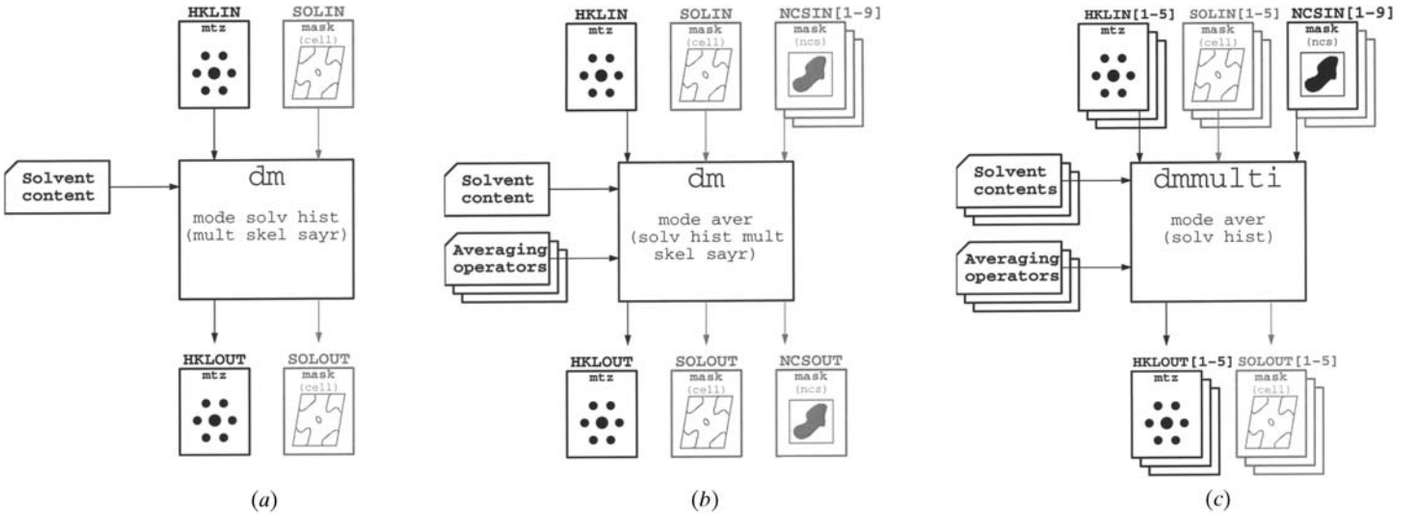

Fig. 25.2.2.1. (*a*) Input and output data for a *DM* calculation with no averaging. Light outlines indicate optional information. (*b*) Input and output data for a *DM* averaging calculation: for a single averaging domain, the averaging mask may be calculated automatically. For multi-domain averaging, all domain masks must be given. (*c*) Input and output data for *DMMULTI*. An averaging mask (or masks, for multiple domains) must be provided.

its default automatic mode for phase extension. The choices of various modes are described in the following sections.

25.2.2.4.1. *Density-modification modes*

The following density-modification modes (specified by the MODE keyword) are provided by *DM*:

(1) Solvent flattening: This is the most common density-modification technique and is powerful for improving phases at fixed resolution, but weaker at extending phases to higher resolution. Its phasing power is highly dependent on the solvent content. Solvent flattening can be applied at comparatively low resolutions, down to around 5.0 Å.

(2) Histogram matching: This method is applied only to the density in the protein region. This method is weaker than solvent flattening for improving phases, but is much more powerful at extending phases to higher resolutions. This is due to a unique feature of histogram matching which uses a resolution-dependent target for phase improvement. The phasing power of histogram matching is inversely related to the solvent content. Therefore, histogram matching plays a more important role in phase improvement when the solvent content is low. Histogram matching works to as low as 4.0 Å, but does no harm below that. Histogram matching should probably be applied as a matter of course in any case where the structure is not dominated by a large proportion of heavy-metal atoms. Even in this case, histogram matching may be applied by defining a solvent mask with solvent, protein and excluded regions.

(3) Multi-resolution modification: This method controls the level of detail in the map as a function of resolution by applying histogram matching and solvent flattening at multiple resolutions. This strengthens phase improvement at fixed resolution, although it generally improves phase-extension calculations too.

(4) Noncrystallographic symmetry averaging: Averaging is one of the most powerful techniques available for improving phases and is applicable even at very low resolutions. In extreme cases, averaging may be used to achieve an *ab initio* structure solution (Chapman *et al.*, 1992; Tsao *et al.*, 1992). It should therefore be applied whenever it is present and the operators can be determined.

(5) Skeletonization: Iterative skeletonization is the process of tracing a 'skeleton' of connected densities through the map and then building a new map by filling density around this skeleton. The implementation in *DM* is adapted for use on poor maps, where it is sometimes but not always of use. To bring out side chains and missing loops, the *ARP* program (Lamzin & Wilson, 1997) is more suitable.

(6) Sayre's equation: This method is more widely used in small-molecule calculations, and is very powerful at better than 2.0 Å resolution and when there are no heavy atoms in the structure. However, its phasing power is lost quickly as resolution decreases beyond 2.0 Å. The calculation takes significantly longer than other density-modification modes.

The most commonly used modes are solvent flattening and histogram matching – these give a good first map in most cases. Recently, multi-resolution modification has been added to this list. Averaging is applied whenever possible. Skeletonization and Sayre's equation are generally only applied in special situations.

25.2.2.4.2. *Phase-combination modes*

Density-modification calculations are somewhat prone to producing grossly overestimated figures of merit (Cowtan & Main, 1996). Users should be aware of this. In general the phases and figures of merit produced by density-modification calculations should only be used for the calculation of weighted F_o maps. They should not be used for the calculation of difference maps or used in refinement or other calculations (the *REFMAC* program is an exception, containing a mechanism to deal with this form of bias). The use of $2F_o - F_c$-type maps should be avoided when the calculated phases are from density modification, since they are dependent on two assumptions, neither of which hold for density modification: that the current phases are very close to being correct and that the calculated amplitudes may only approach the observed values as the phase error approaches zero.

To limit the problems of overestimation, three phase-combination modes are provided (controlled by the COMBINE keyword):

(1) Free-Sim weighting: This is the simplest mode to use. Although convergence is weaker than the reflection-omit mode, the calculation never overshoots the best map. If there is averaging information, then convergence is much stronger and the phase-combination scheme is much less important. In addition, phase relationships in reciprocal space limit the effectiveness of the reflection-omit scheme. Therefore, the free-Sim weighting scheme should usually be used when there is averaging.

references