - \varphi_{2,\mathbf{h}}).\end{aligned} \tag{7}$$

On the right-hand side of this expression, the wavelength dependence is contained in the factors $Q(=f^a_{2,\lambda}/f^n_{2,\mathbf{h}})$ and $\delta_{2,\lambda}[=\tan^{-1}(f''_{2,\lambda}/f'_{2,\lambda})]$. They are either measured or theoretically calculated.

So far most of the applications have been applied to noncentrosymmetric crystals (Kahn, Fourme, Bosshard, Chiadmi, Risler, Dideberg & Wery, 1985; Hendrickson, Smith, Phizackerley & Merritt, 1988; Guss, Merritt, Phizackerley, Hedman, Murata, Hodgson & Freeman, 1988; Murthy, Hendrickson, Orme-Johnson, Merritt & Phizackerley, 1988; Hendrickson, Pahler, Smith, Satow, Merritt & Phizackerley, 1989). For such systems, we have $|F^n_{i,\mathbf{h}}| = |F^n_{i,\bar{\mathbf{h}}}|$ and $\varphi^n_{i,\mathbf{h}} = -\varphi^n_{i,\bar{\mathbf{h}}} \neq 0$ or $\pi$. The quantities $|F^n_{1,\mathbf{h}}|^2$, $|F^n_{2,\mathbf{h}}|^2$, $|F^n_{1,\mathbf{h}}||F^n_{2,\mathbf{h}}|\cos(\varphi_{1,\mathbf{h}} - \varphi_{2,\mathbf{h}})$ and $|F^n_{1,\mathbf{h}}||F^n_{2,\mathbf{h}}|\sin(\varphi_{1,\mathbf{h}} - \varphi_{2,\mathbf{h}})$ can, at least in principle, be determined by solving four equations of the form of (7) generated by measuring $|F_{\lambda\mathbf{h}}|^2$ and $|F_{\lambda\bar{\mathbf{h}}}|^2$ at two different wavelengths. The technique of anomalous dispersion for these applications often makes use of the fact that $|F_{\lambda\mathbf{h}}|^2 \neq |F_{\lambda\bar{\mathbf{h}}}|^2$. Such a method is not applicable to membrane diffraction.

Most membrane samples consist of centrosymmetric bilayers. In this case, we have $\varphi^n_{i,\mathbf{h}} = \varphi^n_{i,\bar{\mathbf{h}}} = 0$ or $\pi$ and $F_{\lambda\mathbf{h}} = F_{\lambda\bar{\mathbf{h}}}$. Since $F^n_{1,\mathbf{h}}$ and $F^n_{2,\mathbf{h}}$ are real quantities, (7) reduces to

$$|F_{\lambda\mathbf{h}}|^2 = F^{n\,2}_{1,\mathbf{h}} + a_{\lambda\mathbf{h}}F^{n\,2}_{2,\mathbf{h}} + b_{\lambda\mathbf{h}}F^n_{1,\mathbf{h}}F^n_{2,\mathbf{h}}, \tag{8}$$

with

$$a_{\lambda\mathbf{h}} = 1 + Q(Q + 2\cos\delta_{2,\lambda}), \tag{9}$$

$$b_{\lambda\mathbf{h}} = 2(1 + Q\cos\delta_{2,\lambda}). \tag{10}$$

It is clear that it is impossible to solve for the signs of $F^n_{1,\mathbf{h}}$ and $F^n_{2,\mathbf{h}}$ from equations of the form of (8). Only their absolute magnitudes and their ratio $g_{\mathbf{h}} = F^n_{2,\mathbf{h}}/F^n_{1,\mathbf{h}}$ can be determined from such equations, as (8) is equivalent to

$$|F_{\lambda\mathbf{h}}|^2 = (1 + a_{\lambda\mathbf{h}}g^2_{\mathbf{h}} + b_{\lambda\mathbf{h}}g_{\mathbf{h}})F^{n\,2}_{1,\mathbf{h}}. \tag{11}$$

Nevertheless, we will show that, for the example we consider in the following, it is possible to solve the phase problem with the use of Patterson's (1934) function.

### Gramicidin channel

Gramicidin is a linear pentadecapeptide which spontaneously inserts into black lipid membranes and forms transmembrane ion channels. Because of its simplicity, the channel has served as a prototypical model for the study of ion permeation across bilayer membranes. It is now generally accepted that the gramicidin channel is a cylindrical pore formed by two monomers, each a single-stranded $\beta^{6.3}$ helix, hydrogen-bonded head-to-head at their N termini (Urry, 1985; Arseniev, Barsukov, Bystrov, Lomize & Ovchinnikov 1985). However, the structural details of the channel are unknown. Recently we used uniformly aligned multilayer samples of membranes containing gramicidin and measured the X-ray diffractions of such samples with and without ions ($Tl^+$, $K^+$, $Ba^{2+}$, $Mg^{2+}$). The phases of the reflections were determined by the swelling method. From the difference electron-density profiles, we found a pair of symmetrically located ion-binding sites for $Tl^+$ at 9·6 (3) Å and for $Ba^{2+}$ at 13·0 (2) Å from the midpoint of the gramicidin channel (Olah *et al.*, 1991). This was the first X-ray diffraction on gramicidin in its membrane-active form. It seemed to us that this would be a good system for testing the anomalous-dispersion method.

### Experiment

*Materials and preparation of samples*

The materials and sample preparation were the same as described by Olah *et al.* (1991). Briefly, a hydrated mixture of gramicidin, dilauroylphosphatidylcholine (DLPC) and thallium acetate (in the molar ratio 1/10/1) was aligned into uniform multilayers between a polished Be plate (314 μm × 10 mm diameter) and a silica plate. The thickness of the multilayers was about 10 μm. The alignment was inspected from the silica side with a polarized reflection microscope (Huang & Olah, 1987). X-ray diffraction of the multilayers was performed from the Be side.

*Synchrotron-radiation source*

The experiment was performed at the F2 station of the Cornell High Energy Synchrotron Source (CHESS). An Si(111) double-crystal monochromator was used to produce a tunable X-ray beam of approximately 1 eV bandwidth over the required range of wavelength. A total-reflection mirror was set in the X-ray path to remove the energetic photons higher than 14 keV. The energy of the monochromatic X-rays was calibrated to be 11·919 keV at the $L_{III}$-absorption edge of Au foil. The wavelength at another angular setting of the monochromator was calculated assuming $d = 3·1352$ Å. The beam path from the source to the center of the experimental hutch is 22 m. Two 0·5 (vertical) × 1 mm (horizontal) slits before the mirror were used to define the incident beam. The incident-beam intensity was monitored by an ion chamber ($N_2$) placed after the mirror.

*X-ray absorption spectra and anomalous scattering factor*

Theoretical values of anomalous scattering factors are available (Cromer, 1983). But the method of computation for these values was crude, particularly near the absorption edges. In principle, the imaginary part can be obtained by measuring the atomic absorption coefficient; the real part can then be calculated by using the Kramers-Kronig relations (James, 1982). However, the normalization of the atomic absorption coefficient is in practice rather difficult. Thus we normalize the measured absorption coefficient to the theoretical values far from the absorption edges.

The absorption spectrum of a Tl atom near its $L_{III}$ edge (12·658 keV) was measured in the fluorescence mode from the same material that was used for diffraction measurements. A solid-state detector with a multichannel analyzer was used to count the fluorescence intensity $I_f(E)$ as the energy $E$ of the X-rays was varied. The incident-beam intensity $I_0(E)$ was monitored by an ion chamber. The spectrum was taken in five segments: pre-edge 12·20 to 12·50 keV in 10 eV steps; 12·50 to 12·62 keV in 5 eV steps; near-edge 12·62 to 12·70 keV in 1 eV steps; 12·70 to 13·00 keV in 3 eV steps; tail 13·00 to 13·80 keV in 10 eV steps. The fluorescence spectrum $R = I_f(E)/I_0(E)$ was reduced to the atomic absorption coefficient $\mu_a(E)$ by fitting it to the theoretical values (Cromer, 1983) outside the edge region as follows.

$$\mu_a(E) = sR - [a + b(E - E_0) + c(E - E_0)^2]. \quad (12)$$

$s$ is the scaling factor and $E_0$ is the edge energy 12·658 keV. The quantity in the square bracket is the background partly due to the scattering effect and

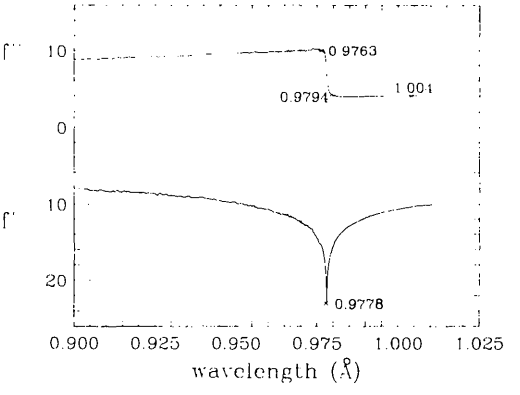



Fig. 1. The anomalous scattering factor of the Tl atom near the $L_{III}$ edge. $f'$ is the real part and $f''$ the imaginary part. The numbers are the wavelengths (Å) at which the reflections were measured.

Table 1. *Structure factors measured at four wavelengths*

| $h$ | $q=4\pi(\sin\theta)/\lambda$ (Å$^{-1}$) | $I$(0·9763 Å) | $I$(0·9778 Å) | $I$(0·9794 Å) | $I$(1·004 Å) | delta *(%) |
|---|---|---|---|---|---|---|
| 1 | 0·1456 | 0·747 | 0·745 | 0·746 | 0·749 | −0·5 |
| 2 | 0·2912 | 0·435 | 0·429 | 0·433 | 0·439 | −2·3 |
| 3 | 0·4368 | 1·48E−02 | 1·61E−02 | 1·49E−02 | 1·39E−02 | 16 |
| 4 | 0·5823 | 2·07E−02 | 2·21E−02 | 2·09E−02 | 1·95E−02 | 14 |
| 5 | 0·7279 | 1·10E−03 | 0·84E−03 | 1·06E−03 | 1·27E−03 | −34 |
| 6 | 0·8735 | 7·13E−03 | 6·93E−03 | 7·13E−03 | 7·30E−03 | −5·1 |
| 7 | 1·0191 | 6·12E−04 | 4·85E−04 | 5·69E−04 | 6·91E−04 | −30 |
| 8 | 1·1647 | 2·26E−04 | 3·10E−04 | 2·87E−04 | 2·75E−04 | 13 |

* delta = [$I$(0·9778 Å) − $I$(1·004 Å)]/$I$(1·004 Å) indicates the percent changes due to the anomalous dispersion.

Table 2. *Atomic scattering factor of thallium near its $L_{III}$-absorption edge and the factors $a_{\lambda h}$, $b_{\lambda h}$*

| λ (Å) | | | 0·9763 | | 0·9778 | | 0·9794 | | 1·004 | |
|---|---|---|---|---|---|---|---|---|---|---|
| $f'_\lambda$ | | | −15·71 | | −23·00 | | −16·00 | | −10·38 | |
| $f''_\lambda$ | | | 10·04 | | 7·52 | | 4·24 | | 4·04 | |
| $f^a_\lambda$ | | | 18·64 | | 24·20 | | 16·64 | | 11·14 | |
| $h$ | $q$ | $f^n_h$ | $a_{\lambda h}$ | $b_{\lambda h}$ | $a_{\lambda h}$ | $b_{\lambda h}$ | $a_{\lambda h}$ | $b_{\lambda h}$ | $a_{\lambda h}$ | $b_{\lambda h}$ |
| 1 | 0·1456 | 79·7 | 0·661 | 1·606 | 0·515 | 1·423 | 0·640 | 1·596 | 0·759 | 1·740 |
| 2 | 0·2912 | 79·4 | 0·659 | 1·604 | 0·513 | 1·421 | 0·639 | 1·595 | 0·758 | 1·739 |
| 3 | 0·4368 | 79·0 | 0·658 | 1·602 | 0·512 | 1·418 | 0·637 | 1·592 | 0·757 | 1·737 |
| 4 | 0·5823 | 78·6 | 0·656 | 1·600 | 0·509 | 1·415 | 0·635 | 1·590 | 0·756 | 1·736 |
| 5 | 0·7279 | 78·1 | 0·655 | 1·598 | 0·507 | 1·411 | 0·633 | 1·588 | 0·754 | 1·734 |
| 6 | 0·8735 | 77·6 | 0·653 | 1·595 | 0·504 | 1·407 | 0·631 | 1·585 | 0·753 | 1·732 |
| 7 | 1·0191 | 77·0 | 0·650 | 1·592 | 0·501 | 1·402 | 0·629 | 1·582 | 0·751 | 1·730 |
| 8 | 1·1647 | 76·3 | 0·648 | 1·589 | 0·498 | 1·397 | 0·626 | 1·578 | 0·749 | 1·728 |

partly due to the energy dependence of the detectors. The fitting of (12) was carried out in the least-squares fashion using $a$, $b$, $c$ and $s$ as free parameters.

The imaginary part of the anomalous scattering factor, $f''$, is directly related to the atomic absorption coefficient (James, 1982):

$$f''(E)=(mc/2e^2h)E\mu_a(E). \quad (13)$$

The symbols $m$, $c$, $e$ and $h$ refer to the fundamental physical constants with the usual meaning. The real part, $f'$, is in turn calculated from the Kramers–Kronig relation

$$f'(E)=(2/\pi)\int_0^\infty [E'f''(E')/(E^2-E'^2)]\,dE'. \quad (14)$$

The results are shown in Fig. 1.

*Diffraction measurement*

The aligned multilayer sample was mounted on a goniometer head. The sample was covered by a humidity chamber equipped with Mylar windows, so that the water content of the sample remained constant during the diffraction measurements. (The Bragg diffraction of the Mylar films would not enter the detector.) Bragg diffraction was measured using the $\theta$–$2\theta$ geometry on a Huber diffractometer. The incident-beam intensity was monitored by an ion chamber filled with $N_2$. The diffracted-beam flux was counted by a solid-state detector with a multichannel analyzer. The detector slit was 0·5 (vertical) × 1 mm (horizontal). (The plane of diffraction was vertical; the distance from the sample to the detector was 40·6 cm.) An $\omega$ scan indicated that the mosaic spread of the sample was about 0·2°. To maximize the effect of anomalous dispersion, reflections were recorded at the wavelength of $f'$ minimum (0·9778 Å), that of $f''$ maximum (0·9763 Å) and two other wavelengths below the edge at 0·9794 and 1·004 Å (Fig. 1). Eight Bragg peaks were recorded at each wavelength.

*Data reduction*

Data reduction included (1) background subtraction, (2) corrections for the Be and sample absorptions and for the scattering volume, (3) corrections for the Lorentz factor and the atomic scattering factor [it should be pointed out that the Lorentz factor depends on the wavelength as well as the scattering angle, *i.e.* $\lambda/\sin 2\theta$ (Warren, 1969)] and (4) corrections for the energy dependence of the air absorption in the X-ray path and the energy dependence of the ion chamber. The reduced magnitudes of the structure factors are given in Table 1.

## Solving the phase problem of centrosymmetric systems

To solve (11), we first calculated the factors $a_{\lambda h}$ and $b_{\lambda h}$ [(9), (10)] by using the anomalous atomic scattering factors for the Tl atom determined above and the normal scattering factors from *International Tables for X-ray Crystallography* (1968) (Table 2). Since we have measured the magnitudes of the structure factors $|F_{\lambda h}|^2$ at four different wavelengths, we solved for $|F^n_{1,h}|$, $|F^n_{2,h}|$ and $g_h$ by using four equations of the form of (11) in a least-squares fashion (Table 3). We

Table 3. $|F^n_{1,\mathbf{h}}|$, $|F^n_{2,\mathbf{h}}|$ *and* $g_{\mathbf{h}}$

| $h$ | $q$ | $\lvert F^n_{1,\mathbf{h}}\rvert$ | $\lvert F^n_{2,\mathbf{h}}\rvert$ | $g_{\mathbf{h}}$ |
|---|---|---|---|---|
| 1 | 0·1456 | 0·850 | 1·70E−02 | 2·0E−02 |
| 2 | 0·2912 | 0·620 | 4·96E−02 | 8·0E−02 |
| 3 | 0·4368 | 0·167 | 5·68E−02 | −0·34 |
| 4 | 0·5823 | 0·189 | 5·66E−02 | −0·30 |
| 5 | 0·7279 | 3·78E−04 | 4·06E−02 | 107 |
| 6 | 0·8735 | 7·33E−02 | 1·39E−02 | 0·19 |
| 7 | 1·0191 | 3·26E−03 | 2·66E−02 | 8·2 |
| 8 | 1·1647 | 2·18E−02 | 0·61E−02 | −0·28 |

note that, because the sign of $g_{\mathbf{h}}$ is solved, the entire phase problem is solved if one can determine the phases of $F^n_{2,\mathbf{h}}$. The special feature of a centrosymmetric system is that whereas in a noncentrosymmetric system the phase factor $\exp(i\varphi)$ can take on any value on a unit circle in the complex plane, in a centrosymmetric system the phase factor can be only one of two discrete values, 1 or −1. Experimentally one needs only to determine the sign of $F^n_{2,\mathbf{h}}$. Thus we put forth the following hypothesis: *Provided there are only a few discrete positions for the anomalous atoms, it is likely that the approximate positions of these atoms will determine the correct signs of* $F^n_{2,\mathbf{h}}$. The justification for this hypothesis will be discussed later. The approximate positions of the anomalous atoms will be obtained from the Patterson function. Once the phases of $F^n_{2,\mathbf{h}}$ are determined, more-precise atomic positions are obtained from the direct Fourier transform of the structure factors.

### *Patterson function of* Tl *atoms*

We construct the Patterson function of Tl atoms as follows:

$$P(x) = \sum_{\mathbf{h}=1}^{8} |F^n_{2,\mathbf{h}}|^2 \cos(2\pi hx/D), \qquad (15)$$

where $D = 43{\cdot}1$ Å is the lamellar spacing. The function (Fig. 2) shows five distinct peaks on the Patterson coordinate, approximately at 0, 11·8, 18·7, 24·4, 31·3 Å. In order to interpret this pattern, let us consider a unit cell consisting of 20 DLPC molecules, 2 gramicidin monomers (which form one channel), 2 thallium acetate molecules and a number of water molecules. If the electron density of the unit cell is plotted on the $x$ axis (normal to the plane of the membrane) with the origin set at the mid-plane of the bilayer, the cell will range from $-D/2$ to $D/2$ and a centrosymmetric bilayer from $-H/2$ to $H/2$ ($H < D$); from $-D/2$ to $-H/2$ and $H/2$ to $D/2$ is water; the gramicidin channel (whose length is less than $H$) is centrosymmetrically embedded in the bilayer. Thus the positions of Tl ions must also be symmetrically distributed; that is, potentially there are pairs of symmetric ion sites ($x$ and $-x$) and unpaired sites at $x = 0$ and $D/2$ (equivalently $-D/2$). It is easy to prove that five peaks in $P(x)$ imply that there are three ion sites; two are a symmetric pair and one either at $x = 0$ or $D/2$. However, it is known

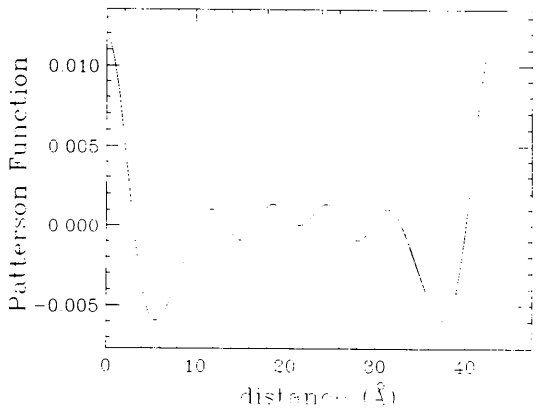
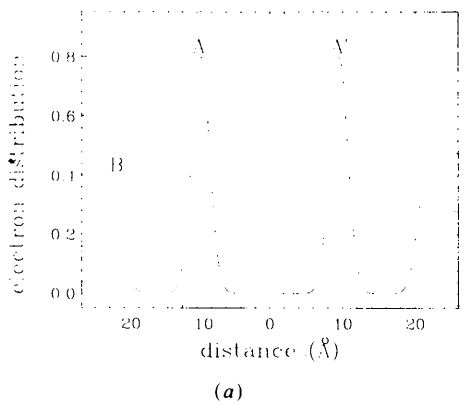
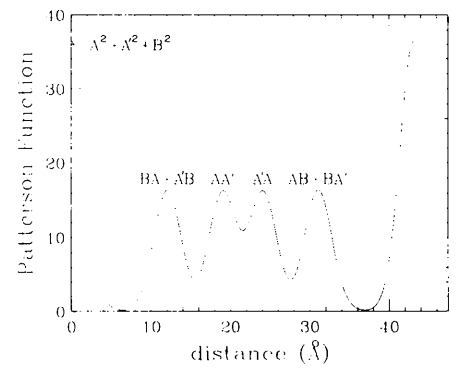



Fig. 2. The Patterson function of the Tl ions.




(*a*)




(*b*)

Fig. 3. (*a*) Schematic diagram of the Tl-ion distribution in the unit cell deduced from the Patterson function in Fig. 2. There are three ion sites. $A$ and $A'$ are located approximately at ±9·5 Å; each on average is occupied by 0·8 atom. $B$ is located at the center of the water layer, $x = D/2$, and is on average occupied by 0·4 atom. The heights of the peaks represent the average occupation numbers and the widths of the peaks (same for all) are arbitrarily chosen. (*b*) The Patterson function constructed from the schematic ion distribution shown in (*a*). This is to be compared qualitatively with Fig. 2.

Table 4. *Signs of structure factors*

| $h$ | $F^n_{2,h}$ | $g_h$ | $F^n_{1,h}$ | $F^n_h$ |
|---|---|---|---|---|
| 1 | − | + | − | − |
| 2 | − | + | − | − |
| 3 | − | − | + | + |
| 4 | + | − | − | − |
| 5 | + | + | + | + |
| 6 | − | + | − | − |
| 7 | − | + | − | − |
| 8 | + | − | − | − |

Table 5. *The range of the position of site A which gives the correct phase of* $F^n_{2h}$

| $h$ | 1 | 2 | 3 |
|---|---|---|---|
| Range of site $A$ ($x$=) | 9·07 to 21·55 | 6·27 to 15·33 | 3·02 to 11·38 |
| $F^n_{2,h}$ | − | − | − |

| 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|
| 7·67 to 13·93 | 6·83 to 10·45 | 9·29 to 12·30 | 7·47 to 11·05 | 9·23 to 12·36 |
| + | + | − | − | + |

experimentally that the gramicidin channel does not transmit anions (Hladky & Haydon, 1984). Model studies show that anions do not enter the channel (Roux & Karplus, 1991); consequently, a cation-binding site at the center of the channel is highly unstable. It is certainly much more likely that the unpaired site is in the water layer at $x = D/2$. Indeed, the best approximate solution (for the ion sites) to reproduce the five peaks of $P(x)$ is one at $x = D/2$ (designated as $B$ in Fig. 3$a$) and a pair at $x = \pm 9{\cdot}5$ Å (designated as $A$ and $A'$ in Fig. 3$a$). These three ion sites would produce the following five Patterson peaks (Fig. 3$b$) located at 0 ($A^2 + A'^2 + B^2$), $D/2 - 9{\cdot}5 \simeq 12{\cdot}1$ ($BA + A'B$), $2 \times 9{\cdot}5 \simeq 19{\cdot}0$ ($AA'$), $D - 2 \times 9{\cdot}5 \simeq 24{\cdot}1$ ($A'A$), $D/2 + 9{\cdot}5 \simeq 31{\cdot}1$ ($BA' + AB$) in the Patterson coordinates.

Let $A$, $A'$ and $B$ be the average numbers of Tl ions at the sites $A$, $A'$ and $B$, respectively. Since there are two ions in the unit cell, we have $A + A' + B = 2$. Since in $P(x)$ (see Figs. 2 and 3$b$) the peak heights of the ($BA + A'B$) peak and the ($AA'$) peak are approximately the same, we have $2B \simeq A$. Hence we get $A = A' \simeq 0{\cdot}8$ and $B \simeq 0{\cdot}4$. This approximate distribution is then used to calculate the phases of $F^n_{2,h}$ (Table 4). Using the signs of $g_h$, one then obtains the phases of $F^n_{1,h}$ and subsequently the complete structure factors of the membrane $F^n_h = F^n_{1,h} + F^n_{2,h}$ (Table 4). The electron-density profile of the membrane obtained from the Fourier transform of $F^n_h$ is the same as the previous result (Olah *et al.*, 1991).

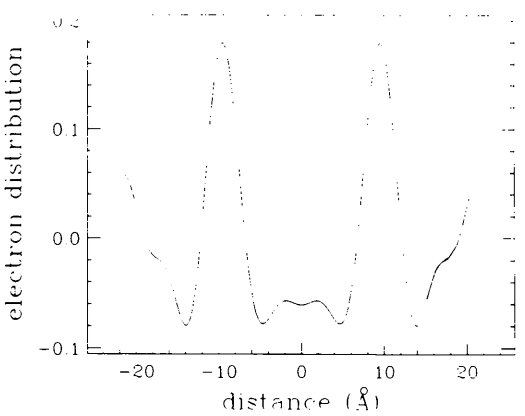



Fig. 4. Fourier transform of $F^n_{2,h}$ gives the distribution function of Tl ions.

The Fourier transform of $F^n_{2,h}$ gives the distribution function of Tl ions on the $x$ axis (Fig. 4). It shows that two symmetric ion-binding sites of the gramicidin channel are located at ±9·5 Å from the center. Our previous study (Olah *et al.*, 1991) using the swelling method and difference electron densities determined the ion-binding sites to be at 9·6 (3) Å. The present result from the anomalous-dispersion technique is in complete agreement.

*Justification of the hypothesis*

We noted above that the atomic positions deduced from the Patterson function are only approximate because the resolution of the Patterson peaks is rather poor. Nevertheless, we hypothesized that the phases determined by these approximate Tl-atom positions are likely to be correct. This is justified by the analysis shown in Table 5. We see that there is a range of position for the ion site $A$ (and the corresponding one for $A'$) that gives the correct phase to a Bragg order [we know the phase is correct from our previous experiment (Olah *et al.*, 1991)]. As long as the Patterson function determines the position of the $A$ site in the range 9·29 to 10·45 Å, we will obtain the correct phases for all Bragg orders. This example demonstrates the applicability as well as the limitation of the anomalous-dispersion method for membrane reflections.

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**References**

ARSENIEV, A. S., BARSUKOV, I. L., BYSTROV, V. F., LOMIZE, A. L. & OVCHINNIKOV, YU. A. (1985). *FEBS Lett.* **186**, 168-174.

CROMER, D. T. (1983). *J. Appl. Cryst.* **16**, 437.

FRANKS, N. P. & LIEB, W. R. (1979). *J. Mol. Biol.* **133**, 469-500.

GUSS, J. M., MERRITT, E. A., PHIZACKERLEY, R. P., HEDMAN, B., MURATA, M., HODGSON, K. O. & FREEMAN, H. C. (1988). *Science*, **241**, 806-811.

HENDRICKSON, W. A., PAHLER, A., SMITH, J. L., SATOW, Y., MERRITT, E. A. & PHIZACKERLEY, R. P. (1989). *Proc. Natl Acad. Sci. USA*, **86**, 2190-2194.

HENDRICKSON, W. A., SMITH, J. L., PHIZACKERLEY, R. P. & MERRITT, E. A. (1988). *Proteins: Struct. Funct. Genet.* **4**, 77-88.
HLADKY, S. B. & HAYDON, D. A. (1984). In *Current Topics in Membranes and Transport*, Vol. 21, edited by W. D. STEIN, p. 327. New York: Academic Press.
HUANG, H. W. & OLAH, G. A. (1987). *Biophys. J.* **51**, 989-992.
*International Tables for X-ray Crystallography* (1968). Vol. III. Birmingham: Kynoch Press. (Present distributor Kluwer Academic Publishers, Dordrecht.)
JAMES, R. W. (1982). *The Optical Principles of the Diffraction of X-rays*, p. 147. Woodbridge, CT: Ox Bow Press.
KAHN, R., FOURME, R., BOSSHARD, R., CHIADMI, M., RISLER, J. L., DIDEBERG, O. & WERY, J. P. (1985). *FEBS Lett.* **179**, 133-137.
KARLE, J. (1989). *Phys. Today*, **42**, No. 6, 22-29.
MURTHY, H. M. K., HENDRICKSON, W. A., ORME-JOHNSON, W. H., MERRITT, E. A. & PHIZACKERLEY, R. P. (1988). *J. Biol. Chem.* **263**, 18430-18436.
OLAH, G. A., HUANG, H. W., LIU, W. & WU, Y. (1991). *J. Mol. Biol.* In the press.
PATTERSON, A. L. (1934). *Phys. Rev.* **46**, 372-375.
ROUX, B. & KARPLUS, M. (1991). *Ion Transport in the Gramicidin Channel: Free Energy Profile in the Right-Handed Dimer.* In preparation.
TORBET, J. & WILKINS, M. H. F. (1976). *J. Theor. Biol.* **62**, 447-458.
URRY, D. W. (1985). In *The Enzymes of Biological Membranes*, Vol. 1, edited by A. N. MARTONOSI, pp. 229-258. New York: Plenum.
WARREN, B. E. (1969). *X-ray Diffraction*, pp. 41-45. New York: Addison-Wesley.
YEAGER, M. J. (1975). *Brookhaven Symp. Biol.* **27**, III3-III36.

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# Domain Structures due to the Non Group–Subgroup Transition in $(C_3H_7NH_3)_2PbCl_4$

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**Abstract**

It is shown that the non group-subgroup transition $Pnam \leftrightarrow P2_1/b$ which the compound bis(propylammonium) lead tetrachloride, $(C_3H_7NH_3)_2PbCl_4$, undergoes must lead to domain structures on each side of the transition point. In both of them, the boundary operators are anticipated.

## 1. Introduction

Very recently (Zangar, Miane, Courseille, Chanh, Couzi & Mlik, 1989), an interesting non group-subgroup transition was observed in bis(propylammonium) lead tetrachloride $(C_3H_7NH_3)_2PbCl_4$. The transition is reversible, occurs at 339 K under 1 atm ($10^5$Pa) and follows the sequence

$$\begin{matrix} Pnam & \rightarrow & P2_1/b \\ (Z=4) & & (Z=2) \end{matrix}$$

with increasing temperature. $Z$ denotes, as usual, the number of formula units within a cell.

Because of the transition, a domain structure is expected to appear (antiphase domains and twins by merohedry). All possible symmetry operators connecting domains can be derived from the sole knowledge of space groups. To each boundary between domains is associated a coset of operators ('boundary operators'), which thus characterizes the type of boundary. *Any* operator belonging to a coset transforms one variant into the variant on the other side of the boundary. Translation (antiphase) boundaries and twin boundaries are commonly observed, especially by electron microscopy or X-ray topography. In addition, group theory predicts in some cases *mixed* (*i.e.* glide reflections and/or screw rotations) boundaries. The possibility of such a kind of boundary was first predicted in group-subgroup transitions (Guymont, Gratias, Portier & Fayard, 1976; Guymont, 1978) and indeed is very rarely observed (see, however, Jiang, Zhang, Hei & Kuo, 1985). A boundary is essentially an antiphase one as soon as at least *one* of the operators of the characteristic coset is a pure translation (Guymont, Gratias, Portier & Fayard, 1976). On the contrary, for a boundary to be essentially mixed (or 'translation-twin'), *all* the operators inside the characteristic coset must be mixed.

The group-theory analysis was afterwards extended to non group-subgroup transitions under rather general conditions (Guymont, 1981) and is applied here to determine the domain structures of $(C_3H_7NH_3)_2PbCl_4$.

## 2. Symmetry analysis of both structures

The orthorhombic room-temperature structure of $(C_3H_7NH_3)_2PbCl_4$ has been determined recently

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