# Methods in Enzymetegy Vehenne 298

## Methods in Enzymology

Volume 298

# Molecular Motors and the Cytoskeleton Part B

**EDITED BY** 

Richard B. Vallee

WORCESTER FOUNDATION FOR BIOMEDICAL RESEARCH SHREWSBURY, MASSACHUSETTS



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# Methods in Enzymology

Volume 298
MOLECULAR MOTORS AND THE CYTOSKELETON
Part B

## METHODS IN ENZYMOLOGY

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Article numbers are in parentheses following the names of contributors.

Affiliations listed are current.

- Bruce Alberts (19), Department of Biochemistry and Biophysics, National Academy of Sciences, Washington, DC 20418
- VIKI J. ALLAN (28), School of Biological Sciences, University of Manchester, Manchester M13 9PT, United Kingdom
- KATHRYN AYSCOUGH (2), Department of Biochemistry, University of Dundee, Dundee DD14HN, Scotland
- RONALD J. BASKIN (35), Section of Molecular and Cellular Biology, University of California, Davis, California 95616
- MARY C. BECKERLE (7), Department of Biology, University of Utah, Salt Lake City, Utah 84112-0840
- JAMES B. BINGHAM (15), Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218
- STEVEN M. BLOCK (38), Department of Molecular Biology and Princeton Materials Institute, Princeton University, Princeton, New Jersey 08544
- MAUREEN BLÖMBERG-WIRSCHELL (20), Program in Molecular Medicine, UMASS Medical Center, Worcester, Massachusetts 01605
- KERRY BLOOM (26), Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599
- GARY G. BORISY (43), University of Wisconsin, Madison, Wisconsin 53706
- Michael C. Brown (8), Department of Anatomy and Cell Biology, State University of New York Health Science Center, Syracuse. New York 13210
- MICHAEL R. BUBB (3), Department of Medicine, University of Florida College of Medicine, Gainesville, Florida 32610
- JANIS K. BURKHARDT (31). Department of Pathology and Committee on Immunology, University of Chicago, Chicago, Illinois 60637

- RICHARD E. CHENEY (1), Department of Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7545
- DUANE A. COMPTON (27), Department of Biochemistry, Dartmouth Medical School. Hanover, New Hampshire 03755
- MICAH DEMBO (40), Department of Biomedical Engineering, Boston University, Boston. Massachusetts 02215
- Arshad Desai (12, 23), Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143
- GEORGIOS S. DIAMANTOPOULOS (17), Department of Cell Biology, University of Geneva. CH-1211 Geneva 4, Switzerland
- STEPHEN J. DOXSEY (20), Program in Molecular Medicine, UMASS Medical Center, Worcester, Massachusetts 01605
- HAROLD P. ERICKSON (25). Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- JOHN E. ERIKSSON (42), Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, BioCity, FIN-20521 Turku, Finland
- URSULA EUTENEUER (34), Adolf-Butenandt-Institute for Cell Biology, Ludwig-Maximilians-University Munich, D-80336 Munich. Germany
- Christine M. Field (23, 41), Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143
- JEFFREY T. FINER (37), Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305
- DAVID C. FUNG (11). Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305-5307

- VLADIMIR I. GELFAND (30), Department of Cell and Structural Biology, University of Illinois at Urbana/Champaign, Urbana, Illinois 61801
- LEAH HAIMO (33), Department of Biology, University of California, Riverside, Riverside, California 92521
- Yung Jin Han (36), Laboratory of Molecular Cardiology, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892
- Ann-Sofi Härmälä-Braskén (42), Department of Biochemistry and Pharmacy, Åbo Akademi University, BioCity, FIN-20520 Turku, Finland
- SUSAN BAND HORWITZ (21), Albert Einstein College of Medicine, Bronx, New York 10461
- KEN JACOBSON (40), Department of Cell Biology and Anatomy, and Lineburger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7090
- THOMAS JARCHAU (10), Medizinische Universitätsklinik, Institut für Klinische Biochemie und Pathobiochemie, D-97078 Würzburg, Germany
- MARY ANN JORDAN (22), Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, California 93106-9610
- Anna Kashina (13), Section of Molecular and Cellular Biology, University of California, Davis, Davis, California 95616
- Joseph F. Kelleher (5), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- Stephen J. King (15), Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218
- DAVID G. I. KINGSTON (21), Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061
- MICHAEL P. KOONCE (34), Division of Molecular Medicine, Wadsworth Center, Albany, New York 12201-0509

- THOMAS E. KREIS (17, 32), Département de Biologie Cellulaire, Sciences III, University of Geneva, CH-1211 Geneva 4. Switzerland
- JANARDAN KUMAR (16, 29), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- JUDITH LACOSTE (9), Department of Microbiology, University of Virginia, Health Sciences Center, Charlottesville, Virginia 22908
- CHUNLIN LU (25), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- ELIZABETH J. LUNA (4), Department of Cell Biology, University of Massachusetts Medical Center, Shrewsbury, Massachusetts 01545
- Joe Lutkenhaus (24), Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, Kansas 66160
- AMY MA (9), Department of Microbiology, University of Virginia, Health Sciences Center, Charlottesville, Virginia 22908
- CAROL McGOLDRICK (29), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- Frank McNally (18), Section of Molecular and Cellular Biology, University of California, Davis, Davis, California 95616
- AMIT D. MEHTA (37), Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305
- DAVID MEYER (13), Section of Molecular and Cellular Biology, University of California, Davis, Davis, California 95616
- Andrey Mikhailov (42), Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, BioCity, FIN-20521 Turku, Finland
- TIMOTHY J. MITCHISON (6, 12, 19, 23, 41), Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115 and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, California 94143-0450

- AMIT MUKHERJEE (24), Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, Kansas 66160
- R. DYCHE MULLINS (5), The Salk Institute for Biological Studies, La Jolla, California 92037
- THOMAS MUND (10), Medizinische Universitatsklinik, Institut für Klinische Biochemie und Pathobiochemie, D-97078 Würzburg, Germany
- KAREN OEGEMA (23, 41), Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, California 94143
- Timothy N. Oliver (40), Physiology Department, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7545
- GEORGE A. ORR (21), Albert Einstein College of Medicine, Bronx, New York 10461
- J. THOMAS PARSONS (9), Department of Microbiology, University of Virginia, Health Sciences Center, Charlottesville, Virginia 22908
- ROBERT J. PELHAM, JR. (39), Department of Physiology, University of Massachusetts Medical School, Shrewsbury, Massachusetts 01545
- Daniel W. Pierce (14), Department of Cellular and Molecular Pharmacology, Howard Hughes Medical Institute, San Francisco, California 94143
- THOMAS D. POLLARD (5), The Salk Institute for Biological Studies, La Jolla, California 92037
- SRINIVASA RAO (21), Albert Einstein College of Medicine, Bronx, New York 10461
- MATTHIAS REINHARD (10), Medizinische Universitätsklinik, Institut für Klinische Biochemie und Pathobiochemie, D-97078 Würzburg, Germany
- Janet E. Rickard (17), Department of Cell Biology, University of Geneva, CH-1211 Geneva 4. Switzerland
- DANIEL R. RINES (13), Section of Molecular and Cellular Biology, University of California. Davis, Davis, California 95616

- STEPHEN L. ROGERS (30), Department of Cell and Structural Biology, University of Illinois at Urbana/Champaign, Urbana, Illinois 61801
- CECILIA SAHLGREN (42). Department of Bioiogy, Åbo Akademi University, BioCity. FIN-20520 Turku. Finland
- E. D. SALMON (26), Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599
- JOCHEN SCHEEL (32), Department of Physical Biology, Max-Planck-Institute for Developmental Biology, Tubingen, Germany
- MANFRED SCHLIWA (34), Adolf-Butenandi-Institute for Cell Biology, Ludwig-Maximilians-University Munich, D-80336 Munich. Germany
- KAREN L. SCHMEICHEL (7), Ernest Orlando Berkeley National Laboratory, Berkeley, California 94720
- JONATHAN M. SCHOLEY (13), Section of Molecular and Cellular Biology, University of California, Davis, Davis, California 95610
- TRINA A. SCHROER (15), Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218
- James R. Sellers (36), Laboratory of Molecular Cardiology, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892
- SID SHAW (26), Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599
- MICHAEL P. SHEETZ (16, 29). Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- Bob Skibbens (26), Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599
- ILAN SPECTOR (3), Department of Physiology and Biophysics, State University of New York, Stony Brook, Stony Brook, New York 11794
- JAMES A. SPUDICH (37), Department of Biochemistry, Stanford University, School of Medicine, Stanford, California 94305

- BETH E. STRONACH (7), Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115
- TATYANA M. SVITKINA (43), University of Wisconsin, Madison, Wisconsin 53706
- CHARLES S. SWINDEL (21), Bryn Mawr College, Bryn Mawr, Pennsylvania 19010
- JULIE A. THERIOT (11), Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305-5307
- IRINA S. TINT (30), Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, Pennsylvania 19122
- DIANA M. TOIVOLA (42), Department of Biology, Åbo Akademi University, BioCity, FIN-20520 Turku, Finland
- CHRISTOPHER E. TURNER (8), Department of Anatomy and Cell Biology, State University of New York Health Science Center, Syracuse, New York 13210
- RONALD D. VALE (14), Department of Cellular and Molecular Pharmacology, Howard Hughes Medical Institute, San Francisco, California 94143
- KOEN VISSCHER (38), Department of Molecular Biology and Princeton Materials Institute, Princeton University, Princeton, New Jersey 08544

- CLAIRE E. WALCZAK (41), Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, California 94143
- ULRICH WALTER (10), Medizinische Universitätsklinik, Institut für Klinische Biochemie und Pathobiochemie, D-97078 Würzburg, Germany
- YU-LI WANG (39), Department of Physiology, University of Massachusetts Medical School, Shrewsbury, Massachusetts 01545
- MATTHEW D. WELCH (6), Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, California 94143-0450
- LESLIE WILSON (22), Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, California 93106-9610
- MEI LIE WONG (19, 23), Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143
- ELAINE YEH (26), Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599
- YIXIAN ZHENG (19, 41), Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210

#### Preface

This volume is the fourth in the *Methods in Enzymology* series on the same general topic. The first two volumes (85 and 134) were entitled "Structural and Contractile Proteins: The Contractile Apparatus and the Cytoskeleton." The title was changed to its current version with the third volume (196). The structural and functional complexity of cytoplasm was undreamed of a few decades ago and has been one of the great surprises of modern cell biology. These volumes represent a compilation of methodological information on the three major filament systems—F-actin, microtubules, and intermediate filaments—as well as on a number of cytoskeletal structures which lie outside these categories. The topics covered reflect the increasing diversity of isoforms, regulatory factors, and motility proteins that have been identified so far.

The rapid pace of discovery of new proteins and novel activities continues unabated and is reflected in the contents of this volume. Much of this information originated from genetic and molecular analysis, and a clear trend has been to produce and characterize polypeptides identified by these approaches and others by recombinant means. Common methods have emerged for the preparation of diverse proteins, making the art of protein purification and handling seem to be of decreasing value. However, many aspects of the behavior of individual proteins are distinctive, and the need for unique methods for the purification of recombinant proteins is becoming more appreciated, as revealed in this volume.

The need to understand protein-protein interactions has also become increasingly evident. Thus, although many interactions can be reproduced using recombinant proteins, the need to visualize, preserve, and isolate complexes with minimal perturbation has become of great interest. Methods to identify and isolate complexes with minimal manipulation are included in this volume. The case of the kinesin and myosin protein families is particularly revealing. Within the past few years there has been a remarkable proliferation of myosin-related and kinesin-related genes and their products, and analysis of recombinant motor domains has proceeded at a rapid pace. Gradually the newly identified proteins are becoming realities in the test tube, and each has its unique behavior and biochemical complexity. This trend is likely to follow for most, if not all, of the kinesin-. mvosin-, and dynein-related genes. Methods to isolate novel mvosin- and kinesin-related holoenzyme complexes are described in this volume, and future volumes promise to reflect a dramatic increase in the purification of these proteins from their cell and tissue sources.

Many of the energy-utilizing proteins of the cytoskeleton catalyze novel enzymatic reactions involving force-producing, filament-severing, and other nonconventional activities. New methods to monitor these activities both *in vivo* and *in vitro* have been essential to progress in the field and are included in this and in previous volumes.

A number of colleagues have provided valuable help in surveying the current state of the field and identifying topics of value for this volume. I am particularly grateful to Drs. Elizabeth Luna, Yu-li Wang, Kevin Vaughan, and Jorge Garces for their advice and assistance.

RICHARD B. VALLEE

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