Preface

For decades it has been known that structured conformations are important for the proper functioning of most cellular proteins. However, appreciation that protein folding to the functional conformations as well as the structural maintenance of protein molecules are very complex processes has only emerged during the last ten years. The intimate interplay uncovered by this scientific development led us to realize that perturbations of the protein folding process and disturbances of conformational maintenance are major disease mechanisms. This development has given rise to the concept of conformational diseases and the broader signature of protein folding diseases, comprising diseases in which mutations or environmental stresses may result in a partial misfolding that leads then to alternative conformations capable of disturbing cellular processes. This may happen by self-association (aggregation), as in prion and Alzheimer's diseases, or by incorporation of alternatively folded subunits into structural entities, as in collagen diseases. Another possibility is that folding to the native structure is impaired or abolished, resulting in decreased steady-state levels of the correctly folded protein, as is observed in cystic fibrosis and α1-antitrypsin deficiency, as well as in many enzyme deficiencies. In addition, deficiencies of proteins that are engaged in assisting and supervising protein folding (protein quality control) may impair the folding of many other proteins, resulting in pathological phenotypes. Examples of this are the spastic paraplegia attributable to mutations in mitochondrial protease/chaperone complexes.

The association of mutation-induced disease with folding defects may be the rule rather than the exception, but the realization of this has been hampered by the still prevailing problem of predicting such an effect based on structural knowledge of the folded conformation. This is possible for active site mutations, which are readily predictable in most cases where structural information and biochemical knowledge are available. In contrast, it is much more complicated to elucidate the byways of protein folding and to reveal misfolding, mislocalization, premature degradation of intermediates, and accumulation of non-native aggregates in order to track down protein folding diseases. New ways of thinking and new methods and techniques have been and are still being devised to tackle the various questions. This, coupled with our recent progress in the understanding of pathways of protein folding in vivo, has greatly promoted research on all aspects of conformational diseases. Although uncertainties remain in our understanding of these pathways, the folding of newly synthesized proteins and the refolding of proteins unfolded

by cellular stresses, such as heat shock, are known to be assisted and supervised by molecular chaperones. If folding of a mutant protein cannot be achieved under the given conditions, or if a protein has attained a "wrong" structure, such proteolytic degradation systems as the proteasome are mobilized to degrade the polypeptide. The interplay of folding helpers and the degradative systems constitutes protein quality control. In disease states the protein quality control systems either remove conformationally aberrant mutant proteins, leading to decrease or lack of function, or fail to remove aberrantly folding proteins, leading to cellular disturbances and cell death.

The tremendous enhancement in the array of techniques available for the discovery of genetic variations and further knowledge of the human genome has led to an increasing number of studies on the effects of newly discovered gene variations. Previously, such investigations were exclusively performed in highly specialized research laboratories. Now, many more laboratories engaged in investigating diseases have begun to use these new techniques to track down mutation effects, and because of their steadily growing availability, tests for genetic variations are marching into clinical laboratories for more routine tasks as well. It is therefore appropriate to compile the core range of techniques applied in this research field in one volume.

As the techniques chapters in *Protein Misfolding and Disease: Principles and Protocols* testify, a remarkable set of analytical tools that can be used to uncover the characteristics of conformational diseases in patient material or cellular model systems is now available. The techniques have usually been developed for the analysis of a particular protein; however, the common basic molecular mechanisms in conformational diseases make it possible to adapt the methods for many other proteins. As we have experienced ourselves—and what also can be seen in the various chapters—the challenge in these types of experiments is often to find the exact conditions that may reveal or alleviate the molecular effects. Since effects often are cell-type specific, and perturbations and mutations may have several effects that contribute to the molecular pathology, it is often necessary to compare results from different systems and different conditions. The present compilation of many different techniques used in a variety of cell-types and subcellular compartments should help in this respect. Our intention has been to deliver to experienced researchers and experts in specific methodologies and techniques a book that will inspire them and generate new ideas. For students and researchers new in the field our ambition was to make *Protein Misfolding and Disease: Principles and Protocols* a broad and sound basis for their further work. Therefore, we have included two introductory chapters that provide short overviews of protein folding diseases and related quality control systems, as well as a number of

chapters on "paradigmatic" case studies in specific diseases that highlight general principles and consider the field from an historical perspective.

Protein Misfolding and Disease: Principles and Protocols attempts to bridge the gap between a large number of very complex diseases and the techniques for their study at the cellular and subcellular level. We acknowledge that a focus on protein folding and misfolding as the principal phenomena may sacrifice some of the complexity. By doing so, however, we gain the detailed knowledge necessary for understanding that complexity. We hope that our book will help the reader in this respect.

Peter Bross
Niels Gregersen