

15.1. PHASE IMPROVEMENT BY ITERATIVE DENSITY MODIFICATION

shown in equation (15.1.3.2). If the origin term of G is set to zero, then the modified structure factor, $F_{\text{mod}}(\mathbf{h})$, will depend on the values of all the structure factors except itself; this is equivalent to performing a reflection-omit calculation with that reflection alone omitted.

Let the origin-removed G be called $G_\gamma(\mathbf{h})$ and its Fourier transform $g_\gamma(\mathbf{x})$:

$$G_\gamma(\mathbf{h}) = \begin{cases} 0, & \mathbf{h} = 0 \\ G(\mathbf{h}), & \mathbf{h} \neq 0 \end{cases}, \quad (15.1.4.7)$$

then

$$g_\gamma(\mathbf{x}) = g(\mathbf{x}) - \overline{g(\mathbf{x})}. \quad (15.1.4.8)$$

The convolution of the reflection data with $G_\gamma(\mathbf{h})$ is equivalent to performing a reflection-omit calculation, omitting every reflection in turn. However, the convolution may still be performed in real space; thus, the full omit calculation becomes a simple multiplication of the map by $g_\gamma(\mathbf{x})$:

$$\rho_{\text{mod}}(\mathbf{x}) = g_\gamma(\mathbf{x}) \times \rho(\mathbf{x}). \quad (15.1.4.9)$$

In a solvent-flattening calculation, $g_\gamma(\mathbf{x})$ will be equal to $g(\mathbf{x})$ minus the fraction of the cell that is protein. In the case of a cell with 50% solvent, $g_\gamma(\mathbf{x})$ has a value of 0.5 in the protein and -0.5 in the solvent. Multiplication of the map by this function results in flipping of the solvent.

If the origin term of the G function, γ , can be determined, then the flipping calculation may alternatively be performed by subtracting a copy of the initial map scaled by γ from the modified map. This is the γ correction of Abrahams (1997). This approach may be generalized to arbitrary density-modification methods by use of the perturbation γ (Cowtan, 1999). In this approach, a random perturbation is applied to the starting data. Density modification is applied to both the perturbed and unperturbed maps. The relative size of the perturbation signal in the modified map gives an estimate for γ . The perturbation γ provides effective bias correction for any combination of solvent flattening, histogram matching and averaging. γ may also be estimated as a function of resolution, allowing successful application to multi-resolution modification and possibly atomization as well.

15.1.5. Combining constraints for phase improvement

The chemical and physical information of the underlying structure that the electron density represents serves as constraints on the phases. For small molecules, the constraints of positivity and atomicity are sufficient to solve the phase problem *ab initio* (Hauptman, 1986; Karle, 1986; Woolfson, 1987), because crystals of small molecules generally diffract to atomic resolution. However, no single constraint at our disposal is powerful enough to render the macromolecular phase problem determinable, because macromolecule crystals rarely diffract to atomic resolution. Therefore, individual constraints are combined to produce a more powerful density-modification protocol. This is because these constraints represent different characteristic features of the electron density and they contain independent phasing information.

The phasing power of a method increases with the number of independent constraints employed, the number of density points affected and the amplitude of changes imposed on the electron density. It also depends on the physical nature and accuracy of the constraints and how the constraints are applied. One obvious way of implementing several constraints is to apply them one after the other to the electron density. This sequential application, although easy to implement, suffers some drawbacks. The cyclic application of all constraints may not converge easily, since some constraints

may contain contradicting information as to how the density should be modified. An alternative way of implementing various constraints is simultaneous application. The density solution that satisfies all the constraints is obtained by a global minimization procedure (Main, 1990b; Zhang & Main, 1990b).

15.1.5.1. The system of nonlinear constraint equations

The constraints used in *SQUASH/DM* can be divided into three categories. The first category comprises the linear constraints, such as solvent flatness, density histogram and equal molecules. The second category comprises the nonlinear constraints, such as the local shape of electron density as expressed in Sayre's equation. The third category comprises the available structural data, such as the observed structure-factor amplitudes and the experimental phases. The first and second categories of constraints are used to solve new electron-density values. The third category of constraints is used as a means to filter the modified phases.

The modification to the density value at a grid point by a linear constraint is independent of the values at other grid points. These constraints include solvent flattening, histogram matching and molecular averaging. These density-modification methods construct an improved map directly from an initial density map as expressed by

$$\rho(\mathbf{x}) = H(\mathbf{x}), \quad (15.1.5.1)$$

where $H(\mathbf{x})$ is the target electron density produced by these linear constraints.

The new electron density that satisfies both the linear constraints represented by equation (15.1.5.1) and the nonlinear constraints expressed by Sayre's equation (15.1.2.31) can be obtained by solving the systems of simultaneous equations (Zhang & Main, 1990b)

$$\begin{cases} (V/N) \sum_{\mathbf{y}} \rho^2(\mathbf{y}) \psi(\mathbf{x} - \mathbf{y}) - \rho(\mathbf{x}) = 0 \\ H(\mathbf{x}) - \rho(\mathbf{x}) = 0 \end{cases}. \quad (15.1.5.2)$$

Equation (15.1.5.2) represents a system of nonlinear simultaneous equations with as many unknowns as the number of grid points in the asymmetric unit of the map and with twice as many equations as unknowns. The functions $H(\mathbf{x})$ and $\psi(\mathbf{x} - \mathbf{y})$ are both known. The least-squares solution, using either the full matrix or the diagonal approximation, is obtained using the Newton–Raphson technique with fast Fourier transforms, as described in the next section (Main, 1990b).

15.1.5.2. Least-squares solution to the system of nonlinear constraint equations

For a system of nonlinear equations of electron density,

$$\mathbf{F}(\rho(\mathbf{x})) = \mathbf{0}, \quad (15.1.5.3)$$

where

$$\mathbf{F}(\rho(\mathbf{x})) = [F_1(\rho(\mathbf{x})) \ F_2(\rho(\mathbf{x})) \ \dots \ F_m(\rho(\mathbf{x}))]^T, \\ \rho(\mathbf{x}) = [\rho_1 \ \rho_2 \ \dots \ \rho_n]^T,$$

$\mathbf{0}$ is a null vector, n is the number of grid points and m is the number of equations, the Newton–Raphson method of solution is to find a set of shifts, $\delta\rho(\mathbf{x})$ to $\rho(\mathbf{x})$, through a system of linear equations,

$$\mathbf{J}\delta\rho(\mathbf{x}) = -\varepsilon, \quad (15.1.5.4)$$

where \mathbf{J} is a matrix of partial derivatives of \mathbf{F} with respect to $\rho(\mathbf{x})$ and is called the Jacobian matrix,