
Preface

Knowledge of the three-dimensional structure of a protein is absolutely required for the complete understanding of its function. The spatial orientation of amino acids in the active site of an enzyme demonstrates how substrate specificity is defined, and assists the medicinal chemist in the design of specific, tight-binding inhibitors. The shape and contour of a protein surface hints at its interaction with other proteins and with its environment. Structural analysis of multiprotein complexes helps to define the role and interaction of each individual component, and can predict the consequences of protein mutation or conditions that promote dissociation and rearrangement of the complex.

Determining the three-dimensional structure of a protein requires milligram quantities of pure material. Such quantities are required to refine crystallization conditions for X-ray analysis, or to overcome the sensitivity limitations of NMR spectroscopy. Historically, structural determination of proteins was limited to those expressed naturally in large amounts, or derived from a tissue or cell source inexpensive enough to warrant the use of large quantities of cells. However, with the advent of the techniques of modern gene expression, many proteins that are constitutively expressed in minute amounts can become accessible to large-scale purification and structural analysis.

Membrane proteins have been resistant to structural analysis for a variety of reasons. First, the proper folding of membrane proteins, and their insertion into a membrane bilayer, has been problematic. Bacterial expression systems are not often useful, and researchers have had to express in eukaryotic systems with lower expression efficiency and added experimental difficulty. Second, even when large amounts of protein are available, the hydrophobic nature of membrane proteins has made them resistant to X-ray or NMR analysis. The proper conditions for solubilizing membrane proteins with retention of structure vary, and discovery of proper experimental conditions can be tedious and frustrating. As a result, the three-dimensional structures of only a small percentage of the population of membrane proteins have been determined at the atomic level.

The complete purpose of *Membrane Protein Protocols: Expression, Purification, and Characterization* is to provide examples of how different membrane proteins have been overexpressed in both prokaryotic and eukaryotic expression systems, how natural and overexpressed proteins have been solubilized from their host membranes, and how the solubilized proteins have been purified in active form. Through examination of each individual system, a researcher may

find some inspiration to overcome problems encountered in their laboratories. The casual reader may gain insight into the difficulties experienced in the study of membrane proteins, and might be led to novel ways to circumvent common roadblocks. As the structures of additional membrane proteins come to light, we may gain a better understanding of the complex nature of biological membranes, and the cell itself.

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