Introduction

After sequencing the human genome and the genome of many other organisms, the age of “structural genomics” has started. It is now possible to take the genetic sequence and infer the amino acid sequence of all proteins from it. Unfortunately this does not necessarily tell us something about the function of these proteins. Even for the organism that is genetically best understood, *Escherischia coli*, the function of ca. 40% of its protein is not known. Under the structural genomics initiative, many groups set out to determine as many protein folds as possible. A protein fold is a stretch of amino acids that fold into a defined secondary structure motif. These structural prototypes adopt similar structures, but do not necessarily have detectable sequence homology. At the moment, about 1,000 different folds are known and it is estimated that a few thousand different folds exist. Often X-ray crystallography of 3D protein crystals and nuclear magnetic resonance (NMR) studies of concentrated protein solutions are the only source of structural information at atomic level, and most of the work in structural genomics is limited to water-soluble proteins. Membrane proteins and larger protein complexes are mostly excluded from these efforts because they are less amenable to this “high-throughput” approach.

Membrane proteins constitute 25% of all proteins, therefore it is crucial to improve our understandings of these proteins. The “Protein Data Base” (PDB) stores ca. 28,000 structures of water-soluble proteins (about 18,000 of these are unrelated), but only 87 structures of membrane proteins are available (of which 51 are unrelated) and only 93 structures originate from proteins with a molecular weight $>$250kDa.

The handling of membrane proteins and large protein complexes is much more demanding than for water-soluble proteins. The reasons for this are:

- Membrane proteins are usually difficult to express in large quantity for structural analysis;
- Monodispersity and stability of the purified protein or large protein complexes are often difficult to control;
- The localization of membrane proteins in the lipid bilayer requires that they exhibit hydrophilic and hydrophobic surfaces.

Therefore solubilization and purification of membrane proteins necessitates the use of detergent for masking the hydrophobic area. As a consequence of this dual property — hydrophobic and hydrophilic — the total hydrophilic surfaces available to provide a good crystal contact necessary for 3D crystallization are very limited. The hydrophobic domain, which is masked by detergent micelles, does not play a major role in the crystal contact. The growth of 3D crystal of membrane proteins is therefore a complicated task. In this context, electron microscopy of single particles (for protein with a molecular weight >250 kDa) and 2D crystals is a powerful technique for which, in some cases, no other alternative approach is possible. Thanks to advances in electron microscopy instrumentation, specimen preparation and image processing, this technology is beginning to satisfy the demand for structures and allow learning about mechanisms at atomic level. The atomic structures of light harvesting complex, bacteriorhodopsin, and tubulin have now been solved, crucial elements of secondary structure have been revealed in several membrane proteins (aquaporin, rhodopsin, gap junctions and Ca^{2+} and H^{+}-ATPase) and a novel viral fold of the hepatitis B core protein has been determined through the application of this technique.

Nevertheless the resolution obtained by this technique is often in the medium range and the ability to combine structures of macromolecular complexes derived by electron microscopy with X-ray or NMR structures of their components allows the reconstruction of molecular machines and large multi-protein complexes in considerable detail. Two-dimensional crystals have been most successfully used to obtain high and medium resolution structural information by electron microscopy.

In this book we offer an overview of the technique of crystallization of proteins on lipid monolayers. The structural information is then obtained by imaging the 2D crystals using electron microscopy and image processing. This method allows any soluble or membrane protein from very small molecular weight to large complexes such as viruses to be crystallized in two dimensions. Strategies to adsorb, concentrate, orient and organize proteins or macromolecules on supports suitable for electron microscopic observation and with fluidity properties similar to biological membranes will be presented and discussed. Biophysical techniques to monitor and improve the process of crystallization will be detailed.