25.2.1.10.2.1. *Simple rotational symmetry*

For 'proper' NC symmetry, only pure *n*-fold rotations are involved with *n* a small integer, *i.e.* twofold, threefold *etc.* In this case, only a single envelope mask encompassing all of the molecules to be averaged is needed for averaging and operator refinement, since one does not have to differentiate between molecules within the aggregate. Initial operator refinement can use a simple spherical mask of appropriate radius, with the sphere centred near the aggregate centre of mass and on the rotation axis. One can also use a mask created either by hand (described below) or created from atomic coordinates, as described earlier. For averaging purposes, however, a mask created by hand is usually desired. The NC symmetry operator refinement is carried out within the program *LSQROT* (batch).

25.2.1.10.2.2. *Complex rotational and/or translational symmetry*

For 'improper' NC symmetry, where there are translational components and/or arbitrary rotation angles involved, separate envelope masks must be assigned to each molecule in the aggregate for both NC symmetry operator refinement and averaging. Initial operator refinement can proceed with spherical masks of an appropriate radius centred on the centre of mass of each molecule in the aggregate. As in the 'proper' NC symmetry case, one can also use masks created by hand or generated from atomic coordinates for operator refinement, but hand-traced masks will be desired for the actual averaging. The NC symmetry operator refinement is carried out within the program *LSQROTGEN* (batch).

25.2.1.10.3. *Averaging mask construction*

Masks encompassing the region(s) to be averaged are usually created by hand in the interactive program *MAPVIEW*. Here, one reads in a submap comprising the desired region of whatever type of map is available, usually an MIR map. An appropriate contour level and initial section are selected and the contoured electron density for that section appears on the screen. One then selects the 'add next section' menu item two or three times to create a projection over several sections of the map, since in the projection the molecular boundary is usually more obvious. Selecting the 'trace mask' menu item then allows the user to hand-contour the molecular envelope by directing the cursor tied to a mouse or other pointing device. One then moves to an adjacent section and repeats the process until the complete 3D mask is obtained. To simplify matters and speed up the process, there are 'copy next mask' and 'copy previous mask' menu items allowing one to take advantage of the fact that the mask is a slowly changing function, particularly when near the centre of the molecule. One can use this feature to copy a mask from the previous or following section and apply it to the current section. Up to twelve distinct 3D masks can be selected. Each mask is colour-coded and can be simultaneously displayed superimposed on the contoured electron-density section. Once the mask is completed, the 'make asu' menu item is selected to apply crystallographic symmetry operations to all points within the generated envelope masks. If these operations generate a point also within the envelope masks, the point is flagged in red to indicate that it is redundant, indicating that when tracing the mask, one inadvertently strayed into a symmetry-related molecule. After this check for redundancy, all points within the submap distinct from but related to points within the molecular envelopes by crystal symmetry are flagged in green. This enables one to detect packing contacts and also to ensure that all significant electron density has been assigned to some envelope. Upon completion, the mask is written to a file suitable for use either in averaging, solvent flattening (after expansion by *BLDCEL*), or operator refinement. In cases of 'proper' NC symmetry, it is often desirable to trace the averaging envelope mask in a 'skewed' map, such that one is looking directly down the NC rotation axis. In this case, it is usually very obvious where the NC symmetry breaks down, simplifying identification of the averaging envelope. If the averaging mask is created in a skewed submap, then the batch program *TRNMSK* can be used to transform it so as to correspond to the original, unskewed submap for use in averaging calculations (which do not require skewing).

25.2.1.10.4. *Map averaging*

All averaging calculations are carried out by the program *MAPAVG* (batch), which requires the submap to be averaged along with the envelope masks and NC symmetry operators. A copy of the input submap is made and each grid point in the mask is examined in turn. If the grid point lies within any averaging envelope, then all points related to it by NC symmetry are generated from the operators and examined. If the generated points also lie within the appropriate envelope mask, the electron density there is interpolated, as described earlier, and the density values for all related points are summed. The average value of the electron density is then inserted at the original point in the submap copy. Upon completion, the averaged version of the submap is written to a file and correlation coefficients for regions related by the various NC symmetry operations are output. The averaged submap is then passed to the program *BLDCEL* along with the averaging mask and the original unaveraged *FSFOUR* map from which the submap was created. For all points within the averaging envelope(s), their electron-density values and those at points related by crystallographic symmetry are inserted into the full-cell map, and it is written to a file. This file then contains the NC symmetry averaged electron density expanded to a full-cell map that obeys space-group symmetry. As an option, the averaging mask can also be expanded in *BLDCEL* to a full-cell mask, which could then be used for solvent flattening.

25.2.1.10.4.1. *Single-crystal averaging*

For NC symmetry averaging within a single crystal, the calculations are exactly as described above. One refines the NC symmetry operators with *LSQROT* or *LSQROTGEN*, creates the appropriate envelope mask(s) with *MAPVIEW*, averages with *MAPAVG* and expands the averaged submap to a full cell with *BLDCEL*.

25.2.1.10.4.2. *Multiple-crystal averaging*

If multiple crystal forms are available and one has a source of phase information for each crystal form, then averaging over the independent molecular copies within all crystal forms is possible. In fact, one may also have NC symmetry *within* some of the crystal forms. One can utilize all of this information during averaging by exactly the same process as previously described. For each form, the appropriate envelope mask(s) must be obtained and any internal NC symmetry operators refined, as described earlier. Then operators relating molecules from one crystal form to another must be obtained and refined. The program *LSQROTGEN* can read in multiple submaps, allowing refinement of the additional operators. The program *MAPAVG* accepts submaps from up to six different crystal forms. Averaging over all copies then proceeds exactly as described above, except that prior to averaging, density in all submaps is placed on a common scale, and upon completion averaged submap files are written for each crystal form.

25.2.1.10.5. *Phase combination and extension*

During NC symmetry averaging, phase combination and extension is carried out precisely as described during solvent flattening and negative-density truncation. The only difference is that after generation of each electron-density map, the NC

references