# Protein Engineering Protocols

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METHODS IN MOLECULAR BIOLOGY™

# Protein Engineering Protocols

*Edited by*

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HUMANA PRESS TOTOWA, NEW JERSEY

© 2007 Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

**www.humanapress.com**

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This publication is printed on acid-free paper. ∞
ANSI Z39.48-1984 (American Standards Institute)

Permanence of Paper for Printed Library Materials.

Production Editor: Melissa Caravella

Cover design by Patricia F. Cleary

Cover illustration: From Fig. 1, Chapter 16, "A General Method of Terminal Truncation, Evolution, and Re-Elongation to Generate Enzymes of Enhanced Stability," by Jochen Hecky, Jody M. Mason, Katja M. Arndt, and Kristian M. Müller

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Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

eISBN 1-59745-187-8

Library of Congress Cataloging in Publication Data

Protein engineering protocols / edited by Kristian M. Müller, Katja M. Arndt.
p. cm. -- (Methods in molecular biology ; v. 352)
Includes bibliographical references and index.
ISBN 1-58829-072-7 (alk. paper)
1. Protein engineering. I. Arndt, Katja M. II. Müller, Kristian M. III. Series: Methods in molecular biology (Clifton, N.J.) ; v. 352.
TP248.65.P76P746 2006
660.6'3--dc22

2006041110

# Preface

Protein engineering is a fascinating mixture of molecular biology, protein structure analysis, computation, and biochemistry, with the goal of developing useful or valuable proteins. *Protein Engineering Protocols* will consider the two general, but not mutually exclusive, strategies for protein engineering. The first is known as rational design, in which the scientist uses detailed knowledge of the structure and function of the protein to make desired changes. The second strategy is known as directed evolution. In this case, random mutagenesis is applied to a protein, and selection or screening is used to pick out variants that have the desired qualities. By several rounds of mutation and selection, this method mimics natural evolution. An additional technique known as DNA shuffling mixes and matches pieces of successful variants to produce better results. This process mimics recombination that occurs naturally during sexual reproduction.

The first section of *Protein Engineering Protocols* describes rational protein design strategies, including computational methods, the use of non-natural amino acids to expand the biological alphabet, as well as impressive examples for the generation of proteins with novel characteristics. Although procedures for the introduction of mutations have become routine, predicting and understanding the effects of these mutations can be very challenging and requires profound knowledge of the system as well as protein structures in general. Consequently, this section focuses on the question of how to design a protein with the desired properties, and examples are chosen to cover a wide range of engineering techniques, such as protein–protein interactions, DNA binding, antibody mimics, and enzymatic activity.

The second section of *Protein Engineering Protocols* deals with evolutionary techniques. In contrast to rational design, directed evolution strategies do not require prior structural knowledge of a protein, nor is it necessary to be able to predict what effect a given mutation will have. Indeed, the results of directed evolution experiments are often surprising in that desired changes are often caused by unexpected mutations. Several factors determine the success of such a strategy: the library design and quality, the choice of the method for evolution and/or DNA recombination, and the selection or screening method. Consequently, this second section of *Protein Engineering Protocols* provides instructions to each of these steps, starting from general ideas of library design and statistical assessment of library quality. New methods for DNA shuffling

as well as different selection strategies are presented. Examples are given for the evolution of different characteristics, such as protein folds, folding, thermostability, and enzyme activity.

This volume provides a comprehensive guide to the methods used at every stage of the engineering process. It combines a thorough theoretical foundation with detailed protocols and will be invaluable to all research workers in the area, from graduate students to senior investigators. We would like to thank all authors for their excellent contributions and Prof. John M. Walker for his editorial guidance, patience, and assistance throughout the editorial process.

***Katja M. Arndt***
***Kristian M. Müller***

# Contents

Preface ..... *v*
Contributors ..... *ix*

**Part I Design and Computational Strategies for Protein Engineering**

1 Combinatorial Protein Design Strategies Using Computational Methods
***Hidetoshi Kono, Wei Wang, and Jeffery G. Saven*** ..... ***3***

2 Global Incorporation of Unnatural Amino Acids in *Escherichia coli*
***Jamie M. Bacher and Andrew D. Ellington*** ..... ***23***

3 Considerations in the Design and Optimization of Coiled Coil Structures
***Jody M. Mason, Kristian M. Müller, and Katja M. Arndt*** ..... ***35***

4 Calcium Indicators Based on Calmodulin–Fluorescent Protein Fusions
***Kevin Truong, Asako Sawano, Atsushi Miyawaki, and Mitsuhiko Ikura*** ..... ***71***

5 Design and Synthesis of Artificial Zinc Finger Proteins
***Wataru Nomura and Yukio Sugiura*** ..... ***83***

6 Monobodies: *Antibody Mimics Based on the Scaffold of the Fibronectin Type III Domain*
***Akiko Koide and Shohei Koide*** ..... ***95***

7 Engineering Site-Specific Endonucleases
***Peter Friedhoff and Alfred Pingoud*** ..... ***111***

**Part II Evolutionary Strategies for Protein Engineering**

8 Protein Library Design and Screening: *Working Out the Probabilities*
***Michel Denault and Joelle N. Pelletier*** ..... ***127***

9 Protein Design by Binary Patterning of Polar and Nonpolar Amino Acids
***Luke H. Bradley, Yinan Wei, Peter Thumfort, Christine Wurth, and Michael H. Hecht*** ..... ***155***

10 Versatile DNA Fragmentation and Directed Evolution With Nucleotide Exchange and Excision Technology
***Sabine C. Stebel, Katja M. Arndt, and Kristian M. Müller*** ..... ***167***

11 Degenerate Oligonucleotide Gene Shuffling
***Peter L. Bergquist and Moreland D. Gibbs*** ..... ***191***

12 M13 Bacteriophage Coat Proteins Engineered for Improved Phage Display
***Sachdev S. Sidhu, Birte K. Feld, and Gregory A. Weiss*** ..... ***205***

13 Ribosome-Inactivation Display System
***Satoshi Fujita, Jing-Min Zhou, and Kazunari Taira*** ..... ***221***

14 Compartmentalized Self-Replication: *A Novel Method for the Directed Evolution of Polymerases and Other Enzymes*
***Farid J. Ghadessy and Philipp Holliger*** ..... ***237***

15 Synthesis of Degenerated Libraries of the *Ras*-Binding Domain of *Raf* and Rapid Selection of Fast-Folding and Stable Clones With the Dihydrofolate Reductase Protein Fragment Complementation Assay
***François-Xavier Campbell-Valois and Stephen W. Michnick*** ..... ***249***

16 A General Method of Terminal Truncation, Evolution, and Re-Elongation to Generate Enzymes of Enhanced Stability
***Jochen Hecky, Jody M. Mason, Katja M. Arndt, and Kristian M. Müller*** ..... ***275***

Index ..... 305

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