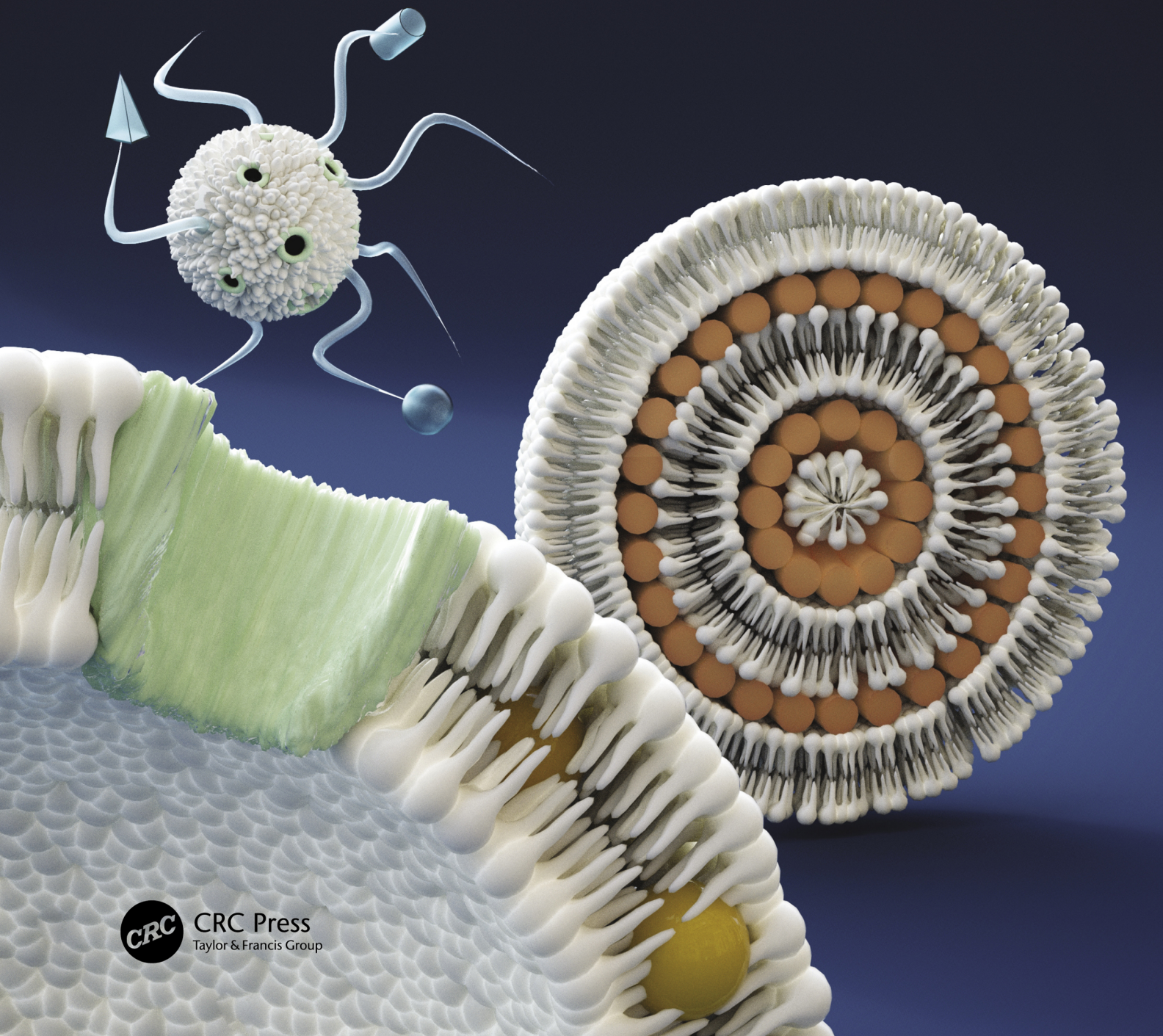
edited by Cyrus R. Safinya & Joachim O. Rädler

Handbook of Lipid Membranes

Molecular, Functional, and Materials Aspects



CRC Press
Taylor & Francis Group

Handbook of Lipid Membranes

Handbook of Lipid Membranes

Molecular, Functional, and Materials Aspects

Edited by
Cyrus R. Safinya
Joachim O. Rädler

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

First Edition published 2021
by CRC Press
6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL 33487-2742

and by CRC Press
2 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN

CRC Press is an imprint of Taylor & Francis Group, LLC

ISBN: 978-1-466-55572-3 (hbk)
ISBN: 978-1-032-01441-8 (pbk)
ISBN: 978-0-429-19407-8 (ebk)

Typeset in Times
by codeMantra

Contents

Preface

Lipid membrane science has evolved from its roots in biochemistry and biophysics to become an essential component in the emerging interdisciplinary fields of nanobioscience and nanobiotechnology, with new everyday applications appearing at an ever-increasing rate. This shift calls for a new type of introductory-level reading material spanning multiple disciplines and appropriate for both beginners and advanced researchers. The *Handbook of Lipid Membranes: Molecular, Functional, and Materials Aspects* offers a multifaceted perspective, leading the reader through membrane-related processes in reconstituted and living matter systems. It teaches how to learn from nature to build artificial and functional membrane-based systems, including those not based on lipids. The emphasis is on the science and technology of lipids and artificial membranes and includes chapters on advanced X-ray and force measurement techniques. The 19 chapters are simultaneously self-contained yet connected with common themes running between the chapters. Included are chapters covering the role of lipids as structural components in determining distinct membrane shapes, topologies and inter-membrane interactions. By making a direct connection to cellular systems, membrane curvature generation, mediated by peptide and protein binding, and biological signaling lipids in concert with cytoskeletal proteins are presented. Beyond the science in lipid biology and biophysics, the chapters reveal how mastering lipid interactions enables novel biomedical and nanotechnological applications with fine-tuned functionality. Subject-based chapters discuss current applications in biomedicine, cosmetics and nanotechnology. This includes lipid vectors in nucleic acid and drug delivery, in dermal applications, and in artificial biointerfaces and lipid-based sensors. A final engaging chapter takes the reader to the beginning of where it all started and explores the role of lipids in the origin of life.

The discovery of liposomes by A. D. Bangham and R. W. Horne in the 1960s with efficient encapsulation properties and striking similarities in electron micrographs to cell plasma membranes led to their hypothesis that lipids are the primary structural components responsible for the permeability barriers of biological membranes. This major discovery led the way for a rapid worldwide increase in research on the biophysical properties of lipid vesicles and on the biological function of membrane-associated proteins, reconstituted in liposomes. The realization that liposomes contain sites for incorporation of hydrophobic, hydrophilic and amphiphilic molecules, led researchers early on to investigate, on the one hand, their promise as components of personal care products and, on the other hand, as carriers of drugs and genes for therapeutic applications. The latter includes over 100 lipid-based currently-ongoing clinical trials worldwide targeting a single gene, such as cystic fibrosis, and multigene cancer diseases. Most remarkably, we now see a flourishing of lipid-based science opening new approaches to vaccines with the Moderna and Pfizer/BioNTech lipid-mRNA nanoparticles saving millions of lives in the worldwide pandemic in 2020 and 2021 due to COVID-19.

This handbook brings together contributions from world experts in lipid and artificial membrane science and applications. In the first chapter, Erich Sackmann and Avinoam Ben-Shaul provide a historical survey of membrane physics from the perspective of two-dimensional self-assembling systems consisting of interacting lipids and membrane-associated proteins. The development of certain key novel experimental techniques and theoretical approaches over the last few decades, which have contributed to our current understanding of membrane physics, is discussed. The significance of lipid mobility, membrane defects and membrane curvature elasticity, to membrane thermomechanical properties, and the impact on biological membrane structure and function are described.

The following two chapters start out by introducing lipid membrane structure and phase behavior. Gregory Smith and Cyrus Safinya introduce the phase diagram of gel-like ordered membranes, consisting of lipids with phosphocholine headgroups, in Chapter 2. X-ray scattering and reflectometry techniques are described that determine the intra- and intermolecular lipid arrangement and probe interlayer interactions by analysis of thermal diffuse scattering. The chapter outlines how structural studies of highly oriented freely suspended multilayer preparations have revealed that the gel phase is comprised of three phases distinguished by the direction of chain tilt within the two-dimensional ordered membrane. Stacks of fluid membranes are analyzed within the context of a Landau–De Gennes type elastic energy displaying characteristics of a low-dimensional Landau–Peierls system. Synchrotron X-ray scattering shows that such very dilute fluid lamellar phases are stabilized by entropically mediated undulation forces elucidated by Wolfgang Helfrich.

Chapter 3 by Charlotte Conn and John Seddon continues with a comprehensive description of the structure and properties of cubic and inverse hexagonal phases. The authors describe how the stability of these non-lamellar phases is derived from forces underlying curvature and topological transformations in lipid membranes. Further, the effect of hydrostatic pressure on phase behavior is discussed and shown to be relevant in marine biology. Numerous illuminating examples are presented that show the occurrence of non-lamellar phases in living matter and in a wide range of basic science studies and bionanotechnological applications. This includes cubic and hexagonal phase structures enabling specific functions, *in vivo*, and, as structured drug delivery vectors, cubic phases to enable membrane protein structure determination.

In Chapter 4, Tim Salditt gives a summary of state-of-the-art X-ray analysis of highly oriented lipid membranes employing X-ray optics, nanoscale focusing, time-resolved X-ray diffraction, and lensless coherent imaging. Illustrations of these powerful new synchrotron X-ray techniques are shown as applied to a wide range of systems of high scientific interest, including nonequilibrium dynamics of membranes, short-lived intermediate structures of membrane fusion, and the asymmetric structures of synaptic vesicles and myelin multilayers. The chapter concludes by presenting coherent X-ray imaging as a powerful new method for membrane structure analysis.

Deborah Leckband introduces the reader to the surface forces apparatus (SFA) in Chapter 5, a unique technique that enables direct measurement of forces between surface-bound membranes containing membrane-associated proteins, including intrinsically disordered proteins. The SFA technique, with biological material contained between surfaces, naturally mimics the confined environment *in vivo*. Thus, measured protein-mediated forces, both attractive and repulsive, are expected to elucidate membrane–protein function at biological interfaces. Forces mediated by adhesion proteins, including lectins that bind carbohydrates, neural cell adhesion molecules, and cadherin adhesion proteins, ubiquitous in most tissues, are highlighted. The chapter further describes cell-binding kinetics employing micropipette measurement techniques.

Chapter 6 by Tomer Markovich, David Andelman and Rudolf Podgornik is an entirely self-contained theoretical chapter covering our current understanding of electrostatic forces in charged membrane systems. Poisson–Boltzmann (PB) theory of charged membranes, in the presence of no added salt and added salt, is described. Ion profiles near single and between two membranes are considered. As becomes evident, the electrostatic forces described in the chapter are relevant to many of the biological systems described in the handbook. The chapter further reviews van der Waals forces and briefly discusses the limitations of PB theory, including the limit of high surface charge and multivalent counterions, where the PB theory breaks down due to its mean-field nature. The material is presented in a manner suitable for instructors teaching at the first-year graduate or upper-division undergraduate levels.

The key physical concepts underlying distinct vesicle shapes are lipid molecule shape, membrane composition, and intermolecular interactions. In Chapter 7, Alexandra Zidovska takes the reader on a broad review of membrane shape evolution in vesicles (i.e., liposomes) as discovered in laboratories worldwide over the last few decades. The chapter includes sections connecting modern elasticity models to shape evolution observed experimentally. This includes the hugely successful Helfrich curvature elastic model and beyond Helfrich theory with models that incorporate the intrinsic area differences between the outer and inner membrane leaflets due to the finite curvature of liposomes. The chapter concludes by connecting *in vitro* to *in vivo* observations where many similar shapes have been observed.

Michelle Lee, Nathan Schmidt and Gerard Wong take the concept of membrane curvature further in Chapter 8 and discuss how peptides modulate membrane morphology. Membrane binding peptides play a vital role as antimicrobial agents, enabling budding and release of viruses and enabling membrane tubulation and fission. The chapter provides a survey of membrane curvature generation mechanisms and illustrates the case of antimicrobial peptides. The authors explain how certain cationic and hydrophobic structural motifs of antimicrobial peptides lead to selective pore-forming activity against bacterial membranes. Thus, as the authors explain, intelligently designed alterations in amino-acid sequence, intended to enhance peptide membrane-permeating ability, may be implemented in the development of novel antibiotics to fight persistent bacterial strains.

In Chapter 9, David Slochower, Yu-Hsiu Wang, Ravi Radhakrishnan and Paul Janmey describe how the shape of eukaryotic cell membranes is controlled by both the plasma membrane lipid bilayer and the underlying cytoskeletal network. At a molecular level, the role of phosphoinositides in regulating the membrane–cytoskeletal interface, by binding cytoskeletal proteins and proteins that cause or sense membrane curvature, is described. In parallel, mechanisms of producing membrane curvature driven by the cytoskeleton are introduced. Combining this information within the context of recent experimental results and multiscale simulations, the authors present an integrated view of how these fascinating lipids perform their many cellular functions and help orchestrate the dynamic changes in membrane curvature.

The effect of osmotic stress on cell membrane topology is the focus of Chapter 10 by James Ho, Bo Liedberg and Atul Parikh. Starting with giant unilamellar vesicles, as model membrane compartments, the permeation of water and the generation of osmotic gradients by hindered permeation of solutes is introduced. The chapter gives experimental and theoretical insights into shape changes of flaccid vesicles in hypertonic media and transient pore formation in response to hypotonic media. Shape deformations due to osmotic stress coupling with phase separation of multicomponent vesicles are also discussed. Finally, the potential relevance of stress relaxation mechanisms is discussed within the context of ubiquitous osmotic challenges that primitive protocells most likely experienced in early life.

Lipid-nucleic acid complexes are self-assembled systems that incorporate key concepts of lipid molecule shape and membrane curvature discussed in previous chapters. Their scientific perspective and applications *in vitro* and *in vivo* are described in three consecutive chapters. Chapter 11, by Cyrus Safinya, Kai Ewert, Youli Li and Joachim Rädler, introduces the self-assembled structures of cationic liposome (CL)-nucleic acid complexes used in gene delivery. The authors explain how concepts of membrane curvature and electrostatic interactions explain the formation of liquid crystalline inverse hexagonal, lamellar and hexagonal phases with DNA residing in one, two, or three dimensions,

respectively. The chapter describes how transfection efficiency, measuring the expression of DNA transferred by CL complexes into cells, depends on the underlying structures of the complexes. The concepts presented in the chapter are expected to apply to ongoing efforts to understand the physics of self-assembly in cationic ionizable lipid-mRNA formulations used in cancer therapeutics and vaccine applications (e.g., Moderna and Pfizer/BioNTech nanoparticle vaccines).

In Chapter 12, Tyler Goodwin and Leaf Huang cover lipids as delivery vehicles of DNA, RNA and peptides in *in vivo* therapeutic applications. The authors describe how lipid vectors may be designed to be highly efficient in the delivery of biomacromolecules. Based on rational design over many years, lipid vectors were developed to overcome extracellular and intracellular barriers. The chapter reviews the improvements and the remaining challenges of current lipid vectors in *in vivo* applications. The survey highlights promising ionizable lipids and composite nanocore-lipid vectors and discusses the progress made in clinical trials.

Chapter 13 turns to theory, where electrostatic models underlying self-assembly of charged membranes and oppositely charged biomacromolecules, including DNA, proteins and peptides, are reviewed by Guilherme Volpe Bossa, Klemen Bohinc and Sylvio May. The influence of lipid mobility, protein-mediated lipid phase separation and charge regulation on the interactions stabilizing proteins adsorbed on membranes is discussed. Theoretical models of the phase behavior of lipid-DNA complexes are reviewed for cationic and zwitterionic lipids. Discussions on important local interactions between neutral zwitterionic lipid headgroups and divalent cations and DNA and membranes containing zwitterionic lipids are presented. The chapter concludes with a general discussion of the stability of membrane pores resulting from the interactions between amphiphilic peptides and membranes.

Lipid membranes are powerful in functionalizing or protecting surfaces. Marta Bally, Hudson Pace and Fredrik Höök give an account of the use of planar lipid membranes as bioanalytical sensors in Chapter 14. The chapter focuses on a broad range of surface-based biosensing techniques. Immobilization of lipid assemblies at a sensor surface is achieved by a supported lipid bilayer, tethered bilayers, or a free-spanning bilayer of surface-tethered vesicles. Moreover, patterned planar membranes are used for high-throughput screening and lab-on-a-chip devices. The authors show how membrane-associated proteins may refine biosensing to detect interactions between membrane proteins and ligands. Discussions are also presented about how measurements of membrane–membrane interactions in biosensors have been enhanced by the invention of single-vesicle assays that determine equilibrium-binding constants from statistics of residence times. An outlook on the use of lipid-based nanoreactors and force-driven manipulation of membrane components in supported lipid bilayers for chip-based bioanalytics is presented.

Chapter 15, by Jérôme Bibette and Abdou Rachid Thiam, consists of a comprehensive overview of the structure and function of the skin and novel dermal applications in cosmetics and pharmaceutics. The role of lipids in dermal health and the downside consequences of lipid disorders on skin function are reviewed. The chapter contains a wealth of information on the molecular components of cosmetic and pharmaceutical products. The authors describe the state of the art in cosmetic and therapeutic dermal applications, through rational design of lipid and surfactant-based vectors, optimized for delivery through the skin. Current lipid nanovectors used worldwide are described. This includes liposome formulations, nano- and micro-emulsions, and solid lipid nanoparticles.

Chapter 16 by Theo Lohmüller, Bert Nickel and Joachim Rädler is dedicated to the properties and applications of so-called supported lipid bilayer systems, which represent planar lipid bilayers that form at the solid–fluid interface through vesicle fusion and in-plane spreading. In these systems, lipids and incorporated proteins retain high lateral mobility. Hence, charged macromolecules that bind to oppositely charged planar-supported bilayer systems exhibit ideal two-dimensional properties and can be manipulated in-plane through external electric fields. In polymer-supported lipid bilayer systems, the membrane is elevated from the solid, providing room for larger transmembrane proteins. The chapter concludes with examples of the use of supported lipid bilayer systems as novel platforms for presenting molecules to living cells.

In the last two decades, increasing research efforts have been directed toward developing artificial membranes that are not based on lipids.

Chapter 17 by Timothy Deming summarizes advances in the synthesis of well-defined block and statistical copolypeptides that can be assembled into stable membrane structures. Polymerization chemistries are described that allow precision copolypeptide synthesis with control over chain length, chain length distribution, and chain-end functionality. The author explains how well-defined copolypeptides of controlled dimensions, including molecular weight, sequence, and composition, can be prepared, which spontaneously assemble into vesicles with polypeptide membranes. Examples of polypeptide membrane vesicles possessing unique properties (due to the amino acid building blocks and ordered conformations of the polypeptide segments) are presented in the context of biomedical application.

In Chapter 18, Daniel Hammer, Zhichun Wang, Ellen Reed, Chen Gao and Kevin Vargo give a comprehensive overview of membranes constructed with synthetic amphiphilic polymers and biopolymers with novel architectures. The focus of the chapter is on the science and technological applications of artificial vesicles made from spontaneous assembly of block copolymers (polymersomes) and copolypeptides, recombinant proteins and amphiphilic dendrimers. The methods for producing self-assembling amphipathic proteins employing modern recombinant

technology are described. The chapter concludes with the latest developments in the construction of artificial vesicles from glycan-based Janus dendrimers. The novel artificial vesicles described in this chapter promise to open new opportunities for the intelligent design of functional nanoparticles with a broad range of applications, including, in molecular delivery and sensing.

The final Chapter 19, by Christos Georgiou and David Deamer, gives a fascinating presentation of some central aspects of the origin of life and, in particular, emphasizes the role played by single-tailed amphiphilic fatty acids and double-tailed lipids. The authors consider alkaline hydrothermal vents and hydrothermal fields as possible geological sites for the prebiotic environment for the spontaneous formation of life around four billion years ago. Discussions are presented about the physical and chemical properties of lipids, which would have been essential in the spontaneous assembly and formation of very simple early membranes with their required barrier properties in comparison with the plasma membrane of current living organisms containing a range of associated proteins designed for a diverse set of functions. The authors conclude with a thought-provoking discussion of the positive and negative aspects of hydrothermal fields versus hydrothermal vents as locations for the onset of the first cellular life on earth.

Cyrus R. Safinya
Joachim O. Rädler

Editors

Cyrus R. Safinya is a Distinguished Professor at the University of California, Santa Barbara (UCSB). His primary appointment is in the Materials Department in the College of Engineering and he has joint appointments in the Molecular, Cellular, and Developmental Biology Department, and, by courtesy, in the Physics Department and the Biomolecular Science and Engineering Program. He received a B.S. in Physics and Mathematics from Bates College in 1975 and a PhD in Physics from the Massachusetts Institute of Technology in 1981 for his studies on liquid crystal phase transitions under the guidance of Robert J. Birgeneau (currently Chancellor Emeritus and Distinguished Professor of Physics and Materials Science at the University of California, Berkeley). Safinya joined the Exxon Research & Engineering Company immediately after obtaining his PhD and started his research studies on the structure of complex fluids and biological membranes before moving to UCSB in 1992. He was a Rothschild Fellow and a Visiting Directeur de Recherche at the Curie Institute in 1994 and, between 2009 and 2013, a Distinguished Visiting Professor at the Korean Advanced Institute of Science & Technology. Safinya is the author or co-author of more than 220 publications, many of which have appeared in journals on research topics in biophysics, chemical physics, physical chemistry, bioengineering, and biomedical sciences. His current research aims to elucidate structures and interactions in soft and biological matter systems, including, in lipid-nucleic acid mixtures and protein assemblies derived from neurons. In parallel, his group works on the development of novel lipid vectors as carriers of nucleic acid (DNA and RNA) and hydrophobic drugs in gene and cancer therapeutics.

Joachim O. Rädler is a Professor of Experimental Physics at Ludwig-Maximilians-University (LMU), and holds the Chair for Soft Condensed Matter. He studied Physics at the Friedrich Wilhelms University in Bonn, at Cambridge University (UK) and at the Technical University of Munich. In 1993, he received his doctoral degree in Biophysics for his work on vesicle adhesion under the guidance of Erich Sackmann. As a post-doctoral fellow during 1993–1996, he studied cationic lipid-DNA complexes with Cyrus Safinya at UC Santa Barbara. Rädler received his habilitation in Experimental Physics at Technical University of Munich, where he worked on supported membranes. In 2000, he was appointed as a senior group leader at the Max Planck Institute for Polymer Research. He was named full professor at LMU in 2001 and became a member of the Center for NanoScience in the same year. From 2008 to 2011, he held a temporary consulting and teaching position for experimental NanoBio physics at the University College Dublin. He served as spokesperson of the Center for NanoScience at LMU, of the collaborative research center "Nanoagents" and of various graduate programs. He is the author or co-author of over 180 publications on research topics in soft matter and biophysics, physical chemistry, chemical physics, bioengineering, and bionanotechnology. His current research focuses on the self-assembly of siRNA and mRNA lipid nanoparticles, the interaction of nanomaterials with living cells, time-resolved studies of single-cell gene expression, and the physics of cell migration.