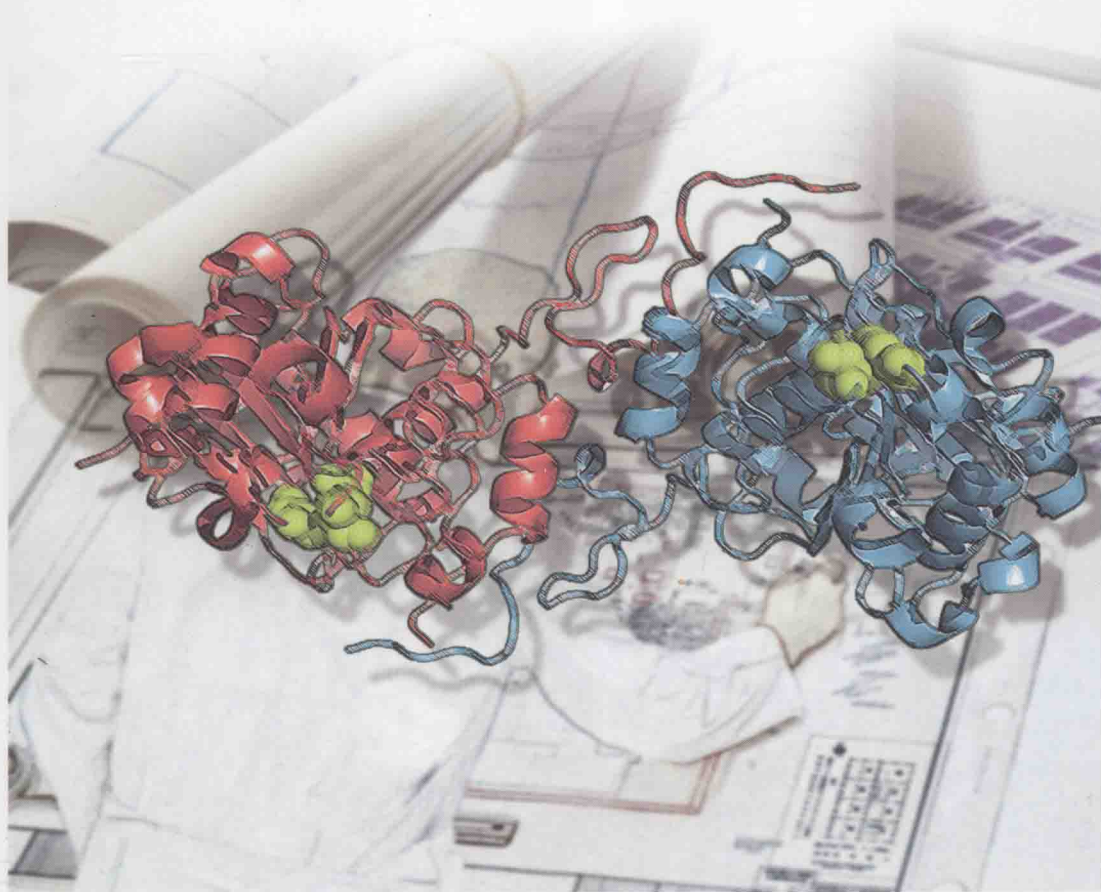


Edited by Stefan Lutz  
and Uwe T. Bornscheuer

 WILEY-VCH

# Protein Engineering Handbook

Volume 2



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*Edited by*

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**Protein Engineering  
Handbook**

Volume 2

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## ***Further Reading***

Cox, M. M., Phillips, G. N. (eds.)

### **Handbook of Proteins**

**Structure, Function and Methods. 2 Volume Set**

2008

Hardcover

ISBN: 978-0-470-06098-8

Miller, L. W. (eds.)

### **Probes and Tags to Study Biomolecular Function**

2008

Hardcover

ISBN: 978-3-527-31566-6

Lengauer, T. (ed.)

### **Bioinformatics – From Genomes to Therapies**

2007

Hardcover

ISBN: 978-3-527-31278-8

Schreiber, S. L., Kapoor, T., Wess, G. (eds.)

### **Chemical Biology**

**From Small Molecules to Systems Biology and Drug Design**

2007

Hardcover

ISBN: 978-0-470-84984-2

Aehle, W. (ed.)

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**Production and Applications**

2007

Hardcover

ISBN: 978-3-527-31689-2

Meyers, R. A. (ed.)

### **Proteins**

**From Analytics to Structural Genomics**

2006

Hardcover

ISBN: 978-3-527-31608-3

## Preface

Protein engineering is pursued by scientists from many different disciplines. Chemists, biochemists, biologists, and engineers alike are engaged in tailoring enzymes. As diverse as their intellectual background is their motivation to do so, varying from a desire to understand the fundamentals of biocatalysis such as the intimate relationship of structure, dynamics and function to questions of evolution, from a need to adjust enzyme properties for industrial processes to the challenge of generating novel proteins for therapeutic and biomedical applications. To meet their objectives, researchers are using highly creative and innovative approaches to introduce beneficial changes to enzymes, focusing on – among other properties – greater activity, altered substrate specificity, improved enantioselectivity, and increased stability.

As a field of research, protein engineering has made significant contributions towards a better understanding of the physical and chemical properties of proteins. In return, it has benefited from advances in traditional areas of biochemistry and biophysics. Insights into the role of protein structure from x-ray crystallography and NMR spectroscopy experiments have been rapidly growing and, together with clever mechanistic studies by enzymologists, have greatly contributed towards a better rationale for function. Separately, the emerging appreciation for protein dynamics, as well as the implementation of single-molecule studies has given us an intimate look at the performance of not just bulk catalyst but individual molecules as they move along the reaction coordinate. Paralleling advances in our understanding of the fundamentals, the last two decades have brought three paradigm shifts on the technological side of protein engineering. Starting with the introduction of the polymerase chain reaction and recombinant gene technology, progress in the field has empowered researchers to manipulate amino acid sequences in a relatively straightforward fashion and obtain vast quantities of selected polypeptides in heterologous expression systems. Next, the recreation of protein evolution processes in the laboratory, using random mutagenesis and *in vitro* recombination techniques, has opened up exciting and powerful new opportunities for protein engineers in all disciplines. Lastly, the recent development of predictive computer algorithms has added an important new tool, complementing experimental approaches by guiding the design and, in some cases, allowing for complete *de novo* construction of enzymes.

Capturing these exciting developments, Volume 1 of this book series focuses on fundamental aspects of protein engineering. While the opening chapter by Edmondson defines some of the terminology related to the characterization of engineered enzymes and the comparison to its natural parents, the contributions of Kazlauskas, Hollfelder and Miller concentrate on the active site, exploring enantioselectivity and substrate promiscuity. An often neglected yet critical aspect of protein engineering is folding of the polypeptide chain. While Fersht highlights the application of protein engineering for the studies of the folding process, DeLisa recapitulates some of the strategies to identify properly folded proteins. Along the same line, protein dynamics is another largely overlooked aspect of protein engineering. The contribution by Romesberg introduces a series of spectrophotometric techniques to capture protein motion while the article by Pelletier summarizes recent findings by NMR spectroscopy and x-ray crystallography. In one example for putting protein folding and dynamics data to work in the context of enzyme engineering, Sagermann presents a simple yet elegant method to explore and exploit conformational changes for creating functional protein switches.

Giving thought to the observation that not all proteins are equally suitable for laboratory evolution, Höcker provides a more practical perspective on the selection of protein frameworks as starting points for enzyme engineering. New activity and promising templates for engineering can also be found in the vastness of the metagenome. Many new opportunities in this emerging research area are discussed in the chapters by Ferrer and Eck. Separately, the contributions by Wood and Horswill review the utilization of intein sequences as protein engineering tools. Finally, the application of computational methods to guide protein engineering and *de novo* design is examined in the section by Saven.

In Volume 2, mutagenesis and shuffling strategies for generating libraries are described in the contributions by Reetz, Jäger and Tawfik while computational and experimental tools for chimeraesis are reviewed in the chapters by Arnold and Lutz. Less conventional but highly useful, Ostermeier discusses the impact of circular permutation on the structure and function of proteins.

As library generation represents only half the challenge in directed evolution, effective methods for searching the often substantial library diversity are necessary. Such screening or selection protocols are commonly performed *in vivo* or with the help of display systems as reported by Hilvert, Withers, Soumillion and Wittrup. The combination of such systems with spectroscopic assays offers a highly versatile screening strategy as outlined by Reymond and Coco. Alternatively, Ueda and Tawfik describe elegant *in vitro* strategies for library analysis. More product-oriented, Zhao and Bornscheuer discuss the application of protein engineering towards altering substrate and cofactor specificity, as well as enantioselectivity in individual enzymes. These strategies are not limited to single-enzyme systems. The chapters by Khosla, Steinbüchel, and Schmidt-Dannert demonstrate their application towards the manipulation of entire pathways. Similarly, protein engineering also offers new opportunities for tailoring biomaterials as described in the contributions of Taguchi, Conticello and Kaplan.

In summary, this book series attempts to capture some of the diverse interests and approaches in protein engineering, reflecting the many different disciplines and individual motivations and objectives in this area. We hope that it offers solutions to existing protein engineering problems and inspires new ideas to tackle the challenges in the field. In today's fast-moving world, it is unrealistic to expect an all-inclusive, up-to-date collection of knowledge and methods in any printed media. The current research literature is a more appropriate source for the latest hypotheses and technology. Aiming for scientists new to the field, we instead emphasize a review of the basics in the field, as well as introduce selected new and promising strategies for protein engineering. We hope that this will provide readers with a comprehensive overview of this highly interdisciplinary research topic. For the experienced protein engineer, the book series might offer some new inspiration as well.

A book project such as this would never succeed without the wonderful support of many individuals that inspired, encouraged, and assisted in its assembly. In addition to thanking all of the authors for their efforts, we would like to acknowledge our colleagues and students at Emory University and the University of Greifswald for their advice in managing such a project, as well as their willingness to review and proof-read the pages that make up the two volumes. Finally, our special thanks also extend to the people at Wiley Publisher, namely Dr. Frank Weinreich and Dr. Heike Nöthe for their editorial assistants, as well as Claudia Zschernitz and Nele Denzau for their help during the printing stage of the books.

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