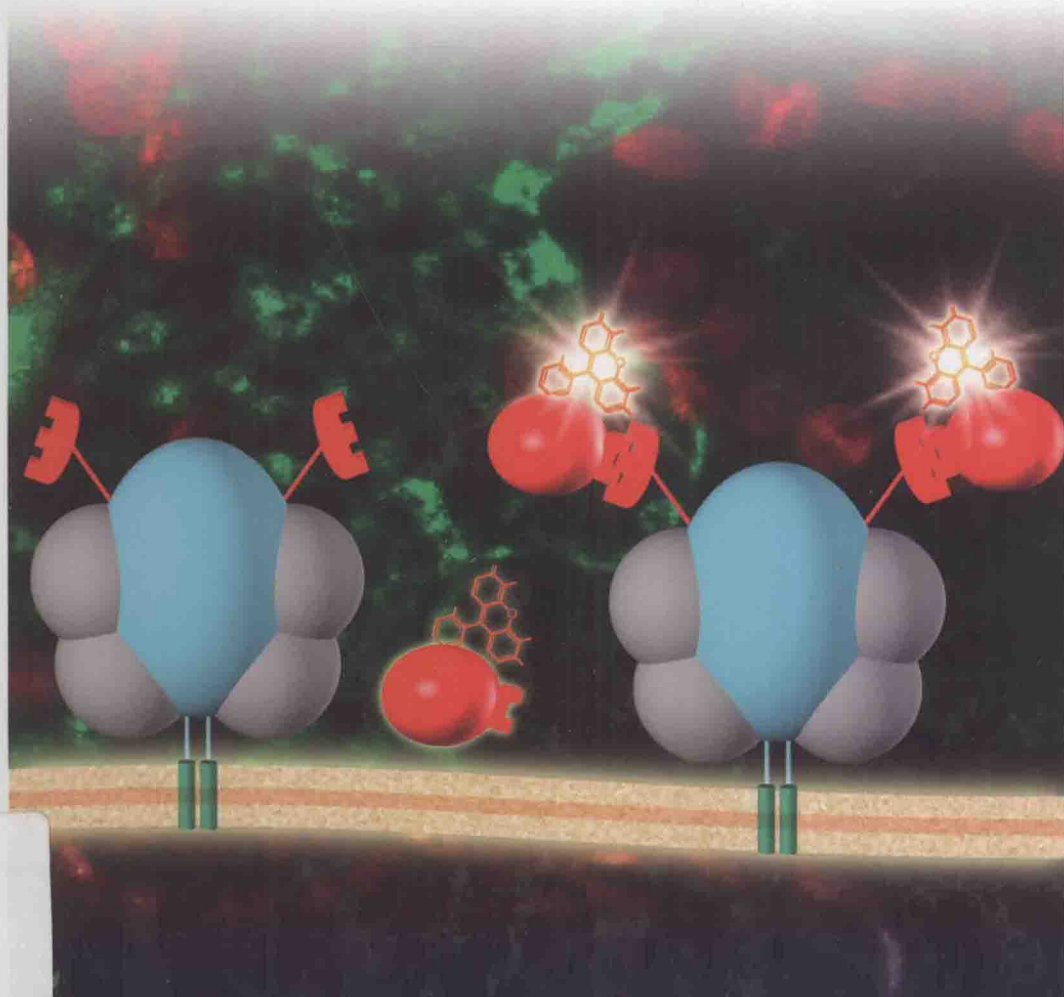


Edited by Lawrence W. Miller

 WILEY-VCH

# Probes and Tags to Study Biomolecular Function

for Proteins, RNA, and Membranes



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*Edited by*  
*Lawrence W. Miller*



WILEY-VCH Verlag GmbH & Co. KGaA

#### The Editor

**Prof. Dr. Lawrence W. Miller**  
Department of Chemistry  
University of Illinois  
845 West Taylor Street  
Chicago, IL 60607-7061  
USA

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**Library of Congress Card No.:** applied for

#### **British Library Cataloguing-in-Publication Data**

A catalogue record for this book is available from the British Library.

#### **Bibliographic information published by the Deutsche Nationalbibliothek**

Die Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at <http://dnb.d-nb.de>.

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

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**Typesetting** Thomson Digital, Noida, India

**Printing** betz-druck GmbH, Darmstadt

**Binding** Litges & Dopf Buchbinderei GmbH, Heppenheim

**Cover Design** Grafik-Design Schulz, Fußgönheim

Printed in the Federal Republic of Germany

Printed on acid-free paper

**ISBN:** 978-3-527-31566-6

**Probes and Tags to Study  
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## Preface

A central challenge of biochemistry and molecular cell biology is to understand how biomolecules interact and react to organize and control cell growth, structure and function. The contributions in this book reveal many ways in which the technologies of specific chemical and genetic labeling can be used in conjunction with optical microscopy to dynamically analyze the spatial and temporal organization of proteins, lipids, and even messenger RNA in single living cells.

More than half a century of progress in biochemistry, genetics, molecular biology and cellular physiology has yielded a rich understanding of the macromolecular basis of structure and information flow in living systems. Complete genomes have been sequenced, tens of thousands of protein structures have been determined, and the activities and functions of thousands of enzymes have been analyzed. Over the past decade, methods that allow for the real-time analysis of molecular function in the environment of the living cell or organism have come to prominence. The ability to dynamically and non-destructively image the translocations, interactions or reactions of one or more chemically unique biomolecules affords a mechanistic understanding of cellular function that is not accessible using traditional biochemical assays.

Selectivity is the unifying idea that runs throughout this book's chapters. *In vivo* studies require non-invasive methods of imparting unique optical or chemical functionalities to particular molecular species. One strategy is to prepare a soluble probe molecule that is fluorescent or otherwise detectable, but which retains the same cellular localization and biological function of the unlabeled species. The other general strategy is to genetically label a protein with a functional tag. This tag can be one of many commonly used autofluorescent proteins, or it can be a receptor protein or polypeptide that binds to a soluble, cell-permeable, small molecule that has the desired functionality.

The first three contributions in this volume describe various strategies for probing the function of the lipid bilayer. The spatiotemporal dynamics of phosphoinositides and their role in activating signaling cascades or membrane trafficking events can be microscopically visualized by expressing fluorescent proteins fused to phosphoinositide-binding motifs. The organization of cholesterol and sphingolipids

into discrete microdomains within the lipid bilayer can be studied with two approaches described herein. Naturally occurring and synthetic fluorescent analogues of cholesterol can be incorporated directly into the lipid bilayer of living cells, allowing microscopic visualization of cholesterol-rich microdomains. Alternatively, lipid-binding toxins can be used to label and detect sphingolipids, cholesterol and GPI-anchored proteins.

Several contributors offer experimental strategies for appending specific proteins with synthetically optimized small molecules. Proteins of biological interest can be genetically encoded as fusions to receptor proteins or polypeptides. Cell-permeable ligands can be synthesized with a variety of functionalities, including enhanced fluorescence, photoaffinity or analyte sensing capability. Upon addition to culture medium, these ligands can bind specifically and stably to the fusion protein chimeras via high-affinity non-covalent interaction or enzyme-mediated covalent linkage. The smallest possible functional tag for a protein is a single amino acid. A comprehensive description of methods for incorporating unnatural amino acids with specific chemical or optical properties into proteins, both *in vitro* and *in vivo*, is included.

There are comparatively few robust methods for the sequence-specific labeling of nucleic acid species in living cells or organisms. Fusion of bacteriophage MS2 coat protein to fluorescent proteins allows labeling of messenger RNA containing the cognate MS2 hairpin binding site. Tagged mRNAs can be microscopically visualized in living mammalian cells with single transcript resolution. A thorough description of this technique is provided in the final chapter of this volume.

The contributions to *Probes and Tags to Study Biomolecular Function* reflect the efforts of chemists and biologists to bring the study of biochemistry from the test tube to the living cell. Taken together, the experimental tools described in this volume reflect the state-of-the-art in technologies to label biomolecules for *in vivo* studies. The step-by-step protocols and illustrations of typical applications will enable researchers to select the best solution for their experimental problems. Looking ahead, the real-time analysis of molecular function within living cells will continue to be an indispensable approach to studying mechanistic biology. Our contributors have laid a foundation for the future development of more robust and selective labeling technologies that should allow for *in vivo* detection with even greater sensitivity and spatio-temporal resolution.

Chicago, 2008

Lawrence W. Miller

## List of Contributors

### **Martin Andresen**

Max Planck Institute for Biophysical  
Chemistry  
Mitochondrial Structure and Dynamics  
Group  
Department of NanoBiophotonics  
Am Fassberg 11  
37077 Goettingen  
Germany

### **Barbara P. Atshaves**

Texas A&M University  
Department of Physiology and  
Pharmacology  
TVMC  
College Station  
TX 77843-4466  
USA

### **Robert Bittman**

Queens College  
Department of Chemistry and  
Biochemistry  
CUNY  
Flushing  
NY 11367-1597  
USA

### **Nediljko Budisa**

Max Planck Institute of Biochemistry  
BioFuture Independent Research Group  
Molecular Biotechnology  
Am Klopferspitz 18  
82152 Martinsried  
Germany

### **Jeffrey A. Chao**

Albert Einstein College of Medicine  
Department of Anatomy and Structural  
Biology  
1300 Morris Park Avenue  
Bronx NY 10461  
USA

### **Virginia W. Cornish**

Columbia University  
Department of Chemistry  
3000 Broadway  
MC 3111  
New York  
NY 10027  
USA

### **Kevin Czaplinski**

Albert Einstein College of Medicine  
Department of Anatomy and Structural  
Biology  
1300 Morris Park Avenue  
Bronx NY 10461  
USA



**Adalberto M. Gallegos**

Texas A&M University  
Department of Pathobiology  
TVMC, College Station  
TX 77843-4466  
USA

**Arnaud Gautier**

École Polytechnique Fédérale de  
Lausanne  
Institute of Chemical Sciences and  
Engineering  
1015 Lausanne  
Switzerland

**Guillaume Halet**

University College London  
Department of Physiology  
Gower Street  
London WC1E 6BT  
UK

**Huan Huang**

Texas A&M University  
Department of Physiology and  
Pharmacology  
TVMC  
College Station  
TX 77843-4466  
USA

**Reiko Ishitsuka**

RIKEN Discovery Research Institute  
Lipid Biology Laboratory  
2-1, Hirosawa  
Wako-shi  
Saitama 351-0198  
Japan

**Stefan Jakobs**

Max Planck Institute for Biophysical  
Chemistry  
Mitochondrial Structure and Dynamics  
Group  
Department of NanoBiophotonics  
Am Fassberg 11  
37077 Goettingen  
Germany

**Kai Johnsson**

École Polytechnique Fédérale de  
Lausanne  
Institute of Chemical Sciences and  
Engineering  
1015 Lausanne  
Switzerland

**Ann B. Kier**

Texas A&M University  
Department of Pathobiology  
TVMC, College Station  
TX 77843-4466  
USA

**Toshihide Kobayashi**

RIKEN Discovery Research Institute  
Lipid Biology Laboratory  
2-1, Hirosawa  
Wako-shi  
Saitama 351-0198  
Japan

**Norman J. Marshall**

The University of Chicago  
Department of Chemistry  
929 E. 57th Street  
GCIS 505A  
Chicago  
IL 60637  
USA

**Avery L. McIntosh**

Texas A&M University  
 Department of Physiology and  
 Pharmacology  
 TVMC  
 College Station  
 TX 77843-4466  
 USA

**Lawrence W. Miller**

University of Illinois at Chicago  
 Department of Chemistry  
 845 W. Taylor Street  
 MC 111  
 Chicago  
 IL 60607  
 USA

**Helen O'Hare**

École Polytechnique Fédérale de  
 Lausanne  
 Institute of Chemical Sciences and  
 Engineering  
 1015 Lausanne  
 Switzerland

**Yoshiko Ohno-Iwashita**

Tokyo Metropolitan Institute of  
 Gerontology  
 Biomembrane Research Group  
 Tokyo 173-0015  
 Japan

**Friedhelm Schroeder**

Texas A&M University  
 Department of Physiology and  
 Pharmacology  
 TVMC  
 College Station  
 TX 77843-4466  
 USA

**Robert H. Singer**

Albert Einstein College of Medicine  
 Department of Anatomy and Structural  
 Biology  
 1300 Morris Park Avenue  
 Bronx NY 10461  
 USA

**Thomas A. Spencer**

Dartmouth College  
 Department of Chemistry  
 Hanover  
 NH 03755-1812  
 USA

**Stephan M. Storey**

Texas A&M University  
 Department of Physiology and  
 Pharmacology  
 TVMC  
 College Station  
 TX 77843-4466  
 USA

**Patricia Viard**

University College London  
 Department of Pharmacology  
 Gower Street  
 London WC1E 6BT  
 UK

**Christian A. Wurm**

Max Planck Institute for Biophysical  
 Chemistry  
 Mitochondrial Structure and Dynamics  
 Group  
 Department of NanoBiophotonics  
 Am Fassberg 11  
 37077 Goettingen  
 Germany

***Birgit Wiltschi***

Max Planck Institute of Biochemistry  
BioFuture Independent Research Group  
Molecular Biotechnology  
Am Klopferspitz 18  
82152 Martinsried  
Germany

***Jun Yin***

The University of Chicago  
Department of Chemistry  
929 E. 57th Street  
GCIS 505A  
Chicago  
IL 60637  
USA

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