

15. DENSITY MODIFICATION AND PHASE COMBINATION

Table 15.1.2.1. Constraints used in density modification

Constraints	Use	Effectiveness and limitation
(1) Solvent flatness	Solvent flattening	Works best at medium resolution. Relatively resolution insensitive. Good for phase refinement. Weak on phase extension.
(2) Ideal electron-density distribution	Histogram matching	Works at a wide range of resolutions. More effective at higher resolution. Very effective for phase extension.
(3) Equal molecules	Molecular averaging	Works better at low to medium resolution. Its phasing power increases with the number of molecules in the asymmetric unit.
(4) Protein backbone connectivity	Skeletonization	Requires near atomic resolution to work.
(5) Local shape of electron density	Sayre's equation	The equation is exact at atomic resolution. It can be used at non-atomic resolution by choosing an appropriate shape function. Its phasing power increases quickly with resolution. Very powerful for phase extension.
(6) Atomicity	Atomization	If the initial map is good enough, iteration could lead to a final model.
(7) Structure-factor amplitudes	Sim weighting	Can be used to estimate the reliability of the calculated phases after density modification. It assumes the random distribution of errors that caused the discrepancy between the calculated and observed structure-factor amplitudes.
(8) Experimental phases	Phase combination	This can be used to filter out the incorrect component of the estimated phases. Most phase-combination procedures assume independence between the calculated and observed phases.

spaces are filled with the solvent in which the crystallization was performed. This solvent is a disordered liquid, and thus the arrangement of atoms in the solvent regions varies between unit cells, except in those small regions near the surface of the protein. The X-ray image forms an average of electron density over many cells, so the electron density over much of the solvent region appears to be constant to a good approximation.

The existence of a flat solvent region in a crystal places strong constraints on the structure-factor phases. The constraint of solvent flatness is implemented by identifying the molecular boundaries and replacing the densities in the solvent region by their mean density value.

When solving a structure, the contents of the unit cell are usually known, and so an estimate can be formed of how much of the cell volume is taken up by solvent (Matthews, 1968). If the solvent region can be located in the cell, then we can improve an electron-density map by setting the electron density in this region to the expected constant solvent density. Once the resulting modified phases are combined with the experimental data, an improvement can often be seen in the protein regions of the map (Bricogne, 1974).

The solvent region of a unit cell may usually be determined even from a poor MIR map using the following features:

(1) The mean electron density in the solvent region should be lower than that in the protein region. Note that this information will come from the low-resolution data, which dictate long-range density variations over the unit cell.

(2) The variation in density in the flat solvent region should be much smaller than that in the ordered protein region containing isolated clumps of density. The 'peakiness' of the protein region comes from the high-resolution data.

A good method for locating the solvent region therefore takes into account information from both low- and high-resolution structure factors. Many methods have been proposed to locate the protein-solvent boundary. The first of these were the visual identification methods. The boundary was identified by digitizing a mini-map with the aid of a graphic tablet (Hendrickson *et al.*, 1975; Schevitz *et al.*, 1981). The hand-digitizing procedure was very time-consuming and prone to subjective judgmental errors. Nevertheless, these methods demonstrated the potential of solvent flattening and stimulated further improvement on boundary-identification methods. An automated method using a linked, high-density approach was first proposed by Bhat & Blow (1982). Based on the fact that the densities are generally higher in the protein region than in the solvent region, they defined the molecular boundary by locating the protein as a region of linked, high-density points.

Convolution techniques were subsequently adopted as an efficient method of molecular-boundary identification. Reynolds *et al.* (1985) proposed a high mean absolute density value approach. The electron density within the protein region was expected to have greater excursions from the mean density value than the solvent region, which is relatively featureless. The molecular boundary was located based on the value of a smoothed 'modulus' electron