

13. MOLECULAR REPLACEMENT

13.2.3.3. Strategies

Since the first attempts to detect subunits within the crystal asymmetric unit, a great deal of experience has been gained and the results collected in several treatises on molecular replacement (Rossmann, 1972; Machin, 1985; Dodson *et al.*, 1992; Carter & Sweet, 1997).

The success of the rotation function relies in part on the choice of the domain of integration, *i.e.*, inner and outer radii of the spherical domain, and limits on data resolution. The outer radius is chosen so as to maximize the ratio between the number of intramolecular and intermolecular vectors. A typical value of this radius is 50–75% of the subunit's diameter for spherical molecules. The choice of the inner radius is less crucial, provided that the origin peaks are subtracted from the Patterson functions; it is often set to zero.

The resolution range of the selected diffraction data depends on whether we are computing self- or cross-rotation functions. For self rotations, data up to the highest available resolution may be used. For cross rotations, the high-resolution limit is dictated by the degree of similarity between the probe and the actual molecules that constitute the crystal. The low-resolution limit is usually chosen so as to skip the solvent contribution.

Another parameter of interest concerns the angular resolution, which is rather specific to the fast rotation function. Although it was derived by expanding the interference function to follow the original reciprocal-space formulation as closely as possible, it may also be obtained by expanding the Patterson functions in spherical harmonics and then performing the integration (Crowther, 1972). In this way, the concept of angular resolution is more evident. In fact, the spherical harmonics play the same role in the angular domain as the imaginary exponentials do in Cartesian coordinate space. The analogue of the Miller indexes here is the label ℓ , directly related to the angular resolution. The term with $\ell = 0$ is invariant under rotations and is thus eliminated from the summation in equation (13.2.3.14). It represents the contribution of the best radial functions, in a least-squares sense, that approximate the Patterson functions within Ω . The origin peak is thus properly removed by omitting the $\ell = 0$ term, as well as a substantial component of the original function. Also, by omitting low ℓ 's, the angular resolution of peaks is enhanced. These eliminations may also be done in other formulations, but never as efficiently.

The parameters discussed above are not all independent. The relationship between them stems from the behaviour of the spherical Bessel functions for small values of their argument (Watson, 1958),

$$j_\ell(2\pi hb) \simeq (2\pi hb)^\ell / (2\ell + 1)!!$$

[see equation (13.2.3.8)]. So, when omitting low ℓ terms, we are also weighting down low-resolution data.

13.2.3.4. Symmetry properties of the rotation function

The overlap integral that defines \mathcal{R} may be calculated by rotating P_t instead of P_s , but with the inverse rotation,

$$\begin{aligned} \mathcal{R}(\mathbf{R}) &= (1/v) \int_{\Omega} P_t(\mathbf{r}) P_s(\mathbf{R}^{-1}\mathbf{r}) d^3\mathbf{r} \\ &= (1/v) \int_{\Omega} P_t(\mathbf{R}\mathbf{r}) P_s(\mathbf{r}) d^3\mathbf{r}. \end{aligned} \quad (13.2.3.18)$$

This property enables the analysis of the consequence of the symmetries of the Patterson functions upon the rotation function (Tollin *et al.*, 1966; Moss, 1985). For example, when the target and search functions are the same, a trivial symmetry of the self-rotation results: its values at \mathbf{R} and \mathbf{R}^{-1} are the same or, in Euler angles, at (α, β, γ) and $(180 - \gamma, \beta, 180 - \alpha)$. More generally, if P_t is invariant under the rotation \mathbf{T} , *i.e.*, $P_t(\mathbf{T}^{-1}\mathbf{r}) = P_t(\mathbf{r})$ [and similarly

for the search function, $P_s(\mathbf{S}^{-1}\mathbf{r}) = P_s(\mathbf{r})$], then

$$\begin{aligned} \mathcal{R}(\mathbf{R}) &= (1/v) \int_{\Omega} P_t(\mathbf{r}) P_s(\mathbf{R}^{-1}\mathbf{r}) d^3\mathbf{r} \\ &= (1/v) \int_{\Omega} P_t(\mathbf{T}^{-1}\mathbf{r}) P_s(\mathbf{S}^{-1}\mathbf{R}^{-1}\mathbf{r}) d^3\mathbf{r} \\ &= (1/v) \int_{\Omega} P_t(\mathbf{r}) P_s(\mathbf{S}^{-1}\mathbf{R}^{-1}\mathbf{T}\mathbf{r}) d^3\mathbf{r} = \mathcal{R}(\mathbf{T}^{-1}\mathbf{RS}). \end{aligned} \quad (13.2.3.19)$$

However, the actual symmetry displayed by \mathcal{R} depends on the parameterization of the rotations and on the orientation of the orthonormal axes with respect to the crystal ones. Within the Euler parameterization, if the target function has an n -fold rotation axis parallel to the orthonormal z axis, then, according to equation (13.2.2.3), \mathcal{R} will have a periodicity of $360/n$ along α . Similarly, a rotation axis of order n along z of P_s gives rise to a periodicity of $360/n$ along γ . Therefore, the amount of calculation is reduced by choosing z along the Patterson functions' highest rotational symmetry axes [see equation (13.2.3.11)].

13.2.4. The locked rotation function

The rotational NCS, determined with the help of the self-rotation function, may be used to enhance the signal-to-noise ratio of cross-rotation functions (Rossmann *et al.*, 1972; Tong & Rossmann, 1990). If $\{\mathbf{S}_n, n = 1, \dots, N\}$ denotes the set of NCS rotations, including the identity, and \mathbf{R} is a correct orientation of the cross rotation, then $\mathbf{S}_n\mathbf{R}$ must also correspond to a correct orientation. Here we are assuming that the rotational NCS forms a group. Otherwise, either $\mathbf{S}_n\mathbf{R}$ or $\mathbf{S}_n^{-1}\mathbf{R}$, but not both, corresponds to another correct orientation. Therefore, a function may be defined, the locked cross rotation, whose values are the average of the cross-rotation values at orientations related by the NCS:

$$\mathcal{R}_{LC}(\mathbf{R}) = \sum_{n=1}^N \mathcal{R}(\mathbf{S}_n\mathbf{R}) / N. \quad (13.2.4.1)$$

By redefining the target function, it can be computed as an ordinary cross rotation. Indeed, \mathcal{R}_{LC} may be written in a form similar to equation (13.2.3.3),

$$\begin{aligned} \mathcal{R}_{LC}(\mathbf{R}) &= \sum_{n=1}^N (1/v) \int_{\Omega} P_t(\mathbf{r}) P_s(\mathbf{R}^{-1}\mathbf{S}_n^{-1}\mathbf{r}) d^3\mathbf{r} / N \\ &= (1/v) \int_{\Omega} \left[\sum_{n=1}^N P_t(\mathbf{S}_n\mathbf{r}) / N \right] P_s(\mathbf{R}^{-1}\mathbf{r}) d^3\mathbf{r}, \end{aligned} \quad (13.2.4.2)$$

with the target Patterson function substituted by the average over the NCS of the rotated target functions. The computation of equation (13.2.4.2) is particularly simple in the case of the fast rotation function. The substitution

$$e_{\ell, m, n}^{(t)} \rightarrow \sum_{m'=-\ell}^{\ell} \left[\sum_{n=1}^N \mathcal{D}_{m, m'}^{\ell}(\mathbf{S}_n) / N \right] e_{\ell, m', n}^{(t)}, \quad (13.2.4.3)$$

where we replaced the sum over \mathbf{S}_n^{-1} by a sum over \mathbf{S}_n , because of the rearrangement theorem of group theory, gives the required target coefficients.

The same ideas may be applied to the self-rotation function. Here the NCS is assumed beforehand, with elements $\{\mathbf{I}_n, n = 1, \dots, N\}$ in a given reference orientation. They are related to the actual NCS elements by

$$\mathbf{S}_n = \mathbf{R}_n \mathbf{I}_n \mathbf{R}_n^{-1} \quad (13.2.4.4)$$

[see equation (13.2.2.2)]. Since each \mathbf{S}_n should correspond to a local