16.1. *AB INITIO* PHASING

used passively as the basis for a figure of merit (DeTitta *et al.*, 1975). However, the reliability of these invariants, as given by their conditional probability distribution (Hauptman, 1975), is proportional to $1/N$, and they have not as yet been shown to be useful for macromolecular phasing. The reliability of higher-order invariants decreases even more rapidly as structure size increases.

16.1.3.2. 'Multisolution' methods and trial structures

Successful crystal structure determination requires that sufficient phases be found such that a Fourier map computed using the corresponding structure factors will reveal the atomic positions. It is particularly important that the biggest terms (*i.e.*, largest $|E|$) be included in the Fourier series. Thus, the first step in the phasing process is to sort the reflections in decreasing order according to their $|E|$ values and to choose the number of large $|E|$ reflections that are to be phased. The second step is to generate the possible invariants involving these intense reflections and then to sort them in decreasing order according to their A_{HK} values. Those invariants with the largest A_{HK} values are retained in sufficient number to achieve the desired over-determination. *Ab initio* phase determination by direct methods requires not only a set of invariants, the average values of the cosines of which are presumed to be known, but also a set of starting phases. Therefore, the third step in the phasing process is the assignment of initial phase values. If enough pairs of phases, φ_K and φ_{-H-K}, are known, the structure invariants can then be used to generate further phases (φ_H) which, in turn, can be used to evaluate still more phases. Repeated iterations will permit most reflections with large $|E_H|$ to be phased.

Depending on the space group, a small number of phases can be assigned arbitrarily in order to fix the origin position and, in noncentrosymmetric space groups, the enantiomorph or polar-axis direction. However, except for the simplest structures, these reflections provide an inadequate foundation for further phase development. Historically, a 'multisolution' or multi-trial approach (Germain & Woolfson, 1968) was taken in which other reflections are each assigned many different starting values in the hope that one or more of the resultant phase combinations will lead to a solution. Solutions, if they occurred, were identified on the basis of some suitable figure of merit. Although phases can be evaluated sequentially, the order determined by a so-called convergence map (Germain *et al.*, 1970), it has become standard in recent years to use a random-number generator to assign initial values to all available phases from the outset (Baggio *et al.*, 1978; Yao, 1981). A variant of this procedure is to use the random-number generator to assign initial coordinates to the atoms in the trial structures and then to obtain initial phases from a structure-factor calculation. In addition, some dual-space programs [*SHELXD* (Schneider & Sheldrick, 2002), *HySS* (Grosse-Kunstleve & Adams, 2003)] can also use the Patterson function to generate starting atoms or phases (see Section 16.1.8).

16.1.4. Reciprocal-space phase refinement or expansion (*shaking*)

Once a set of initial phases has been chosen, it must be refined against the set of structure invariants whose values are presumed known. In theory, any of a variety of optimization methods could be used to extract phase information in this way. However, so far only three purely reciprocal-space methods have been shown to be of practical value: tangent refinement, parameter-shift opti-

mization of the minimal function, and maximization of Karle–Hauptman determinants (Section 16.1.12.7).

16.1.4.1. The tangent formula

The *tangent formula*,

$$\tan(\varphi_H) = \frac{-\sum_K |E_K E_{-H-K}| \sin(\varphi_K + \varphi_{-H-K})}{\sum_K |E_K E_{-H-K}| \cos(\varphi_K + \varphi_{-H-K})}, \qquad (16.1.4.1)$$

(Karle & Hauptman, 1956), is the relationship used in conventional direct-methods programs to compute φ_H given a sufficient number of pairs ($\varphi_K, \varphi_{-H-K}$) of known phases. It can also be used within the phase-refinement portion of the dual-space *Shake-and-Bake* procedure (Weeks, Hauptman *et al.*, 1994; Usón & Sheldrick, 1999). The variance associated with φ_H depends on $\sum_K E_H E_K E_{-H-K}/N^{1/2}$ and, in practice, the estimate is only reliable for $|E_H| \gg 1$ and for structures with a limited number of atoms (N). If equation (16.1.4.1) is used to redetermine previously known phases, the phasing process is referred to as *tangent-formula refinement*; if only new phases are determined, the phasing process is *tangent expansion*.

The tangent formula can be derived using the assumption of equal resolved atoms. Nevertheless, it suffers from the disadvantage that, in space groups without translational symmetry, it is perfectly fulfilled by a false solution with all phases equal to zero, thereby giving rise to the so-called 'uranium-atom' solution with one dominant peak in the corresponding Fourier synthesis. In conventional direct-methods programs, the tangent formula is often modified in various ways to include (explicitly or implicitly) information from the so-called 'negative' quartet invariants (Schenk, 1974; Hauptman, 1974; Giacovazzo, 1976) that are dependent on the smallest as well as the largest E magnitudes. Such modified tangent formulas do indeed largely overcome the problem of pseudosymmetric solutions for small N, but because of the dependence of quartet-term probabilities on $1/N$, they are little more effective than the normal tangent formula for large N.

16.1.4.2. The minimal function

Constrained minimization of an objective function like the *minimal function*,

$$R(\Phi) = \sum_{H,K} A_{HK} \left\{ \cos \Phi_{HK} - [I_1(A_{HK})/I_0(A_{HK})] \right\}^2 \Big/ \sum_{H,K} A_{HK}$$

$$(16.1.4.2)$$

(Debaerdemaeker & Woolfson, 1983; Hauptman, 1991; DeTitta *et al.*, 1994), provides an alternative approach to phase refinement or phase expansion. $R(\Phi)$ is a measure of the mean-square difference between the values of the triplets calculated using a particular set of phases and the expected values of the same triplets as given by the ratio of modified Bessel functions. The minimal function is expected to have a constrained global minimum when the phases are equal to their correct values for some choice of origin and enantiomorph (the minimal principle). Experimentation has thus far confirmed that, when the minimal function is used actively in the phasing process and solutions are produced, the final trial structure corresponding to the smallest value of $R(\Phi)$ is a solution provided that $R(\Phi)$ is calculated directly from the atomic positions before the phase-refinement step (Weeks, DeTitta *et al.*, 1994). Therefore, $R(\Phi)$ is also an extremely useful figure of merit. The minimal function can also include contributions from higher-order (*e.g.* quartet) invariants, although their use is not as imperative as with the tangent formula because the minimal function does not have a minimum

when all phases are zero. In practice, quartets are rarely used in the minimal function because they increase the CPU time while adding little useful information for large structures.

The cosine function in equation (16.1.4.2) can also be replaced by other functions of the phases giving rise to alternative minimal functions. Examples include an exponential expression that has been found to give superior results for several $P1$ structures (Hauptman *et al.*, 1999). In addition, substructure determination using a very simple and computationally efficient modified minimal function,

$$m(\varphi) = 1 - (N_I/N_T) \qquad (16.1.4.3)$$

(where I is an arbitrary interval $[-r, r]$, N_I is the number of triplets whose values lie in I and N_T is the total number of triplets), has been reported (Xu & Hauptman, 2004, 2006; Xu *et al.*, 2005) and incorporated into the *BnP* software (see Section 16.1.12.4).

16.1.4.3. Parameter shift

In principle, any minimization technique could be used to minimize $R(\Phi)$ by varying the phases. So far, a seemingly simple algorithm, known as parameter shift (Bhuiya & Stanley, 1963), has proven to be quite powerful and efficient as an optimization method when used within the *Shake-and-Bake* context to reduce the value of the minimal function. For example, a typical phase-refinement stage consists of three iterations or scans through the reflection list, with each phase being shifted a maximum of two times by 90° in either the positive or negative direction during each iteration. The refined value for each phase is selected, in turn, through a process which involves evaluating the minimal function using the original phase and each of its shifted values (Weeks, DeTitta *et al.*, 1994). The phase value that results in the lowest minimal-function value is chosen at each step. Refined phases are used immediately in the subsequent refinement of other phases. It should be noted that the parameter-shift routine is similar to that used in ψ-map refinement (White & Woolfson, 1975) and *XMY* (Debaerdemaeker & Woolfson, 1989).

16.1.5. Real-space constraints (*baking*)

For several decades, classical direct methods operated exclusively in reciprocal space, determining phases through statistical relationships between them. Only when this process had converged did the method move into real space by calculating one or more electron-density maps that were examined using stereochemical criteria. In macromolecular crystallography, density modification has always played a central role in phasing. A major advance in direct methods for macromolecules (and large molecules in general) occurred when density-modification methods were incorporated and adapted into the phasing procedure. They are often very simple: peaks which give rise to unrealistic geometries or which are too weak are removed, new structure factors are calculated and hence new phase angles are derived in an iterative process. (They can also be quite sophisticated as in *ACORN2*, which we will discuss in Section 16.1.12.1.) A consequence of this is that the once-clear dividing line between direct methods and other structure-solution techniques has become somewhat blurred.

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 Karle (1968). He found that even a relatively small, chemically sensible, fragment extracted by manual inter-

pretation 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 within the dual-space direct-methods framework to replace peak picking by alternative density-modification procedures such as low-density elimination (Shiono & Woolfson, 1992; Refaat & Woolfson, 1993) or solvent flattening (Wang, 1985). 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 study by Weeks & Miller (1999*b*) 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, provided that care is taken with disulfide bridges (Section 16.1.11).

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 has been used for

references