Abstract: Electron density modification methods are an indispensable part of any de novo macromolecular crystal structure determination and can be of crucial importance for determining structures solved by the molecular replacement method. In MAIN, a number of density modification procedures have been implemented. They encompass tools like solvent flattening and electron density averaging. In addition, maps can be generated using maximum likelihood weighting as well as by the “kick map” approach. Kick maps have been shown to be a good alternative to maximum-likelihood maps, when model bias has to be revealed. These approaches are integrated with the MAIN model building and refinement tools, which also allow multicrystal averaging and refinement with non-crystallographic constraints across a variety of crystal forms.

Keywords: density modification; density averaging; map calculation; kick maps.

1. Introduction

Density modification is understood as a procedure in which density of solvent as well as molecular regions are (iteratively) modified. Historically, electron density averaging routines were developed immediately after the model building tools and solvent picking procedures in the early 1990s as a part of my Ph.D. thesis [1]. Cathepsin B was the first structure where averaging with MAIN has been applied [2]. The other features such as map calculation, solvent flattening and refinement, model validation, and autobuilding tools have been gradually appended later.

The phase problem must be solved before MAIN can become useful. This implies that the initial density map should be either constructed from contributions of heavy atoms or calculated from an appropriately positioned molecular model. In the cases of large structures containing a variety of identical subunits with known position, internal symmetry, and approximate size, phases can be derived by density averaging within MAIN too.
Input to MAIN are molecular models, space group and unit cell data, structure factors, and density maps. From these data density maps can be calculated and manipulated. Molecular models, structure factors, and density maps are interoperable forms of data within the algebra of the MAIN program.

From the perspective of a user density modification procedures are triggered within MAIN by clicking the four menu items (RMS_FIT, MAK_MASK, DM_PREP, DM_NEXT). The macros underlying them are configured with the help of the “main_config” tools. In the user perspective, the solvent flattening procedure differs from density averaging in the “RMS_FIT” part, the use of which is confined to the non-crystallographic symmetry (NCS) tools, density averaging, and model building.

Maps are input to density modification procedures; therefore, their calculation is described first. Density averaging is considered as a special case of solvent flattening, therefore, the latter is described before the density averaging.

2. Map calculations

Fo, phase-combined and difference maps (2Fo–Fc, Fo–Fc) can be computed using least squares and maximum-likelihood weighting schemes and averaged kick map and kick omit maps schemes. The maximum likelihood functions for map calculations and refinement entered MAIN with the help of Urzhumtzev and Afonine in 2002 [3]. Reciprocal space anisotropic B-factor and bulk-solvent correction terms based on contribution of solvent derived from the molecular envelope [4] are also included.

The map calculations can be activated as a part of the MAP_CALC menu or configured by the density modification scripts in the case of density modification procedures.

Phase combination is performed using Hendrickson—Lattman coefficients and structure factors from the model. The structure factors from the model are calculated in MAIN followed by phase combination in the external “Sigma A” program [5].

The MAP_CALC menu items BULK_SOL, ANISO_B, ML_MAPS, RE_PHASE, PHAS_CMB, FOBS_MAP, 2FO_FC_M, FO_FC_MA cover map calculations apart from kick maps. The BULK_SOL, ANISO_B, ML_MAPS are flags, which turn on and off the corresponding options.

Among these, only kick maps and kick omit maps are unique to MAIN, therefore they are briefly described below.

2.1. KICK MAPS

The procedure that randomly displaces atoms from their original positions is called “kicking”. The concept of kick maps was introduced in MAIN 97