

16. DIRECT METHODS

16.1.2.1. SIR differences

Given the individual normalized structure-factor magnitudes for native structures ($|E_{\text{nat}}|$) and for structures containing heavy atoms ($|E_{\text{der}}|$), as well as the atomic scattering factors $|f_j| = |f_j^0 + f_j' + if_j''| = [(f_j^0 + f_j')^2 + (f_j'')^2]^{1/2}$ which allow for the possibility of anomalous scattering, then greatest-lower-bound estimates of SIR difference- E magnitudes are

$$|E_{\Delta}| = \frac{\left| \left(\sum_{j=1}^{N_{\text{der}}} |f_j|^2 \right)^{1/2} |E_{\text{der}}| - \left(\sum_{j=1}^{N_{\text{nat}}} |f_j|^2 \right)^{1/2} |E_{\text{nat}}| \right|}{q \left[\left(\sum_{j=1}^{N_{\text{der}}} |f_j|^2 \right) - \left(\sum_{j=1}^{N_{\text{nat}}} |f_j|^2 \right) \right]^{1/2}}, \quad (16.1.2.2)$$

where $q = q_0 \exp(q_1 s^2 + q_2 s^4)$ is a least-squares-fitted empirical renormalization scaling function, dependent on $\sin(\theta)/\lambda$, that imposes the condition $\langle |E_{\Delta}|^2 \rangle = 1$ and serves to define q_0 , q_1 and q_2 .

16.1.2.2. SAD differences

Given Friedel pairs of normalized structure-factor magnitudes ($|E_{+\mathbf{H}}|$, $|E_{-\mathbf{H}}|$) and the atomic scattering factors (f^0 , f_j' and f_j''), then the greatest-lower-bound estimates of SAD difference $|E|$'s are

$$|E_{\Delta}| = \frac{\left[\sum_{j=1}^N (f_j^0 + f_j')^2 + (f_j'')^2 \right]^{1/2} \| |E_{+\mathbf{H}}| - |E_{-\mathbf{H}}| \|}{2q \left[\sum_{j=1}^N (f_j'')^2 \right]^{1/2}}, \quad (16.1.2.3)$$

where, again, q is an empirical renormalization scaling function that imposes the condition $\langle |E_{\Delta}|^2 \rangle = 1$.

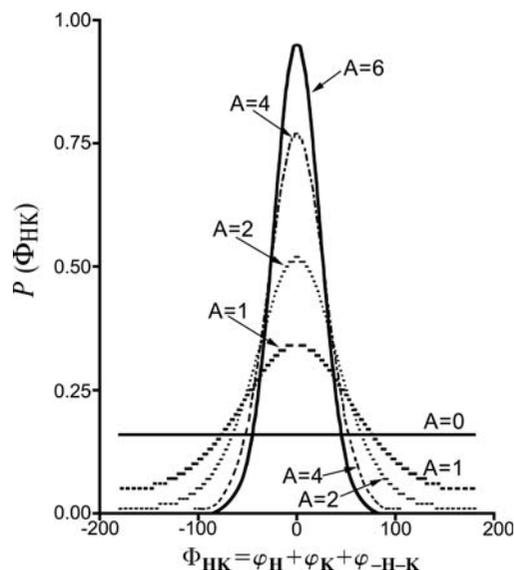
In the case of MAD data, the anomalous differences for all wavelengths can be combined in the form of F_A structure factors as described in Chapter 14.2 of this volume by Smith & Hendrickson. In the *SHELX* system, the program *SHELXC* prepares the $|F_A|$ values and the phase shifts α for SAD, SIR, SIRAS, RIP (radiation-damage-induced phasing) and MAD experimental phasing (Sheldrick, 2008, 2010), and the corresponding $|E_A|$ values are derived by *SHELXD*.

16.1.2.3. Difference intensities and direct methods

There are, of course, difficulties with normalized difference intensities. Direct methods normalize the data to give difference E magnitudes, and the macromolecular temperature factors incorporated in this process can be a source of error. The temperature-factor correction, often large for macromolecules, can take small, statistically dubious, amplitude differences at high resolution when working with anomalous difference data and magnify them into large normalized structure-factor differences that are then used in direct methods. These methods are very sensitive to such errors and will often fail unless a suitable resolution truncation limit is imposed. Usually this is around 0.5 Å larger than the experimental diffraction limit of the data or up to the resolution shell with $I/\sigma(I) = 10$. Morris *et al.* (2004) have extended their work on profiles to devise a method of normalization based on $\langle |E|^2 \rangle$ profiles, and this should be more robust than traditional methods.

16.1.3. Starting the phasing process

The phase problem of X-ray crystallography may be defined as the problem of determining the phases φ of the normalized structure factors E when only the magnitudes $|E|$ are given. Owing to the atomicity of crystal structures and the redundancy of the known magnitudes, the phase problem is overdetermined


Figure 16.1.3.1

The conditional probability distribution, $P(\Phi_{\mathbf{HK}})$, of the three-phase structure invariants, $\Phi_{\mathbf{HK}}$, having associated parameters $A_{\mathbf{HK}}$ with values of 0, 1, 2, 4 and 6. When $A \approx 0$, all values of $\Phi_{\mathbf{HK}}$ are equally likely, and no information useful for phase determination is available. However, the sum of the three phases for most invariants with $A \approx 6$ is close to 0° , and an estimate of one phase can be made if the other two are known.

and is, therefore, solvable in principle. This overdetermination implies the existence of relationships among the E 's and, since the magnitudes $|E|$ are presumed to be known, there exist identities among the phases that are dependent on the known magnitudes alone. The techniques of probability theory lead to the joint probability distributions of arbitrary collections of E from which the conditional probability distributions of selected sets of phases, given the values of suitably chosen magnitudes $|E|$, may be inferred.

16.1.3.1. Structure invariants

The magnitude-dependent entities that constitute the foundation of direct methods are linear combinations of phases called *structure invariants*. The term 'structure invariant' stems from the fact that the values of these quantities are independent of the choice of origin. The most useful of the structure invariants are the three-phase or *triplet invariants*,

$$\Phi_{\mathbf{HK}} = \varphi_{\mathbf{H}} + \varphi_{\mathbf{K}} + \varphi_{-\mathbf{H}-\mathbf{K}}, \quad (16.1.3.1)$$

the conditional probability distribution (Cochran, 1955), given $A_{\mathbf{HK}}$, of which is

$$P(\Phi_{\mathbf{HK}}) = [2\pi I_0(A_{\mathbf{HK}})]^{-1} \exp(A_{\mathbf{HK}} \cos \Phi_{\mathbf{HK}}), \quad (16.1.3.2)$$

where

$$A_{\mathbf{HK}} = (2/N^{1/2}) |E_{\mathbf{H}} E_{\mathbf{K}} E_{\mathbf{H}+\mathbf{K}}| \quad (16.1.3.3)$$

and N is the number of atoms, here presumed to be identical, in the primitive unit cell. This distribution is illustrated in Fig. 16.1.3.1. The expected value of the cosine of a particular triplet, $\Phi_{\mathbf{HK}}$, is given by the ratio of modified Bessel functions, $I_1(A_{\mathbf{HK}})/I_0(A_{\mathbf{HK}})$.

Estimates of the invariant values are most reliable when the normalized structure-factor magnitudes ($|E_{\mathbf{H}}|$, $|E_{\mathbf{K}}|$ and $|E_{-\mathbf{H}-\mathbf{K}}|$) are large and the number of atoms in the corresponding primitive unit cell, N , is small. This is one important reason why direct phasing is more difficult for macromolecules than it is for small molecules. Four-phase or quartet invariants have proven helpful in small-molecule structure determination, particularly when

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 $A_{\mathbf{H}\mathbf{K}}$ values. Those invariants with the largest $A_{\mathbf{H}\mathbf{K}}$ values are retained in sufficient number to achieve the desired overdetermination. *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, $\varphi_{\mathbf{K}}$ and $\varphi_{-\mathbf{H}-\mathbf{K}}$, are known, the structure invariants can then be used to generate further phases ($\varphi_{\mathbf{H}}$) which, in turn, can be used to evaluate still more phases. Repeated iterations will permit most reflections with large $|E_{\mathbf{H}}|$ 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,

$$\tan(\varphi_{\mathbf{H}}) = \frac{-\sum_{\mathbf{K}} |E_{\mathbf{K}} E_{-\mathbf{H}-\mathbf{K}}| \sin(\varphi_{\mathbf{K}} + \varphi_{-\mathbf{H}-\mathbf{K}})}{\sum_{\mathbf{K}} |E_{\mathbf{K}} E_{-\mathbf{H}-\mathbf{K}}| \cos(\varphi_{\mathbf{K}} + \varphi_{-\mathbf{H}-\mathbf{K}})}, \quad (16.1.4.1)$$

(Karle & Hauptman, 1956), is the relationship used in conventional direct-methods programs to compute $\varphi_{\mathbf{H}}$ given a sufficient number of pairs ($\varphi_{\mathbf{K}}$, $\varphi_{-\mathbf{H}-\mathbf{K}}$) 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 $\varphi_{\mathbf{H}}$ depends on $\sum_{\mathbf{K}} E_{\mathbf{H}} E_{\mathbf{K}} E_{-\mathbf{H}-\mathbf{K}} / N^{1/2}$ and, in practice, the estimate is only reliable for $|E_{\mathbf{H}}| \gg 1$ 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; Giacobozzo, 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*,

$$R(\Phi) = \sum_{\mathbf{H}, \mathbf{K}} A_{\mathbf{H}\mathbf{K}} \left\{ \cos \Phi_{\mathbf{H}\mathbf{K}} - [I_1(A_{\mathbf{H}\mathbf{K}}) / I_0(A_{\mathbf{H}\mathbf{K}})] \right\}^2 / \sum_{\mathbf{H}, \mathbf{K}} A_{\mathbf{H}\mathbf{K}} \quad (16.1.4.2)$$

(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