25. MACROMOLECULAR CRYSTALLOGRAPHY PROGRAMS

$$[F_{H_{(\mathrm{obs})}} - F_{H_{(\mathrm{calc})}}]\exp[i\varphi_{H_{(\mathrm{calc})}}], \qquad (25.2.1.16)$$

where $F_{H_{(\mathrm{calc})}}$ and $\varphi_{H_{(\mathrm{calc})}}$ are the calculated heavy-atom structure-factor amplitude and phase, respectively, and $F_{H_{(\mathrm{obs})}}$ is computed from

$$F_{H_{(\mathrm{obs})}}^2 = F_{PH}^2 + F_P^2 - 2F_{PH}F_P\cos(\varphi_{PH} - \varphi_P) \qquad (25.2.1.17)$$

where φ_{PH} and φ_P are the current derivative and native phases, respectively. These coefficients are more accurate than using simple isomorphous difference magnitudes to approximate $F_{H_{(\mathrm{obs})}}$ and can be computed once phasing has begun, since estimates of the required phase differences are then available. Alternatively, the program *MRGDF* (interactive) can be used to produce Fourier coefficients of the form

$$m(F_{PH} - F_P)\exp(i\varphi_P), \qquad (25.2.1.18)$$

where m is the current figure of merit. This method suffers somewhat as phase differences are ignored, but it has the advantage that the amplitude difference does not necessarily involve any derivative previously used in the computation of φ_P. If amplitudes from a new derivative and from the native are used, then peaks in the resulting 'cross-difference' Fourier synthesis for the new derivative will automatically correspond to the same origin and hand as prior sites used in the phasing process, although the hand may still be incorrect. Finally, *GREF* can be used to generate the Fourier coefficients

$$(|F_{PH} - F_P|)\exp[i\varphi_{H_{(\mathrm{calc})}}] \quad \text{or} \quad [|F_{PH} - F_P| - F_{H_{(\mathrm{calc})}}]\exp[i\varphi_{H_{(\mathrm{calc})}}], \qquad (25.2.1.19)$$

with the second set producing a map similar to that obtained using equation (25.2.1.16). Both coefficient sets in equation (25.2.1.19) are lacking in that the phase difference is ignored, but the second set [and also those in equation (25.2.1.16)] has the advantage that heavy-atom sites already in the model are subtracted away, allowing any remaining minor sites to stand out in the resulting map.

25.2.1.7.2. *Bijvoet difference and cross-Bijvoet difference Fourier syntheses*

The files produced by *PHASIT* for anomalous-scattering data sets contain the information needed to produce the Fourier coefficients

$$(F_{PH}^+ - F_{PH}^-)_{\mathrm{obs}}\exp[i(\varphi_P^+ - \pi/2)] \qquad (25.2.1.20)$$

or

$$[(F_{PH}^+ - F_{PH}^-)_{\mathrm{obs}} - (F_{PH}^+ - F_{PH}^-)_{\mathrm{calc}}]\exp[i(\varphi_P^+ - \pi/2)], \qquad (25.2.1.21)$$

where φ_P^+ is the protein phase used when computing F_{PH}^+. The coefficients in equation (25.2.1.20) correspond to a conventional Bijvoet difference Fourier map, which should show large positive peaks at the locations of anomalous-scattering sites when the hand is correct. The coefficients in equation (25.2.1.21) correspond to the case in which contributions from known anomalous scatterers are subtracted out. As in the isomorphous-replacement case, a program *MRGBDF* (interactive) is also provided to generate the Fourier coefficients

$$m(F_{PH}^+ - F_{PH}^-)_{\mathrm{obs}}\exp[i(\varphi_P^+ - \pi/2)], \qquad (25.2.1.22)$$

where the Bijvoet difference does not necessarily have to come from a derivative used in the phasing. If the difference doesn't come from a derivative used in phasing, then a 'cross-Bijvoet difference' Fourier map is obtained, which should produce large positive peaks at anomalous-scatterer locations in the new derivative when the

original hand is correct. Additionally, *GREF* can be used to generate the Fourier coefficients

$$(|F_{PH}^+ - F_{PH}^-|_{\mathrm{obs}})\exp(i\varphi_H^+) \quad \text{or} \quad [|F_{PH}^+ - F_{PH}^-|_{\mathrm{obs}} - F_{H_{(\mathrm{calc})}}^+]\exp(i\varphi_H^+), \qquad (25.2.1.23)$$

where F_H^+ and φ_H^+ are the heavy-atom structure-factor amplitude and phase, used when computing F_{PH}^+. These coefficients can also be used to identify additional anomalous-scatterer sites, but they are insensitive to the hand. As in equation (25.2.1.21), if the second set in equation (25.2.1.23) is used, then contributions from anomalous scatterers already included in the phasing will be subtracted out.

Finally, the program *HNDCHK* (interactive) is provided to determine the enantiomorph by examination of a Bijvoet difference Fourier map. One inputs the map along with the anomalous-scatterer positions used in the phasing. The program then uses a 64-point cubic spline interpolation algorithm to obtain the density precisely at the input coordinates and also at coordinates related to them by a centre of symmetry. If the input heavy-atom configuration had the correct hand, large positive peaks should occur exactly at the input locations. If the hand is incorrect, even larger *negative* peaks occur at the true positions, *i.e.* those related to the input positions by a centre of symmetry.

25.2.1.8. *Solvent flattening and negative-density truncation*

Solvent flattening with negative-density truncation is efficiently carried out by the programs *BNDRY*, *FSFOUR*, *MAPINV* and *RMHEAVY*, all of which are run in batch mode with multiple iterations under the control of a command procedure or shell script. The various aspects of the process as implemented are described below.

25.2.1.8.1. *Mask construction*

Solvent-mask construction follows the procedure suggested by Wang (1985), with the exception that electron density in the vicinity of heavy-atom sites is temporarily ignored during the mask-building process. This allows one to use a tight solvent mask, which maximizes the phasing power of the method while preventing artificial extension of the protein envelope into the solvent region in the vicinity of surface-bound heavy-atom sites. Failure to do this has occasionally been found to deplete the protein region elsewhere to compensate for the incorrectly extended region.

25.2.1.8.1.1. *Automated mask construction*

An electron-density map produced by *FSFOUR* is passed to the program *RMHEAVY* along with a set of heavy-atom locations and a blanking radius. A copy of the map is then made that is identical to the original except that density values within the blanking radius of any heavy-atom site are set to zero. The modified map is then passed to program *MAPINV*, which sets to zero all density values that were negative (note that the F_{000} coefficient is *not* included in program *FSFOUR*) and then computes the corresponding set of structure factors by Fourier inversion. These structure factors are then passed to program *BNDRY* along with a resolution-dependent averaging radius R to compute the Fourier transform of the direct-space weighting function,

$$W(r) = 1 - r/R \text{ if } r \le R \quad \text{and} \quad W(r) = 0 \text{ if } r > R, \qquad (25.2.1.24)$$

where $W(r)$ is the weighting function and r is the distance from the map grid point being evaluated. R is typically 2.5–3 times the minimum d spacing in the data set. Each unique structure factor obtained from map inversion is then multiplied by the transform of $W(r)$, $f(s)$, given by

references