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A Phased Translation Function

BY RANDY J. READ AND ABRAHAM J. SCHIERBEEK

Laboratory of Chemical Physics, University of Groningen, Nijenborgh 16, 9747 *AG Groningen, The Netherlands*

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Abstract

A phased translation function, which takes advantage of prior phase information to determine the position of an oriented molecular replacement model, is examined. The function is the coefficient of correlation between the electron density computed with the prior phases and the electron density of the translated model, evaluated in reciprocal space as a Fourier transform. The correlation coefficient used in this work is closely related to an overlap function devised by Colman, Fehlhammer & Bartels [in *Crystallographic Computing Techniques* (1976), edited by F. R. Ahmed, K. Huml & B. Sedlacek, pp. 248–258. Copenhagen: Munksgaard]. Tests with two protein structures, one of which was solved with the help of the phased translation function, show that little phase information is required to resolve the translation problem, and that the function is relatively insensitive to misorientation of the model.

I. Introduction

Protein crystal structures are solved using phase information from either isomorphous replacement (Blow & Crick, 1959) or molecular replacement (Rossmann, 1972). In some circumstances, neither method is in itself sufficient to solve a structure, and it is desirable to be able to use both simultaneously. We will deal here with the particular question of how to use prior phase information, normally from isomorphous replacement, to solve the translation part of the molecular replacement problem.

It is not uncommon to find that the rotation function (Rossmann & Blow, 1962) gives an apparently unambiguous orientation for a search model, but that the translation problem still cannot be solved. This can arise because the orientation is too much in error; in this case, it can be useful to combine the translation search with a limited systematic variation of the orientation parameters (Lifchitz, Bally & Mornon, 1982; Fujinaga & Read, 1987). If the translation search is still not clear, it is necessary to obtain additional phase information from isomorphous replacement. We will show that one poor derivative can be sufficient to resolve the translation problem; it is not necessary to be able to recognize the molecule in a map computed with the isomorphous replacement phases.

There are a number of examples in the literature of the use of isomorphous replacement data to clarify molecular replacement. Most recent examples involve some form of real-space search. Reynolds, Remington, Weaver, Fisher, Anderson, Ammon & Matthews (1985) identified in a twofold averaged single isomorphous replacement (SIR) map of rat mast cell protease the density corresponding to a single molecule; a six-dimensional real-space search within this density found the orientation and position for an α-chymotrypsin model. Taylor (1983) used interactive computer graphics techniques to position a molecular replacement model in a 5 Å resolution map of *Mucor pusillus* pepsin. Similarly, Bode, Chen, Bartels, Kutzbach, Schmidt-Kastner & Bartunik (1983) could recognize features of a trypsin-like molecule in a multiple isomorphous replacement (MIR) map of kallikrein. A novel method of exploiting isomorphous replacement data has been devised by Cygler & Anderson (1988). They used oriented molecular replacement models of domains of an immunoglobulin Fab to phase heavy-atom differences, expanded to space group $P1$; from the relationships among the heavy-atom peaks the positions of the symmetry elements, and thus the translation vector, could be deduced.

The background theory to a reciprocal-space approach has existed for some time. Rossmann & Blow (1962) described an overlap function that finds non-crystallographic symmetry operations relating regions of electron density within a single map. With the help of that function the non-crystallographic twofold axis relating molecules of α-chymotrypsin could be found (Blow, Rossmann & Jeffery, 1964). A straightforward generalization leads to a general molecular replacement equation (Rossmann, 1972; Argos & Rossmann, 1980) computing the overlap of regions of density in two maps with different unit cells. The reciprocal-space expression [equation (10·20) of Argos & Rossmann (1980)] involves degrees of freedom for the position and shape of the integration volume, as well as the relative orientations of the two coordinate systems. However, it reduces to a simple Fourier

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transform when the two unit cells have the same dimensions and the same orientation, and when the overlap integral is evaluated over the entire unit cell. These conditions are easily arranged for an oriented molecular replacement model. Colman, Fehlhammer & Bartels (1976) have derived such a phased translation function. To the best of our knowledge, this function has been used in only two structure determinations (Colman, Deisenhofer, Huber & Palm, 1976; Deisenhofer, Colman, Huber, Haupt & Schwick, 1976). Perhaps this valuable technique has gone unnoticed because it was described only in the proceedings of a school on crystallographic computing.

Our version of the phased translation function differs in being placed on an absolute scale, which we feel is valuable in interpreting the results. We have also derived a full-symmetry version, which should have increased sensitivity to the correct translation. The derivations of these functions are given in § II. In § III we summarize the results of some tests, which demonstrate the power of the phased translation function even in the presence of large phase errors.

II. Derivation of the phased translation function

When the oriented model is translated to the correct position in the unit cell, its electron density should agree optimally with the density in the map computed using the prior phase information. A reasonable measure of agreement is the coefficient of correlation between the two sets of electron density. This measure has two key advantages: it is on an absolute scale and, more important, it can be evaluated as a Fourier transform.

The correlation coefficient is the following function of the translation to be applied to the model:

$$C(t) = \int_V [\rho_P(\mathbf{x}) - \bar{\rho}_P][\rho_M(\mathbf{x}-\mathbf{t}) - \bar{\rho}_M]\,d\mathbf{x} \times \left\{ \int_V [\rho_P(\mathbf{x}) - \bar{\rho}_P]^2\,d\mathbf{x} \times \int_V [\rho_M(\mathbf{x}-\mathbf{t}) - \bar{\rho}_M]^2\,d\mathbf{x} \right\}^{-1/2}. \quad (1)$$

In this equation, ρ_P is the electron density computed using prior phase information, ρ_M is the density of a single molecule, oriented but arbitrarily positioned in the unit cell, $\mathbf{t}$ is the translation vector and $\bar{\rho}$ indicates the mean density in the cell. The integral is taken over the volume of the unit cell, V. We can easily ensure that the mean density is zero by omitting the $F(000)$ term from the corresponding Fourier summations. Note also that the mean square model density is independent of $\mathbf{t}$, so that (1) becomes

$$C(t) = \int_V \rho_P(\mathbf{x})\rho_M(\mathbf{x}-\mathbf{t})\,d\mathbf{x} \times \left[\int_V \rho_P(\mathbf{x})^2\,d\mathbf{x} \int_V \rho_M(\mathbf{x})^2\,d\mathbf{x} \right]^{-1/2}. \quad (2)$$

The numerator of (2) is the convolution of $\rho_P(\mathbf{x})$ with $\rho_M(-\mathbf{x})$, which can be evaluated as a Fourier transform. The two integrals in the denominator can be evaluated using Parseval's theorem. First we note that

$$\rho_P(\mathbf{x}) = V^{-1} \sum_{\mathbf{h}} m_P |F_o(\mathbf{h})| \exp(i\alpha_P) \exp(-2\pi i \mathbf{h}.\mathbf{x})$$

$$\rho_M(-\mathbf{x}) = V^{-1} \sum_{\mathbf{h}} F_M^*(\mathbf{h}) \exp(-2\pi i \mathbf{h}.\mathbf{x})$$

where m_P is the figure of merit associated with the phase α_P, and F_M^* is the complex conjugate of the structure factor computed from the oriented model. Sums are taken over a sphere in reciprocal space, excluding the origin term. Then

$$C(t) = V^{-1} \sum_{\mathbf{h}} m_P |F_o(\mathbf{h})| \exp(i\alpha_P) F_M^*(\mathbf{h}) \exp(-2\pi i \mathbf{h}.\mathbf{t}) \times (1/V)^{-1} \left[\sum_{\mathbf{h}} (m_P |F_o(\mathbf{h})|)^2 \sum_{\mathbf{h}} |F_M(\mathbf{h})|^2 \right]^{-1/2} \quad (3)$$

or

$$C(t) = (k/V) \sum_{\mathbf{h}} m_P |F_o(\mathbf{h})| |F_M(\mathbf{h})| \exp[i(\alpha_P - \alpha_M)] \times \exp(-2\pi i \mathbf{h}.\mathbf{t}) \quad (4a)$$

where

$$k = V/[\sum_{\mathbf{h}} (m_P |F_o(\mathbf{h})|)^2 \sum_{\mathbf{h}} |F_M(\mathbf{h})|^2]^{1/2}. \quad (4b)$$

The reciprocal-space expression for the numerator in (3) is in essence the phased translation function of Colman, Fehlhammer & Bartels (1976). Doesburg & Beurskens (1983) also use a similar overlap function in their program *TRADIR*.

The phased translation function defined by (4) is simple and efficient to evaluate. The observed amplitudes and prior phases must be expanded to space group $P1$ (or the centered-lattice equivalent) to generate a hemisphere of data, and structure factors must be computed for the oriented model in a $P1$ cell (or the centered-lattice equivalent) of the same dimensions. Then the amplitudes $[m_P|F_o(\mathbf{h})||F_M(\mathbf{h})|]$ and phases $(\alpha_P - \alpha_M)$ serve as input to a $P1$ map calculation. The scale factor k normalizes the results to give correlation coefficients. [Note that the sums in (4*b*) must be corrected by a factor of 2 when they are taken over a hemisphere in reciprocal space.] Phases from isomorphous replacement may have the incorrect hand, so a second map should be computed with phases $(-\alpha_P - \alpha_M)$. The highest peak should indicate the translation vector to be applied to the model.

Full-symmetry phased translation function

It is worth considering what would be the result of including the rotational symmetry operations in the model electron density. Referring to (2), we can see that the numerator would increase by the factor N, the

number of rotational symmetry operations, because we would be summing N symmetry-related integrals. If the symmetry-related model densities did not interpenetrate, the second integral in the denominator of (2), the integral of the squared model density, would similarly increase by a factor of N. Thus, for the correct translation, where we expect little overlap of the models, the correlation would increase by a factor of $N^{1/2}$ if the full symmetry were included. (This fact is useful for comparing results in space groups with different values of N.) However, overlap of the symmetry-related models will increase the squared-density integral in the denominator of (2), thus reducing the correlation coefficient. Since overlap is more likely to occur for incorrect translations, this should increase the signal for the correct translation.

The derivation of the full-symmetry correlation coefficient can be carried out as for (3), leading to the result

$$C(t) = (N/V)\sum_{\mathbf{h}} m_P|F_o(\mathbf{h})|\exp(i\alpha_P)F_M^*(\mathbf{h})\exp(-2\pi i\mathbf{h}.\mathbf{t}) \times (1/V)^{-1}\left[\sum_{\mathbf{h}}(m_P|F_o(\mathbf{h})|)^2\sum_{\mathbf{h}}|F_c(\mathbf{h},\mathbf{t})|^2\right]^{-1/2} \quad (5)$$

where $F_c(\mathbf{h}, \mathbf{t})$ is the structure factor calculated, including symmetry, as a function of the translation applied to the model. Harada, Lifchitz, Berthou & Jolles (1981) have shown that $\sum_{\mathbf{h}}|F_c(\mathbf{h}, \mathbf{t})|^2$ can be evaluated as a Fourier transform. We have not tested (5), primarily because (3) has been sufficient for our problems. However, as Harada *et al.* (1981) have shown for their translation function, some improvement in signal would be expected from the correction for overlap of model densities.

III. Applications of the phased translation function

The phased translation function has been tested on *Streptomyces griseus* trypsin (SGT) and has been used in the structure solution of lipoamide dehydrogenase (LIPDH). Information on these proteins, and on the molecular replacement search models, is summarized in Table 1.

Two factors were considered in choosing the resolution range of data for the tests. First, one might hope to optimize the signal by using resolutions in which a properly positioned molecular replacement model will give the best agreement with the observed data. Use of the resolution shell from 4–8 Å should avoid most problems arising from coordinate errors and from the omission of disordered solvent. Second, comparisons with results from the program *BRUTE* (Fujinaga & Read, 1987) would be most meaningful if the same data were used. Because of the large size of the LIPDH unit cell (Table 1), the computer-intensive *BRUTE* runs were carried out using data in the 5–8 Å resolution shell. No attempt was made to evaluate the effect of different choices of the resolution limits.

SGT

The structure of SGT was solved by molecular replacement with bovine trypsin (BT), though with some difficulty (Read & James, 1988). Some phase information had been available from heavy-atom derivatives, so we were interested to see if this information could have facilitated the solution of the molecular replacement problem for SGT.

The results in Table 2 show that this structure solution could have been much more straightforward. The correct translation can be recognized easily even with the original orientation of the molecular replacement model, which was in error by 6·9°. All other translation functions that have been tested fail with this orientation for BT (Fujinaga & Read, 1987). As the orientation improves, the signal from the phased translation function also improves. Results from the program *BRUTE*, which computes intensity correlation coefficients (Fujinaga & Read, 1987), are shown for comparison in Table 2. *BRUTE* is more sensitive to misorientations and, while the answer is clear for sufficiently accurate orientations, it gives a much weaker signal than the phased translation function.

LIPDH

Extensive efforts to solve the molecular replacement problem for LIPDH met with little success before phase information was available from isomorphous replacement. The fast rotation function of Crowther (1972) indicated the orientation of a glutathione reductase (GR) dimer in the LIPDH crystal, but numerous attempts to solve the translation problem with the Crowther & Blow (1967) translation function or with intensity correlation coefficients computed by the program *BRUTE* failed to give a clear answer.

Two useful heavy-atom derivatives were found, and the resulting MIR phases were improved by the solvent flattening procedure of Wang (1985). The electron density map was not of sufficient quality to trace the polypeptide chain, but the phased translation function using the correct hand of these phases was absolutely unambiguous. (Results of this and other tests are summarized in Table 2.) A local six-dimensional correlation search with *BRUTE* improved the molecular replacement solution, changing the orientation of the GR dimer by 2·9°. A full report of the LIPDH structure determination will be published elsewhere.

In retrospect it seemed that the position of the oriented GR dimer could have been determined by inspection of the density at low resolution. We were therefore interested in learning how sensitive the phased translation function would be to the accuracy

Table 1. *Structures used in test calculations*

	Space group	Cell parameters (Å)	Search model	Sequence identity
Streptomyces griseus trypsin (SGT)* (223 a.a.)	$C222_1$	$a = 72{\cdot}3$ $b = 51{\cdot}0$ $c = 120{\cdot}1$	Bovine trypsin (BT)‡ (223 a.a.)	33%
Lipoamide dehydrogenase (LIPDH)† (476 a.a.)	$P2_12_12_1$	$a = 64{\cdot}1$ $b = 83{\cdot}8$ $c = 192$	Glutathione reductase (GR)‡ (478 a.a.)	26%§

*Structure report: Read & James (1988).
†Crystallization report: Schierbeek, Van der Laan, Groendijk, Wierenga & Drenth (1983).
‡Coordinates of BT (Chambers & Stroud, 1979) and of GR (Thieme, Pai, Schirmer & Schulz, 1981) were obtained from the Brookhaven Protein Data Bank (Bernstein *et al.*, 1977).
§LIPDH sequence and alignment with GR: Westphal & de Kok (1988).

Table 2. *Results of test calculations*

All calculations were performed with data between 4 and 8 Å resolution for SGT and between 5 and 8 Å for LIPDH.

(*a*) Phased translation function

				Correct hand for phases		Incorrect hand for phases	
Structure	Source of phases	Mean figure of merit	Orientation error* ( )	First peak (r.m.s. units)†	Second peak (r.m.s. units)	First peak (r.m.s. units)	Second peak (r.m.s. units)
SGT	MIR‡	0·68	6·9	0·053(5·8)**	0·042(4·6)	0·042(4·5)	0·039(4·2)
SGT	MIR	0·68	3·5	0·089(9·5)**	0·042(4·4)	0·042(4·5)	0·040(4·2)
SGT	MIR	0·68	0·0	0·094(10·0)**	0·046(4·9)	0·042(4·4)	0·041(4·4)
LIPDH	MIRSF§	0·80	2·9	0·068(11·0)**	0·036(5·7)	0·028(4·4)	0·027(4·4)
LIPDH	MIRSF	0·80	0·0	0·093(14·8)**	0·031(5·0)	0·033(5·2)	0·032(5·1)
LIPDH	MIR§	0·60	2·9	0·065(10·2)**	0·030(4·8)	0·030(4.7)	0·029(4·5)
LIPDH	MIR	0·60	0·0	0·093(14·4)**	0·032(5·0)	0·030(4·6)	0·030(4·6)
LIPDH	SIR¶	0·43	2·9	0·056(8·2)**	0·034(4·9)	0·030(4·4)	0·030(4·4)
LIPDH	SIR	0·43	0·0	0·077(11·8)**	0·032(4·8)	0·031(4·7)	0·029(4·4)

(*b*) *BRUTE* (correlation of intensities)

Structure	Orientation error (°)	First peak (r.m.s. units)	Second peak (r.m.s. units)
SGT	6·9	0·14(4·3)	0·13(4·0)
SGT	3·5	0·22(5·7)**	0·19(4·5)
SGT	0·0	0·26(6·6)**	0·21(4·5)
LIPDH	2·9	0·088(4·2)	0·086(3·9)**
LIPDH	0·0	0·113(4·2)**	0·109(4·0)

*Error defined relative to orientation from final six-dimensional *BRUTE* search.
†R.m.s. units = peak height measured in terms of root mean square deviation from the mean.
‡SGT MIR phases obtained from three derivatives, described by Read & James (1988). All derivatives have the same major site, so the phase accuracy is probably overestimated.
§LIPDH MIR phases obtained from ethylmercury phosphate (EMP) and *p*-chloromercury benzenesulfonate derivatives. MIRSF denotes MIR phases improved by the solvent flattening procedure of Wang (1985); the apparent figure of merit for these phases is not strictly comparable to the other figures of merit.
¶LIPDH SIR phases obtained from EMP derivative, without solvent flattening.
**Peaks giving the correct translation vector.

of the phases and of the model orientation. The results in Table 2 demonstrate that the correct translation is clear even with single isomorphous replacement (SIR) phases and the initial less accurate orientation. As one would expect, the clarity of the solution improves with the quality of the phases and of the orientation. For comparison, Table 2 also presents the results of *BRUTE* translation searches using the initial and final orientations. The correct translation gives the highest correlation only for the final orientation, but even then the discrimination from the highest noise peak is small.

The key conclusion to be drawn from the experience with LIPDH is that very little phase information was actually needed to resolve the translation problem: a single derivative would have been sufficient. The SIR map in which the GR dimer could be recognized by the phased translation function is quite uninterpretable by eye. Fig. 1(*a*) shows part of GR in the SIR electron density; though the relationship between model and density is not random, it is also not obvious. In contrast, Fig. 1(*b*) shows that the fit of model to density is much better in the current electron density map. The work required for further improvements to both model and density is in progress.

IV. Summary

The phased translation function is a simple and efficient algorithm that exploits prior phase information to solve the translation part of the molecular replacement problem. The prior phase information comes from isomorphous replacement in the cases we have tested; with the addition of even weak phase information, difficult translation problems become straightforward. We anticipate that this procedure would also be effective if the phase information came from a partial molecular replacement model, for example a protein model omitting a flexible domain, or one member of a complex of two proteins.

Because the phased translation function is computed as a Fourier transform, it is quite efficient to evaluate. For example, one run with SGT, including structure-factor calculation and map calculations for both hands, requires about 25 min on a VAX 11/750. This efficiency, coupled with the relatively low sensitivity to errors in the orientation of the model, implies that fairly extensive six-dimensional molecular replacement searches can be performed. Searches of the orientational parameters could be centered on peaks in the rotation function but, with some optimization of the algorithm on a supercomputer, even a full six-dimensional search could be contemplated. However, if the boundaries of a single molecule are visible, the domain rotation function of Colman, Fehlhammer & Bartels (1976) probably gives a more efficient method of exploiting prior phase information to solve the rotation problem.

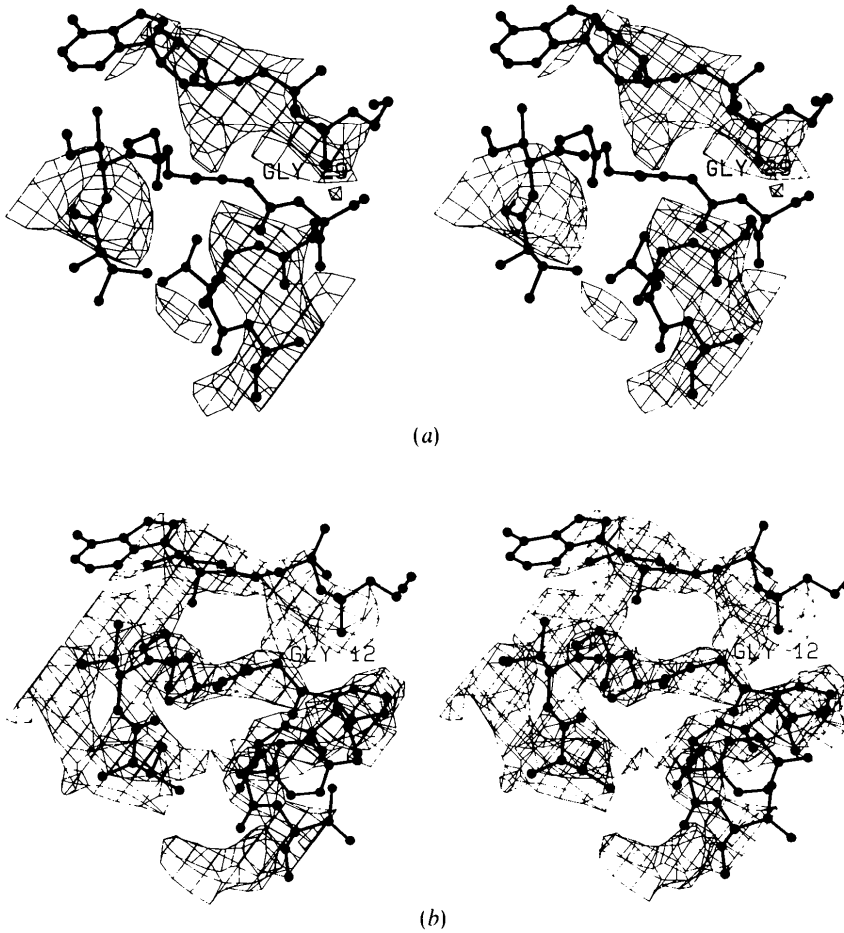

Fig. 1. (*a*) A portion of the GR model in the SIR electron density map of LIPDH. This region, part of the flavine-adenine dinucleotide (FAD) coenzyme binding site in the interior of the molecule, is highly conserved between GR and LIPDH. Thus, one would expect the model to fit the density as well here as in any other region of the map. The electron density is contoured at the level of the root mean square (r.m.s.) value of the map. (*b*) The same region of LIPDH in the current electron density map, contoured at the r.m.s. value of the map.

It is notoriously difficult to be convinced that molecular replacement is failing. Many parameters can be varied in the attempt to obtain a clear result. There is a strong incentive to continue because, when successful, molecular replacement requires much less work than isomorphous replacement. Our experiences suggest that an entire structure determination by isomorphous replacement is not the only alternative. A single poor derivative might well provide all the extra information that is needed.

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