

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

Liposomes

Methods and Protocols Volume 2: Biological Membrane Models

Edited by

Volkmar Weissig

*Department of Pharmaceutical Sciences, Midwestern University College of Pharmacy Glendale,
Glendale, AZ, USA*

Editor
Volkmar Weissig
Department of Pharmaceutical Sciences
Midwestern University College of Pharmacy Glendale
Glendale, AZ
USA
vweiss@midwestern.edu

ISSN 1064-3745 e-ISSN 1940-6029
ISBN 978-1-60761-446-3 e-ISBN 978-1-60761-447-0
DOI 10.1007/978-1-60761-447-0
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2009933261

© Humana Press, a part of Springer Science+Business Media, LLC 2010

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Humana Press, c/o Springer Science+Business Media, LLC, 233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

While the advice and information in this book are believed to be true and accurate at the date of going to press, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Cover illustration: Background art is derived from Figure 6c in Chapter 21

Printed on acid-free paper

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

Preface

Efforts to describe and model the molecular structure of biological membranes go back to the beginning of the last century. In 1917, Langmuir described membranes as a layer of lipids one molecule thick [1]. Eight years later, Gorter and Grendel concluded from their studies that “the phospholipid molecules that formed the cell membrane were arranged in two layers to form a lipid bilayer” [2]. Danielli and Robertson proposed, in 1935, a model in which the bilayer of lipids is sequestered between two monolayers of unfolded proteins [3], and the currently still accepted fluid mosaic model was proposed by Singer and Nicolson in 1972 [4].

Among those landmarks of biomembrane history, a serendipitous observation made by Alex Bangham during the early 1960s deserves undoubtedly a special place. His finding that exposure of dry phospholipids to an excess of water gives rise to lamellar structures [5] has opened versatile experimental access to studying the biophysics and biochemistry of biological phospholipid membranes.

Although during the following 4 decades biological membrane models have grown in complexity and functionality [6], liposomes are, besides supported bilayers, membrane nanodiscs, and hybrid membranes, still an indisputably important tool for membrane biophysicists and biochemists. In vol. II of this book, the reader will find detailed methods for the use of liposomes in studying a variety of biochemical and biophysical membrane phenomena concomitant with chapters describing a great palette of state-of-the-art analytical technologies.

Moreover, besides providing membrane biophysicists and biochemists with an immeasurably valuable experimental tool, Alex Bangham’s discovery has triggered the launch of an entirely new subdiscipline in pharmaceutical science and technology. His observation that the lamellar structures formed by phospholipids exposed to aqueous buffers are able to sequester small molecules has led to the development of the colloidal drug delivery concept. Following initial studies of enzyme encapsulation in liposomes as an approach towards the treatment of storage diseases [7, 8], a few years later in two *New England Journal of Medicine* landmark papers, Gregory Gregoriadis outlined the huge carrier potential of liposomes in biology and medicine [9, 10]. The following 2 decades saw immense efforts in academia and in soon-to-be-founded start-up companies to turn Gregoriadis’ vision into clinical reality. These 20 years of intense work in liposome laboratories around the world finally culminated with the FDA (USA) approval of the first injectable liposomal drug, Doxil, in February of 1995. Today, liposomes present the prototype of all nanoscale drug delivery vectors currently under development. Lessons learned in the history of over 40 years of Liposome Technology should be heeded by new investigators in the emerging field of pharmaceutical and biomedical nanotechnology. Volume I of this book is dedicated to state-of-the-art aspects of developing liposome-based pharmaceutical nanocarriers.

All chapters were written by leading experts in their particular fields, and I am extremely grateful to them for having spent parts of their valuable time to contribute to this book. It is my hope that together we have succeeded in providing an essential source of practical

know-how for every investigator, young and seasoned ones alike, whose research area involves in one way or another phospholipids, glycolipids, and cholesterol.

Last but not least, I would like to thank John Walker, the series editor of “Methods in Molecular Biology,” for having invited me to assemble this book and above all for his unlimited guidance and help throughout the whole process.

Glendale, AZ

Volkmar Weissig

References

1. Bangham AD, Standish MM, Watkins JC (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol* 13(1):238–252
2. Chan YH, Boxer SG (2007) Model membrane systems and their applications. *Curr Opin Chem Biol* 11(6):581–587
3. Danielli JF, Davson H (1935) A contribution to the theory of permeability of thin films. *J Cell Comp Physiol* 5:495–508
4. Gorter E, Grendel F (1925) On bimolecular layers of lipoids on the chromocytes of the blood. *J Exp Med* 41:439–443
5. Gregoriadis G (1976) The carrier potential of liposomes in biology and medicine (second of two parts). *N Engl J Med* 295(14):765–770
6. Gregoriadis G (1976) The carrier potential of liposomes in biology and medicine (first of two parts). *N Engl J Med* 295(13):704–710
7. Gregoriadis G, Ryman BE (1971) Liposomes as carriers of enzymes or drugs: a new approach to the treatment of storage diseases. *Biochem J* 124(5):58P
8. Gregoriadis G, Leathwood PD, Ryman BE (1971) Enzyme entrapment in liposomes. *FEBS Lett* 14(2):95–99
9. Langmuir I (1917) The constitution and structural properties of solids and liquids. II. Liquids. *J Am Chem Soc* 39:1848–1906
10. Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes. *Science* 175(23):720–731

Contents

<i>Preface</i>	<i>v</i>
<i>Contributors</i>	<i>xi</i>
1 Utilization of Liposomes for Studying Drug Transfer and Uptake <i>Alfred Fahr and Xiangli Liu</i>	1
2 The Use of Liposomes in the Study of Drug Metabolism: A Method to Incorporate the Enzymes of the Cytochrome P450 Monooxygenase System into Phospholipid, Bilayer Vesicles. <i>James R. Reed</i>	11
3 Use of Liposomes to Study Cellular Osmosensors <i>Reinhard Krämer, Sascha Nicklisch, and Vera Ott</i>	21
4 Studying Mechanosensitive Ion Channels Using Liposomes <i>Boris Martinac, Paul R. Rohde, Andrew R. Battle, Evgeny Petrov, Prithwish Pal, Alexander Fook Weng Foo, Valeria Vásquez, Thuan Huynh, and Anna Kloda</i>	31
5 Studying Amino Acid Transport Using Liposomes <i>Cesare Indiveri</i>	55
6 Use of Liposomes for Studying Interactions of Soluble Proteins with Cellular Membranes <i>Chris T. Höfer, Andreas Herrmann, and Peter Müller</i>	69
7 Liposomal Reconstitution of Monotopic Integral Membrane Proteins. <i>Zahra MirAfzali and David L. DeWitt</i>	83
8 The Reconstitution of Actin Polymerization on Liposomes <i>Mark Stamnes and Weidong Xu</i>	95
9 Electroformation of Giant Unilamellar Vesicles from Native Membranes and Organic Lipid Mixtures for the Study of Lipid Domains under Physiological Ionic-Strength Conditions. <i>L.-Ruth Montes, Hasna Abyayauch, Maitane Ibarguren, Jesus Sot, Alicia Alonso, Luis A. Bagatolli, and Felix M. Goñi</i>	105
10 Visualization of Lipid Domain-Specific Protein Sorting in Giant Unilamellar Vesicles <i>Martin Stöckl, Jörg Nikolaus, and Andreas Herrmann</i>	115
11 Biosynthesis of Proteins Inside Liposomes. <i>Pasquale Stano, Yutetsu Kuruma, Tereza Pereira de Souza, and Pier Luigi Luisi</i>	127
12 Study of Respiratory Cytochromes in Liposomes. <i>Iseli L. Nantes, Cintia Kawai, Felipe S. Pessoto, and Katia C.U. Mugnol</i>	147
13 Use of Liposomes to Evaluate the Role of Membrane Interactions on Antioxidant Activity. <i>Salette Reis, Marlene Lúcio, Marcela Segundo, and José L.F.C. Lima</i>	167

14	Studying Colloidal Aggregation Using Liposomes.	189
	<i>Juan Sabín, Gerardo Prieto, and Félix Sarmiento</i>	
15	Assessment of Liposome–Cell Interactions	199
	<i>Jan A.A.M. Kamps</i>	
16	Methods to Monitor Liposome Fusion, Permeability, and Interaction with Cells.	209
	<i>Nejat Düzgüneş, Henrique Faneca, and Maria C. Pedroso de Lima</i>	
17	The Use of Isothermal Titration Calorimetry to Study Multidrug Transport Proteins in Liposomes.	233
	<i>David Miller and Paula J. Booth</i>	
18	Studying Lipid Organization in Biological Membranes Using Liposomes and EPR Spin Labeling	247
	<i>Witold K. Subczynski, Marija Raguz, and Justyna Widomska</i>	
19	Membrane Translocation Assayed by Fluorescence Spectroscopy	271
	<i>J. Broecker and S. Keller</i>	
20	Interaction of Lipids and Ligands with Nicotinic Acetylcholine Receptor Vesicles Assessed by Electron Paramagnetic Resonance Spectroscopy.	291
	<i>Hugo Rubén Arias</i>	
21	Environmental Scanning Electron Microscope Imaging of Vesicle Systems	319
	<i>Yvonne Perrie, Habib Ali, Daniel J. Kirby, Afzal U.R. Mohammed, Sarah E. McNeil, and Anil Vangala</i>	
22	Freeze-Fracture Electron Microscopy on Domains in Lipid Mono- and Bilayer on Nano-Resolution Scale.	333
	<i>Brigitte Papahadjopoulos-Sternberg</i>	
23	Atomic Force Microscopy for the Characterization of Proteoliposomes	351
	<i>Johannes Sitterberg, Maria Manuela Gaspar, Carsten Ehrhardt, and Udo Bakowsky</i>	
24	Method of Simultaneous Analysis of Liposome Components Using HPTLC/FID.	363
	<i>Sophia Hatziantoniou and Costas Demetzos</i>	
25	Viscometric Analysis of DNA-Lipid Complexes.	369
	<i>Sadao Hirota and Nejat Düzgüneş</i>	
26	Fluorometric Analysis of Individual Cationic Lipid-DNA Complexes.	385
	<i>Edwin Pozharski</i>	
27	Fluorescence Resonance Energy Transfer-Based Analysis of Lipoplexes	393
	<i>Edwin Pozharski</i>	
28	Analysis of Lipoplex Structure and Lipid Phase Changes	399
	<i>Rumiana Koynova</i>	
29	Fluorescence Methods for Evaluating Lipoplex-Mediated Gene Delivery.	425
	<i>Henrique Faneca, Nejat Düzgüneş, and Maria C. Pedroso de Lima</i>	
30	FRET Imaging of Cells Transfected with siRNA/Liposome Complexes	439
	<i>Il-Han Kim, Anne Järve, Markus Hirsch, Roger Fischer, Michael F. Trendelenburg, Ulrich Massing, Karl Rohr, and Mark Helm</i>	
31	Spectral Bio-Imaging and Confocal Imaging of the Intracellular Distribution of Lipoplexes	457
	<i>Sebastian Schneider and Regine Süß</i>	

32	Techniques for Loading Technetium-99m and Rhenium-186/188 Radionuclides into Pre-formed Liposomes for Diagnostic Imaging and Radionuclide Therapy	469
	<i>Beth Goins, Ande Bao, and William T. Phillips</i>	
33	Fluorescence Correlation Spectroscopy for the Study of Membrane Dynamics and Organization in Giant Unilamellar Vesicles	493
	<i>Ana J. García-Sáez, Dolores C. Carrer, and Petra Schwille</i>	
34	Liposome Biodistribution via Europium Complexes	509
	<i>Nathalie Mignet and Daniel Scherman</i>	
35	Biosensor-Based Evaluation of Liposomal Binding Behavior	519
	<i>Gerd Bendas</i>	
36	Use of Liposomes to Study Vesicular Transport.	531
	<i>Kohji Takei, Hiroshi Yamada, and Tadashi Abe</i>	
	<i>Index</i>	543

Contributors

- TADASHI ABE • *Department of Neuroscience, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*
- HASNA AHYAYAUCH • *Unidad de Biofísica (CSIC-UPV/EHU), Leioa, Spain*
- HABIB ALI • *School of Life and Health Sciences, Aston University, Birmingham, UK*
- ALICIA ALONSO • *Unidad de Biofísica (CSIC-UPV/EHU), Leioa, Spain*
- HUGO RUBÉN ARIAS • *Department of Pharmaceutical Sciences, College of Pharmacy, Midwestern University, Glendale, AZ, USA*
- LUIS A. BAGATOLLI • *Unidad de Biofísica (CSIC-UPV/EHU), Leioa, Spain*
- UDO BAKOWSKY • *Department of Pharmaceutical Technology and Biopharmacy, Philipps-Universität Marburg, Marburg, Germany*
- ANDE BAO • *Department of Radiology, University of Texas Health Science Center, San Antonio, TX, USA*
- ANDREW R. BATTLE • *Molecular Biophysics Laboratory, School of Biomedical Sciences and Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia*
- GERD BENDAS • *Department of Pharmacy, Rheinische Friedrich Wilhelms University Bonn, Bonn, Germany*
- PAULA J. BOOTH • *Department of Biochemistry, University of Bristol, Bristol, UK*
- JANA BROECKER • *Leibniz Institute of Molecular Pharmacology FMP, Berlin, Germany*
- DOLORES C. CARRER • *BIOTEC, Technische Universität Dresden, Dresden, Germany*
- COSTAS DEMETZOS • *Department of Pharmaceutical Technology, School of Pharmacy, University of Athens, Athens, Greece*
- DAVID L. DEWITT • *Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA*
- NEJAT DÜZGÜNEŞ • *Department of Microbiology, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA, USA*
- CARSTEN EHRHARDT • *School of Pharmacy and Pharmaceutical Sciences, University of Dublin, Trinity College Dublin, Dublin, Ireland*
- ALFRED FAHR • *Department of Pharmaceutics, Friedrich-Schiller-University, Jena, Germany*
- HENRIQUE FANECA • *Faculty of Science and Technology, Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal*
- ROGER FISCHER • *German Cancer Research Center (DKFZ), Heidelberg, Germany*
- ALEXANDER FOOK WENG FOO • *Molecular Biophysics Laboratory, School of Biomedical Sciences and Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia*
- ANA J. GARCÍA-SÁEZ • *BIOTEC, Technische Universität Dresden, Dresden, Germany*
- MARIA MANUELA GASPAR • *Unidade Novas Formas de Agentes Bioativos, iMed, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal*

- BETH GOINS • *Department of Radiology, University of Texas Health Science Center, San Antonio, TX, USA*
- FELIX M. GOÑI • *Unidad de Biofísica (CSIC-UPV/EHU), Leioa, Spain*
- SOPHIA HATZIANTONIOU • *Department of Pharmaceutical Technology, School of Pharmacy, University of Athens, Athens, Greece*
- MARK HELM • *Department of Chemistry, Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Heidelberg, Germany*
- ANDREAS HERRMANN • *Mathematisch-Naturwissenschaftliche Fakultät I, Institut für Biologie/Biophysik, Humboldt Universität zu Berlin, Berlin, Germany*
- SADAO HIROTA • *Department of Material Science, School of Engineering, Tokyo Denki University, Tokyo, Japan*
- MARKUS HIRSCH • *Department of Chemistry, Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Heidelberg, Germany*
- CHRIS HÖFER • *Institut für Biologie/Biophysik, Humboldt Universität zu Berlin, Berlin, Germany*
- THUAN HUYNH • *Molecular Biophysics Laboratory, School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia*
- MAITANE IBARGUREN • *Unidad de Biofísica (CSIC-UPV/EHU), Leioa, Spain*
- CESARE INDIVERI • *Dipartimento di Biologia Cellulare, Università della Calabria, Arcavacata di Rende, CS, Italy*
- ANNE JÄRVE • *Department of Chemistry, Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Heidelberg, Germany*
- JAN A.A.M. KAMPS • *Laboratory for Endothelial Biomedicine & Vascular Drug Targeting Research, Medical Biology Section, Department Pathology & Medical Biology, University Medical Center Groningen, Groningen, The Netherlands*
- CINTIA KAWAI • *Centro Interdisciplinar de Investigação Bioquímica CIIB, Universidade de Mogi das Cruzes, Mogi das Cruzes, S.P., Brazil*
- SANDRO KELLER • *Leibniz Institute of Molecular Pharmacology FMP, Berlin, Germany*
- IL-HAN KIM • *Department of Bioinformatics and Functional Genomics, German Cancer Research Center (DKFZ), Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Heidelberg, Germany*
- DANIEL J. KIRBY • *School of Life and Health Sciences, Aston University, Birmingham, UK*
- ANNA KLODA • *Molecular Biophysics Laboratory, School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia*
- RUMIANA KOYNOVA • *Northwestern University, Evanston, IL, USA*
- REINHARD KRÄMER • *Institute of Biochemistry, University of Cologne, Cologne, Germany*
- YUTETSU KURUMA • *“Enrico Fermi” Study and Research Center, Rome, Italy*
- JOSÉ L.F.C. LIMA • *REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal*
- XIANGLI LIU • *Department of Pharmaceutics, Friedrich-Schiller-University, Jena, Germany*
- MARLENE LÚCIO • *REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal*
- PIER LUIGI LUISI • *Biology Department, University of RomaTre, Rome, Italy*

- BORIS MARTINAC • *Molecular Biophysics Laboratory, School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia*
- ULRICH MASSING • *Department of Clinical Research, Tumor Biology Center, Freiburg, Germany*
- SARAH E. MCNEIL • *School of Life and Health Sciences, Aston University, Birmingham, UK*
- NATHALIE MIGNET • *Unité de Pharmacologie Chimique et Génétique; CNRS, UMR 8151, Paris, France; Inserm, U 640, Paris, France; Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes, Paris, France; ENSCP, Paris, France*
- DAVID MILLER • *Department of Biochemistry, University of Bristol, Bristol, UK*
- ZAHRA MIRAFZALI • *Encapsula NanoSciences LLC, 441 Donelson, Pike, Suite 345, Nashville, TN 37214, USA*
- AFZAL U. R. MOHAMMED • *School of Life and Health Sciences, Aston University, Birmingham, UK*
- L.-RUTH MONTES • *Unidad de Biofísica (CSIC-UPV/EHU), Leioa, Spain*
- KATIA C.U. MUGNOL • *Centro Interdisciplinar de Investigação Bioquímica CIIB, Universidade de Mogi das Cruzes, Mogi das Cruzes, S.P., Brazil*
- PETER MÜLLER • *Institut für Biologie/Biophysik, Humboldt Universität zu Berlin, Berlin, Germany*
- ISELI L. NANTES • *Centro Interdisciplinar de Investigação Bioquímica CIIB, Universidade de Mogi das Cruzes, Mogi das Cruzes, S.P., Brazil*
- SASCHA NICKLISCH • *Institute of Biochemistry, University of Cologne, Cologne, Germany*
- JÖRG NIKOLAUS • *Mathematisch-Naturwissenschaftliche Fakultät I, Institut für Biologie/Biophysik, Humboldt-Universität zu Berlin, Berlin, Germany*
- VERA OTT • *Institute of Biochemistry, University of Cologne, Cologne, Germany*
- PRITHWISH PAL • *Molecular Biophysics Laboratory, School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia*
- BRIGITTE PAPAHAJDOPOULOS-STERNBERG • *NanoAnalytical Laboratory, San Francisco, CA, USA*
- MARIA C. PEDROSO DE LIMA • *Department of Biochemistry, Faculty of Science and Technology, Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal*
- TEREZA PEREIRA DE SOUZA • *Biology Department, University of RomaTre, Rome, Italy*
- YVONNE PERRIE • *School of Life and Health Sciences, Aston University, Birmingham, UK*
- REGINE SÜSS • *Department of Pharmaceutical Technology and Biopharmacy, Albert-Ludwigs University, Freiburg, Germany*
- FELIPE S. PESSOTO • *Centro Interdisciplinar de Investigação Bioquímica CIIB, Universidade de Mogi das Cruzes, Mogi das Cruzes, S.P., Brazil*
- EVGENY PETROV • *Molecular Biophysics Laboratory, School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia*
- WILLIAM T. PHILLIPS • *Department of Radiology, University of Texas Health Science Center, San Antonio, TX, USA*
- EDWIN POZHARSKI • *Department of Pharmaceutical Sciences, University of Maryland, Baltimore, MD, USA*

- GERARDO PRIETO • *Biophysics and Interfaces Group, Department of Applied Physics, Faculty of Physics, University of Santiago de Compostela, Santiago de Compostela, Spain*
- MARIJA RAGUZ • *Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI, USA*
- JAMES R. REED • *Department of Pharmacology, Louisiana State University Health Science Center, New Orleans, LA, USA*
- SALETTE REIS • *REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal*
- PAUL R. ROHDE • *Molecular Biophysics Laboratory, School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia*
- KARL ROHR • *Department of Bioinformatics and Functional Genomics, German Cancer Research Center (DKFZ), Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Heidelberg, Germany*
- JUAN SABÍN • *Biophysics and Interfaces Group, Department of Applied Physics, Faculty of Physics, University of Santiago de Compostela, Santiago de Compostela, Spain*
- FÉLIX SARMIENTO • *Biophysics and Interfaces Group, Department of Applied Physics, Faculty of Physics, University of Santiago de Compostela, Santiago de Compostela, Spain*
- DANIEL SCHERMAN • *Unité de Pharmacologie Chimique et Génétique; CNRS, UMR 8151, Paris, France; Inserm, U 640, Paris, France; Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes, Paris, France; ENSCP, Paris, France*
- SEBASTIAN SCHNEIDER • *Department of Pharmaceutical Technology and Biopharmacy, Albert-Ludwigs University, Freiburg, Germany*
- PETRA SCHWILLE • *BIOTEC, Technische Universität Dresden, Dresden, Germany*
- MARCELA SEGUNDO • *REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal*
- JOHANNES SITTERBERG • *Department of Pharmaceutical Technology and Biopharmacy, Philipps-Universität Marburg, Marburg, Germany*
- JESUS SOT • *Unidad de Biofísica (CSIC-UPV/EHU), Leioa, Spain*
- MARK STAMNES • *Department of Molecular Physiology & Biophysics, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA*
- PASQUALE STANO • *Biology Department, University of RomaTre, Rome, Italy*
- MARTIN STÖCKL • *Mathematisch-Naturwissenschaftliche Fakultät I, Institut für Biologie/Biophysik, Humboldt-Universität zu Berlin, Berlin, Germany*
- WITOLD K. SUBCZYNSKI • *Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI, USA*
- KOHI TAKEI • *Department of Neuroscience, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*
- MICHAEL F. TRENDLENBURG • *German Cancer Research Center (DKFZ), Heidelberg, Germany*

ANIL VANGALA • *School of Pharmacy and Chemistry, Kingston University,
London, UK*

VALERIA VÁSQUEZ • *Biochemistry Department, Gordon Center for Integrative Science,
The University of Chicago, Chicago, IL, USA*

JUSTYNA WIDOMSKA • *Department of Plant Physiology and Biochemistry, Faculty of
Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland*

WEIDONG XU • *Department of Molecular Physiology & Biophysics, Roy J. and Lucille
A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA*

HIROSHI YAMADA • *Department of Neuroscience, Okayama University Graduate
School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*