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Multiprotein Complexes

Methods and Protocols

Edited by

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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 switch-SENSE 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

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