# 15. DENSITY MODIFICATION AND PHASE COMBINATION

## 15.1. Phase improvement by iterative density modification

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### 15.1.1. Introduction

Density modification is a technique for improving the quality of an approximate electron-density map based on some conserved features of the correct electron-density map. These conserved features are independent of the unknown fine detail of the structural conformation. They are often expressed as constraints on the electron density in various forms, either in real or reciprocal space. Since the structure-factor amplitudes are known, these constraints restrict the values of phases and can therefore be used for phase improvement.

The structure-factor amplitudes and phases are independent of each other if we know nothing about the electron density. Therefore, the phases are indeterminable given only the amplitudes (Baker, Krukowski & Agard, 1993). The information about the electron density provides the missing link between structure-factor amplitudes and phases. It is only through the knowledge of the chemical or physical properties of the electron density that the phases can be retrieved. Density modification is usually the most straightforward application of the constraints on electron density. However, this is only a matter of convenience in implementation. Sometimes the constraints can be more readily implemented in reciprocal space on structure factors.

Density-modification methods are usually implemented as an iterative procedure that alternates between density modification in real space and phase combination in reciprocal space. This paradigm was first proposed by Hoppe & Gassmann (1968) in their 'phase correction' method. This approach takes advantage of the particular properties of the constraints and uses them in a way that is most convenient to implement.

Density-modification methods usually require an initial map with substantial phase information. In most cases, these phases are obtained from multiple isomorphous replacement (MIR) or multi-wavelength anomalous dispersion (MAD), but it is also possible to improve maps from other sources, such as molecular replacement. The amount of information in the initial map is dependent on phase accuracy, data resolution and completeness. As more powerful constraints are incorporated, the density modification can be initiated from lower-resolution maps with less accurate phases. *Ab initio* phasing would be achieved if a density-modification method could start from a map generated from random phases. Therefore, density modification can potentially lead to *ab initio* phasing methods, although it does not seek direct solution to the phase problem as its immediate goal.

There are two major components in a density-modification procedure. One is the type of electron-density constraints. The other is the way the constraints are exploited. These two components combined determine the phasing power of the procedure. In this chapter, we will review various electron-density constraints and the way they are exploited for phase improvement.

### 15.1.2. Density-modification methods

The aim of density-modification calculations is to obtain new or improved phase estimates for observed structure-factor amplitudes. Often, this includes calculation of phases for previously unphased reflections, for example, in the case of phase extension. The calculation of weights, which indicate the degree of confidence in the new phase estimates, is also an important part of the calculation. Improved phase estimates are obtained by bringing the initial phase estimates into consistency with additional sources of structural information.

One difficulty in combining information from various sources is that the amplitudes and phases are represented in reciprocal space and include good estimates of error, whereas the other constraints are in real space and in general, represent expectations about the structure which may be hard to quantify. As a result, the method that has been adopted is iterative and divided into real- and reciprocal-space steps. A weighted map is calculated and used as a basis for applying all the real-space modifications. The modified map is then back-transformed to produce a set of amplitudes and phases. The agreement between the observed amplitudes and the amplitudes calculated from the modified map is then used to estimate weights for the modified phases, which are used to combine the modified phases with experimental phases to produce new phases. This process is shown diagrammatically in Fig. 15.1.2.1.

A broad range of techniques have been applied to electron-density maps to impose chemical or physical information. Some sources of information used in density modification are summarized in Table 15.1.2.1. The list included here is not exhaustive, but covers the most widely used methods. Here, we describe some of the constraints and the techniques through which these constraints are implemented for phase improvement.

#### 15.1.2.1. *Solvent flattening*

Solvent flattening exploits the fact that the electron density in the solvent region is flat at medium resolution, owing to the high thermal motion and disorder of solvent molecules. The flattening of the solvent region suppresses noise in the map and therefore improves phases.

##### 15.1.2.1.1. *Introduction*

Biological molecules are typically irregular in shape, often taking roughly globular forms. When they are packed regularly to form a crystal lattice, there are gaps left between them, and these

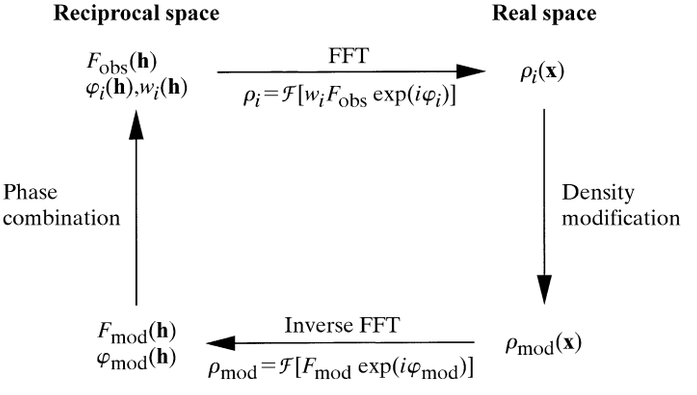


Fig. 15.1.2.1. Density-modification calculation showing iterative application of real-space and reciprocal-space constraints.





references