Biophysical Techniques in Photosynthesis
Volume II

Advances in Photosynthesis and Respiration

VOLUME 26

The scope of our series, beginning with volume 11, reflects the concept that photosynthesis and respiration are intertwined with respect to both the protein complexes involved and to the entire bioenergetic machinery of all life. *Advances in Photosynthesis and Respiration* is a book series that provides a comprehensive and state-of-the-art account of research in photosynthesis and respiration. Photosynthesis is the process by which higher plants, algae, and certain species of bacteria transform and store solar energy in the form of energy-rich organic molecules. These compounds are in turn used as the energy source for all growth and reproduction in these and almost all other organisms. As such, virtually all life on the planet ultimately depends on photosynthetic energy conversion. Respiration, which occurs in mitochondrial and bacterial membranes, utilizes energy present in organic molecules to fuel a wide range of metabolic reactions critical for cell growth and development. In addition, many photosynthetic organisms engage in energetically wasteful photorespiration that begins in the chloroplast with an oxygenation reaction catalyzed by the same enzyme responsible for capturing carbon dioxide in photosynthesis. This series of books spans topics from physics to agronomy and medicine, from femtosecond processes to season long production, from the photophysics of reaction centers, through the electrochemistry of intermediate electron transfer, to the physiology of whole orgamisms, and from X-ray crystallography of proteins to the morphology or organelles and intact organisms. The goal of the series is to offer beginning researchers, advanced undergraduate students, graduate students, and even research specialists, a comprehensive, up-to-date picture of the remarkable advances across the full scope of research on photosynthesis, respiration and related processes.

The titles published in the Series are listed at the end of this volume.

Biophysical Techniques in Photosynthesis

Volume II

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Springer

Library of Congress Control Number: 2007943526

ISBN 978-1-4020-8249-8 (HB)
ISBN 978-1-4020-8250-4 (e-book)

Published by Springer,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

www.springer.com

Cover illustration: The figure on the cover is a courtesy of Petra Fromme and Ingo Grotjohann.
It summarizes significant advances in our understanding of the process of photosynthesis
in the recent years.

The camera ready text was prepared by Lawrence A. Orr,
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Printed on acid-free paper

In Memoriam

Jan Amesz

March 11, 1934 – January 29, 2001

For a tribute, see Photosynth Res (2002) 71: 1–4

Arnold J. Hoff

April 30, 1939 – April 22, 2002

For a tribute, see Chem Phys (2003) 294: 223–225

From the Series Editor

Advances in Photosynthesis and Respiration

Volume 26: Biophysical Techniques in Photosynthesis, Volume II

I am delighted to announce the publication, in *Advances in Photosynthesis and Respiration* (AIPH) Series, of a book ***Biophysical Techniques in Photosynthesis II***. Two distinguished authorities (Thijs Aartsma and Jörg Matysik, both of Leiden University, The Netherlands) have edited this Volume: Aartsma is an authority on the photophysical properties of biological molecules, using innovative optical spectroscopic techniques, whereas Matysik is an authority on photochemistry and spin chemistry as well as in the development of complementary magnetic resonance methods. This book is produced as a sequel to the outstanding Volume 3 of the Series (*Biophysical Techniques in Photosynthesis*), published in 1996, and edited by Jan Amesz and Arnold Hoff, also of Leiden University.

Published Volumes (2006–1994)

- *Volume 25* (2006): ***Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications***, edited by Bernhard Grimm, Robert J. Porra, Wolfhart Rüdiger, and Hugo Scheer, from Germany and Australia. 37 Chapters, 603 pp, Hardcover. ISBN: 978-1-4020-4515-8
- *Volume 24* (2006): ***Photosystem I: The Light-Driven Plastocyanin: Ferredoxin Oxidoreductase***, edited by John H. Golbeck, from USA. 40 Chapters, 716 pp, Hardcover. ISBN: 978-1-4020-4255-3
- *Volume 23* (2006): ***The Structure and Function of Plastids***, edited by Robert R. Wise and J. Kenneth Wise, from USA. 27 Chapters, 575 pp, Softcover: ISBN: 978-1-4020-6570-6, Hardcover, ISBN: 978-1-4020-4060-3
- *Volume 22* (2005): ***Photosystem II: The Light-Driven Water:Plastoquinone Oxidoreductase***, edited by Thomas J. Wydrzynski and Kimiyuki Satoh, from Australia and Japan. 34 Chapters, 786 pp, Hardcover. ISBN: 978-1-4020-4249-2
- *Volume 21* (2005): ***Photoprotection, Photoinhibition, Gene Regulation, and Environment***, edited by Barbara Demmig-Adams, William W. III Adams and Autar K. Mattoo, from USA. 21 Chapters, 380 pp, Hardcover. ISBN: 978-1-4020-3564-7
- *Volume 20* (2006): ***Discoveries in Photosynthesis***, edited by Govindjee, J. Thomas Beatty, Howard Gest and John F. Allen, from USA, Canada and UK. 111 Chapters, 1304 pp, Hardcover. ISBN: 978-1-4020-3323-0
- *Volume 19* (2004): ***Chlorophyll a Fluorescence: A Signature of Photosynthesis***, edited by George C. Papageorgiou and Govindjee, from Greece and USA. 31 Chapters, 820 pp, Hardcover. ISBN: 978-1-4020-3217-2
- *Volume 18* (2005): ***Plant Respiration: From Cell to Ecosystem***, edited by Hans Lambers and Miquel Ribas-Carbo, from Australia and Spain. 13 Chapters, 250 pp, Hardcover. ISBN: 978-1-4020-3588-3
- *Volume 17* (2004): ***Plant Mitochondria: From Genome to Function***, edited by David Day, A. Harvey Millar and James Whelan, from Australia. 14 Chapters, 325 pp, Hardcover. ISBN: 978-1-4020-2399-6
- *Volume 16* (2004): ***Respiration in Archaea and Bacteria:Diversity of Prokaryotic Respiratory Systems***, edited by Davide Zannoni, from Italy. 13 Chapters, 310 pp, Hardcover. ISBN: 978-1-4020-2002-5
- *Volume 15* (2004): ***Respiration in Archaea and Bacteria: Diversity of Prokaryotic Electron Transport Carriers***, edited by Davide Zannoni, from Italy. 13 Chapters, 350 pp, Hardcover. ISBN: 978-1-4020-2001-8
- *Volume 14* (2004): ***Photosynthesis in Algae***, edited by Anthony W. Larkum, Susan Douglas and John A. Raven, from Australia, Canada and UK. 19 Chapters, 500 pp, Hardcover. ISBN:

978-0-7923-6333-0

- *Volume 13* (2003): ***Light-Harvesting Antennas in Photosynthesis***, edited by Beverley R. Green and William W. Parson, from Canada and USA. 17 Chapters, 544 pp, Hardcover. ISBN: 978-0-7923-6335-4
- *Volume 12* (2003): ***Photosynthetic Nitrogen Assimilation and Associated Carbon and Respiratory Metabolism***, edited by Christine H. Foyer and Graham Noctor, from UK and France. 16 Chapters, 304 pp, Hardcover. ISBN: 978-0-7923-6336-1
- *Volume 11* (2001): ***Regulation of Photosynthesis***, edited by Eva-Mari Aro and Bertil Andersson, from Finland and Sweden. 32 Chapters, 640 pp, Hardcover. ISBN: 978-0-7923-6332-3
- *Volume 10* (2001): ***Photosynthesis Photobiochemistry and Photobiophysics***, by Bacon Ke, from USA. 36 Chapters, 792 pp, Softcover: ISBN: 978-0-7923-6791-8. Hardcover: ISBN: 978-0-7923-6334-7
- *Volume 9* (2000): ***Photosynthesis: Physiology and Metabolism***, edited by Richard C. Leegood, Thomas D. Sharkey and Susanne von Caemmerer, from UK, USA and Australia. 24 Chapters, 644 pp, Hardcover. ISBN: 978-0-7923-6143-5
- *Volume 8* (1999): ***The Photochemistry of Carotenoids***, edited by Harry A. Frank, Andrew J. Young, George Britton and Richard J. Cogdell, from UK and USA. 20 Chapters, 420 pp, Hardcover. ISBN: 978-0-7923-5942-5
- *Volume 7* (1998): ***The Molecular Biology of Chloroplasts and Mitochondria in Chlamydomonas***, edited by Jean David Rochaix, Michel Goldschmidt-Clermont and Sabeeha Merchant, from Switzerland and USA. 36 Chapters, 760 pp, Hardcover. ISBN: 978-0-7923-5174-0
- *Volume 6* (1998): ***Lipids in Photosynthesis: Structure, Function and Genetics***, edited by Paul-André Siegenthaler and Norio Murata, from Switzerland and Japan. 15 Chapters, 332 pp, Hardcover. ISBN: 978-0-7923-5173-3
- *Volume 5* (1997): ***Photosynthesis and the Environment***, edited by Neil R. Baker, from UK. 20 Chapters, 508 pp, Hardcover. ISBN: 978-0-7923-4316-5
- *Volume 4* (1996): ***Oxygenic Photosynthesis: The Light Reactions***, edited by Donald R. Ort, and Charles F. Yocum, from USA. 34 Chapters, 696 pp, Softcover: ISBN: 978-0-7923-3684-6. Hardcover: ISBN: 978-0-7923-3683-9
- *Volume 3* (1996): ***Biophysical Techniques in Photosynthesis***, edited by Jan Amesz and Arnold J. Hoff, from The Netherlands. 24 Chapters, 426 pp, Hardcover. ISBN: 978-0-7923-3642-6
- *Volume 2* (1995): ***Anoxygenic Photosynthetic Bacteria***, edited by Robert E. Blankenship, Michael T. Madigan and Carl E. Bauer, from USA. 62 Chapters, 1331 pp, Hardcover. ISBN: 978-0-7923-3682-8
- *Volume 1* (1994): ***The Molecular Biology of Cyanobacteria***, edited by Donald R. Bryant, from USA. 28 Chapters, 916 pp, Hardcover. ISBN: 978-0-7923-3222-0

Further information on these books and ordering instructions can be found at <http://www.springeronline.com> under the Book Series 'Advances in Photosynthesis and Respiration.' Table of Contents of Volumes 1–25 can be found at <http://www.life.uiuc.edu/govindjee/photosynSeries/ttocs.html>. Special discounts are available to members of the International Society of Photosynthesis Research, ISPR (<http://www.photosynthesisresearch.org/>).

About the Volume 26: Biophysical Techniques in Photosynthesis II

Biophysical Techniques in Photosynthesis II has 24 authoritative Chapters, and is authored by 54 international authorities from 10 countries (Australia, Canada, China, France, Germany, Israel, Japan, The Netherlands, United Kingdom, and the United States of America). It is a truly international book and the editors deserve our thanks and our congratulations for giving this gift for our future. Readers of other Volumes in the Series, particularly Volume 22 (Photosystem II), Volume 24 (Photosystem I), Volume 25 (Chlorophylls and Bacteriochlorophylls) and the forthcoming Volume on Purple Phototrophic Bacteria will benefit a great deal by using this Volume as an accompanying Volume.

Since 1996, when the first Volume on *Biophysical Techniques in Photosynthesis*, was published, several new experimental techniques and methods have been devised at a rapid pace. The present book is a sequel to the 1996 book, which was edited by Jan Amesz and Arnold Hoff, to whom the current book is dedicated. It complements that Volume by providing a comprehensive overview of the most important new techniques developed over the past ten years, especially those

that are relevant for research on the mechanism and fundamental aspects of photosynthesis. The contributions are written by leading scientists in their field. The book has five sections: Imaging (4 chapters); Structure (5 chapters); Optical and laser spectroscopy (4 chapters); Magnetic resonance (6 chapters); and Theory (5 chapters). Each chapter describes the basic concepts of the technique, practical applications and scientific results. Possibilities and limitations from a technical as well as a scientific point of view are addressed, allowing the reader not only to recognize the potential of a particular method for his/her own quest, but also to assess the resources that are required for implementation. The book is intended for use by both the beginning graduate students and the researchers in photosynthesis as well as in (bio)physics, (bio)chemistry and biology in general.

The readers can easily find the titles and the authors of the individual chapters in the Table of Contents of this book. Instead of repeating this information here, I prefer to thank each and every author by name (listed in alphabetical order) that reads like a "Who's Who in Principles of Biophysical Techniques":

Thijs J. **A**artsma; James P. Allen; Andrei V. Astashkin; Virginijus **B**arzda; Igor V. Borovykh; Claudia Büchel, Francesco Buda; Wenrui **C**hang; Paula C.A. **d**a Fonseca; Eugenio Daviso; Huub J. M. de Groot; Graham R. **F**leming; Petra Fromme; Bas **G**obets; Daniella Goldfarb; Marie Louise Groot; Warwick **H**illier; Martin F. Hohmann-Marriott; Alfred R. Holzwarth; Gunnar **J**eschke; Asako **K**awamori; Jürgen Köhler; Lars Konermann; Ioan Kosztin; Gerd Kothe; Zhenfeng **L**iu; Ying-Zhong **M**a; Nancy Makri; Jörg Matysik; Johannes Messinger; Klaus Möbius; Edward P. Morris; James R. **N**orris; William W. **P**arson; Oleg G. **P**oluektov; Elizabeth L. **R**ead; Thomas Renger; Robert W. Roberson; Simon **S**cheuring; Klaus Schulten; Heinz-Jürgen Steinhoff; Marion C. **T**hurnauer; David M. Tiede; Herbert **v**an Amerongen; Henk Van As; Allison M. L. van de Meene; Rienk van Grondelle; Frank van Mourik; Bart van Oort; Ivo H. M. van Stokkum; Arieh **W**arshel; Carel W. Windt; Donatas **Z**igmantas; and Xiaobing Zuo.

Complete List of Chapters in Biophysical Techniques in Photosynthesis, AIPH Volume 3, edited by Jan Amesz and Arnold Hoff

As Volume 26 is a sequel to Volume 3, it is beneficial for the readers of the new volume to consult and cite chapters in the earlier volume, I present below complete references to all the chapters in that book. Please note that this volume was published by Kluwer Academic Publishers which was later acquired by Springer, the publishers of the current volumes.

Chapter 1: Amesz J (1996) Developments in classical optical spectroscopy. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 3–10. Kluwer Academic Publishers, Dordrecht

Chapter 2: Garab G (1996) Linear and circular dichroism. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 11–40. Kluwer Academic Publishers, Dordrecht

Chapter 3: Sauer K and Debreczeny M (1996) Fluorescence. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 41–61. Kluwer Academic Publishers, Dordrecht

Chapter 4: Jimenez R and Fleming GR (1996) Ultrafast spectroscopy of photosynthetic systems. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 63–73. Kluwer Academic Publishers, Dordrecht

Chapter 5: Holzwarth AR (1996) Data analysis of time-resolved measurements. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 75–92. Kluwer Academic Publishers, Dordrecht

Chapter 6: Inoue Y (1996) Photosynthetic thermoluminescence as a simple probe of Photosystem II election transport. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 93–107. Kluwer Academic Publishers, Dordrecht

Chapter 7: Aartsma TJ, Louwe RJW and Schellenberg P (1996) Accumulated photon echo measurements of excited state dynamics in pigment-protein complexes. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 109–122. Kluwer Academic Publishers, Dordrecht

Chapter 8: Reddy NRS and Small GJ (1996) Spectral hole burning: Methods and applications to photosynthesis. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 123–136. Kluwer Academic Publishers, Dordrecht

Chapter 9: Mäntele W (1996) Infrared and Fou-

rier-transform infrared spectroscopy. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 137–160. Kluwer Academic Publishers, Dordrecht

Chapter 10: Robert B (1996) Resonance Raman studies in photosynthesis — chlorophyll and carotenoid molecules. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 161–176. Kluwer Academic Publishers, Dordrecht

Chapter 11: Boxer SG (1996) Stark spectroscopy of photosynthetic systems. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 177–189. Kluwer Academic Publishers, Dordrecht

Chapter 12: Malkin S (1996) The photoacoustic method in photosynthesis—monitoring and analysis of phenomena which lead to pressure changes following light excitation. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 191–206. Kluwer Academic Publishers, Dordrecht

Chapter 13: Hoff AJ (1996) Magnetic resonance: An introduction. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 209–210. Kluwer Academic Publishers, Dordrecht

Chapter 14: Levanon H (1996) Time-resolved electron paramagnetic resonance spectroscopy—principles and applications. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 211–233. Kluwer Academic Publishers, Dordrecht

Chapter 15: Britt RD (1996) Electron spin echo methods in photosynthesis research. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 235–253. Kluwer Academic Publishers, Dordrecht

Chapter 16: Lubitz W and Lendzian F (1996) ENDOR spectroscopy. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 255–275. Kluwer Academic Publishers, Dordrecht

Chapter 17: Hoff AJ (1996) Optically detected magnetic resonance (ODMR) of triplet states in photosynthesis. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 277–298. Kluwer Academic Publishers, Dordrecht

Chapter 18: De Groot HJM (1996) Magic angle spinning nuclear magnetic resonance of photosynthetic components. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 299–313. Kluwer Academic Publishers, Dordrecht

Chapter 19: Schiffer M (1996) Structure determination of proteins by X-ray diffraction. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 317–324. Kluwer Academic Publishers, Dordrecht

Chapter 20: Boekema EJ and Rögner M (1996) Electron microscopy. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 325–336. Kluwer Academic Publishers, Dordrecht

Chapter 21: Yachandra VK and Klein MP (1996) X-ray absorption spectroscopy: determination of transition metal site structures in photosynthesis. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 337–354. Kluwer Academic Publishers, Dordrecht

Chapter 22: Debrunner PG (1996) Mössbauer spectroscopy. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 355–373. Kluwer Academic Publishers, Dordrecht

Chapter 23: Tiede DM and Thiyagarajan P (1996) Characterization of photosynthetic supramolecular assemblies using small angle neutron scattering. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 375–390. Kluwer Academic Publishers, Dordrecht

Chapter 24: Van Gorkom HJ and Gast P (1996) Measurement of photosynthetic oxygen evolution. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis (Advances in Photosynthesis and Respiration, Vol 3), pp 391–405. Kluwer Academic Publishers, Dordrecht

Future AIPH and Other Related Books

The readers of the current series are encouraged to watch for the publication of the forthcoming books (not necessarily arranged in the order of future appearance):

- *Sulfur Metabolism in Phototrophic Organisms* (Editors: Rüdiger Hell, Christiane Dahl, David B. Knaff and Thomas Leustek);

- *The Purple Phototrophic Bacteria* (Editors: C. Neil Hunter, Fevzi Daldal, Marion Thurnauer and J. Thomas Beatty);
- *C-4 Photosynthesis and Related CO_2 Concentrating Mechanisms* (Editors: Agepati S. Raghavendra and Rowan Sage);
- *Photosynthesis: Biochemistry, Biophysics, Physiology and Molecular Biology* (Editors: Julian Eaton-Rye and Baishnab Tripathy);
- *Abiotic Stress Adaptation in Plants: Physiological, Molecular and Genomic Foundation* (Editors: Ashwani Pareek, Sudhir K. Sopory, Hans J. Bohnert and Govindjee);
- *The Chloroplast Biochemistry, Molecular Biology and Bioengineering, Part 1. The Chloroplast System: Pigments, Lipids, Pigment-Proteins and Macromolecular Complexes; Part 2. Genes, Genomes, Proteomes, Regulation, Transformation, Bioengineering and Stress* (Editors: Constantin Rebeiz, Hans Bohnert, Christoph Benning, Henry Daniell, Beverley R. Green, J. Kenneth Hoober, Hartmut Lichtenthaler, Archie R. Portis and Baishnab C. Tripathy);
- *Photosynthesis In Silico: Understanding Complexity from Molecules to Ecosystems* (Editors: Agu Laisk, Ladislav Nedbal and Govindjee); and
- *Lipids in Photosynthesis: Essential and Regulatory Functions,* (Editors: Hajime Wada and Norio Murata).

In addition to these contracted books, the following topics, among others, are under consideration:

- Cyanobacteria
- Genomics, Proteomics and Evolution
- Biohydrogen Production
- ATP Synthase and Proton Translocation
- Interactions between Photosynthesis and other Metabolic Processes
- Carotenoids II
- Green Bacteria and Heliobacteria
- Ecophysiology
- Photosynthesis, Biomass and Bioenergy
- Global Aspects of Photosynthesis
- Artificial Photosynthesis

Readers are encouraged to send their suggestions for these and future Volumes (topics, names of future editors, and of future authors) to me by E-mail (gov@uiuc.edu) or fax (1-217-244-7246).

In view of the interdisciplinary character of research in photosynthesis and respiration, it is my earnest hope that this series of books will be used in educating students and researchers not only in Plant Sciences, Molecular and Cell Biology, Integrative Biology, Biotechnology, Agricultural Sciences, Microbiology, Biochemistry, and Biophysics, but also in Bioengineering, Chemistry, and Physics.

I take this opportunity to thank and congratulate both Thijs Aartsma and Jörg Matysik for their outstanding and painstaking editorial work. I thank all the 54 authors (see the list above) of this book in our AIPH Series: without their authoritative chapters, there would be no such Volume. I give special thanks to Larry Orr for typesetting this book: his expertise has been crucial in bringing this book out to completion. We owe Jacco Flipsen and Noeline Gibson (both of Springer) thanks for their friendly working relation with us that led to the production of this book. Thanks are also due to Jeff Haas (Director of Information Technology, Life Sciences, University of Illinois at Urbana-Champaign, UIUC), Evan DeLucia (Head, Department of Plant Biology, UIUC) and my dear wife Rajni Govindjee for constant support.

November 29, 2007

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Govindjee

A 2006 photograph of the Series Editor Govindjee in front of the Natural History Building (one of the places where the concept of the two light reaction and two pigment system, in oxygenic photosynthesis, arose during 1956-1960), and the plaque at Urbana, Illinois, honoring two of the early pioneers in Biophysics of Photosynthesis: Robert Emerson (1903–1959) and Eugene I. Rabinowitch [born as Evgenii Isaakovich Rabinovich] (1898–1973). Left to right: Rajni Govindjee (1961 PhD, under Eugene Rabinowitch), Govindjee (1960 PhD, also under Rabinowitch) and Rita Khanna (1980 PhD, under Govindjee)

Govindjee, born in 1932, obtained his B.Sc. (Chemistry, Biology) and M.Sc. (Botany, Plant Physiology) in 1952 and 1954, from the University of Allahabad, India, and his Ph.D. (Biophysics, under Prof. Eugene Rabinowitch), in 1960, from the University of Illinois at Urbana-Champaign (UIUC), IL, U.S.A. He is best known for his research on the excitation energy transfer, light emission, the primary photochemistry and the electron transfer in Photosystem II (PS II). His research, with many collaborators, has included the discovery of a short-wavelength form of chlorophyll (Chl) *a* functioning in the Chl *b*-containing system, now called PS II; of the two-light effects in Chl *a* fluorescence and in NADP (nicotinamide dinucleotide phosphate) reduction in chloroplasts (Emerson Enhancement). Further, he has worked on the existence of different spectral fluorescing forms of Chl *a* and the temperature dependence of excitation energy transfer down to 4 K; basic relationships between Chl *a* fluorescence and photosynthetic reactions; unique role of bicarbonate on the acceptor side of PS II, particularly in protonation events involving the Q_B binding region; the theory of thermoluminescence in plants; picosecond measurement on the primary photochemistry of PS II; and the use of Fluorescence Lifetime Imaging Microscopy (FLIM) of Chl *a* fluorescence in understanding photoprotection against excess light. His current focus is on the 'History of Photosynthesis Research,' in 'Photosynthesis Education,' and in the 'Possible Existence of Extraterrestrial Life.' He has served on the faculty of the UIUC for ~40 years. Since 1999, he has been Professor Emeritus of Biochemistry, Biophysics and Plant Biology at the same institution. His honors include: Fellow of the American Association of Advancement of Science; Distinguished Lecturer of the School of Life Sciences, UIUC; Fellow and Lifetime member of the National Academy of Sciences (India); President of the American Society for Photobiology (1980–1981); Fulbright Scholar and Fulbright Senior Lecturer; Honorary President of the 2004 International Photosynthesis Congress (Montréal, Canada); and the 2006 Recipient of the Lifetime Achievement Award from the Rebeiz Foundation for Basic Biology.

Contents

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Preface

Eleven years ago, Jan Amesz and Arnold Hoff edited the book *Biophysical Techniques in Photosynthesis* as volume 3 in the Series *Advances in Photosynthesis and Respiration* (Kluwer Academic Publishers, now Springer). These methods are now well established in the photosynthesis community and beyond, and are used by many laboratories in various scientific fields. At the 13th Photosynthesis Congress in Montréal (2004) it became evident that over the past decade a range of new biophysical methods had been developed at a remarkable pace. Many of these are not covered in the book by Amesz and Hoff. This provided the inspiration and the incentive for the publication of a second volume on biophysical techniques in photosynthesis research, with specific emphasis on the developments over the past ten years.

The present book is intended to continue the successful concept of the previous one. This means that it will also focus on the techniques rather than on the scientific questions involved. The purpose is to make the basic techniques and underlying principles of these newly developed methods accessible to young and experienced researchers alike, and to give information about the practical aspects of the methods. In addition, the discussion of selected results obtained in photosynthesis research provides insight into their potential.

Care has been taken to avoid overlap with and duplication of topics that were already covered in the previous volume. Hence the two books are highly complementary, and together they will provide an excellent entry into biophysical techniques in photosynthesis research. The methods discussed in this book are divided in five categories:

1. Imaging: It is becoming increasingly important in the life sciences, and many of the advancements quickly find their way in photosynthesis research. Methods that are discussed in this section are atomic force microscopy (AFM), nonlinear optical microscopy, three-dimensional electron microscopy, and magnetic resonance imaging.

2. Structure: The structure of proteins is a key to the functional and mechanistic aspects of biological processes at the molecular level. It provides the basis for a quantitative approach in terms of theory and modeling of key parameters of the photosynthetic process. This section reviews major developments in methods for structure determination, from the crystallization of membrane proteins to electron and X-ray crystallography.

3. Optical and Laser Spectroscopy: These have been prime tools for investigating photosynthetic systems, e.g., for the analysis of pigment composition and for unraveling pathways for and dynamics of energy and electron transfer. In the past ten years we have seen the development of femtosecond time-resolved infrared and nonlinear optical spectroscopy, of the simultaneous measurement of the time- and spectral evolution of fluorescence, and of single-molecule techniques, all with important applications in photosynthesis research.

4. Magnetic Resonance: Developments in this technique have revolved on high-field techniques in EPR (Electron Paramagnetic Resonance) and NMR (Nuclear Magnetic Resonance), high time-resolution in EPR, distance measurements by EPR in combination with spin labeling, and magic angle spinning techniques in NMR in conjunction with chemically induced nuclear polarization. The dynamic and structural details revealed by these techniques provide access to the driving forces of photosynthesis at the molecular level.

5. Theory: It plays an increasingly important role in the understanding of photosynthetic mechanisms, a development spurred by the rapidly increasing and detailed structural information that is becoming available. Advancements are found in the calculations of electrostatic energies, the theory for excitation energy transfer, molecular dynamics simulations, and new mathematical and quantum-chemical methods.

Many of the techniques and methods discussed in this book were not in existence ten years ago, providing a rationale for this volume. It is interesting to note the growing importance of the relationship between structure, experiment and theory.

This book has been made possible by the help and effort of many. First of all, we are indebted to the contributing authors for their willingness to spend precious time and energy. Furthermore, we thank Govindjee, the Series Editor, who engendered the idea of this book. He has provided invaluable support and advice along the way. Finally, we acknowledge

the assistance of Larry Orr, not only for typesetting, but also for many helpful suggestions, and of Jacco Flipsen and his staff at Springer in producing this book. Finally, we thank our families for their patience when we spent many odd hours on preparing this book.

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Thijs J. Aartsma, born in 1948, has a long term interest in the study of the photophysical properties of biomolecules using optical spectroscopic techniques. After obtaining his M.Sc. degree in 1973 in physical chemistry, he received his PhD degree in 1978 on photon echo relaxation in molecular mixed crystals, at the University of Groningen, The Netherlands, under supervision of Prof. D.A. Wiersma. From 1978–1981 he was a postdoctoral fellow at the University of Washington, Seattle, in the group of Prof. A.L. Kwiram, after which he became an assistant professor at the Florida State University in Tallahassee. He joined the Department of Biophysics at the Leiden University in 1986, first as an associate professor and since 2004 as a full professor. His research in photosynthesis has centered on time-resolved spectroscopy of energy and electron transfer. He has studied the effect of intermolecular interactions on the optical spectra of light-harvesting complexes, and has, among other, established the signature of exciton coherence in the optical properties of the FMO (Fenna-Mathews-Olson) -complex, with successful modeling of the optical spectra. He initiated the application if single-molecule techniques in the investigation of antenna complexes from purple bacteria, providing a unique view on the exciton structure in these systems. His recent work also involves the investigation of the redox turn-over of single metalloproteins and enzymes by fluorescence detection.

Jörg Matysik was born in 1964 in Essen (Germany). His research interest is in photochemistry and spin-chemistry as well as in the development of complementary optical magnetic resonance methods. Of especial interest is the spin evolution in the early light-reaction in photosynthesis. Initially, Matysik went for vocational training as Chemielaborant in the Bergbau-Forschung (Institute for Coal Mining Research) in Essen-Kray before he studied Chemistry at the Universität-Gesamthochschule in Essen where he obtained his Chemie-Diplom (1992) in the group of Prof. Bernhard Schrader, working with Fourier-Transform (FT) Raman spectroscopy on tetrapyrroles. For his PhD (1995), he investigated phytochrome with FT-Raman spectroscopy at the Max-Planck-Institut für Stahlenchemie in Mülheim an der Ruhr in the group of Prof. Peter Hildebrandt in the department headed by Prof. Kurt Schaffner. As Japanese Society for Promotion of Science (JSPS) and Humboldt fellow he worked with Raman spectroscopy on heme proteins in Prof. Teizo Kitagawa's group at the Institute for Molecular Sciences in Okazaki. Since 1997 he is at the Leiden Institute of Chemistry, first as Marie-Curie fellow and Casimir-Ziegler awardee in the solid-state nuclear magnetic resonance (NMR) group of Prof. Huub de Groot. He now has his own research group on optical solid-state NMR. He is a recipient of the Jonge Chemici award (2001) and the Vidi award (2003) of the Nederlandse Organisatie voor Wetenschappelijk Onderzoek.

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Color Plates

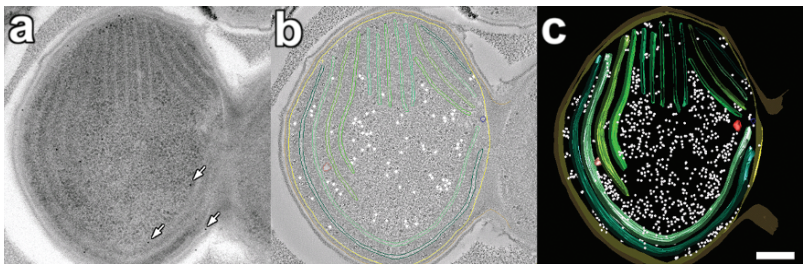

Fig. 1. Steps involved in the recording, reconstruction and modeling of a tomogram of a thin section of the cyanobacterium *Synechocystis* PCC 6803. (a) Image that is part of a tilt series. Fiducial markers appear as dark circles and are indicated by arrows. (b) Slice through reconstructed volume with model contours (white: ribosomes; yellow: cytoplasmic membrane; green: thylakoid membranes. (c) Model of section based on the reconstructed volume. Scale ~100 nm. See Chapter 2, p. 26.

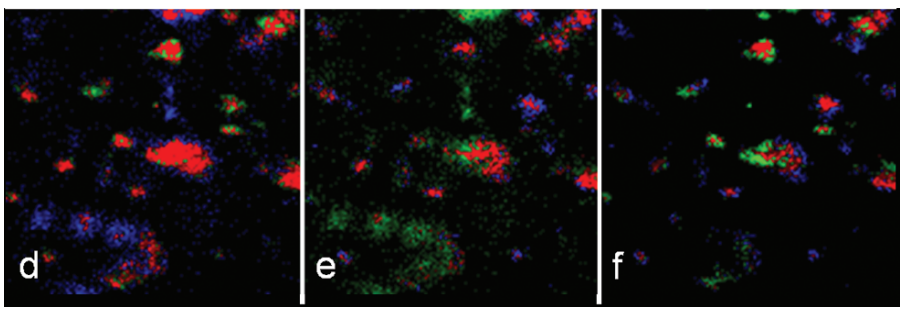

Fig. 2. Imaging isolated LHCII with a multimodal microscope. Panels d, e and f show colocalized images obtained by structural image cross-correlation analysis. Panel d compares MPF and SHG images (panels a and b; see Fig. 3, Chapter 3, p. 48), where correlated pixels are presented in red, uncorrelated fluorescence is presented in green and uncorrelated SHG is shown in blue. Panel e compares MPF and THG images (panels a and c; see Fig. 3, Chapter 3, p. 48). The red color represents correlation between MPF and THG while the green and blue shows uncorrelated MPF and THG signals, respectively. The panel f compares SHG and THG (panels b and c). The red color represents correlation between SHG and THG, while the green and blue represents uncorrelated SHG and THG, respectively.

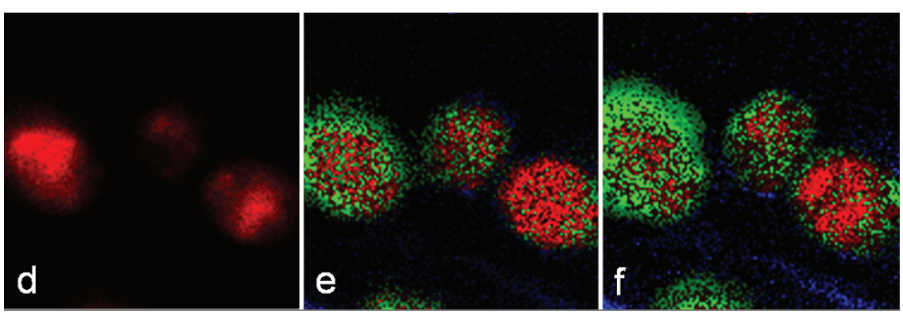

Fig. 3. Imaging in situ chloroplasts of *Clivia mineata* with a multimodal microscope. Panels d, e and f show colocalized images obtained by structural image cross-correlation analysis. Panel d compares MPF and SHG images (panels a and b; see Fig. 4, Chapter 3, p. 50), where correlated pixels are presented in red, uncorrelated fluorescence is presented in green and uncorrelated SHG is shown in blue. Panel e compares MPF and THG images (panels a and c; see Fig. 4, Chapter 3, p. 50). The red color represents correlation between MPF and THG while the green and blue shows uncorrelated MPF and THG signals, respectively. The panel f compares SHG and THG (panels b and c). The red color represents correlation between SHG and THG, while the green and blue represents uncorrelated SHG and THG, respectively.

Thijs J. Aartsma and Jörg Matysik (eds): Biophysical Techniques in Photosynthesis II, pp. CP1–CP7.

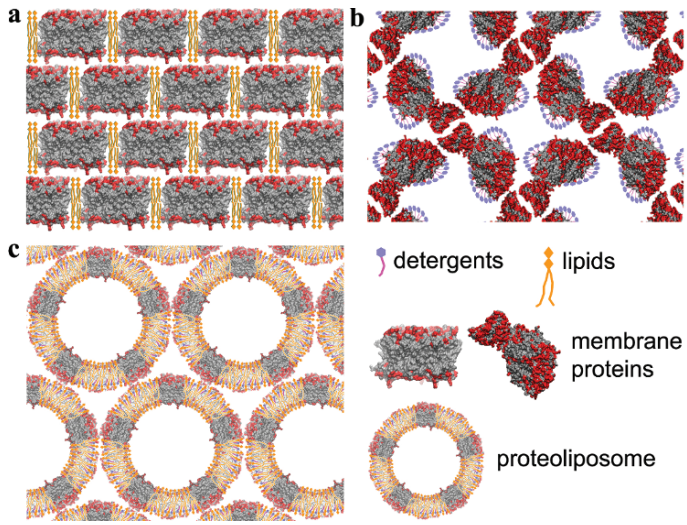

Fig. 1. The packing diagrams of three basic types of membrane protein crystals. (a) Type I, stacks of two-dimensional crystals. (b) Type II membrane proteins are crystallized in complex with detergent micelles. (c) Type III, crystals built from proteoliposome vesicles. The nonpolar regions of membrane proteins are in light gray while the polar regions are shown in darker gray. See Chapter 5, p. 80.

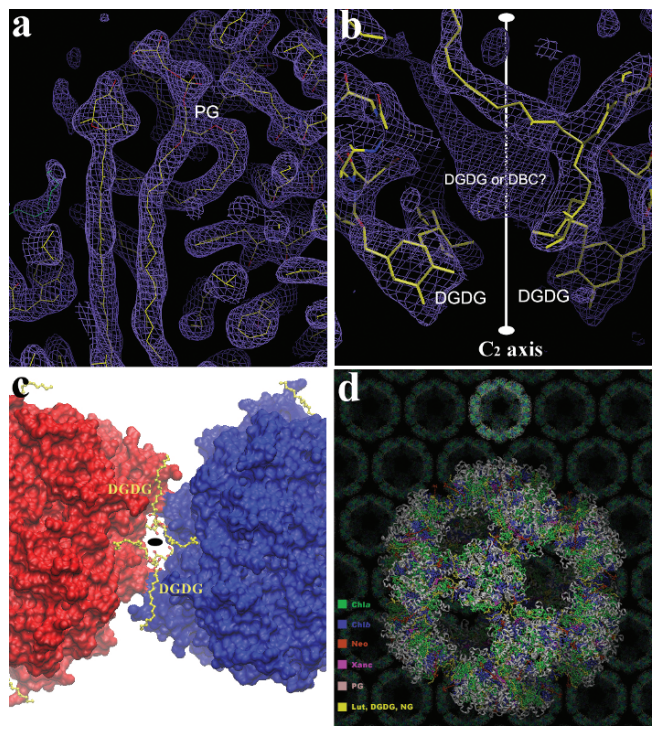

Fig. 2. The roles of lipids in the structure of LHC-II-DGDG proteoliposome. (a), (b) The well-defined 2Fo-Fc electron densities (1.5 × σ level) of PG and DGDG, respectively. In between the two DGDG molecules, there is a piece of electron density remaining unassigned. The icosahedral C_2 axis runs through it. (c) Top view of the trimer-trimer interface along the icosahedral C_2 axis. The two trimers are represented as surface models in the left and right halves of the panel, while the DGDG molecules are shown as ball-and-stick models in light gray. The black ellipse indicates the projection of C_2 axis on the paper plane. (d) The detailed structure of LHC-II-DGDG proteoliposome determined at 2.72 Å resolution. The apoproteins are shown as white ribbons. Prosthetic groups are drawn as ball-and-stick models in different gray levels. Chla, chlorophyll *a*; Chlb, chlorophyll *b*; Lut, lutein; Neo, neoxanthin; Xanc, xanthophyll-cycle carotenoid. The background shows a portion of the crystal lattice. See Chapter 5, p. 94.

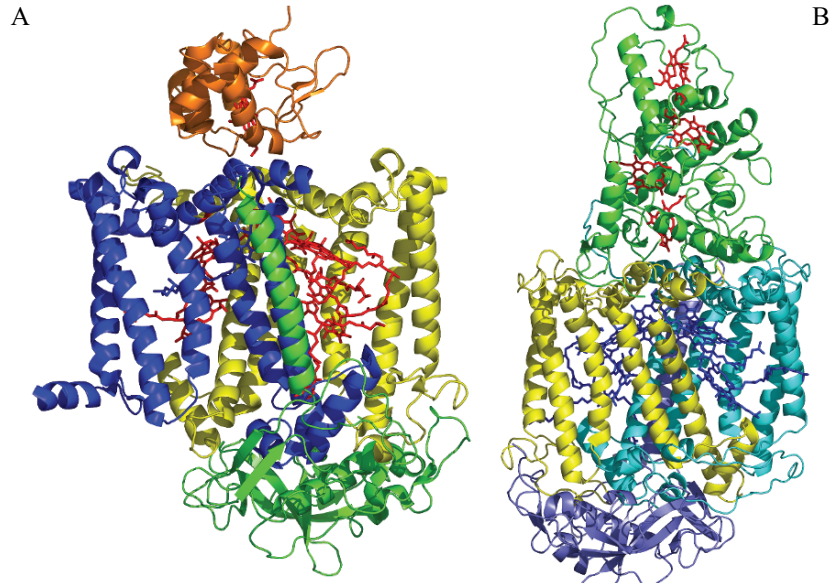

Fig. 1. A. Structure of the reaction center from *Rhodobacter sphaeroides* with cytochrome c_2 bound (PDB entry 1L9J). The three subunits of the reaction center, L (yellow), M (blue), and H (green) and the bound cytochrome c_2 (red) are shown as ribbon diagrams. Cofactors are shown in red. Also shown are the cofactors of the reaction center and the cytochrome heme (red). B. Structure of the reaction center from *Blastochloris viridis* (PDB entry 1PRC). The reaction center consists of four subunits, L (yellow), M (blue-green), H (blue), and a tetraheme cytochrome. Hemes are shown in red, other cofactors in blue. See Chapter 6, p. 115.

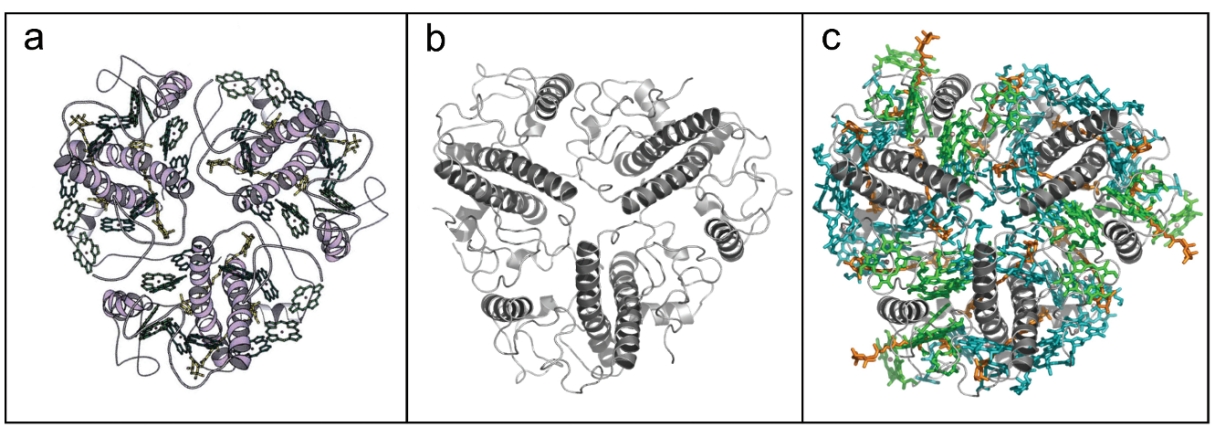

Fig. 2. Comparison of the structure of LHC2 as determined by electron crystallography (a) and X-ray crystallography (b and c). For clarity, all pigments were omitted in (b). (Figures courtesy of Professor W. Kühlbrandt). See Chapter 7, p. 143.

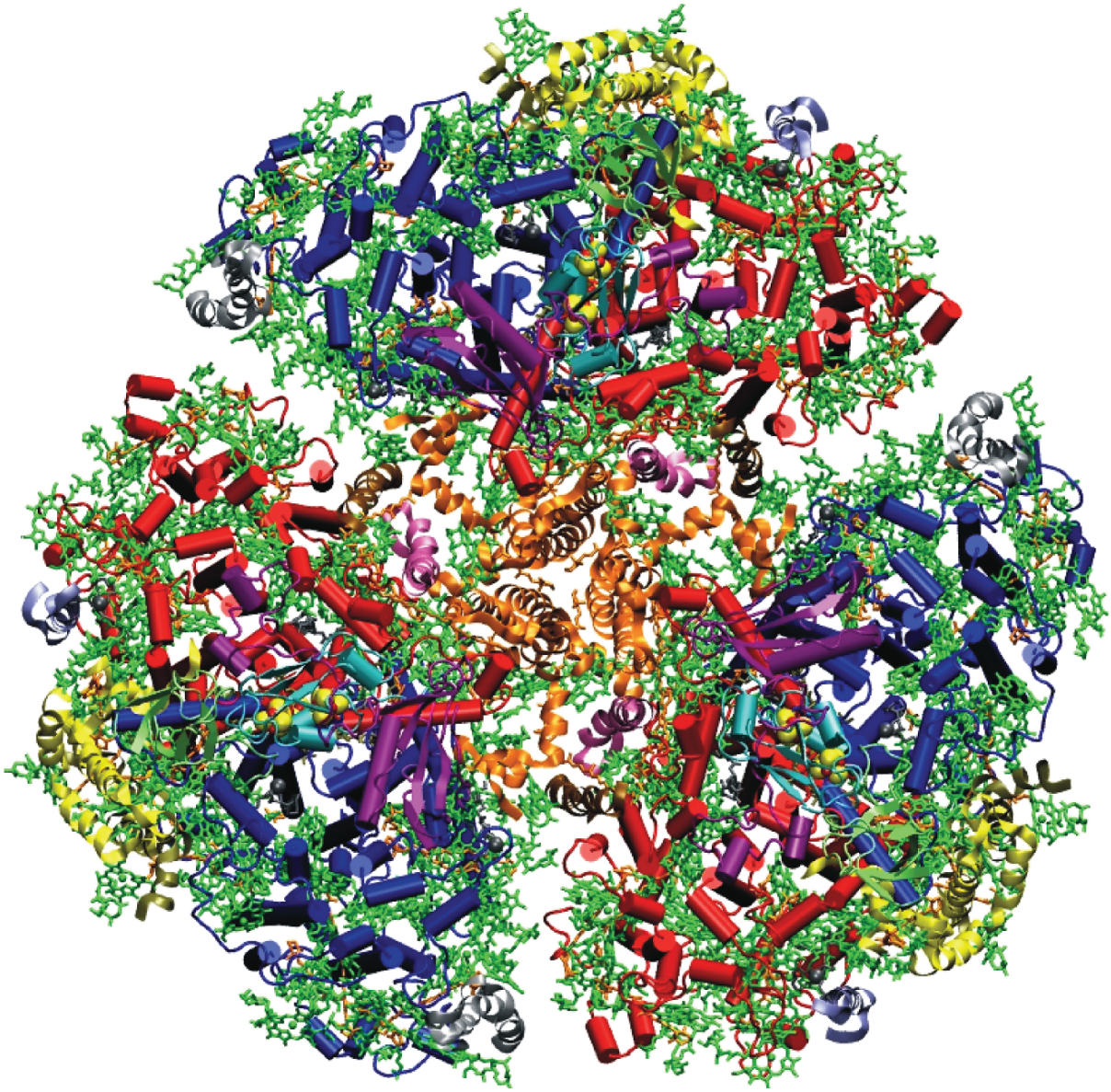

Fig. 1. Structure of Photosystem I (PDB entry 1JB0). The molecule is a trimer. View direction is from the stromal side onto the membrane plane. Each monomer consists of 12 proteins subunits, shown in a ribbon presentation. The large subunit PsaA and PsaB are forming the center of each monomer. PsaA and PsaB contain 11 transmembrane helices, each, which are shown as solid columns. These large subunits carry the electron transfer chain and coordinate most of the antenna chlorophylls and carotenoids. 127 cofactors are bound to one monomer of PS I: 96 chlorophylls, 22 carotenoids, 3 4Fe4S clusters, 2 phylloquinones and 1 Ca. PS I contains 7 small membrane intrinsic proteins that are all located peripheral to the core of PsaA and PsaB. They contain 1 to 3 transmembrane helices which are shown in a ribbon representation. The three stromal subunits, PsaC, D and E provide the docking site for ferredoxin. Modified from Jordan et al., 2001. See Chapter 6, p. 119.

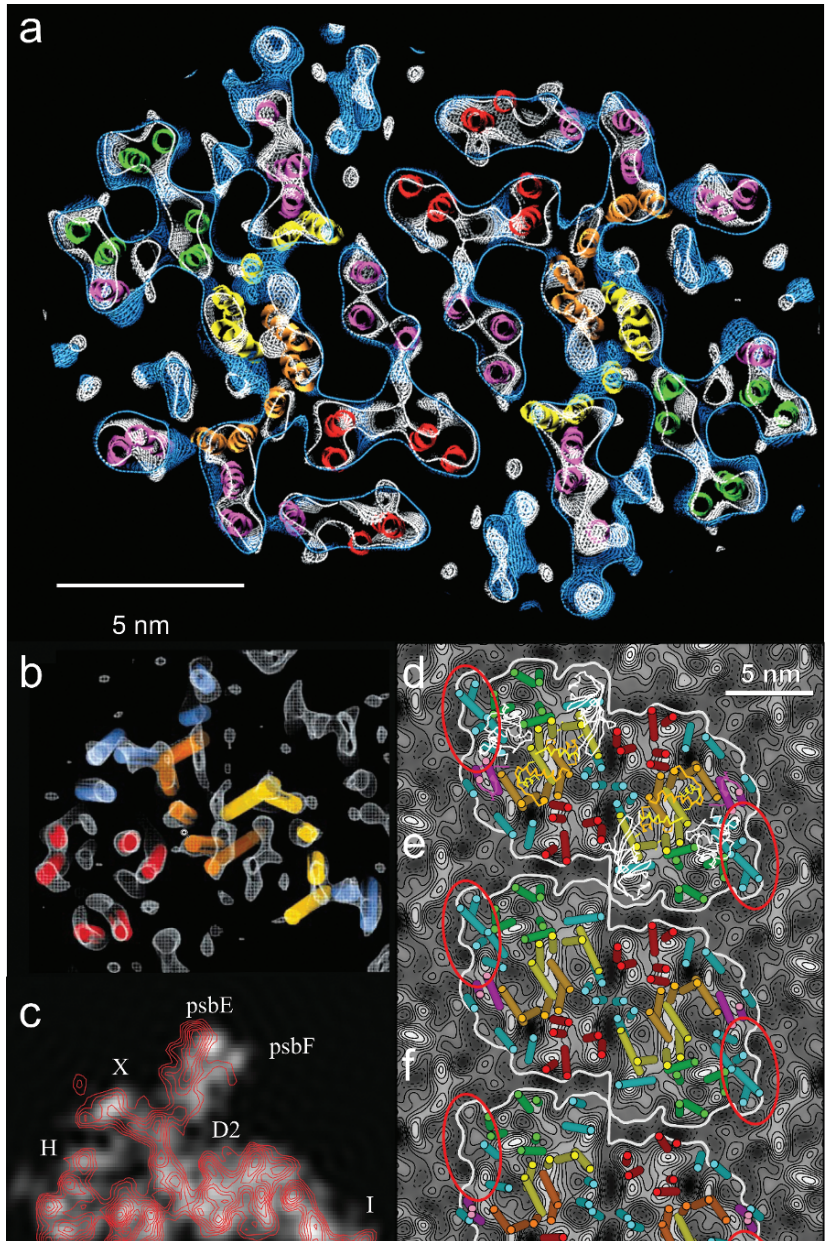

Fig. 2. Electron crystallographic studies of PS II. (a) Section of the 10Å 3D electron crystallographic structure of spinach PS II dimeric core complex (Hankamer et al., 2001) viewed from the lumen. Protein density is shown as chicken-wire and fitted transmembrane helices as ribbons which are color coded to identify subunits as follows: D1, yellow; D2, orange; CP47, red; CP43, green; α- and β-subunits of Cyt *b*559 and low-molecular-weight subunits, magenta. (b) Lumenal view of the 8Å 3D electron crystallographic structure of the spinach CP47-RC PS II subcomplex (Rhee et al., 1998). Protein density is shown as chicken wire and fitted transmembrane helices as cylinders, color coded as in (a) except that Cyt *b*559 and the low-molecular-weight subunits are colored blue. (c) 5.5Å electron crystallographic projection structure of the spinach CP47-RC PS II subcomplex (red contours) (Büchel and Kühlbrandt, 2005) compared with a simulated projection map at the same resolution calculated from the relevant subunits in the X-ray crystallographic structure of cyanobacterial PS II (Ferreira et al., 2004). (d-f) Comparison of the structural data on the PS II core dimer from *Synechococcus elongatus* and spinach (da Fonseca et al., 2002). The electron crystallographic 16Å projection structure of PS II from *Synechococcus elongatus*, represented as contoured grayscale, is overlaid with: (d) the full X-ray crystallography structure and (e) the transmembrane helices of *Synechococcus elongatus* (Zouni et al., 2001); (f) the transmembrane helices of the spinach core dimer deduced from electron crystallography (Hankamer et al., 2001). Color coding as in (a) except that small subunits are shown as blue and, Cyt *b*559 as magenta and extrinsic subunits as white ribbon. See Chapter 7, p. 144.

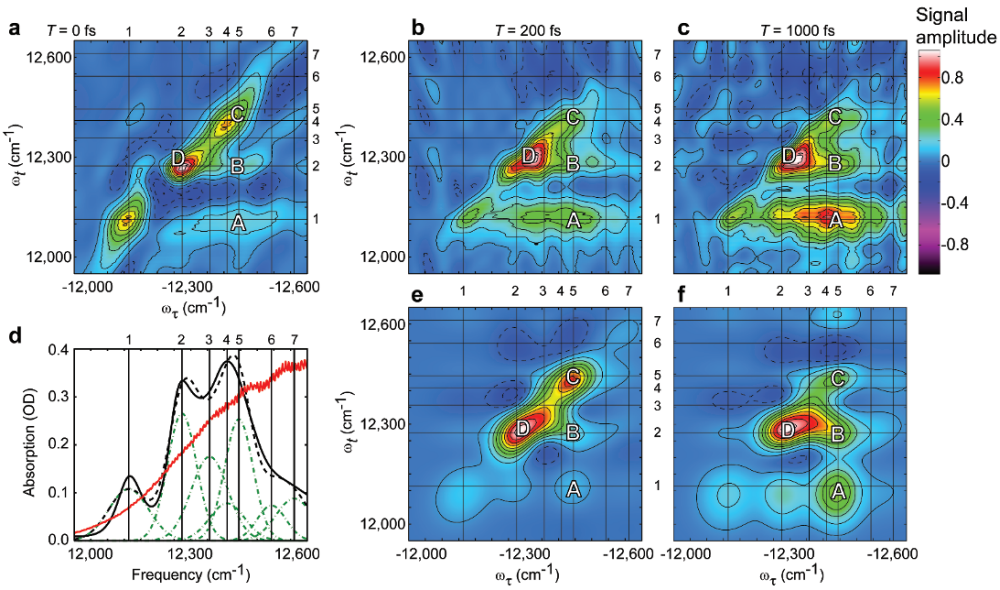

Fig. 1. Experimental and simulated real part of the electric field, corrected for radiative line-shape distortions, of the FMO complex at 77K. (a–c), The experimental 2D spectra (upper three panels) are shown for population times $T = 0$ fs (a), $T = 200$ fs (b) and $T = 1$ ps (c). Contour lines are drawn in 10% intervals of the peak amplitude, with solid lines representing positive features and dashed lines negative features. Horizontal and vertical grid lines indicate excitonic levels 1−7 as labeled. (d), The experimental (solid black) and simulated (dashed black) linear absorption spectra with individual exciton contributions as shown (dashed-dotted green). The laser spectrum (red) covers all transition frequencies. (e,f), Simulation of 2D spectra are shown for $T = 200$ fs (e) and $T = 1$ ps (f). Two off-diagonal peaks marked as A and B are indicators of electronic coupling and energy transport, and two diagonal peaks are marked as C and D (Brixner et al., 2005). See Chapter 11, p. 217.

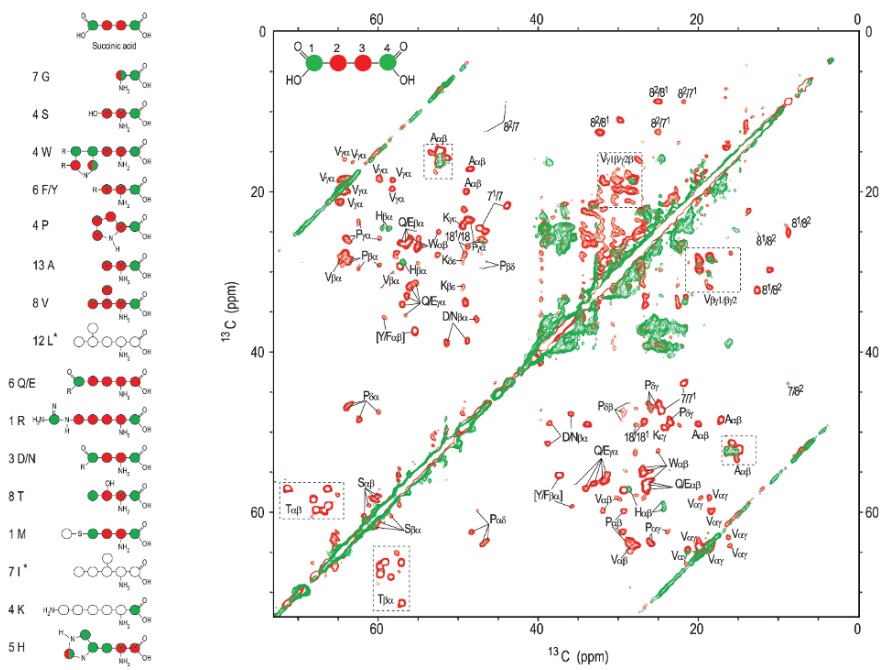

Fig. 2. Pattern labeling of LH2 starting from 1,4 (black) or 2,3 (grey) ^{13}C labeled succinic acid in *R. acidophila*. On the left the enrichment of the residues is shown, while on the right a superposition of the aliphatic region of two ^{13}C-^{13}C PDSD datasets is shown, illustrating the spectral simplifications that can be obtained with pattern labeling. See Chapter 18, p. 379.

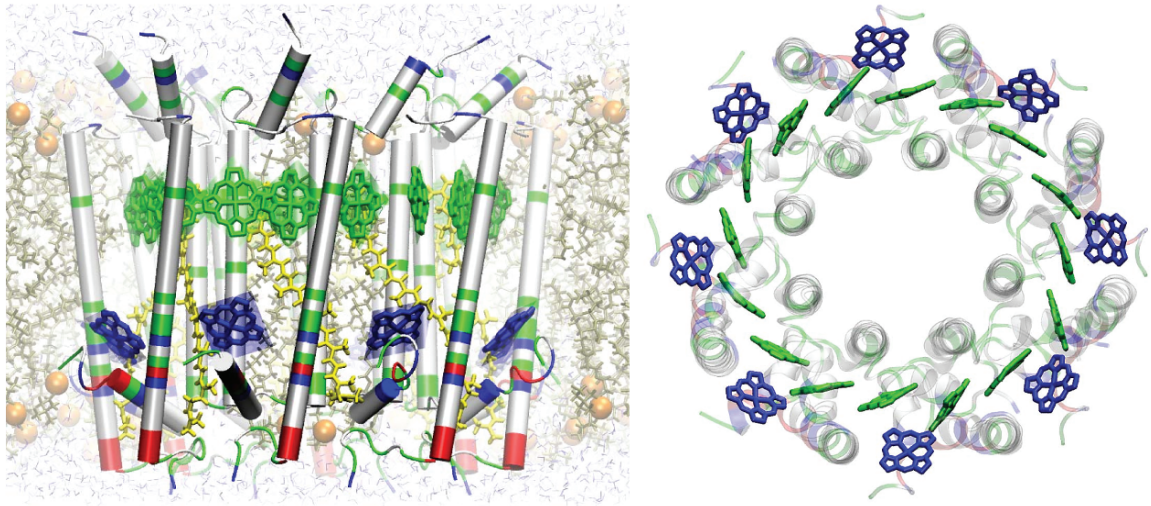

Fig. 1. Left: Side view of the LH2 complex from *Rs. molischianum* (PDB entry 1LGH) embedded in a fully solvated POPC lipid bilayer. The transmembrane helices of the apoprotein subunits are shown as cylinders (cartoon representation) and are colored by residue type; dark (light) colors represent hydrophilic (hydrophobic) residues. For clarity only the BChl macrocycles and the back half of the lipids are shown. The clearly visible B800 (B850) ring is surrounded mostly by polar and charged (nonpolar) protein residues. Right: Tilted side view of the quantum system formed by the optically active B800 and B850 rings. Graphics rendered with the program VMD (Humphrey et al., 1996). See Chapter 22, p. 448 and Chapter 23, p. 473.

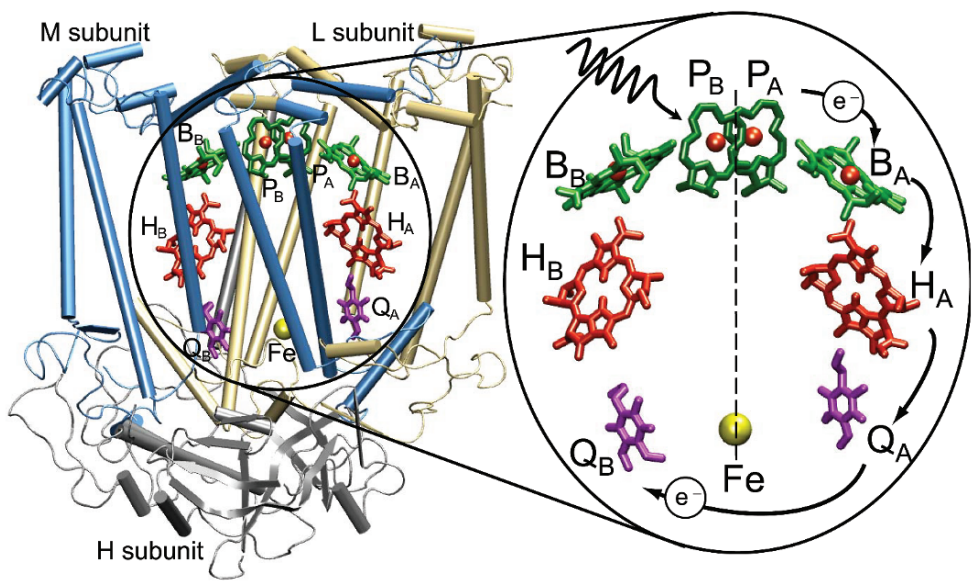

Fig. 2. Left: Structure of the PRC of *Rb. sphaeroides* (PDF entry 1PCR). For clarity only the backbone of the protein subunits L, M and H are shown. The protein helices are represented by cylinders. The cofactors are labeled, and for clarity their phytyl tails are not shown. Right: Spatial distribution of the cofactors in the PRC. The path of the electrons through the PRC is indicated by the arrows. Graphics rendered with the program VMD (Humphrey et al., 1996). See Chapter 22, p. 453 and Chapter 23, p. 477.

Chapter 1

The Supramolecular Architecture of the Bacterial Photosynthetic Apparatus Studied by Atomic Force Microscopy (AFM)

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Summary

The atomic force microscope (AFM) has developed into a powerful tool in structural biology allowing topographical information of membrane proteins at submolecular resolution to be acquired. Recently, AFM has been demonstrated to be the unique tool to image the photosynthetic apparatus in native membranes from different photosynthetic bacteria species. This chapter provides rationales how to image at high resolution a native membrane using the AFM, and summarizes the recent results concerning the structure and the supramolecular assembly of the photosynthetic complexes. On the single molecule level, membrane proteins directly studied in the native membrane were never subject to extraction, purification, reconstitution, or crystallization. Hence structural data in a native state and information concerning structural heterogeneity of the individual photosynthetic complexes are contributed. On the level of multi-protein assemblies, experimental images of the supramolecular architecture of the photosynthetic apparatus, its adaptation to environmental factors, and its particularities among species are reported.

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Thijs J. Aartsma and Jörg Matysik (eds): Biophysical Techniques in Photosynthesis II, pp. 1–11.