Protein Engineering

Methods and Protocols

Edited by

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Preface

Since the discovery of proteins and their numerous roles in life, scientists are fascinated to study the molecular basis of how proteins function. It is amazing to see the plethora of protein structures and mechanisms that appeared during evolution, and the creativity, which is operating in nature’s continuing process of tailoring and fine-tuning proteins and, thus, life itself.

Proteins, especially enzymes, are also the key players of biocatalysis and biotechnology and thus they are linked to the wealth of our modern society. Besides deepening basic understanding, scientists are attracted by the possibility of knowledge-guided tailoring of proteins to suit the needs of biotechnological applications (rational protein design) or to create novel protein functions. As an alternative to this rationally inspired approach, scientists mimic the process of evolution by introducing random mutations in the laboratory (directed evolution). Although we are far away from understanding and reliably predicting protein folding and function \textit{de novo}, there are remarkable success stories in the field of protein engineering: Enzymes were created that catalyze reactions not observed in nature, they were highly stabilized for robustness in industrial processes, and proteins having superior pharmacological profiles have been successfully created. Hence, protein engineering has become an indispensable tool for pharmaceutical and industrial biotechnology.

Protein engineering is a complex and versatile process. With this book we aim to collect basic and advanced protocols for both stages of protein engineering: (i) the library design phase and (ii) the identification of improved variants by screening and selection. The focus of the book lies on enzyme engineering using rational and semirational approaches. Library creation protocols for random mutagenesis and recombining methods are a very diverse field, and a collection of protocols for this approach has been published recently in the excellent volume \textit{Directed Evolution Library Creation} of this series. Hence, this area is not covered in this edition.

As an introduction, Chapter 1 presents a general introduction into protein engineering. The book is then structured into three parts: \textit{Part I} describes computational protocols for rational protein engineering with the aid of case studies. A review (Chapter 2) summarizes different design approaches and methodologies. Protein tunnel inspection and basic steps of molecular modeling are exemplified using the user-friendly software packages CAVER (Chapter 3) and YASARA (Chapter 4). Chapter 5 demonstrates how to use the FRESCO algorithm to stabilize proteins. The presented guide allows to follow this more complex, but very powerful computational engineering protocol. To study structure–function relationships, one useful experimental approach is to study the so-called mutability landscape of a protein. By characterizing every possible single variant of each amino acid position of a protein, beneficial substitutions and nonmutable residues can be identified. Chapter 6 presents a laboratory protocol for an efficient way how to construct and analyze such a library.

\textit{Part II} focuses on the high-throughput expression of libraries and summarizes common solutions for various problems (Chapters 7 and 8). As a more advanced technique, Chapter 9 presents the split-GFP complementation assay. This approach allows determining the amount of the desired protein via fluorescence measurements in the presence of the entire host proteins. Activity data can then be normalized to the amount of total proteins...
without the need of enzyme purification. Chapter 10 covers expression and functional studies of membrane proteins using *E. coli* and insect cell-free expression systems.

High-throughput screening and selection assays are covered in Part III of this book. This is a very broad research area. Consequently, only exemplary screening protocols can be given as an inspiration for the development of alternative screening assays. An introductory review (Chapter 11) provides an overview of currently existing approaches. The following chapters deal with microplate assays: Chapter 12 describes the design of photometric screening protocols with emphasis on hydrolytic enzymes. Exemplary protocols for screening transaminases, laccases, and β-glucosidase are presented in Chapters 13–15. As screening campaigns have to be well planned and need an efficient way to collect, process, and visualize the data, Chapter 16 describes an open-source software solution that aids experimental planning, but especially data processing and visualization.

The last protocols present solutions for screening and selection procedures. This part of the book covers techniques like solid phase agar plate assays (Chapter 17), droplet sorting (Chapter 18), selection by FACS (Chapter 19), and a growth assays for active and thermostable variants (Chapter 20).

We very much hope that this compilation of concepts, methods, and protocols will help readers to facilitate the planning and performance of their experiments, but most importantly, that they will easily create and discover the desired improved proteins or enzymes. We keep our fingers crossed for success!

*Greifswald, Germany*  
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