Three-Dimensional Reconstruction of Nonperiodic Macromolecular Assemblies from Electron Micrographs

J. Frank and M. Radermacher

1 Introduction

1.1 General

Through the pioneering work of a few laboratories, the electron microscope has been transformed into a tool of quantitative structure research. Its role in the three-dimensional analysis of biomolecular assemblies having high complexity has been firmly established, and was recently recognized in the award of the Nobel prize in Chemistry to Aaron Klug. Molecular electron microscopy and electron crystallography have helped to unravel, to name but a few examples, the calcium-dependent structure of the gap junction (Unwin and Zampighi 1980; Unwin and Ennis 1984), the organization of DNA into nucleosomes (Klug et al. 1980; Klug 1983), and the architecture of numerous viruses (see literature survey by Baker 1981 and reviews by Vainshtein 1978 and Mellema 1980).

The methods used in accomplishing these results are based to a large extent on methods of X-ray crystallography. Indeed, all examples mentioned above have in common that they relate to specimens possessing crystalline order or high symmetry. Both properties make multiple measurements of the molecule projection available, which can be used to obtain highly accurate averaged projections within defined error limits. These, in turn, form the basis for the reconstruction of the object in three dimensions (De Rosier and Klug 1968; Henderson and Unwin 1975).

The topic of this article is the three-dimensional analysis, by electron microscopy and image processing, of macromolecules and biomolecular assemblies that exist as single unordered particles or fibers. At the outset, we must make a distinction between structures that allow structural information to be collected from different particles and those that, due to their flexibility or morphological heterogeneity, must be separately reconstructed. The first case leads, quite naturally, to an averaging approach akin to that used in elec-
tron crystallography, and lets us expect results with high statistical significance. The other case may be compared to the medical tomography (HERMAN 1980; EDHOLM 1960) of the body of an individual patient, who is different in detail from other patients, even though the building principle of the human body and the kinds of organ and tissue are the same.

Essentially, our distinction divides specimens according to their level of ultrastructure:

1. Macromolecular assemblies for which function is related to a defined 3-D configuration, allowing no variations among individual, functionally equivalent particles, may be modeled as solid bodies. When prepared in the same way and viewed in the same direction, their projections are identical apart from superimposed components. This property allows coordinate transformations corresponding to 2-D and 3-D rigid body movements to be used when combining data from different particles, and thereby facilitates noise reduction through averaging.

2. In contrast, on the level of cellular ultrastructure, the biological function is often related to the architecture "in the short range" only, allowing a certain amount of variation in shape (example: chromatin fiber studied by SUBIRANA et al. 1983). In these applications, the combination of different particles or fiber segments would only be achieved by using general curvilinear transformations. However, the prohibitive computational expense of procedures for combining data from different, arbitrarily bent, and distorted structures means that, in practice, the powerful averaging methods cannot be applied, and that the result of three-dimensional reconstruction is limited in resolution due to the limited statistical significance of the individual unaveraged projections.

The field reviewed is clearly just being demarcated; as yet, few groups have the necessary computer hardware, software flexibility, and experience. The first macromolecular structure reconstructed without use of symmetries was the fatty acid synthetase (HOPPE et al. 1974), although some exploratory work was done earlier (HART 1968; BENDER et al. 1970). The more recent development is marked by experimental studies of DOVER et al. (1981), SUBIRANA et al. (1983, 1984), OLINS et al. (1983, 1984), KNAUER et al. (1983) and VERSCHOOR et al. (1984). (A more exhaustive survey of work in this area is contained in Sect. 4.4.) Some of these results will be briefly discussed in this chapter, which is primarily focused on methods of reconstruction and data collection.

1.2 The Three-Dimensional Structure as an Average

An important consideration in the reconstruction of noncrystalline objects is the way in which the projection data are combined (Fig. 1). For crys-