Membrane Protein Protocols

METHODS IN MOLECULAR BIOLOGY™

John M. Walker, SERIES EDITOR

249. **Cytokine Protocols**, edited by Marc De Ley, 2004
248. **Antibody Engineering: Methods and Protocols**, edited by Benny K. C. Lo, 2004
247. **Drosophila Cytogenetics Protocols**, edited by Daryl S. Henderson, 2004
246. **Gene Delivery to Mammalian Cells: Volume 2: Viral Gene Transfer Techniques**, edited by William C. Heiser, 2004
245. **Gene Delivery to Mammalian Cells: Volume 1: Nonviral Gene Transfer Techniques**, edited by William C. Heiser, 2004
244. **Protein Purification Protocols, Second Edition**, edited by Paul Cutler, 2004
243. **Chiral Separations: Methods and Protocols**, edited by Gerald Gübitz and Martin G. Schmid, 2004
242. **Atomic Force Microscopy: Methods and Applications**, edited by Pier Carlo Braga and Davide Ricci, 2004
241. **Cell Cycle Checkpoint Control Protocols**, edited by Howard B. Lieberman, 2004
240. **Mammalian Artificial Chromosomes: Methods and Protocols**, edited by Vittorio Sgaramella and Sandro Eridani, 2003
239. **Cell Migration in Inflammation and Immunity: Methods and Protocols**, edited by Daniele D'Ambrosio and Francesco Sinigaglia, 2003
238. **Biopolymer Methods in Tissue Engineering**, edited by Anthony P. Hollander and Paul V. Hatton, 2003
237. **G Protein Signaling: Methods and Protocols**, edited by Alan V. Smrcka, 2003
236. **Plant Functional Genomics: Methods and Protocols**, edited by Erich Grotewold, 2003
235. **E. coli Plasmid Vectors: Methods and Applications**, edited by Nicola Casali and Andrew Preston, 2003
234. **p53 Protocols**, edited by Sumitra Deb and Swati Palit Deb, 2003
233. **Protein Kinase C Protocols**, edited by Alexandra C. Newton, 2003
232. **Protein Misfolding and Disease: Principles and Protocols**, edited by Peter Bross and Niels Gregersen, 2003
231. **Directed Evolution Library Creation: Methods and Protocols**, edited by Frances H. Arnold and George Georgiou, 2003
230. **Directed Enzyme Evolution: Screening and Selection Methods**, edited by Frances H. Arnold and George Georgiou, 2003
229. **Lentivirus Gene Engineering Protocols**, edited by Maurizio Federico, 2003
228. **Membrane Protein Protocols: Expression, Purification, and Characterization**, edited by Barry S. Selinsky, 2003
227. **Membrane Transporters: Methods and Protocols**, edited by Qing Yan, 2003
226. **PCR Protocols, Second Edition**, edited by John M. S. Bartlett and David Stirling, 2003
225. **Inflammation Protocols**, edited by Paul G. Winyard and Derek A. Willoughby, 2003
224. **Functional Genomics: Methods and Protocols**, edited by Michael J. Brownstein and Arkady Khodursky, 2003
223. **Tumor Suppressor Genes: Volume 2: Regulation, Function, and Medicinal Applications**, edited by Wafik S. El-Deiry, 2003
222. **Tumor Suppressor Genes: Volume 1: Pathways and Isolation Strategies**, edited by Wafik S. El-Deiry, 2003
221. **Generation of cDNA Libraries: Methods and Protocols**, edited by Shao-Yao Ying, 2003
220. **Cancer Cytogenetics: Methods and Protocols**, edited by John Swansbury, 2003
219. **Cardiac Cell and Gene Transfer: Principles, Protocols, and Applications**, edited by Joseph M. Metzger, 2003
218. **Cancer Cell Signaling: Methods and Protocols**, edited by David M. Terrian, 2003
217. **Neurogenetics: Methods and Protocols**, edited by Nicholas T. Potter, 2003
216. **PCR Detection of Microbial Pathogens: Methods and Protocols**, edited by Konrad Sachse and Joachim Frey, 2003
215. **Cytokines and Colony Stimulating Factors: Methods and Protocols**, edited by Dieter Köhrholz and Wieland Kiess, 2003
214. **Superantigen Protocols**, edited by Teresa Krakauer, 2003
213. **Capillary Electrophoresis of Carbohydrates**, edited by Pierre Thibault and Susumu Honda, 2003
212. **Single Nucleotide Polymorphisms: Methods and Protocols**, edited by Pui-Yan Kwok, 2003
211. **Protein Sequencing Protocols, Second Edition**, edited by Bryan John Smith, 2003
210. **MHC Protocols**, edited by Stephen H. Powis and Robert W. Vaughan, 2003
209. **Transgenic Mouse Methods and Protocols**, edited by Marten Hofker and Jan van Deursen, 2003
208. **Peptide Nucleic Acids: Methods and Protocols**, edited by Peter E. Nielsen, 2002
207. **Recombinant Antibodies for Cancer Therapy: Methods and Protocols**, edited by Martin Welschof and Jürgen Krauss, 2002
206. **Endothelin Protocols**, edited by Janet J. Maguire and Anthony P. Davenport, 2002
205. **E. coli Gene Expression Protocols**, edited by Peter E. Vaillancourt, 2002
204. **Molecular Cytogenetics: Protocols and Applications**, edited by Yao-Shan Fan, 2002
203. **In Situ Detection of DNA Damage: Methods and Protocols**, edited by Vladimir V. Didenko, 2002
202. **Thyroid Hormone Receptors: Methods and Protocols**, edited by Aria Baniahmad, 2002
201. **Combinatorial Library Methods and Protocols**, edited by Lisa B. English, 2002
200. **DNA Methylation Protocols**, edited by Ken I. Mills and Bernie H. Ramsahoye, 2002
199. **Liposome Methods and Protocols**, edited by Subhash C. Basu and Manju Basu, 2002
198. **Neural Stem Cells: Methods and Protocols**, edited by Tanja Zigova, Juan R. Sanchez-Ramos, and Paul R. Sanberg, 2002
197. **Mitochondrial DNA: Methods and Protocols**, edited by William C. Copeland, 2002

METHODS IN MOLECULAR BIOLOGY™

Membrane Protein Protocols

Expression, Purification, and Characterization

Edited by

Barry S. Selinsky

*Department of Chemistry, Villanova University,
Villanova, PA*

Humana Press Totowa, New Jersey

© 2003 Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

www.humanapress.com

All rights reserved. No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from the Publisher. Methods in Molecular Biology™ is a trademark of The Humana Press Inc.

All papers, comments, opinions, conclusions, or recommendations are those of the author(s), and do not necessarily reflect the views of the publisher.

This publication is printed on acid-free paper. (∞)
ANSI Z39.48-1984 (American Standards Institute)
Permanence of Paper for Printed Library Materials.

Cover illustration: From Fig. 5 in Chapter 17 "Purification, Reconstitution, and Functional Characterization of Zinc Transporter from Rat Renal Brush Border Membranes," by Rajendra Prasad.

Production Editor: Kim Hoather-Potter.
Cover design by Patricia F. Cleary.

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel.: 973-256-1699; Fax: 973-256-8341; E-mail: humana@humanapr.com; or visit our Website: www.humanapress.com

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Humana Press Inc., provided that the base fee of US \$20.00 per copy is paid directly to the Copyright Clearance Center at 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license from the CCC, a separate system of payment has been arranged and is acceptable to Humana Press Inc. The fee code for users of the Transactional Reporting Service is: [1-58829-124-3/03 \$20.00].

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

Library of Congress Cataloging in Publication Data

Membrane protein protocols: expression, purification, and characterization / edited by

Barry S. Selinsky.

p. cm. -- (Methods in molecular biology ; v.228)

Includes bibliographical references and index.

ISBN 1-58829-124-3 (alk. paper); E-ISBN 1-59259-400-X

ISSN 1064-3745

1. Membrane proteins--Laboratory manuals. I. Selinsky, Barry Steven. II. Methods in molecular biology (Totowa, N.J.) ; v.228.

QP552.M44M433 2003
572'.69--dc21

2003041743

Preface

Knowledge of the three-dimensional structure of a protein is absolutely required for the complete understanding of its function. The spatial orientation of amino acids in the active site of an enzyme demonstrates how substrate specificity is defined, and assists the medicinal chemist in the design of specific, tight-binding inhibitors. The shape and contour of a protein surface hints at its interaction with other proteins and with its environment. Structural analysis of multiprotein complexes helps to define the role and interaction of each individual component, and can predict the consequences of protein mutation or conditions that promote dissociation and rearrangement of the complex.

Determining the three-dimensional structure of a protein requires milligram quantities of pure material. Such quantities are required to refine crystallization conditions for X-ray analysis, or to overcome the sensitivity limitations of NMR spectroscopy. Historically, structural determination of proteins was limited to those expressed naturally in large amounts, or derived from a tissue or cell source inexpensive enough to warrant the use of large quantities of cells. However, with the advent of the techniques of modern gene expression, many proteins that are constitutively expressed in minute amounts can become accessible to large-scale purification and structural analysis.

Membrane proteins have been resistant to structural analysis for a variety of reasons. First, the proper folding of membrane proteins, and their insertion into a membrane bilayer, has been problematic. Bacterial expression systems are not often useful, and researchers have had to express in eukaryotic systems with lower expression efficiency and added experimental difficulty. Second, even when large amounts of protein are available, the hydrophobic nature of membrane proteins has made them resistant to X-ray or NMR analysis. The proper conditions for solubilizing membrane proteins with retention of structure vary, and discovery of proper experimental conditions can be tedious and frustrating. As a result, the three-dimensional structures of only a small percentage of the population of membrane proteins have been determined at the atomic level.

The complete purpose of *Membrane Protein Protocols: Expression, Purification, and Characterization* is to provide examples of how different membrane proteins have been overexpressed in both prokaryotic and eukaryotic expression systems, how natural and overexpressed proteins have been solubilized from their host membranes, and how the solubilized proteins have been purified in active form. Through examination of each individual system, a researcher may

find some inspiration to overcome problems encountered in their laboratories. The casual reader may gain insight into the difficulties experienced in the study of membrane proteins, and might be led to novel ways to circumvent common roadblocks. As the structures of additional membrane proteins come to light, we may gain a better understanding of the complex nature of biological membranes, and the cell itself.

I would like to thank the authors who have contributed to this work for their enthusiasm, to John Walker, the series editor, for his help and encouragement, and to the staff at Humana Press who helped to produce this volume.

Barry S. Selinsky

Contents

Preface	v
Contributors	xi
PART I. EXPRESSION OF MEMBRANE PROTEINS	
1 Expression and Purification of the Amphipathic Form of Rabbit Cytochrome b_5 in <i>Escherichia coli</i> Lucy Waskell	3
2 Dihydroorotate Dehydrogenase of <i>Escherichia coli</i> Kaj Frank Jensen and Sine Larsen	11
3 A General Approach for Heterologous Membrane Protein Expression in <i>Escherichia coli</i> : <i>The Uncoupling Protein,</i> <i>UCP1, as an Example</i> Alison Z. Shaw and Bruno Miroux	23
4 Expression of Membrane-Bound Iron–Sulfur Proteins Dirk Schneider, Christian L. Schmidt, and Andreas Seidler	37
5 Heterologous Expression of Human Scavenger Receptor Class B Type I (SR-BI) in <i>Pichia pastoris</i> Chang-Hoon Han, Moritz Werder, Wilk von Gustedt, and Helmut Hauser	49
6 Expression of Oligomeric Amiloride-Sensitive Epithelial Sodium Channel in Sf9 Insect Cells U. Subrahmanyeswara Rao	65
7 Functional Expression of His-Tagged Rhodopsin Sf9 Insect Cells Giel J.C.G.M. Bosman, Jenny van Oostrum, Githa Breikers, Petra H.M. Bovee-Geurts, Corne H.W. Klaassen, and Willem J. DeGrip	73
PART II. DETERGENT SELECTION IN MEMBRANE PROTEIN PURIFICATION	
8 Preparation of Glycerol Facilitator for Protein Structure and Folding Studies in Solution Darren Manley and Joe D. O’Neil	89
9 Solubilization of Chemokine Receptors from Cell Membranes Robert Staudinger and Juan C. Bandrés	103

10	A Systematic Approach for the Solubilization of the Integral Membrane Protein Lysophospholipid: <i>Acyl-CoA Acyltransferase (LAT)</i> Claus Kerkhoff and Volkhard Kaever	111
PART III. MEMBRANE PROTEIN PURIFICATION AND RECONSTITUTION		
11	Purification of Omp50, a Minor Porin of <i>Campylobacter jejuni</i> Jean Michel Bolla	131
12	Purification of Porins from <i>Mycobacterium smegmatis</i> Christian Heinz, Eva Roth, and Michael Niederweis	139
13	Isolation of the Melanocortin 5 Receptor: <i>From cDNA Sequence to Isolating an Integral Membrane Protein</i> Benjamin L. Clarke	151
14	Purification of Mammalian Serine Palmitoyltransferase, a Hetero-Subunit Enzyme for Sphingolipid Biosynthesis, by Affinity-Peptide Chromatography Kentaro Hanada and Masahiro Nishijima	163
15	Purification of Phosphatidylglycerophosphate Synthase from Cultured Mammalian Cells Kiyoshi Kawasaki and Masahiro Nishijima	175
16	Purification of Pancreastatin Receptor from Rat Liver Membranes Víctor Sánchez-Margalet, José Santos-Alvarez, and Sandra Díaz-Troya	187
17	Purification, Reconstitution, and Functional Characterization of Zinc Transporter from Rat Renal Brush Border Membranes Rajendra Prasad	195
18	Isolation of Lipid Raft-Associated Proteolipids Jaime Millán, María C. de Marco, Mohammed Qaidi, Alicia Batista, Fernando Martín-Belmonte, and Miguel A. Alonso	223
19	Purification of Membrane-Bound Catechol-O-Methyltransferase Maria João Bonifácio and Patrício Soares-da-Silva	231
20	Purification and Characterization of Transporter Proteins from Human Erythrocyte Membrane Da-Neng Wang, M. Joanne Lemieux, and Jonathan M. Boulter	239
21	Purification of the Human Erythrocyte PS-Stimulated Mg^{2+} -ATPase: <i>A Putative PS Flippase</i> Jill V. Lyles, Kathleen Cornely-Moss, Christine M. Smith, and David L. Daleke	257

22	Reconstitution and Assay of Biogenic Membrane-Derived Phospholipid Flippase Activity in Proteoliposomes Sathyanarayana N. Gummadi, Sigrún Hrafnisdóttir, Jane Walent, William E. Watkins, and Anant K. Menon	271
23	Expression, Purification, and Reconstitution of Rat Liver Carnitine Palmitoyltransferase I Nicholas F. Brown	281
PART IV. STRUCTURAL ANALYSIS OF MEMBRANE PROTEINS		
24	Crystallization in Lipidic Cubic Phases: A Case Study with <i>Bacteriorhodopsin</i> Valentin I. Gordeliy, Ramona Schlesinger, Rouslan Efremov, Georg Büldt, and Joachim Heberle	305
25	Optical Biosensor Assay Using Retroviral Receptor Pseudotypes Joseph Rucker	317
	Index	329

Contributors

- MIGUEL A. ALONSO • *Centro de Biología Molecular “Severo Ochoa,”
Universidad Autónoma de Madrid, Madrid, Spain*
- JUAN C. BANDRÉS • *Mount Sinai School of Medicine, Bronx, New York, NY, USA*
- ALICIA BATISTA • *Centro de Biología Molecular “Severo Ochoa,” Universidad
Autónoma de Madrid, Madrid, Spain*
- JEAN MICHEL BOLLA • *INSERM, Faculté de Médecine, Marseille, France*
- MARIA JOÃO BONIFÁCIO • *Department of Research and Development, BIAL,
São Mamede do Coronado, Portugal*
- GIEL J.C.G.M. BOSMAN • *Department of Biochemistry, Nijmegen Center for
Molecular Life Sciences, Nijmegen, The Netherlands*
- JONATHAN M. BOULTER • *Skirball Institute of Biomolecular Medicine and
Department of Cell Biology, New York University School of Medicine,
New York, NY, USA*
- PETRA H.M. BOVEE-GEURTS • *Department of Biochemistry, Nijmegen Center
for Molecular Life Sciences, Nijmegen, The Netherlands*
- GITHA BREIKERS • *Department of Biochemistry, Nijmegen Center for Molecular
Life Sciences, Nijmegen, The Netherlands*
- NICHOLAS F. BROWN • *Division of Endocrinology and Metabolism, Department
of Medicine, University of Pittsburgh, Pittsburgh, PA, USA*
- GEORG BÜLDT • *Research Center Jülich, Jülich, Germany*
- BENJAMIN L. CLARKE • *Department of Medical Microbiology and Immunology,
University of Minnesota Duluth, Duluth, MN, USA*
- KATHLEEN CORNELLY-MOSS • *Department of Chemistry and Biochemistry,
Providence College, Providence, RI, USA*
- DAVID L. DALEKE • *Department of Biochemistry and Molecular Biology,
Medical Sciences, Indiana University, Bloomington, IN, USA*
- WILLEM J. DEGRIP • *Department of Biochemistry, Nijmegen Center for
Molecular Life Sciences, Nijmegen, The Netherlands*
- MARÍA C. DE MARCO • *Centro de Biología Molecular “Severo Ochoa,”
Universidad Autónoma de Madrid, Madrid, Spain*
- SANDRA DÍAZ-TROYA • *Department of Medical Biochemistry and Molecular
Biology, Virgen Macarena University Hospital, Seville, Spain*
- ROUSLAN EFREMOV • *Centre for Biophysics and Physical Chemistry of
Supramolecular Structures, Moscow Institute for Physics and Technology,
Dolgoprudny, Russia*

- VALENTIN I. GORDELIY • *Centre for Biophysics and Physical Chemistry of Supramolecular Structures, Moscow Institute for Physics and Technology, Dolgoprudny, Russia*
- SATHYANARAYANA N. GUMMADI • *Department of Chemical Engineering, Indian Institute of Technology-Madras, Chennai, India*
- CHANG-HOON HAN • *Institute of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland*
- KENTARO HANADA • *Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases, Tokyo, Japan*
- HELMUT HAUSER • *Institute of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland*
- JOACHIM HEBERLE • *Research Center Jülich, Jülich, Germany*
- CHRISTIAN HEINZ • *Lehrstuhl für Mikrobiologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany*
- SIGRÚN HRAFNSDÓTTIR • *Department of Membrane Enzymology, Center for Biomembranes and Lipid Enzymology, Utrecht, The Netherlands*
- KAJ FRANK JENSEN • *Institute of Molecular Biology, Department of Biological Chemistry, University of Copenhagen, Copenhagen, Denmark*
- VOLKHARD KAEVER • *Institute of Pharmacology, Medical School of Hannover, Hannover, Germany*
- KIYOSHI KAWASAKI • *Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases, Tokyo, Japan*
- CLAUS KERKHOFF • *Institute of Experimental Dermatology, University of Münster, Germany*
- CORNE H.W. KLAASSEN • *Department of Biochemistry, Nijmegen Center for Molecular Life Sciences, Nijmegen, The Netherlands*
- SINE LARSEN • *Centre for Crystallographic Studies, Department of Chemistry, University of Copenhagen, Copenhagen, Denmark*
- M. JOANNE LEMIEUX • *Skirball Institute of Biomolecular Medicine and Department of Cell Biology, New York University School of Medicine, New York, NY, USA*
- JILL V. LYLES • *Department of Chemistry, Indiana University, Bloomington, IN, USA*
- DARREN MANLEY • *Department of Chemistry, University of Manitoba, Winnipeg, MB, Canada*
- FERNANDO MARTÍN-BELMONTE • *Centro de Biología Molecular "Severo Ochoa," Universidad Autónoma de Madrid, Madrid, Spain*
- ANANT K. MENON • *Department of Biochemistry, University of Wisconsin-Madison, WI, USA*
- JAIME MILLÁN • *Centro de Biología Molecular "Severo Ochoa," Universidad Autónoma de Madrid, Madrid, Spain*

- BRUNO MIROUX • *Faculté de Médecine Necker Enfants-Malades, Paris, France*
- MICHAEL NIEDERWEIS • *Lehrstuhl für Mikrobiologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany*
- MASAHIRO NISHIJIMA • *Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases, Tokyo, Japan*
- JOE D. O'NEIL • *Department of Chemistry, University of Manitoba, Winnipeg, MB, Canada*
- RAJENDRA PRASAD • *Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh, India*
- MOHAMMED QAIDI • *Centro de Biología Molecular "Severo Ochoa," Universidad Autónoma de Madrid, Madrid, Spain*
- U. SUBRAHMANYESWARA RAO • *Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA*
- EVA ROTH • *Lehrstuhl für Mikrobiologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany*
- JOSEPH RUCKER • *Department of Chemistry, Villanova University, Villanova, PA, USA*
- VÍCTOR SÁNCHEZ-MARGALET • *Department of Medical Biochemistry and Molecular Biology, Virgen Macarena University Hospital, Seville, Spain*
- JOSÉ SANTOS-ALVAREZ • *Department of Medical Biochemistry and Molecular Biology, Virgen Macarena University Hospital, Seville, Spain*
- RAMONA SCHLESINGER • *Research Center Jülich, Jülich, Germany*
- CHRISTIAN L. SCHMIDT • *Institut für Biochemie, Medizinische Universität zu Lübeck, Lübeck, Germany*
- DIRK SCHNEIDER • *Fakultät für Biologie, Ruhr-Universität Bochum, Bochum, Germany*
- ANDREAS SEIDLER • *Fakultät für Biologie, Ruhr-Universität Bochum, Bochum, Germany*
- ALISON Z. SHAW • *ICREA, Barcelona, Spain*
- CHRISTINE M. SMITH • *Department of Chemistry, Indiana University, Bloomington, IN, USA*
- PATRÍCIO SOARES-DA-SILVA • *Department of Research and Development, BIAL, São Mamede do Coronado, Portugal*
- ROBERT STAUDINGER • *Department of Neurology, New York University School of Medicine, New York, NY, USA*
- JENNY VAN OOSTRUM • *Department of Biochemistry, Nijmegen Center for Molecular Life Sciences, Nijmegen, The Netherlands*
- WILK VON GUSTEDT • *Institute of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland*
- JANE WALENT • *Department of Biochemistry, University of Wisconsin-Madison, WI, USA*

DA-NENG WANG • *Skirball Institute of Biomolecular Medicine and Department of Cell Biology, New York University School of Medicine, New York, NY, USA*

LUCY WASKELL • *Department of Anesthesiology, University of Michigan, Ann Arbor, MI, USA*

WILLIAM E. WATKINS • *Department of Biochemistry, University of Wisconsin-Madison, WI, USA*

MORITZ WERDER • *Institute of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland*