PREFACE

It has been a privilege to edit the first *Methods in Enzymology* volume focused on protein design, a field that embraces both computational and experimental methods for creating new proteins with interesting properties. Experts who develop and use diverse approaches have generously contributed their insights and protocols to this book. There are many other talented scientists making important advances and discoveries, and I look forward to future volumes in this series that will build on what is presented here.

Protein design occupies a niche between engineering and science. As engineers, protein designers strive to make new molecules with desired characteristics. In doing so, they draw on accumulated scientific knowledge about protein structure, stability, folding, and function. The iterative process of design and redesign tests and further deepens our understanding of these relationships. Thus, even in the earliest days of computational design, when it was not clear whether this approach would ever deliver useful new proteins, the science was rich and exciting. This volume, which is focused on methods, addresses the "how" of protein design more than it does scientific questions about "why does it (or does it not) work?" Yet, in the chapters presented, the reader can find wonderful insights and ideas about ways to grow our understanding of molecular energetics, structural flexibility, and protein evolution, and how information is encoded in protein sequences.

In the past 20 years, significant progress has been made in methods for designing proteins. Advances in experimental protein library screening, for example, using phage or yeast-surface display now enable discovery efforts in many companies and have led to the development of protein therapeutics used in the clinic. Computational protein design, which was until quite recently a purely academic pursuit, is being widely used outside of the laboratories of the original developers. Chemical strategies for diversifying protein backbones, for example, making them less susceptible to proteolysis, are maturing. Applications for these methods abound and include design of novel protein interaction reagents based on non-antibody scaffolds, design of peptide-based inhibitors, design of highly stabilized proteins, design of new enzymatic functions, and prediction of the effects of mutations on stability, binding affinity and specificity, and drug resistance.

Despite progress, protein design remains very challenging. The fundamental problem of how to search vast sequence and conformational spaces

efficiently, using either experimental screens/selections or computational sampling, still demands innovative solutions. And our ability to predict the relative stabilities of different proteins from their sequences is quite primitive. The chapters presented here represent the state of the art in several ways. First, they demonstrate what is possible using existing methods. In cases where methods have matured to the point that they are routinely applied in their developers' labs, formal protocols are presented so that others can use these techniques. This is the case for several powerful experimental screening and selection methods. Second, where capabilities are not as mature, important tools are presented. Several chapters describe software packages, algorithms, or scripts that are available to help users try their own versions of protein design. Third, assessment methods are presented for judging and potentially improving the quality of existing energy functions and for assessing the quality of candidate designs. Interspersed throughout, there are ideas and suggestions about how to confront challenging problems including target selection, conformational sampling, multistate design, and library design.

In the future, many of the methods presented here will be combined and applied to ever more challenging design problems. There are numerous synergies to be realized among the computational methods presented, for example, between structure-based methods and sequence-guided methods. Better integration of computational design and experimental screening is expected to dramatically improve hit rates in future design studies. Exciting innovations can be expected. For now, thanks are due to the authors who contributed to this volume, particularly those who routinely make their code and reagents widely available, thus accelerating progress in this dynamic field.

Amy E. Keating