16. DIRECT METHODS

(White & Woolfson, 1975) and *XMY* (Debaerdemaeker & Woolfson, 1989).

16.1.5. Real-space constraints (*baking*)

Peak picking is a simple but powerful way of imposing an atomicity constraint. The potential for real-space phase improvement in the context of small-molecule direct methods was recognized by Jerome Karle (1968). He found that even a relatively small, chemically sensible, fragment extracted by manual interpretation of an electron-density map could be expanded into a complete solution by transformation back to reciprocal space and then performing additional iterations of phase refinement with the tangent formula. Automatic real-space electron-density map interpretation in the *Shake-and-Bake* procedure consists of selecting an appropriate number of the largest peaks in each cycle to be used as an updated trial structure without regard to chemical constraints other than a minimum allowed distance between atoms. If markedly unequal atoms are present, appropriate numbers of peaks (atoms) can be weighted by the proper atomic numbers during transformation back to reciprocal space in a subsequent structure-factor calculation. Thus, *a priori* knowledge concerning the chemical composition of the crystal is utilized, but no knowledge of constitution is required or used during peak selection. It is useful to think of peak picking in this context as simply an extreme form of density modification appropriate when atomic resolution data are available. In theory, under appropriate conditions it should be possible to substitute alternative density-modification procedures such as low-density elimination (Shiono & Woolfson, 1992; Refaat & Woolfson, 1993) or solvent flattening (Wang, 1985), but no practical applications of such procedures have yet been made. The imposition of physical constraints counteracts the tendency of phase refinement to propagate errors or produce overly consistent phase sets. Several variants of peak picking, which are discussed below, have been successfully employed within the framework of *Shake-and-Bake*.

16.1.5.1. *Simple peak picking*

In its simplest form, peak picking consists of simply selecting the top N_u E-map peaks where N_u is the number of unique non-H atoms in the asymmetric unit. This is adequate for true small-molecule structures. It has also been shown to work well for heavy-atom or anomalously scattering substructures where N_u is taken to be the number of expected substructure atoms (Smith *et al.*, 1998; Turner *et al.*, 1998). For larger structures ($N_u > 100$), it is likely to be better to select about $0.8N_u$ peaks, thereby taking into account the probable presence of some atoms that, owing to high thermal motion or disorder, will not be visible during the early stages of a structure determination. Furthermore, a recent study (Weeks & Miller, 1999b) has shown that structures in the 250–1000-atom range which contain a half dozen or more moderately heavy atoms (*i.e.*, S, Cl, Fe) are more easily solved if only $0.4N_u$ peaks are selected. The only chemical information used at this stage is a minimum inter-peak distance, generally taken to be 1.0 Å. For substructure applications, a larger minimum distance (*e.g.* 3 Å) is more appropriate.

16.1.5.2. *Iterative peaklist optimization*

An alternative approach to peak picking is to select approximately N_u peaks as potential atoms and then eliminate some of them, one by one, while maximizing a suitable figure of merit such as

$$P = \sum_{\mathbf{H}} |E_c^2|(|E_o^2| - 1). \qquad (16.1.5.1)$$

The top N_u peaks are used as potential atoms to compute $|E_c|$. The atom that leaves the highest value of P is then eliminated. Typically, this procedure, which has been termed *iterative peaklist optimization* (Sheldrick & Gould, 1995), is repeated until only $2N_u/3$ atoms remain. Use of equation (16.1.5.1) may be regarded as a reciprocal-space method of maximizing the fit to the origin-removed sharpened Patterson function, and it is used for this purpose in molecular replacement (Beurskens, 1981). Subject to various approximations, maximum-likelihood considerations also indicate that it is an appropriate function to maximize (Bricogne, 1998). Iterative peaklist optimization provides a higher percentage of solutions than simple peak picking, but it suffers from the disadvantage of requiring much more CPU time.

16.1.5.3. *Random omit maps*

A third peak-picking strategy also involves selecting approximately N_u of the top peaks and eliminating some, but, in this case, the deleted peaks are chosen at random. Typically, one-third of the potential atoms are removed, and the remaining atoms are used to compute E_c. By analogy to the common practice in macromolecular crystallography of omitting part of a structure from a Fourier calculation in hopes of finding an improved position for the deleted fragment, this version of peak picking is described as making a *random omit map*. This procedure is a little faster than simply picking N_u atoms because fewer atoms are used in the structure-factor calculation. More important is the fact that, like iterative peaklist optimization, it has the potential for being a more efficient search algorithm.

16.1.6. Fourier refinement (*twice baking*)

E-map recycling, but without phase refinement (Sheldrick, 1982, 1990; Kinneging & de Graaff, 1984), has been frequently used in conventional direct-methods programs to improve the completeness of the solutions after phase refinement. It is important to apply Fourier refinement to *Shake-and-Bake* solutions also because such processing significantly increases the number of resolved atoms, thereby making the job of map interpretation much easier. Since phase refinement *via* either the tangent formula or the minimal function requires relatively accurate invariants that can only be generated using the larger *E* magnitudes, a limited number of reflections are phased during the actual dual-space cycles. Working with a limited amount of data has the added advantage that less CPU time is required. However, if the current trial structure is the 'best' so far based on a figure of merit (either the minimal function or a real-space criterion), then it makes sense to subject this structure to Fourier refinement using additional data, thereby reducing series-termination errors. The correlation coefficient

$$\begin{aligned} \mathrm{CC} = & \left[\left(\sum w E_o^2 E_c^2 \sum w \right) - \left(\sum w E_o^2 \sum w E_c^2 \right) \right] \\ & \times \left\{ \left[\left(\sum w E_o^4 \sum w \right) - \left(\sum w E_o^2 \right)^2 \right] \right. \\ & \left. \times \left[\left(\sum w E_c^4 \sum w \right) - \left(\sum w E_c^2 \right)^2 \right] \right\}^{-1/2} \qquad (16.1.6.1) \end{aligned}$$

(Fujinaga & Read, 1987), where weights $w = 1/[0.04 + \sigma^2(E_o)]$, has been found to be an especially effective figure of merit when used with all the data and is, therefore, suited for identifying the most promising trial structure at the end of Fourier refinement. Either simple peak picking or iterative peaklist optimization can be employed during the Fourier-refinement cycles in conjunction with weighted *E* maps (Sim, 1959). The final model can be further improved by isotropic displacement parameter (B_{iso}) refinement for the individual atoms (Usón *et al.*, 1999) followed by calculation of the Sim (1959) or sigma-A (Read, 1986) weighted map. This is

336