METHODS IN MOLECULAR BIOLOGY™

Series Editor
John M. Walker
School of Life Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

For other titles published in this series, go to
www.springer.com/series/7651

Heterologous Expression of Membrane Proteins

Methods and Protocols

Edited by

Isabelle Mus-Veteau

Institut of Developmental Biology and Cancer, UMR CNRS 6543
Université de Nice-Sophia Antipolis, Parc Valrose, Nice, France

Humana Press

Editor
Isabelle Mus-Veteau, Ph.D.
Institut of Developmental Biology and Cancer, UMR CNRS 6543
Université de Nice-Sophia Antipolis
Parc Valrose, Nice
France
isabelle.mus-veteau@unice.fr

ISSN 1064-3745 e-ISSN 1940-6029
ISBN 978-1-60761-343-5 e-ISBN 978-1-60761-344-2
DOI 10.1007/978-1-60761-344-2
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2009932113

Cover illustration: The background art is derived from Figure 5 in Chapter 12.

Printed on acid-free paper

Humana Press is a part of Springer Science+Business Media (www.springer.com)

Preface

Membrane proteins account for roughly 30% of all open reading frames in fully sequenced genomes. These proteins are of central importance to living cells. They are required for transport processes, sensing changes in the cellular environment, transmission of signals, and control of cell–cell contacts. These proteins are implicated in numerous pathologies, like cancer, cystic fibrosis, epilepsy, hyperinsulinism, heart failure, hypertension and Alzheimer disease, but studies of these and other disorders are hampered by a lack of information about the proteins involved. Knowing the structure of membrane proteins is an essential prerequisite for understanding how these proteins function and, further, how their functions can be modified by small molecules. This is of paramount importance in the pharmaceutical industry, which produces many drugs that bind to membrane proteins (50% of all drug targets are G protein-coupled receptors [GPCRs]), and recognizes the potential of many recently identified GPCRs, ion channels and transporters as targets for future drugs. However, whereas high-resolution structures are available for myriad soluble proteins (more than 42,000 in the Protein Data Bank), three-dimensional (3D) structures have so far been obtained for only 204 membrane proteins, the majority of which are from prokaryotic organisms, with only 25 from mammalian membrane proteins (see http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html). The first membrane proteins were crystallized owing to their natural abundance, circumventing all the difficulties associated with overexpression. However, the majority of medically and pharmaceutically relevant membrane proteins are present in tissues at very low concentration, making overexpression of recombinant membrane proteins in heterologous cells suitable for large-scale production a prerequisite for structural studies. The development of heterologous expression systems capable of delivering high-quality recombinant protein for structural studies remains an essential goal. In 2005, the two first atomic structures of recombinant mammalian membrane proteins expressed in yeast were resolved. Since that time, extensive optimization of heterologous expression systems has begun to bear fruit, and 11 eukaryotic membrane protein structures have been solved to high resolution using recombinant material.

This volume proposes an overview of different heterologous expression systems that produce an adequate amount of membrane proteins for structural analysis. Methods and protocols are described for each heterologous expression system proposed. Some chapters of this volume treat membrane protein solubilization, purification and instability in solution and comment on the strategies that allowed the determination of the structure of the first heterologously expressed mammalian membrane proteins.

Isabelle Mus-Veteau
Nice, France

Contents

Contributors

Bertrand Arnou • *CEA, iBiTecS – Institut de Biologie et Technologies de Saclay, University of Paris-Sud, Gif-sur-Yvette, France*

Jean-Louis Banères • *Institut des Biomolécules Max Mousseron, Université Montpellier I, Université Montpellier II, Faculté de Pharmacie, Montpellier, France*

Frank Bernhard • *Centre for Biomolecular Magnetic Resonance, University of Frankfurt/Main, Institute for Biophysical Chemistry, Frankfurt/Main, Germany*

Michel Bidet • *Institut of Developmental Biology and Cancer, UMR CNRS 6543, Université de Nice-Sophia Antipolis, Parc Valrose, Nice, France*

Sylvain Boutigny • *Laboratoire de Physiologie Cellulaire Végétale, Grenoble, France; and CEA, DSV, iRTSV, Grenoble, France; INRA, Grenoble, France; Université Joseph Fourier, Grenoble, France*

Cécile Breyton • *Laboratoire de Physico-Chimie Moléculaire des Membranes Biologiques and Université Paris-7, Institut de Biologie Structurale, UMR 5075 CNRS/CEA/UJF, 41, rue Jules Horowitz, 38027 Grenoble France*

Karl Brillet • *Dpt Récepteurs et des Protéines Membranaires, Illkirch, France*

Delphine Cardi • *CEA, iBiTecS – Institut de Biologie et Technologies de Saclay, University of Paris-Sud, Gif-sur-Yvette, France*

Volker Dötsch • *Centre for Biomolecular Magnetic Resonance, University of Frankfurt/Main, Institute for Biophysical Chemistry, Frankfurt/Main, Germany*

Katia Duquesne • *Laboratoire d'Ingenierie des Systèmes Maromoléculaire, Institut de Biologie Structurale et Microbiologie, CNRS and Aix Marseille Université, Marseille, France*

Florian Durst • *Centre for Biomolecular Magnetic Resonance, University of Frankfurt/Main, Institute for Biophysical Chemistry, Frankfurt/Main, Germany*

Pierre Falson • *Laboratoire des Protéines de Résistance aux Agents Chimiothérapeutiques (LPRAC), Institut de Biologie et Chimie des Protéines (IBCP), Unité Mixte du Centre National de la Recherche Scientifique (CNRS) et de l'Université Lyon I, Institut Fédératif de Recherches Lyon, France*

Annie Frelet-Barrand • *Laboratoire de Physiologie Cellulaire Végétale, CNRS (UMR-5168)/CEA/INRA (UMR-1200), Université Joseph Fourier, iRTSV, CEA-Grenoble, France*

Eric R. Geertsma • *Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Netherlands Proteomics Centre (NPC), and Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands*

Christine Jaxel • *CEA, iBiTecS – Institut de Biologie et Technologies de Saclay, University of Paris-Sud, Gif-sur-Yvette, France*

Marie Jidenko • *CEA, iBiTecS – Institut de Biologie et Technologies de Saclay, University of Paris-Sud, Gif-sur-Yvette, France*

Olivier Joubert • *Laboratoire de Biologie et Physiopathologie des Systèmes Intégrés, Université Henri Poincaré- Nancy 1, Faculté de Pharmacie, 5 rue Albert Lebrun, BP 80403, F-54001 NANCY CEDEX*

Friederike Junge • *Centre for Biomolecular Magnetic Resonance, University of Frankfurt/Main, Institute for Biophysical Chemistry, Frankfurt/Main, Germany*
Edmund R.S. Kunji • *The Medical Research Council, Dunn Human Nutrition Unit, Cambridge, UK*
Marc le Maire • *CEA, iBiTecS – Institut de Biologie et Technologies de Saclay, University of Paris-Sud, Gif-sur-Yvette, France*
Kenneth Lundstrom • *PanTherapeutics, Lutry, Switzerland*
Estelle Marchal • *CEA, iBiTecS – Institut de Biologie et Technologies de Saclay, University of Paris-Sud, Gif-sur-Yvette, France*
Bruno Miroux • *Université Paris-7, Laboratoire de Biologie Physico-Chimique des Proteines Membranaires, Institut de Biologie Physico-Chimique, Paris, France*
Cédric Montigny • *CEA, iBiTecS – Institut de Biologie et Technologies de Saclay, University of Paris-Sud, Gif-sur-Yvette, France*
Bernard Mouillac • *Institut de Génomique Fonctionnelle, Montpellier, France; INSERM U661, Montpellier, France; Université Montpellier I, Montpellier, France; Université Montpellier II, Montpellier, France*
Isabelle MUS-VETEAU • *Institut of Developmental Biology and Cancer, UMR CNRS 6543, Université de Nice-Sophia Antipolis, Parc Valrose, Nice, France*
Rony Nehmé • *Institut of Developmental Biology and Cancer, UMR CNRS 6543, Université de Nice-Sophia Antipolis, Parc Valrose, Nice, France*
Carlos A. Pereira • *Laboratório de Imunologia Viral, Instituto Butantan, São Paulo, Brasil*
Valérie Panneels • *Heidelberg University Biochemistry Center (BZH), Heidelberg, Germany*
Bert Poolman • *Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Netherlands Proteomics Centre (NPC), and Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands*
Jean-Luc Popot • *Laboratoire de Physico-Chimie Moléculaire des Membranes Biologiques, CNRS and Université Paris-7, Institut de Biologie Physico-Chimique, Paris, France*
Bernard Pucci • *Laboratoire de Chimie Bioorganique et des Systèmes Moléculaires Vectoriels, Université d'Avignon et des Pays de Vaucluse, Faculté des Sciences, Avignon, France*
Daniel Schwarz • *Centre for Biomolecular Magnetic Resonance, University of Frankfurt/Main, Institute for Biophysical Chemistry, Frankfurt/Main, Germany*
Birgit Schneider • *Centre for Biomolecular Magnetic Resonance, University of Frankfurt/Main, Institute for Biophysical Chemistry, Frankfurt/Main, Germany*
Vladimir A. Shirokov • *Institute of Protein Research, Russian Academy of Sciences, Pushchino, Moscow Region, Russia*
Irmgard Sinning • *Heidelberg University Biochemistry Center (BZH), Heidelberg, Germany*
James N. Sturgis • *Laboratoire d'Ingenierie des Systèmes Maromoléculaire, Institut de Biologie Structurale et Microbiologie, CNRS and Aix Marseille Université, Marseille, France*
Christopher G. Tate • *MRC Laboratory of Molecular Biology, Cambridge, UK*

Céline Trometer • *Laboratoire des Protéines de Résistance aux Agents Chimiothérapeutiques (LPRAC), Institut de Biologie et Chimie des Protéies (IBCP), Unité Mixte du Centre National de la Recherche Scientifique (CNRS) et de l'Université Lyon, Institut Fédératif de Recherches, Lyon, France*

Norbert Rolland • *Laboratoire de Physiologie Cellulaire Végétale, CNRS, Grenoble, France; and CEA, DSV, iRTSVGrenoble, France; INRA, Grenoble, France; Université Joseph Fourier, Grenoble, France*

Renaud Wagner • *Dpt Récepteurs et des Protéines Membranaires, Illkirc, France*

Manuela Zoonens • *Université Paris-7, Laboratoire de Biologie Physico-Chimique des Proteines Membranaires, Institut de Biologie Physico-Chimique, Paris, France*