crystal form, which in turn provided phases of sufficient quality to determine heavy-atom sites in derivatives of the second form. Phase-combination and density-modification techniques in the two crystal forms allowed the solution of the structure.

12.2.5.2. *Space-group problems*

Although the macromolecular crystallographer is rarely confronted with the problems facing their small-molecule colleagues with regard to determining the correct space group, the simplified heavy-atom structure may often throw some surprises. Certain pseudosymmetries may become 'exact' for the heavy-atom difference Patterson map. Thus, cross peaks between different heavy atoms may occur on a Harker section (or 'pseudo-Harker section'), complicating interpretation of the Patterson map. Such was the case with azurin (Adman *et al.*, 1978; Nar *et al.*, 1991), where the heavy-atom structure gave rise to a pseudo-homometric Patterson function, *i.e.* one in which two possible (nonequivalent) choices were available for the heavy-atom structure, only one of which was correct. This arose from a pseudo-centring of the lattice that became almost exact for the heavy-atom structure.

In the case of human NC1 (Stubbs *et al.*, 1990), all heavy-atom derivatives appeared to lie on or near the crystallographic twofold axis. This resulted in a partially centrosymmetric heavy-atom structure that failed to deliver sufficient phase information for noncentrosymmetric reflections. To check for problems with the native data set, anomalous difference Patterson maps $\{\text{coefficients } [F_{PH}(\mathbf{S}) - F_{PH}(-\mathbf{S})]^2\}$ were calculated. Coincidence of the peaks obtained from conventional and anomalous Patterson syntheses showed that the heavy-atom positions were correct, but unfortunately did not lead to a structure solution.

12.2.5.3. *High levels of substitution; noncrystallographic symmetry*

Most problematic are the cases where many heavy atoms have become incorporated in the asymmetric unit. Not only does this cause difficulties in the scaling of derivative to native data, but also the large number of peaks results in ambiguities in the solution of the Patterson function. In such cases, it may be necessary to obtain primary phase information from a different source (such as, for example, another low-substitution-site derivative). One important subclass of high-level substitution is when the native asymmetric unit contains several copies of a single molecule (noncrystallographic symmetry or NCS).

A major problem in locating complex noncrystallographic axes is that the geometrical relationship between NCS peaks in the Patterson map is nontrivial. Under certain conditions, NCS results in a recognizable local symmetry within the Patterson map (Stubbs *et al.*, 1996). In many cases, however, these conditions (that the NCS axes of crystallographic symmetry-related molecules are parallel) are not fulfilled. Under such circumstances, all heavy-atom sites (including all crystallographic symmetry-related positions) must be checked carefully with the rotation function in order to pinpoint the NCS axis. This is relatively trivial for low-order NCS (twofold, threefold), but becomes increasingly complicated for higher orders. It should also always be borne in mind that the heavy-atom positions might not necessarily follow the NCS constraints due to crystal packing. If there is reason to suspect that sites are related by local symmetry, then the orientation of this axis can be used in the initial Harker searches; in practice, however, such searches are extremely sensitive to the correct orientation of the axis.

In the case of high-order NCS (such as, *e.g.*, with icosahedral virus structures or symmetric macromolecular complexes), an alternative approach to the usual initial Harker-vector search can be provided by the self-rotation function. Knowledge of the orientation of the NCS axis (from the rotation function) can be used to determine the relative positions of heavy atoms to the NCS axis (Argos & Rossmann, 1976; Arnold *et al.*, 1987; Tong & Rossmann, 1993). The orientation can be refined and the resulting peaks can be used as input in a subsequent translation search of the Harker sections.

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