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Multiprotein Complexes

Methods and Protocols

Edited by

Arnaud Poterszman

Institut de Génétique et de Biologie Moléculaire et Cellulaire, Center for Integrated Biology, Integrated Structural Biology Department, Equipe labellisée Ligue Contre le Cancer, CNRS UMR 7104 - Inserm U 1258, University of Strasbourg, Illkirch, France

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Editor
Arnaud Poterszman
Institut de Génétique et de Biologie
Moléculaire et Cellulaire, Center for
Integrated Biology, Integrated
Structural Biology Department, Equipe
labellisée Ligue Contre le Cancer
CNRS UMR 7104 - Inserm U 1258
University of Strasbourg
Illkirch, France

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Preface

Molecular complexes of interacting proteins govern virtually all biological processes such as metabolism, cell signaling, DNA repair, or gene expression. Macromolecular assemblies are also of great biomedical relevance as factors that perturb biomolecular interaction networks underlie a number of diseases, and deliberate inhibition of protein–protein interactions is an increasingly common strategy in drug discovery initiatives. Unraveling their functions and mechanisms of action is often only potentially accessible through a detailed structural description and the integration of dynamic information. This volume of the *Methods in Molecular Biology* series aims to provide the scientific community with strategies and detailed protocols for the preparation of macromolecular complexes and their characterization in view of structural analysis.

Protein engineering and production are essential tools for structural, biophysical, and functional studies as well as for biotechnology and medical applications. Strategies to prepare proteins and protein complexes have tremendously been improved in part thanks to recent structural genomic programs. Yet, no universal solution has been implemented, and the production and/or reconstitution of protein complexes remains a major bottleneck. This is in particular the case for complexes composed of many subunits which are often incompletely characterized. Additional difficulties result from their low natural abundance or their versatile nature, in part because regulation often involves the formation of transient complexes with low binding constants and in part because their composition varies with the physiological context.

The first section of this book focuses on sample preparation. While Chapters 1 and 2 concentrate on strategies for recombinant expression of multiprotein complexes in prokaryotic and eukaryotic hosts, Chapter 3 illustrates how genome editing with the CRISPR-Cas9 system can be used to precisely modify protein coding genes in mammalian cells; this offers the possibility to replace any coding gene by a reshaped version fused to an affinity tag protein or to a fluorescent reporter, enabling the characterization of endogenous macromolecular complexes expressed under near physiological conditions. In the first section, the production of recombinant antibodies and artificial binding proteins which emerge as key reagents in multiprotein complex research, as inhibitors of protein–protein interactions to modulate activity, as probes for cellular imaging or to facilitate structural determination is also discussed. Chapter 4 details the production of recombinant antibodies in different formats, Chapter 5 the intervening removable affinity tag (iRAT) system for the production of recombinant antibody fragments, and Chapter 6 the isolation of artificial binding proteins (Affimer reagents) based on a non-antibody scaffold.

The second section of the book details a set of biophysical methods that can provide useful indicators for sample optimization and often complement structural information obtained with core technologies for structure determination (X-ray crystallography, nuclear magnetic resonance, and cryo-electron microscopy) by quantitative solution data, helping to understand how biological systems function. Three chapters focus on techniques for the biophysical characterization of biological macromolecules and their complexes. Chapter 7 details interaction measurements of protein–DNA complexes by isothermal titration calorimetry (ITC) and microscale thermophoresis (MST), Chapter 8 the use of the switchSENSE technology for the analysis of enzyme kinetics, and Chapter 9 the sedimentation

velocity methods for the characterization of protein heterogeneity and protein affinity interactions. Another set of articles describe mass spectrometry (MS)-based approaches for macromolecular complex analysis. Chapter 10 focuses on the applications of native mass spectrometry for the characterization of multiprotein complexes ranging from 16 to 801 kDa, Chapter 11 on hydrogen/deuterium exchange mass spectrometry for analyzing protein–DNA interactions, and Chapter 12 on integrative mass spectrometry-based approaches for modeling macromolecular assemblies.

Although high-resolution structure determination using X-ray crystallography or single-particle cryo-electron microscopy (cryo-EM) is now producing a rapid stream of breakthroughs in structural biology, the preparation of suitable crystals or hydrated frozen samples on EM grids is often quite challenging. Purified samples, intact and structurally homogeneous in the test tube, may not crystallize or survive the standard methods of preparing thin aqueous films on grids. In the case of cryo-EM, optimization of sample stability and extensive screening of parameters for grid preparation are often required to collect high-quality datasets. The two last chapters of this section address sample optimization. Chapter 13 provides detailed protocols for preparing negatively stained and hydrated frozen EM-grid while Chapter 14 addresses solubilization screening using membrane proteins as model system.

Finally, the third section of this book addresses the characterization of multiprotein complexes in a cellular environment using state-of-the-art imaging technologies and in vivo approaches. Chapter 15 describes practical aspects of super-resolution imaging and Chapter 16 multi-color FRET-FLIM microscopy in live cells. Chapters 17 and 18 present applications of directed evolution systems and of context-specific and proximity-dependent labeling using the BioID technology.

This book is expected to be used not only by structural/molecular biologists who need to prepare multi-components complexes for their own applications but also by scientists from other fields who are working on macromolecular assemblies from other standpoints and need an overview of state-of-art approaches. I am especially thankful to all the authors for their great contributions, devoting their valuable time to the preparation of the manuscripts. I am also indebted to the Series Editor John M. Walker, to the editorial staff members of Springer and to Marie Christine Poterszman for their kind support in making this book publishable. I hope that this volume provides a useful overview preparation and structural analysis of macromolecular complexes and fills a need for well-described hands-on protocols.

Illkirch, France *Arnaud Poterszman*

Contents

Contributors

Abdullah Ahmed • *Central Laser Facility, Science and Technology Facilities Council (STFC) Rutherford Appleton Laboratory, Research Complex at Harwell, Oxford, UK; Plant Cell Biology, Biological and Medical Sciences, Oxford Brookes University, Oxford, UK*

Leonid Andronov • *Centre for Integrative Biology (CBI), Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*

Magali Aumont-Nicaise • *Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, Gif-s-Yvette, France*

Guillaume Bec • *Institut de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique (CNRS), Université de Strasbourg, Strasbourg, France*

Julien Béthune • *Hamburg University of Applied Sciences, Hamburg, Germany; Cluster of Excellence CellNetworks, Heidelberg University, Heidelberg, Germany; Heidelberg University Biochemistry Center (BZH), Heidelberg, Germany*

Catherine Birck • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*

Stanley W. Botchway • *Central Laser Facility, Science and Technology Facilities Council (STFC) Rutherford Appleton Laboratory, Research Complex at Harwell, Oxford, UK; Plant Cell Biology, Biological and Medical Sciences, Oxford Brookes University, Oxford, UK*

Alice Brion • *Laboratoire Structure et Instabilité des Génomes, Muséum National d'Histoire Naturelle (MNHN), Institut National de la Santé et de la Recherche Médicale (INSERM), U1154, Centre National de la Recherche Scientifique (CNRS), UMR7196, Paris, France*

Jean-Baptiste Charbonnier • *Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, Gif-s-Yvette, France*

Sarah Cianférani • *Laboratoire de Spectrométrie de Masse BioOrganique, Institut Pluridisciplinaire Hubbert Curien (IPHC), Centre National de la Recherche Scientifique (CNRS), UMR 7178, Université de Strasbourg, Strasbourg, France*

Jean-Paul Concordet • *Laboratoire Structure et Instabilité des Génomes, Muséum National d'Histoire Naturelle (MNHN), Institut National de la Santé et de la Recherche Médicale (INSERM), U1154, Centre National de la Recherche Scientifique (CNRS), UMR7196, Paris, France*

Emily Cooke • *Central Laser Facility, Science and Technology Facilities Council (STFC) Rutherford Appleton Laboratory, Research Complex at Harwell, Oxford, UK*

Vincent Corvest • *CALIXAR, Lyon, France*

Michel Desmadril • *Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, Gif-s-Yvette, France*

Christine Ebel • *Institut de Biologie Structurale (IBS), Univ Grenoble Alpes, Commissariat à l'énergie atomique et aux énergies alternatives (CEA), Centre National de la Recherche Scientifique (CNRS), Grenoble, France*

Maria Ecke • *Department of Structural Biochemistry, Max-Planck-Institute of Molecular Physiology, Dortmund, Germany*
Stefanie Egetemaier • *Cluster of Excellence CellNetworks, Heidelberg University, Heidelberg, Germany; Heidelberg University Biochemistry Center (BZH), Heidelberg, Germany*
Eric Ennifar • *Institut de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique (CNRS), Université de Strasbourg, Strasbourg, France*
Stéphane Erb • *Laboratoire de Spectroméérie de Masse BioOrganique, Universitéde Strasbourg, CNRS, IPHC UMR7178, Strasbourg, France*
Karim Essabri • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*
Ruzena Filandrova • *Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic; Faculty of Sciences, Charles University, Prague, Czech Republic*
Alexandre Frechard • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Integrated Structural Biology, Equipe labellisée Ligue Contre le Cancer, Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*
Sylvain Geny • *Laboratoire Structure et Instabilité des Génomes, Muséum National d'Histoire Naturelle (MNHN), Institut National de la Santé et de la Recherche Médicale (INSERM), U1154, Centre National de la Recherche Scientifique (CNRS), UMR7196, Paris, France*
Amandine Gontier • *Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, Gif-s-Yvette, France; Structural Biology and Biophysics (SBB), Bio Structure and Biophysics (BSB), Sanofi, Vitry-sur-Seine, France*
Michael Hust • *Technische Unversität Braunschweig, Institut für Biochemie, Biotechnologie und Bioinformatik, Abteilung Biotechnologie, Braunschweig, Germany; YUMAB GmbH, Braunschweig, Germany*
So Iwata • *Department of Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan*
Sophie Jacquemin • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Equipe labellisée Ligue Contre le Cancer, Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*
Anass Jawhari • *Independent Advisor, Lyon, France*
Qiyang Jiang • *Viva Biotech (Shanghai) Limited, Shanghai, China*
Burak V. Kabasakal • *School of Biochemistry, University of Bristol, Bristol, UK; Institute of Accelerator Technologies, Ankara University, Gölbaçı, Ankara, Turkey*
Alan Kadek • *Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic; Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany*
Daniel Kavan • *Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*
Bruno P. Klaholz • *Centre for Integrative Biology (CBI), Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*

Olga Kolesnikova • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Département of Integrated Structural Biology, Equipe Labellisée Ligue, Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France; Structural and Computational Biology Unit, EMBL Heidelberg, Heidelberg, Germany*

Jens König • *Department of Structure and Function of Proteins, Helmholtz Zentrum für Infektionsforschung GmbH, Braunschweig, Germany*

Andy M. Lau • *Department of Chemistry, King's College London, London, UK*

Petr Man • *Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*

Julien Marcoux • *Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse, CNRS, UPS, Toulouse, France*

Michael J. McPherson • *Astbury Centre for Structural and Molecular Biology, School of Molecular and Cellular Biology, University of Leeds, Leeds, UK*

Clément Nemoz • *Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, Gif-s-Yvette, France*

Heinz Neumann • *Department of Structural Biochemistry, Max-Planck-Institute of Molecular Physiology, Dortmund, Germany; Department of Chemical Engineering and Biotechnology, University of Applied Sciences, Darmstadt, Germany*

Norimichi Nomura • *Department of Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

Yayoi Nomura • *Department of Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

Petr Novak • *Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*

Simon Pichard • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Equipe labellisée Ligue Contre le Cancer, Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*

Argyris Politis • *Department of Chemistry, King's College London, London, UK*

Arnaud Poterszman • *Institut de Génétique et de Biologie Moléculaire et Cellulaire, Center for Integrated Biology, Integrated Structural Biology Department, Equipe labellisée Ligue Contre le Cancer, CNRS UMR 7104 - Inserm U 1258, University of Strasbourg, Illkirch, France*

Cinthia Amaya Ramirez • *Cluster of Excellence CellNetworks, Heidelberg University, Heidelberg, Germany; Heidelberg University Biochemistry Center (BZH), Heidelberg, Germany*

Jean-Baptiste Renaud • *Laboratoire Structure et Instabilité des Génomes, Muséum National d'Histoire Naturelle (MNHN), Institut National de la Santé et de la Recherche Médicale (INSERM), U1154, Centre National de la Recherche Scientifique (CNRS), UMR7196, Paris, France*

Virginie Ropars • *Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, Gif-s-Yvette, France*

Paola Rossolillo • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*

Yumi Sato • *Department of Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan*
Christiane Schaffitzel • *School of Biochemistry, University of Bristol, Bristol, UK*
Damian Schiller • *Department of Structural Biochemistry, Max-Planck-Institute of Molecular Physiology, Dortmund, Germany*
Jennifer Schoberer • *Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, BOKU Vienna, Vienna, Austria*
Patrick Schultz • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Integrated Structural Biology, Equipe labellisée Ligue Contre le Cancer, Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*
Grigory Sharov • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Integrated Structural Biology, Equipe labellisée Ligue Contre le Cancer, Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France; EM Facility, MRC Laboratory of Molecular Biology, Cambridge, UK*
Martin Spinck • *Department of Structural Biochemistry, Max-Planck-Institute of Molecular Physiology, Dortmund, Germany*
Anna A. S. Tang • *Astbury Centre for Structural and Molecular Biology, School of Molecular and Cellular Biology, University of Leeds, Leeds, UK*
Christian Tiede • *Astbury Centre for Structural and Molecular Biology, School of Molecular and Cellular Biology, University of Leeds, Leeds, UK*
Darren C. Tomlinson • *Astbury Centre for Structural and Molecular Biology, School of Molecular and Cellular Biology, University of Leeds, Leeds, UK*
Joop van den Heuvel • *Department of Structure and Function of Proteins, Helmholtz Zentrum für Infektionsforschung GmbH, Braunschweig, Germany*
Paloma F. Varela • *Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, Gif-s-Yvette, France*
Jean-Luc Vonesch • *Centre for Integrative Biology (CBI), Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*
Maximilien Werderer • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Integrated Structural Biology, Equipe labellisée Ligue Contre le Cancer, Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France*
Gala Ramon Zamorano • *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Département of Integrated Structural Biology, Equipe Labellisée Ligue, Centre National de la Recherche Scientifique (CNRS), UMR 7104, Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Université de Strasbourg, Illkirch, France; Department of Life and Medical Sciences, University of Hertfordshire, Hatfield, UK*