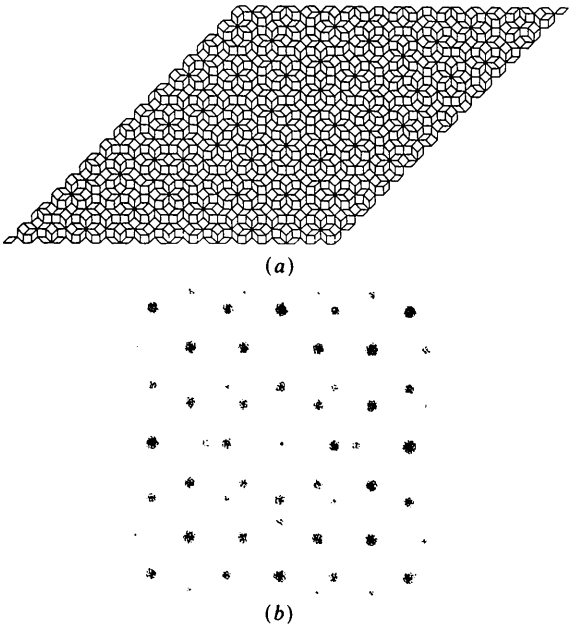

(*a*)

(*b*)

Fig. 2. (*a*) Octagonal tessellation in rhombic cell. (*b*) Diffraction pattern of lattice points about the center of Fig. 2(*a*).

octagonal tessellation pattern is non-periodic, as in the case of pentagonal tessellation (Mackay, 1981). The ratio of similarity from *n*th to $(n+1)$th generation for this tessellation is $1/(2+\sqrt{2})$.

As far as we know the eightfold symmetry diffraction pattern of a non-periodic structure has not yet been reported. The diffraction pattern (Fig. 2*b*) of the tessellation shown in Fig. 2(*a*) was calculated by a FFT algorithm assuming that point-like atoms of 1423 are located at lattice points about the center of the rhombic cell. All the coordinates of lattice points in the *n*th-generation pattern can be computed from those of the zeroth-generation pattern by applying the self-similar subdividing operation recursively.

In Fig. 2(*b*) we can also see sharp Bragg-like peaks with eightfold symmetry as expected, which might prove that the tessellation is a two-dimensional quasi-lattice.

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A reciprocal-space method for calculating a molecular envelope using the algorithm of B. C. Wang.

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Abstract

A method is described to determine the molecular envelope from an isomorphous replacement phased electron density map using the reciprocal-space equivalent of B. C. Wang's algorithm [Wang (1985). In *Methods in Enzymology*, Vol. 115: *Diffraction Methods for Biological Macromolecules*, edited by H. Wyckoff, C. H. W. Hirs & S. N. Timasheff. New York: Academic Press.]. In the case of chloramphenicol acetyl transferase the computation time was reduced from 35 h (using the real-space algorithm) to 40 min.

A suite of programs designed to improve the quality of protein electron density maps has recently been developed and distributed by B. C. Wang and colleagues (Wang, 1985). The basis of their method is to use the electron density map to determine a molecular envelope and then to set the electron density in the solvent region to a constant value (solvent flattening) and apply a positivity constraint to the electron density in the protein region. The modified electron density map is Fourier transformed, and the resulting phases combined with the original single isomorphous replacement (or multiple isomorphous replacement) phase information. The combined phases are then used to calculate a new electron density map, and the whole procedure is repeated iteratively until there is no further improvement in the quality of the electron density.

The solvent flattening part of this procedure has been used successfully in the structure determination of human alpha-1 proteinase inhibitor (Loebermann, Tokuoka, Deisenhofer & Huber, 1984), the photosynthetic reaction centre (Deisenhofer, Epp, Miki, Huber & Michel, 1984) and a light-harvesting biliprotein (Schirmer, Bode, Huber, Sidler & Zuber, 1985), all at 3 Å resolution, and similar results have been obtained by Wang and colleagues in the structure determination of cytochrome c5 at 2·5 Å resolution (Carter, Melis, O'Donnell, Burgess, Furey, Wang & Stout, 1985) as well as a number of structures at lower

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resolution. As expected, the method is most powerful when the solvent content of the crystals is high (70% for the first three examples).

The concept of using solvent flattening to improve isomorphous replacement phases is not new, all the necessary programs are available in Bricogne's molecular averaging package (Bricogne, 1976). Sigler and colleagues (Schevitz, Podjarny, Zwick, Hughes & Sigler, 1981) used the same approach to produce a dramatic improvement in the electron density map of *f*Met-tRNA at 4 Å resolution (also 70% solvent). What is novel about Wang's approach is the algorithm that he uses to determine the molecular envelope from the original electron density map. Instead of relying on visual inspection of the map (usually using an interactive graphics display), Wang's procedure has the advantage of being fully automatic. [An alternative automated procedure has also been proposed by Bhat & Blow (1982).] The first step in Wang's procedure is to calculate an 'averaged' map from the isomorphous replacement map, by replacing the electron density at each grid point by the weighted average of the electron density at all surrounding grid points within a sphere of radius R.

The weighting function used is

$$w(i) = 1 - r(i)/R \quad \text{for } \rho(i) > 0$$
$$= 0 \quad \text{for } \rho(i) < 0$$

where $\rho(i)$ is the electron density at grid point i at a distance $r(i)$ from the centre of the sphere. It is important to realize that because negative densities are ignored (*i.e.* given a weight of zero), the result is *not* the same as simply calculating a map at low resolution. The second step is to compute a histogram of the electron densities in the resulting averaged map, and to choose a 'solvent level' so that the number of grid points with density less than this solvent level corresponds to the expected solvent content of the crystal. [The solvent content can be estimated using the formula given by Matthews (1968) based on the unit-cell contents and the protein molecular weight.] All grid points in the averaged map with a density less than the solvent level are then considered to be in the solvent, while the remainder define the protein.

The optimum value of the averaging radius R depends on both the resolution of the electron density map and on its quality (*i.e.* the noise level in the solvent region). Typically a value between 8 and 10 Å is used to average a 3 Å resolution isomorphous replacement map.

The calculation of the averaged map can be extremely expensive in computing time, particularly since Wang's distributed programs require that the calculation be done in space group $P1$. As an example, chloramphenicol acetyl transferase (CAT) crystallizes in space group $R32$ with equivalent hexagonal cell parameters $a = 107.6$, $c = 123{\cdot}4$ Å. A 3 Å resolution map calculated on a 1·1 Å grid was averaged using a radius $R = 10$ Å; this calculation required 35 h CPU time on a VAX 11/750.

The calculation can be made very much faster by using reciprocal-space methods based on the fast Fourier transform (FFT). A similar approach has been described by Namba & Stubbs (1985) to improve isomorphous phase information derived from fibre diffraction experiments. The authors use a Gaussian weighting function in reciprocal space which, as shown below, will produce very similar results to Wang's procedure.

Wang's averaging procedure in real space is *directly equivalent* to convoluting the truncated isomorphous replacement map (*i.e.* the map with all negative electron density values set to zero) with the weighting function $w(r)$ given by

$$w(r) = 1 - r/R \quad r < R$$
$$= 0 \quad r > R.$$

This may be written as

$$\rho_{av}(i, j, k) = \rho_{tr}(i, j, k) \wedge w(r)$$

where ρ_{av} is the averaged map, ρ_{tr} is the truncated map and $\wedge$ denotes convolution.

From the convolution theorem it follows that

$$\mathrm{FT}[\rho_{av}(i, j, k)] = \mathrm{FT}[\rho_{tr}(i, j, k)] * \mathrm{FT}[w(r)]$$

where FT[ ] denotes the Fourier transform. The Fourier transform of the truncated map is readily calculated using standard FFT programs and it can be shown that the Fourier transform of $w(r)$ is given by

$$g(s) = \mathrm{FT}[w(r)] = Y(uR) - Z(uR)$$

where

$$s = 2\sin\theta/\lambda$$
$$u = 2\pi s$$
$$Y(x) = 3(\sin x - x\cos x)/x^3$$
$$Z(x) = 3[2x\sin x - (x^2 - 2)\cos x - 2]/x^4.$$

[See James (1948), p. 466, for a similar example.]

Thus, to compute the averaged map, the structure factors obtained by back-transforming the truncated map are multiplied by the function $g(s)$ and the modified coefficients are used to calculate a new map which will be identical to that produced by averaging in real space. In the case of CAT, the CPU time was reduced from 35 h to 40 min, even though (in the absence of an $R32$ FFT program) the calculation was performed in space group $P1$.

The function $g(s)$ is similar in form to the transform of a sphere (which would correspond to the weighting function $w = 1$ for $r < R$, $w = 0$ for $r > R$) but falls off rather less rapidly. For $R = 10$ Å, the function has values less than 0·001 for Bragg spacings less than 5 Å, and therefore Fourier terms corresponding to spacings less than 5 Å will make no significant contribution to the averaged map.

The averaging procedure can easily be modified to use different weighting functions $w(r)$, provided that the Fourier transform $g(s)$ can be calculated analytically. Tests using the function

$$w = 1 - (r/R)^2$$

gave very similar results to the original weighting function, suggesting that the averaged map, and hence the molecular envelope, is rather insensitive to the precise form of the weighting function.

Namba & Stubbs (1985) used a Gaussian weighting function with an artificial temperature factor of 300 Å^2 in place of the function $g(s)$. This gives weights very similar to those given by the function $g(s)$ with a radius $R = 10$ Å.

Two practical points are worthy of mention:

(1). It is common practice to omit low-resolution terms (Bragg spacings greater than about 10 Å) from isomorphous

replacement maps, either because the data have not been measured or because the phasing is poor (possibly due to non-isomorphism arising from changes in solvent structure). However, when using the reciprocal-space algorithm, it is *essential* that *all* low-resolution terms are calculated from the back-transform of the truncated map and included in the calculation of the averaged map. This is because it is precisely these terms that contain the information on the gross shape of the molecular envelope.

(2). The step which truncates the isomorphous replacement map can also be used to eliminate large positive peaks in the map which could otherwise distort the local molecular boundary. Such peaks can arise from several sources, such as ripples around heavy-atom positions, build up of errors on crystallographic symmetry axes or the presence of metal ions in the protein structure.

The programs required for the application of the reciprocal-space algorithm are written in Fortran 77 suitable for a VAX computer. The programs and documentation are available on request from the author.

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