Acta Cryst. (1998). D**54**, 1336–1342

Molecular Replacement: the Revival of the Molecular Fourier Transform Method

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(*Received 1 January 1998; accepted 25 March 1998*)

Abstract

The molecular Fourier transform method, perhaps the first application of the molecular-replacement approach, used in the 1950s for the two-dimensional structure determination of small molecules, has been modernised for the efficient solution of complex structures. In the modern application of the molecular Fourier transform (*MFT*), the three-dimensional transform of the molecular model is calculated and fitting is achieved by rotating the weighted reciprocal lattice with respect to the calculated transform. The fit between the transform and the weighted reciprocal lattice is gauged by three different criteria corresponding to *R* factor, correlation coefficient and product function. Since the procedure involves the rotation of indices and is, therefore, independent of the number of atoms, it is much faster than other methods which employ the rotation of the molecular model. This feature enabled the renovation of the rotation–translation search method *ULTIMA*, which utilizes low-order data and packing considerations for the efficient solution of large structures.

1. Introduction

Molecular replacement is now a well established method for solving macromolecular crystal structures when the structure of a similar molecule or fragment is available (*e.g.* Rossmann & Arnold, 1993). Several efficient computer programs have been written to implement the various molecular-replacement techniques. These include: *ULTIMA* (Rabinovich & Shakked, 1984), *MERLOT* (Fitzgerald, 1988), *GLRF-REPLACE* (Tong & Rossmann, 1990; Tong, 1993) *X-PLOR* (Brünger, 1992) and *AMoRe* (Navaza, 1994). Regrettably, however, one of the oldest molecular-replacement methods, the molecular Fourier transform (*MFT*), has not been modernized to take advantage of the power of contemporary computers.

Structure determination by *MFT* is essentially the fitting of the weighted reciprocal lattice onto the Fourier transform of the molecular model. In principle this is performed by rotating the former with respect to the latter until the best fit is obtained between the structure-factor amplitudes and the moduli of the transform at the reciprocal-lattice points (Lipson & Cochran, 1966). The calculation of structure factors is, in fact, the calculation of the Fourier transform of the contents of the unit cell at the reciprocal-lattice points. Molecular Fourier transforms can be evaluated in much the same manner as structure factors except that they are calculated for the molecular model rather than for the whole unit cell.

In the absence of powerful computers, the application of the method was in general limited to a planar contour map of a projection of the model and to one of the main zones of the weighted reciprocal lattice, and the weighted reciprocal plane was manually fitted to this map. One of the first applications of *MFT* was the determination of the crystal structure of naphthalene using a two-dimensional Fourier transform (Knott, 1940). In the 1950s, Klug (1950) studied the structure of triphenylene and Stadler (1953) solved the structure of flavanthrone, both by using two-dimensional *MFT* methods. In the 1960s, the method was used by Bolton & Stadler (Bolton, 1964; Bolton & Stadler, 1964) to solve the structures of dibenzanthrone and isodibenzanthrone and by Rabinovich & Schmidt (1964) to solve the structure of 2,5-dimethyl-1,4-benzoquinone.

Before the era of powerful computers, three-dimensional calculations, even for medium-sized molecular models, were prohibitive and to the best of our knowledge were not performed, except for one case: the crystal structure of triclinic lysozyme with one molecule in the unit cell using 6 Å data (Joynson *et al.*, 1970). Here we present the results of a revived application of the classical molecular Fourier transform method (*MFT*), either as an independent rotation search or combined with a translation search, and show that it competes most favourably with other molecular-replacement methods.

2. Theory

The molecular transform $T(\xi\eta\zeta)$ of a molecule with atoms at (x_i, y_i, z_i) and scattering factors f_i is defined as

$$T(\xi\eta\zeta) = \sum f_i \exp[2\pi i(\xi x_i + \eta y_i + \zeta z_i)],$$

where $(\xi\eta\zeta)$ are the coordinates in the space reciprocal to (xyz) and the summation is over all atoms in the molecule (*e.g.* Cruickshank, 1967). The transform T is a continuous and complex function of $\xi\eta\zeta$. The moduli of

Table 1. *MFT searches*

(*a*) *MFT* search of protein–DNA complex

Space group $P2_12_12_1$; cell dimensions, $a = 38.5$, $b = 95.7$, $c = 120.5$ Å; $G = F$; RF = R_f; 5° increments for the three angles; resolution range, 6.0–1.9 Å.

φ	ψ	θ	RF	S	S_1/S_n
55	80	60	0.421	4.2	1.00
150	80	80	0.419	3.5	1.18
155	80	55	0.419	3.5	1.19
50	90	85	0.419	3.4	1.25
85	85	75	0.419	3.3	1.26

(*b*) Fine *MFT* search of protein–DNA complex

Top unique solutions obtained by a fine search using 1° increments in a grid (10 × 10 × 10) around the rotational parameters above. All other parameters as above.

φ	ψ	θ	RF	Error (°)
51	82	58	0.422	1.7
150	83	59	0.420	2.5

T, $F_c(h'k'l')$, are sampled on a reciprocal-lattice grid whose direct-space dimensions are chosen to be twice to three times the maximum molecular dimension. The weighted reciprocal lattice of the crystal, $F_o(hkl)$, expanded to symmetry $P1$, is rotated and scaled with respect to the calculated *MFT* grid. The rotation is performed by using the three Eulerian angles φ, ψ, θ (see definition by Rabinovich & Shakked, 1984) or the quasi-orthogonal Eulerian angles $\theta_+ = \varphi + \psi$, $\theta_- = \varphi - \psi$, θ (Lattman, 1972). The correspondence between φ, ψ, θ and θ_1, θ_2, and θ_3 used in the direct-rotation function (DeLano & Brünger, 1995) is the following: $\theta_1 = 180 - \psi$, $\theta_2 = \theta$, $\theta_3 = 180 - \varphi$.

The non-integer indices *pqr* obtained after rotation of *hkl* are rounded off to integer values. The agreement between the observed structure factors, $F_o(pqr)$, and the moduli of T, $F_c(h'k'l')$, is gauged by three rotation-function (RF) criteria that correspond to the conventional R factor (R_f), correlation coefficient (C_c) and product function (P_f) as follows:

$$R_f = 1 - \sum |G_o - kG_c| / \sum G_o, \quad k = \sum G_o G_c / \sum G_c^2,$$

$$C_c = \frac{(n \sum G_o G_c - \sum G_o \sum G_c)}{\{[n \sum G_o^2 - (\sum G_o)^2][n \sum G_c^2 - (\sum G_c)^2]\}^{1/2}},$$

$$P_f = (\sum G_o G_c)^2 / (\sum G_o^2 \sum G_c^2).$$

The summation is over the n observed reflections and G_o and G_c are the observed and calculated magnitudes, which can assume values

$$G_o = F_o, F_o^2, E_o, E_o^2,$$

and analogously for G_c.

The rotation of *MFT* is equivalent to the rotation of the model coordinates followed by the calculation of the corresponding structure factors. However, the former is much faster (several orders of magnitude) than the latter as it involves only rotation of the indices and, hence, is independent of the number of atoms in the model. This efficiency led us to renovate our multi-dimensional search method *ULTIMA* (Rabinovich & Shakked, 1984) by replacing the rotation of the model by the rotation of the weighted reciprocal lattice with respect to *MFT*. In this case, the A and B components of the transform are calculated for each of the rotational symmetry elements and the translational contribution to A and B is obtained by the corresponding fringe function (see Rabinovich & Shakked, 1984).

The algorithm used here for matching the weighted reciprocal lattice to the calculated transform can be applied to matching the rotated reciprocal lattice onto itself. This is equivalent to self-rotation procedures used in other methods for determining non-crystallographic symmetry axes.

3. Results and discussion

3.1. *Test cases*

3.1.1. *Protein–DNA complex: E/TR$_{GR}$–GRE.* The structure of this protein–DNA complex has been studied by Gewirth & Sigler (1995) and solved by the direct-rotation function (DeLano & Brünger, 1995). We chose this as a test case for two reasons. Firstly, as pointed out by DeLano & Brünger (1995), all other available procedures failed to solve the structure. Secondly, both the direct-rotation function and *MFT* are based on reciprocal-space search rather than Patterson methods. The search model used by them was one half of the previously determined GR–GRE dimeric complex (Luisi *et al.*, 1991). Also, in their model, residues that differed between GR and E/TR$_{GR}$ were modified to alanine or glycine residues.

In our procedure, the search model was taken as half of the GR–GRE complex (PDB entry 1GLU). The high-resolution data of E/TR$_{GR}$–GRE (PDB entry 1LAT) were used. Only the protein component was used as the search model without any modification of the amino acids (a total of 630 non-H atoms). The resolution range used was 6–1.9 Å. The *MFT* of the model was calculated in a unit cell with dimensions of 100 × 100 × 100 Å and an overall B factor of 15 Å^2. The rotational search was performed with increments of 5° for each Eulerian angle. The angular ranges used for this case and the following ones were determined by both the symmetry of the crystal lattice and the symmetry of the molecular model (Hirshfeld, 1968).

The top trial solutions on the basis of the three criteria (R_f, C_c and P_f) using the four different magnitudes for G (see §2) were examined. The discrimination on the basis of signal to noise was similar for the three criteria with a slight preference for RF = R_f using $G = F$. The results based on this criterion are given in Table 1(*a*). For each orientation, Eulerian angles (φ, ψ, θ) are

given along with the RF value, the RF value in units of standard deviation above the average (S) and the signal-to-noise ratio (S_1/S_n). Since the discrimination on the basis of signal to noise was not sufficient to identify the two monomers in the asymmetric unit, a finer rotational search was performed, using 1° increments in the angles around the parameters of the top solutions of Table 1(*a*). The top seven solutions obtained by the finer search were found to correspond to either of the two correct orientations of the monomer which are related by a non-crystallographic twofold symmetry. The two unique solutions, with the corresponding errors from the known orientation, are given in Table 1(*b*). An attempt to solve the rotational parameters by using a modified model, where the amino acids that differed between the two proteins were omitted (a total of 560 atoms), yielded unambiguously the correct orientations as the top two solutions (results not shown). All computations were performed on a Silicon Graphics workstation (model INDIGO 2, Power Extreme R8000). The total CPU time was 50 min for the first search and a few minutes for the second.

Our attempts to solve the translational parameters of the two independent protein monomers failed, as also reported by DeLano & Brünger (1995).

3.1.2. *Lactate dehydrogenase*. The crystal structures of this enzyme from two different organisms (mouse and *Bacillus stearothermophilus*) were determined by Rossmann and co-workers (Donald *et al.*, 1979; Hogrefe *et al.*, 1987; Piontek *et al.*, 1990; PDB entry codes 2LDX and 1LDB). The two proteins are tetrameric with a total of 9108 and 10060 non-H atoms for 1LDB and 2LDX, respectively. The search model was the tetrameric structure of 2LDX, using 6–3 Å data from 1LDB. The *MFT* model was calculated in a unit cell with dimensions 200 × 200 × 200 Å and the RF search was performed with 5° increments in the Eulerian angles. The *MFT* search yielded two solutions with the highest S values which correspond to the correct structure and are related to each other by one of the model dyads (Table 2*a*). The best discrimination in this case was obtained by using $G = E^2$ and RF = C_c for the rotation function. The best orientation was used for a translation search by *ULTIMA* with 8–4 Å data and 2 Å increments along the a and b axes. The search yielded the correct translation as the top solution shown in Table 2(*b*). The corresponding CPU times for *MFT* and translation searches were 20 and 2 min, respectively.

Table 2. *Lactate dehydrogenase*

(*a*) *MFT* search

Space group $P61$; cell dimensions, $a = b = 86.9$, $c = 357.3$ Å; $G = E^2$; RF = C_c; 5° increments for the three angles; resolution range, 6.0–3.0 Å.

φ	ψ	θ	RF	S	S_1/S_n	Error (°)
315	5	85	0.024	8.3	1.00	1.8
315	175	90	0.019	6.6	1.26	11.0
320	135	65	0.010	3.6	2.29	48.9
305	110	85	0.010	3.4	2.43	76.4
340	0	50	0.010	3.4	2.48	42.5

(*b*) Translation search

2 Å increments along the a and b axes; resolution range, 8.0–4.0 Å.

x	y	z	R factor	S	S_1/S_n	Error (Å)
0.463	0.562	0.000	0.470	11.2	1.00	0.2
0.475	0.663	0.000	0.497	3.0	3.74	8.3
0.525	0.275	0.000	0.497	2.8	3.95	28.1
0.538	0.600	0.000	0.498	2.7	4.14	5.8
0.625	0.512	0.000	0.498	2.7	4.14	10.8

3.2. *New structures*

3.2.1. *Alcohol dehydrogenases*. The crystal structure of alcohol dehydrogenase from *Clostridium beijerinckii* has been solved by SIR (Korkhin *et al.*, 1998; PDB entry 1PED). The molecular structure 1PED has been used to solve the crystal structure of alcohol dehydrogenase from *Thermoanaerobacter brockii* (denoted here as ADH1) and the crystal structure of the enzyme from *C. beijerinckii* complexed to NADP (denoted here as ADH2) (diffraction data provided by Y. Korkhin). All enzymes are tetrameric, the number of non-H atoms being approximately 10700. The *MFT* model based on 1PED was calculated within a cell of dimensions 200 × 200 × 200 Å. The *MFT* search was performed in 5° angular steps using 6–2.5 Å data for each structure.

In the case of ADH1, the best discrimination in terms of signal to noise was obtained by using either $G = E^2$ with RF = C_c or $G = F$ with RF = R_f. The *MFT* results based on R_f are given in Table 3(*a*). The top two orientations are related to each other by one of the non-crystallographic dyads of the tetramer. The first orientation was subjected to a translation search by *ULTIMA*, where both enantiomorphic space groups, $P6_1$ and $P6_5$, were tried. The top solutions for both space groups on the basis of conventional R factor are given in Table 3(*b*), establishing the space group as $P6_5$. The correctness of the top solution was confirmed by a further refinement of the structure. The errors in orientation and translational parameters are based on comparison with the refined structure (Korkhin *et al.*, 1998). The corresponding CPU times for *MFT* and each translation search were 30 and 2 min.

For comparison, the total CPU time required to solve this structure by *AMoRe* (Navaza, 1994), using data between 15 and 4 Å, was 12 min for each space group. Also, the correct rotation was located at position 21 with a σ of 7.6 compared to 16.8 for the top rotation.

The structure of ADH1 was also solved by the combined *MFT*/translation search, *MFTULT*, using 15–10 Å data and increments of 10° and 2 Å for the rotational and translational grids, respectively. Both space groups were tried. The top 20 solutions on the basis of R-factor values for space group $P6_5$ (ranging from 0.37

Table 3. *Alcohol dehydrogenase (ADH1)*

(*a*) *MFT* search

Space group $P6_1$ or $P6_5$; cell dimensions, $a = b = 81.5$, $c = 399.7$ Å; $G = F$; RF = R_f; 5° increments for the three angles; resolution range, 6.0–2.5 Å.

φ	ψ	θ	RF	S	S_1/S_n	Error (°)
60	60	90	0.515	12.3	1.00	1.5
60	120	90	0.507	6.0	2.06	0.8
115	5	10	0.505	3.4	3.61	34.7
60	0	10	0.504	3.0	4.04	41.9

(*b*) Translation search

2 Å increments along the *a* and *b* axes; resolution range, 8.0–4.0 Å.

$P6_1$						
x	y	z	R factor	S	S_1/S_n	Error (°)
0.112	0.738	0.000	0.445	5.5	1.00	
0.613	0.237	0.000	0.447	5.0	1.11	
0.613	0.738	0.000	0.450	4.0	1.37	
$P6_5$						
0.625	0.250	0.000	0.404	16.0	1.00	1.5
0.287	0.575	0.000	0.435	7.5	2.14	
0.762	0.387	0.000	0.435	7.4	2.16	

Table 4. *Alcohol dehydrogenase/NADP (ADH2)*

(*a*) *MFT* search

Space group $P2_12_12_1$; cell dimensions, $a = 90.4$, $b = 151.4$, $c = 127.9$ Å; $G = E_2$; RF = P_f; 5° increments for the three angles; resolution range, 6.0–2.5 Å.

φ	ψ	θ	RF	S	S_1/S_n	Error (°)
110	50	35	0.259	11.5	1.00	8.0
75	90	35	1.245	3.6	3.18	18.3
55	75	5	0.245	3.4	3.36	30.5
25	20	5	0.245	3.2	3.56	60.5
95	35	5	0.244	2.9	3.99	41.0

(*b*) Translation search

3 Å increments along the three axes; resolution range, 8.0–4.0 Å

x	y	z	R factor	S	S_1/S_n	Error (°)
0.433	0.200	0.225	0.444	7.7	1.00	1.5
0.433	0.260	0.225	0.487	4.2	1.84	8.0
0.433	0.320	0.225	0.491	3.9	1.98	17.0
0.367	0.200	0.225	0.492	3.8	2.03	5.8
0.433	0.140	0.225	0.498	3.3	2.34	10.3

to 0.42) yielded several trial structures (including the top one) with rotational and translational parameters close to the values of the correct structure. CPU time for *MFTULT* was 2 min.

In the case of ADH2, the best discrimination was obtained by using $G = E^2$ for either correlation coefficient (C_c) or product function (P_f). The results based on RF = P_f are given in Table 4(*a*). The top orientation was subjected to translation search (Table 4*b*). The correctness of the top solution was confirmed by further refinement (Y. Korkhin and F. Frolow, private communication). The structure of ADH2 was also solved by *MFTULT* using data in the range 15–12 Å and angular and translational steps of 10° and 3 Å, respectively. CPU time was 30 min.

It should be emphasized that the use of low-order data is advantageous or even essential when the molecular model is approximate, as discussed by Rabinovich & Shakked (1984) and by Urzhumtsev & Podjarny (1995). It allows the use of relatively coarse rotational and translational grids, thereby reducing dramatically the computing time.

3.2.2. *DNA dodecamer d(ACCGACGTCGGT)*. This DNA fragment is the central region of a 16 bp DNA target of the E2 protein from bovine papillomavirus type 1 (BPV-1) whose structure has been determined in its complex with the DNA-binding domain of the E2 protein (Hegde *et al.*, 1992). The free DNA dodecamer has been crystallized in two crystal forms R3 and P1. The former was solved by *ULTIMA* as it contained one molecule in the asymmetric unit (Rozenberg *et al.*, 1997). The unit-cell dimensions of the triclinic crystal and the strong reflections at 3.3–3.4 Å spacing indicated the presence of three B-DNA-type molecules in the unit cell (3 × 486 non-H atoms) oriented approximately along the three unit-cell axes. The *MFT* model was constructed from fibre-diffraction-based coordinates of B-DNA (Chandrasekaran & Arnott, 1989). The axis associated with the Eulerian angle ψ was chosen to coincide with the DNA-helix axis, and the transform was calculated in a unit cell of dimensions 130 × 130 × 130 Å.

In this case, the *MFT* search was expected to give three orientations corresponding to the three unique molecules. However, due to the pseudo-tenfold symmetry of the B-DNA duplex with respect to the helix axis, three families of orientation peaks, each at well defined values of φ and θ, were obtained. In each family, ten peaks were spaced apart by about 36° in ψ which is the average rotation per base-pair in a B-DNA helix. A contour map of the peaks based on correlation coefficient (RF = C_c) for one of the three families is shown in Fig. 1. The highest peaks on the basis of the three criteria (C_c, R_f and P_f) using $G = E^2$ in the range of 8–4 Å, are given in Tables 5(*a*), 5(*b*) and 5(*c*), respectively.

Since it was not possible at this stage to identify the three correct orientations with regard to ψ, we attempted to find simultaneously the ψ values of three molecules and the translational parameters (x, y, z) of two molecules. The translation of one molecule can be arbitrarily fixed at (0, 0, 0). For the combined search, *MFTULT* was modified to handle a rotation–translation search of two molecules and partial structure factors calculated from a third molecule. Ten sets of partial structure factors were calculated for a molecule fixed at position (0, 0, 0) and two Eulerian angles ($\varphi = 170°$ and $\theta = 75°$), but differing in the rotation around the helix axis (ψ) according to the results of the *MFT* search. Each set of partial structure factors was used for a multi-dimensional search where the varied parameters were

the ten ψ values of each molecule and the fractional coordinates of its molecular centre.

Several trials were performed by varying the resolution range and grid size. Low-resolution data (19–8 Å) and a relatively coarse translational grid (4 Å steps in each direction) were found to be adequate for this search. The top 20 trial structures on the basis of *R*-factor values (ranging from 0.33 to 0.37), were subjected to rigid-body least-squares refinement at the same resolution (19–8 Å). Several solutions converged to essentially one structure displaying the lowest *R* factor (0.26) and the highest correlation coefficient (0.81). The refined rigid-body parameters of the three molecules in the unit-cell are given in Table 5(*d*). Further refinement of this structure using higher order data and *X-PLOR* (Brünger, 1992) confirmed this as the correct structure (Rozenberg *et al.*, 1997). CPU time for the *MFT* search and the subsequent rotation–translation (*MFTULT*) search were 4 and 160 min, respectively. Examination of the *MFT* search results shows that the three correct orientations are found within the top ten solutions for each of the three RF criteria (marked by † in Table 5). By accepting only rotational grid points within the top solutions an approximately 50-fold reduction in the running time could have been achieved for the combined rotation–translation search.

4. Summary

In the present study we applied the *MFT* method to several structures including test cases where the crystal structures were known (protein–DNA complex and lactate dehydrogenase) and new structures (two alcohol dehydrogenases and a DNA dodecamer). Two general conclusions emerge from these studies: (i) the *MFT* method is faster by several orders of magnitude than any other method based on reciprocal-space search (*e.g.* Rabinovich & Shakked, 1984; DeLano & Brünger, 1995) since the calculated transform is derived only once

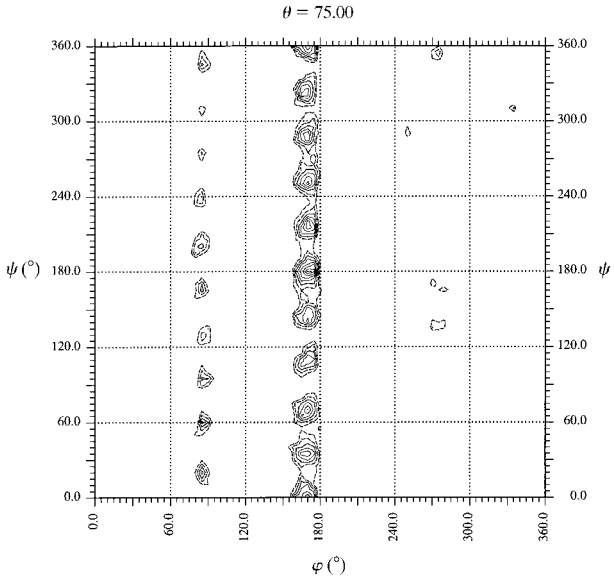

Fig. 1. A section of a contour map at $\theta = 75^\circ$ based on $G = E^2$ and RF = C_c, using data in the range of 8–4 Å. Contour level cutoff, 2σ; contour interval, 0.5σ. The figure was generated with the program *CMap* (Rozenberg, 1997).

Table 5. *DNA dodecamer*

Space group *P*1; cell dimensions, $a = 40.5$, $b = 40.1$, $c = 40.5$ Å; $\alpha = 82.6$, $\beta = 116.2$, $\gamma = 80.7^\circ$. This triclinic system was used, rather than the one where all angles are greater than 90°, to facilitate comparison with the *R*3 structure of the same DNA sequence. $G = E^2$; 5° increments for the three angles; resolution range, 8.0–4.0 Å.

(*a*) *MFT* search, RF = C_c

φ	ψ	θ	RF	S	S_1/S_n
270	20	90	0.206	5.7	1.00
270	10	25	0.203	5.7	1.01
275	330	25	0.199†	5.6	1.03
170	180	75	0.199†	5.6	1.03
270	60	90	0.199	5.6	1.03
90	195	90	0.198	5.5	1.04
265	195	25	0.193	5.4	1.06
90	85	90	0.193	5.4	1.07
90	125	90	0.191	5.3	1.08
90	335	90	0.191†	5.3	1.08

(*b*) *MFT* search, RF = R_f

φ	ψ	θ	RF	S	S_1/S_n
170	215	75	0.215	6.2	1.00
170	180	80	0.211†	5.8	1.07
260	240	20	0.210	5.7	1.10
270	340	25	0.207†	5.5	1.14
280	215	25	0.205	5.2	1.19
90	340	90	0.203†	5.1	1.23
235	195	25	0.203	5.0	1.24
270	10	25	0.202	5.0	1.25
170	250	80	0.202	5.0	1.26
165	35	80	0.202	4.9	1.27

(*c*) *MFT* search, RF = P_f

φ	ψ	θ	RF	S	S_1/S_n
270	20	90	0.318	5.9	1.00
170	180	75	0.316†	5.8	1.02
270	10	25	0.313	5.6	1.05
270	340	25	0.312†	5.5	1.07
90	340	90	0.310†	5.4	1.08
270	23	20	0.310	5.4	1.08
90	15	90	0.310	5.4	1.09
270	60	90	0.309	5.3	1.10
265	195	25	0.309	5.3	1.10
170	215	75	0.307	5.2	1.12

(*d*) Refined parameters after rigid-body refinement. Resolution range, 19.0–8.0 Å

φ	ψ	θ	x	y	z	*R* factor	Correction
170.7	181.9	75.1	0.000	0.000	0.000	0.26	0.81
270.1	336.3	27.1	0.401	−0.034	0.528		
87.7	333.1	91.1	0.551	0.562	0.532		

† Correct orientation.

rather than for each rotation of the molecular model, and (ii) the fit of the weighted reciprocal lattice to the calculated transform can be gauged not only by the Patterson correlation coefficient (Hauptman, 1982) but also by other criteria such as R factor and product function. High discrimination based on several different criteria is in general the hallmark of a correct structure.

In cases where the molecular model comprised a fraction of the contents of the asymmetric unit, discrimination between correct and false orientation, on the basis of signal-to-noise ratio, was found to be significantly weaker than in cases where the whole asymmetric unit contents was used for the *MFT* search. Hence, additional searches were performed. In the case of the protein–DNA complex, a finer rotational search around the parameters of the top solutions of the coarse search unambiguously yielded the correct orientations of the two unique monomers in the asymmetric unit. In the case of the DNA dodecamer with three unique molecules in the unit cell, additional complexity was imposed by the pseudo-tenfold helical symmetry of the double helix. As a consequence, only two rotational parameters (φ, θ) could be determined with confidence for each molecule. The third rotation (ψ) could be identified only after a combined rotation–translation search.

When the search model comprised the major part of the asymmetric unit, the simultaneous rotation–translation method, *MFTULT*, competed favourably with *MFT* followed by translation search. *MFTULT* allows the use of lower order data and hence coarser rotational and translational grids. Also, the use of packing criteria reduces dramatically the number of translational grid points searched for each rotational grid point. As a result, the corresponding runs are very fast, as demonstrated by the two alcohol-dehydrogenase structures. The use of low-order data is particularly advantageous when the model is not highly similar to the crystal structure as discussed above. In cases where the asymmetric unit contains more than one molecule or when a partial model is available, the use of *MFT* followed by either *ULTIMA* or *MFTULT* appears to be the method of choice. All three programs can be obtained from the authors upon request.

5. Concluding remarks

At the end of their chapter on Fourier transforms, Lipson & Cochran (1966) wrote:

> The transform approach is so different from other approaches that one is tempted to ask why it is thought worthwhile introducing it at all when so many other methods appear to be available.

They also proposed several answers to the question. Today, however, after 30 years of major advances in X-ray crystallography and computing facilities, the appropriate answer would be the following: in an effort to solve a macromolecular structure, every approach should be tried until the desired goal is achieved.

We thank Yakov Korkhin for providing us with the data of the various alcohol dehydrogenase structures prior to publication. We also thank Joseph Gilboa for comments on the manuscript. This work was supported by grants from the Basic Research Foundation administered by the Israel Academy of Sciences and Humanities and from the United States–Israel Binational Science Foundation, BSF. ZS holds the Helena Rubinstein Professorial Chair of Structural Biology.

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