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H. Vogel (Eds.)

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Editors

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Professor Dr. Rudolf Rigler
Laboratory of Medical Biophysics, MBB
Karolinska Institute
S-171 77 Stockolm
Sweden

Professor Dr. Horst Vogel
Laboratory of Physical Chemistry of Polymers and Membranes
Institute of Chemical Sciences and Engineering
Swiss Federal Institute of Technology (EPFL)
CH-1015 Lausanne
Switzerland

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Preface

The investigation of molecules as individuals has grown rapidly in recent years, and in the process has uncovered molecular properties not normally accessible by ensemble experiments. In particular, the direct characterization of biologically important molecules such as enzymes, molecular motors, or receptors and entire signaling complexes in action, for example in a live biological cell, yielded unexpected insights. Common approaches for studying single molecules include the electrical detection of ion channels in membranes, the measurement of the dynamics of (bio)chemical reactions between individual molecules, the imaging of individual molecules by scanning probe techniques or by fluorescence correlation spectroscopy, and the direct monitoring of single molecules by optical microscopies, to mention a few. The application of these techniques in physics, chemistry, and biology has opened new areas of nanotechnology. This book provides a representative selection of recent developments in the rapidly evolving field of single molecule techniques of importance in life sciences and will have future impact on the quantitative description of biological processes. The editors of this book hope that the chapters, written by leading scientists in the field, will attract students and scientists from different disciplines, provide them with an authentic insight into this young field of research, allow them to evaluate experimental methods and results, and thereby give them support for their own research.

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Rudolf Rigler
Horst Vogel

Contents

Contributors

Bayley, Hagan
Department of Chemistry, University of Oxford, Oxford OX1 3TA, UK,
hagan.bayley@chem.ox.ac.uk

Bentolila, Laurent A.
Department of Chemistry and Biochemistry, University of California
at Los Angeles, Los Angeles, CA 90095-1569, USA, lbento@chem.ucla.edu
California NanoSystems Institute, University of California at Los Angeles,
Los Angeles, CA 90095-1569, USA,
LBENTO@chem.ucla.edu

Cisneros, David
Center of Biotechnology, University of Technology, Tatzberg 47-51, Dresden,
Germany

De Schryver, Frans C.
Department of Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200F,
3001 Heverlee, Belgium,
frans.deschyver@chem.kuleuven.ac.be

Engel, Andreas
M.E. Müller Institute for Structural Biology, Biozentrum, University of Basel,
Klingelbergstrasse 70, 4056 Basel, Switzerland,
andreas.engel@unibas.ch

Fotiadis, Dimtrios
M.E. Müller Institute for Structural Biology, Biozentrum, University of Basel,
Klingelbergstrasse 70, 4056 Basel, Switzerland

Fromm, David P.
Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA

Haran, Gilad
Department of Chemical Physics, Weizmann Institute of Science, Rehovot 76100,
Israel, gilad.haran@weizmann.ac.il

He, Meng
Department of Chemistry, Kent State University, Kent, OH 44242, USA

Hofkens, Johan
Department of Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200F, 3001 Heverlee, Belgium
Unité Chimie des Matériaux Inorganiques et Organiques, Université Catholique de Louvain, Bâtiment Lavoisier Place L. Pasteur 1, 1348 Louvain-la-Neuve, Belgium

Hofmann, Clemens
Molecular Nano-Optics and Spins (MoNOS), Huygens Laboratory, Leiden University, Postbus 9504, 2333 CA Leiden, The Netherlands

Janovjak, Harald
Center of Biotechnology, University of Technology, Tatzberg 47-51, Dresden, Germany

Jesorka, Aldo
Department of Chemistry and Bioscience, Chalmers University of Technology, Kemivägen 10, SE-41296 Göteborg, Sweden

Kedrov, Alexej
Center of Biotechnology, University of Technology, Tatzberg 47-51, Dresden, Germany

Keijzer, Sandra de
Physics of Life Processes, Leiden Institute of Physics, Leiden University, P.O. Box 9504, 2300 RA, The Netherlands
Cell Biology, Leiden Institute of Biology, Leiden University, P.O. Box 9504, 2300 RA, The Netherlands

Kinkhabwala, Anika
Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA

Kino, Gordon
Department of Electrical Engineering, Stanford University, Stanford, CA 94305-5080, USA

Köhler, Jürgen
Experimental Physics IV and BIMF, University of Bayreuth, 95440 Bayreuth, Germany, juergen.koehler@uni-bayreuth.de

Kulzer, Florian
Molecular Nano-Optics and Spins (MoNOS), Huygens Laboratory, Leiden University, Postbus 9504, 2333 CA Leiden, The Netherlands

Lord, Samuel J.
Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA

Loos, Davey
Department of Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200F, 3001 Heverlee, Belgium

Lu, Zhikuan
Department of Chemistry, Kent State University, Kent, OH 44242, USA

Luchian, Tudor
Dept. of Biophysics & Medical Physics, Alexandru I. Cuza University Iasi-6, Romania

Michalet, Xavier
Department of Chemistry and Biochemistry, University of California at Los Angeles, Los Angeles, CA 90095-1569, USA

Moerner, W.E.
Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA, wmoerner@stanford.edu

Müller, Daniel J.
Center of Biotechnology, University of Technology, Tatzberg 47-51, Dresden, Germany

Nishimura, Stefanie Y.
Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA

Orrit, Michel
Molecular Nano-Optics and Spins (MoNOS), Huygens Laboratory, Leiden University, Postbus 9504, 2333 CA Leiden, The Netherlands, orrit@molphys.leidenuniv.nl

Orwar, Owe
Department of Chemistry and Bioscience, Chalmers University of Technology, Kemivägen 10, SE-41296 Göteborg, Sweden, orwar@chembio.chalmers.se

Petrášek, Zdeněk
Institut für Biophysik, Technische Universität Dresden, Tatzberg 47–51, 01307 Dresden, Germany

Prummer, Michael
Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), CH-1015 Lausanne, Switzerland

Schmidt, Thomas
Physics of Life Processes, Leiden Institute of Physics, Leiden University, P.O. Box 9504, 2300 RA, The Netherlands, Schmidt@Physics.LeidenUniv.nl

Schuck, P. James
Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA

Schuler, Benjamin
Biochemisches Institut, Universität Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland, schuler@bioc.unizh.ch

Schwille, Petra
Institut für Biophysik, Technische Universität Dresden, Tatzberg 47–51, 01307 Dresden, Germany, petra.schwille@biotec.tu-dresden.de

Shin, Seong-Ho
Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

Snaar-Jagalska, B. Ewa
Cell Biology, Leiden Institute of Biology, Leiden University, P.O. Box 9504, 2300 RA, The Netherlands

Spaink, Herman P.
Cell Biology, Leiden Institute of Biology, Leiden University, P.O. Box 9504, 2300 RA, The Netherlands

Steffensen, Mackay
Department of Chemistry, University of Oxford, Oxford, OX1 3TA, UK

Sundaramurthy, Arvind
Department of Electrical Engineering, Stanford University, Stanford, CA 94305-5080, USA

Tokarz, Michal
Department of Chemistry and Bioscience, Chalmers University of Technology, Kemivägen 10, SE-41296 Göteborg, Sweden

Twieg, Robert J.
Department of Chemistry, Kent State University, Kent, OH 44242, USA

Verheijen, Wendy
Department of Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200F, 3001 Heverlee, Belgium

Vogel, Horst
Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), CH-1015 Lausanne, Switzerland, horst.vogel@epfl.ch

Weiss, Shimon
Department of Chemistry and Biochemistry, University of California at Los Angeles, Los Angeles, CA 90095-1569, USA, sweiss@chem.ucla.edu
California NanoSystems Institute, University of California at Los Angeles, Los Angeles, CA 90095-1569, United States of America

Department of Physiology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA 90095-1569, USA

A. Willets, Katherine
Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA

Zondervan, Rob
Molecular Nano-Optics and Spins (MoNOS), Huygens Laboratory, Leiden University, Postbus 9504, 2333 CA Leiden, The Netherlands

Chapter 1
Nanophotonics and Single Molecules

W.E. Moerner(✉), P. James Schuck, David P. Fromm, Anika Kinkhabwala, Samuel J. Lord, Stefanie Y. Nishimura, Katherine A. Willets, Arvind Sundaramurthy, Gordon Kino, Meng He, Zhikuan Lu, and Robert J. Twieg

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Abstract Single emitting molecules are currently providing a new window into nanoscale systems ranging from biology to materials science. The amount of information that can be extracted from each single molecule depends upon the specific photophysical properties of the fluorophore and how these properties are affected by the nearby environment. For this reason, it is necessary to develop single-molecule emitters with as many different reporter functions as possible. The first part of this chapter describes a relatively new class of single-molecule fluorophores which offer

W.E. Moerner
Department of Chemistry, Stanford University, Stanford, CA, 94305-5080 USA
wmoerner@stanford.edu

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tunable photophysical properties and, in turn, improved local reporting functionality on the nanometer length scale. The second part of this chapter presents metallic nanostructures which can address a second issue: the mismatch between the typical size of a single fluorophore (~1 nm along a long dimension) and the wavelength of light (~500 nm). Such nanostructures could lead to more efficient excitation of single molecules, in particular, higher excitation probability as well as reduced backgrounds, and effectively higher spatial resolution. Metallic nanostructures based on two triangles formed into a bowtie shape feature large enhancements of the local electromagnetic field and give rise to strong surface-enhanced Raman scattering of molecules. In future work, enhanced electromagnetic structures can be combined with single-molecule reporters in a variety of applications.

1.1 Introduction

In recent decades, the ability to perform optical spectroscopy and microscopy of single molecules in condensed phase samples (Moerner and Orrit 1999) has fascinated scientists in fields ranging from biophysics (Zhuang and Rief 2000; Weiss 1999; Lu 2005; Rasnik et al. 2005), to cellular biology (Moerner 2003), to materials and polymer science (Cotlet et al. 2004; Barbara et al. 2005; Lee et al. 2005), and even to quantum-mechanical single-photon sources (Moerner 2004; Lounis and Orrit 2005). Using now-standard experimental methods (Ha et al. 1999; Ha 2001; Moerner and Fromm 2003), information on local interactions can be extracted, molecule by molecule, by the measurement of excited state lifetimes, spectra, orientations, brightness, degree of energy transfer, and photon correlations, thus removing ensemble averaging and allowing exploration of heterogeneity. Single-molecule studies often reveal complex statistical fluctuations, which allow useful comparison with theoretical models in a variety of cases (Barkai et al. 2004; Watkins and Yang 2004; Hummer and Szabo 2005).

A continuing need exists for the improvement and extension of these efforts in order to increase the variety and amount of information that may be obtained from single-molecule studies. For example, at room temperature, eventual photobleaching limits the knowledge that may be extracted from each individual molecule. To compensate for this, it is necessary to continually develop new reporter functions for robust fluorophores that provide sufficiently strong signals at the single-molecule level.

The first part of this chapter describes a relatively new class of single-molecule fluorophores which offer tunable photophysical properties and, in turn, improved local reporting functionality on the nanometer length scale. While a single molecule can report on its immediate nanoenvironment, in most experiments, there is a large mismatch between the size of a single fluorophore (~1 nm along a long dimension) and the wavelength of light (~500 nm). Developing ways to overcome this would lead to more efficient excitation of single molecules, in particular, higher excitation probability as well as reduced backgrounds, and effectively higher spatial resolution.