6 The Self–Rotation Function

Multi–subunit proteins often exhibit local or non–crystallographic symmetry (NCS) relating the subunits. The self–rotation function can be used to determine the orientation and order of local symmetry. The Patterson function contains a set of peaks representing intramolecular vectors (self–vectors) for each molecular orientation found in the crystallographic cell. These peaks are distributed around the Patterson origin within a volume of double the molecular diameter, differentiating them from the intermolecular (cross–vectors) which, depending on the molecular packing, can occur anywhere in the Patterson function. The self–rotation function $R(C)$ (Rossmann & Blow, 1962) is the auto–correlation function of the native Patterson, $P(x)$, with a rotated version of itself:

$$R(C) = \int P(x) \cdot P(Cx) dx \tag{4}$$

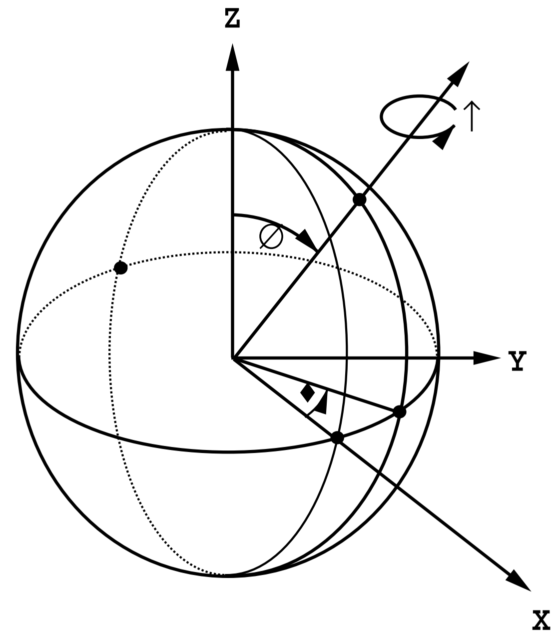
where C is the three dimensional rotation matrix that relates the rotated coordinate system to the stationary one and

$$P(x, y, z) = \frac{1}{V} \sum_h \sum_k \sum_l |F|^2 \cos 2\pi(hx + ky + lz). \tag{5}$$

This function $[R(C)]$ will have a maximum at orientations which superimposes two molecules in the unit cell. The self–rotation function is evaluated on an angular grid. In the Fourier transform used to evaluate the Patterson, the sampling needs to be much finer than half the resolution limit so that the linear interpolation between grid points is possible (Shannon, 1949). The rotation function can also be calculated by expanding the Patterson in spherical harmonics and the associated Legendre polynomials (Crowther, 1972). In the rotation function computation, the crystallographic system is transformed into a Cartesian coordinate system. The orientation space is scanned in a system of Eulerian angles (θ_1, θ_2 and θ_3), where θ_1 is the counterclockwise rotation about the z–axis looking down the positive z–axis toward the origin. θ_2 and θ_3 are the rotations about the new x– and z–axes respectively. The spherical polar angle convention is easier to visualise and accomplishes any rotation by an appropriate spin κ about a properly chosen axis, specified by two angles ϕ and ω. A molecular n–fold rotation will be found in this system in a $\kappa = \frac{360^o}{n}$ section. Thus, self–rotation function peaks corresponding to a two–fold axis occur in the $\kappa = 180^o$, a three–fold in the $\kappa = 120^o$ section, etc. The cross–rotation function involves a rotational search for agreement between the Patterson functions of the search and target structures as a function of their relative orientation. The Eulerian and spherical polar angle conventions are illustrated in Figure 11.

The rotational search was carried out using the `CCP4` (1994) program `POLARRFN` (written by Wolfgang Kabsch) which is a fast rotation function employing a polar angle convention. It produces rotation function map sections of constant rotation angle κ; each section containing all values of ω and ϕ which are the angles from the pole and about the equator, respectively. The highest peak in the relevant κ section indicates the ω and ϕ angles for the orientation of the

(a)



(b)

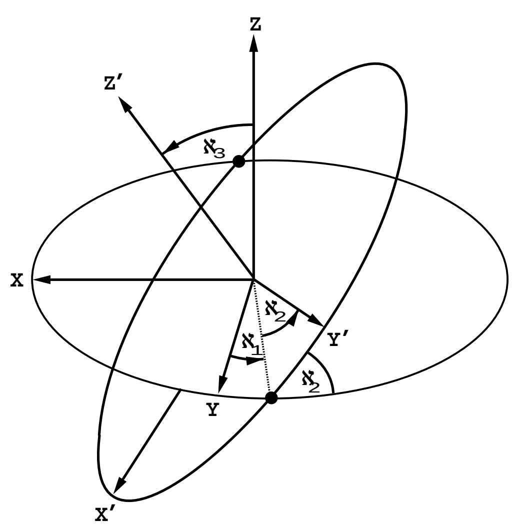
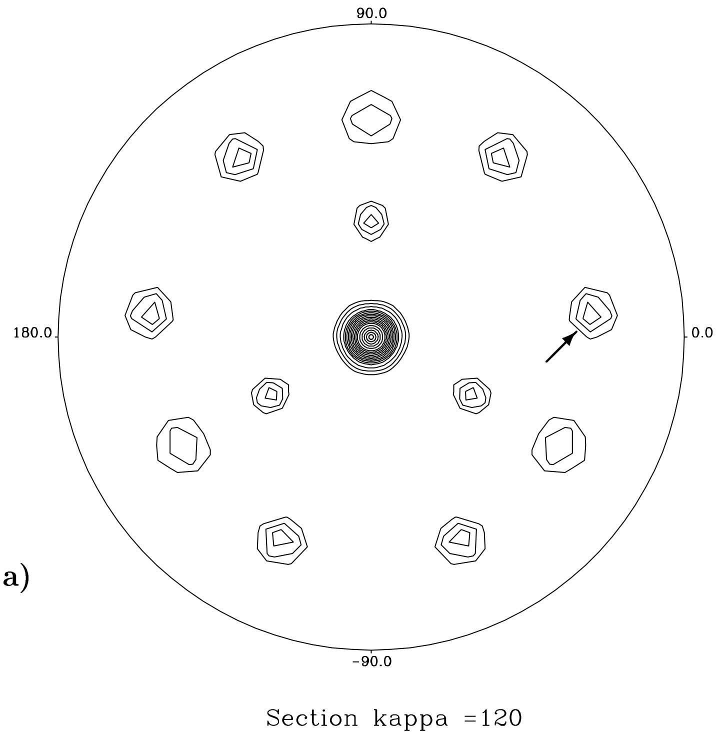


Figure 11: Definition of (a) the spherical polar angles and (b) the Eulerian conventions adopted from Rossmann (1962). In the Eulerian convention, the unprimed system represents the fixed coordinate system.

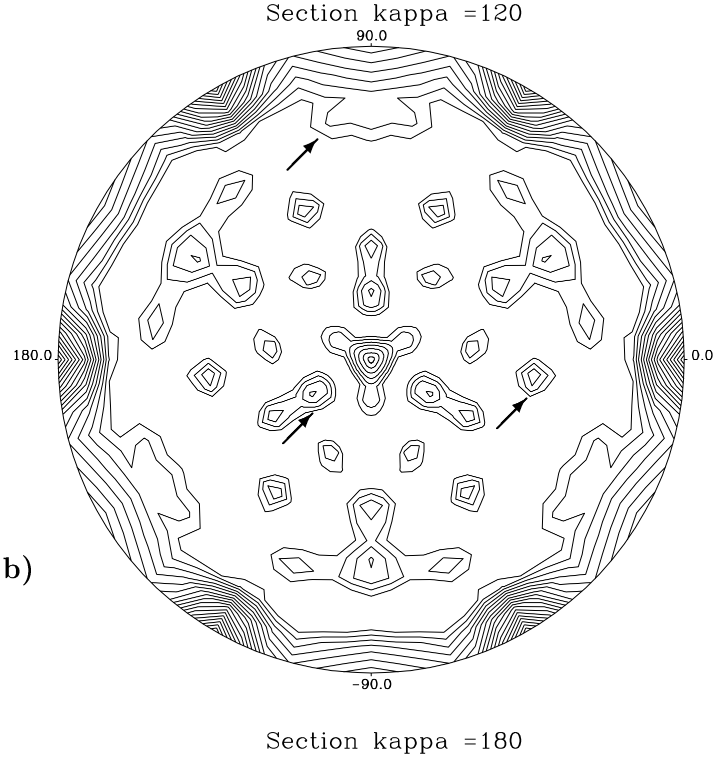
non–crystallographic axis. The direction cosines of the axis are defined as

$$\begin{pmatrix} \sin(\omega)\cos(\phi) \\ \sin(\omega)\sin(\phi) \\ \cos(\omega) \end{pmatrix}. \tag{6}$$

The program can calculate the rotation function on a grid as small as 1^o in ω and ϕ. Typically, a coarse rotation search (grid of 5^o) is followed by a fine search (1^o) in the vicinity of the solution. Due to the limit of 30 Bessel functions in the program, the integration radius cannot be greater than 5.83 times the resolution limit. Calculations were performed using diffraction data from several different resolution ranges with varying Patterson search radii and yielded consistent results. Best results were obtained using a Patterson integration radius of 18 Å and diffraction data between 10 and 3 Å resolution. The rotation functions calculated at different resolution ranges and/or different maximal vector lengths show peaks of slightly varying height located at the same positions. Significant peaks not lying on special positions (which are found in the trigonal Patterson symmetry on the mirror planes at $\omega = n \cdot 30^o$ and two–folds at $\omega = n \cdot 30^o + 15^o$ for all values of ϕ and $n = 0, 1, 2, ...$) were found on the $\kappa = 120^o$ and $\kappa = 180^o$ sections, relating molecules within the oligomer by a three– and a two–fold local rotation axis respectively. Figure 12 shows the contoured stereographic projection of these sections. The $\kappa = 180^o$ section shows three orthogonal two–fold axes with (ω, ϕ) values of $(126^o, 174^o)$; $(42^o, 210^o)$ and $(107^o, 280^o)$ and heights of 0.16, 0.21 and 0.14, respectively, relative to the origin peak height and with interdyad angles of 89.9^o, 89.9^o and 92.3^o. The results from the $\kappa = 180^o$ section therefore suggest that the



(a)



(b)

Figure 12: The (a) $\kappa = 120^o$ and (b) $\kappa = 180^o$ sections of the self–rotation function contoured at 10% of the origin peak with increments of 5%. The local non–special peak in the $\kappa = 120^o$ section and three peaks corresponding to the 222 set in the $\kappa = 180^o$ section are marked with arrows. The peak with (ω, ϕ) values of (40^o, 210^o) lies on a mirror plane and has a peak height significantly greater (0.21) than the other two peaks forming the 222 set (0.14 and 0.16).

enzyme may be a tetramer with 222 point group symmetry. Other studies (Uchida *et al.*, 1984; Ohta *et al.*, 1986; Aisaka *et al.*, 1991), which employed gel filtration chromatography in aqueous buffer, indicated Neu5Ac lyase could be a trimer (M_r 90,000) composed of 32 kDa subunits. The self–rotation function peak found on the $\kappa = 120^o$ section with a height of 0.17 times the origin peak height initially misleadingly suggested confirmation of these results. As became evident later, this peak results from a crystal packing operation relating a pair of tetramers only by a 120^o rotation. The correct identification of the oligomeric state as tetrameric with point group 222 explains all the significant peaks found by the self–rotation function in all κ (*i.e.* 0^o to 180^o) sections, whereas a trimeric model is only consistent with the peaks found on the $\kappa = 120^o$ section.