Methods in ENZYMOLOGY

Volume 330

Hyperthermophilic Enzymes
Part A

Edited by

Michael W. W. Adams
Robert M. Kelly

ACADEMIC PRESS

Methods in Enzymology

Volume 330

Hyperthermophilic Enzymes Part A

EDITED BY

Michael W. W. Adams

THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA

Robert M. Kelly

NORTH CAROLINA STATE UNIVERSITY
RALEIGH, NORTH CAROLINA

ACADEMIC PRESS
San Diego London Boston New York Sydney Tokyo Toronto

This book is printed on acid-free paper. ♾

Academic Press
A Harcourt Science and Technology Company
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.academicpress.com

Academic Press
Harcourt Place, 32 Jamestown Road, London NW1 7BY, UK
http://www.academicpress.com

International Standard Book Number: 0-12-182231-1

PRINTED IN THE UNITED STATES OF AMERICA
01 02 03 04 05 06 07 SB 9 8 7 6 5 4 3 2 1

Methods in Enzymology

Volume 330

HYPERTHERMOPHILIC ENZYMES

Part A

METHODS IN ENZYMOLOGY

EDITORS-IN-CHIEF

John N. Abelson Melvin I. Simon

DIVISION OF BIOLOGY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

FOUNDING EDITORS

Sidney P. Colowick and Nathan O. Kaplan

Contributors to Volume 330

Article numbers are in parentheses following the names of contributors.
Affiliations listed are current.

MICHAEL W. W. ADAMS (3, 30), *Department of Biochemistry and Molecular Biology, Center for Metalloenzyme Studies, University of Georgia, Athens, Georgia 30602*

IAIN J. ANDERSON (5), *Integrated Genomics, Inc., Chicago, Illinois 60612*

GARABED ANTRANIKIAN (17), *Institute of Technical Microbiology, Technische Universität Hamburg-Harburg, Hamburg 21073, Germany*

ROBERT D. BARBER (28), *Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, Pennsylvania 16802*

MICHAEL W. BAUER (16, 22), *Novartis Agricultural Biotechnology Research Institute, Research Triangle Park, North Carolina 27709*

MARKE M. BEERTHUYZEN (21), *NIZO Food Research, Ede NL-6701 BA, The Netherlands*

PETER L. BERGQUIST (19), *Department of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia, and Department of Molecular Medicine, University of Auckland Medical School, Auckland 92019, Australia*

COSTANZO BERTOLDO (17), *Institute of Technical Microbiology, Technische Universität Hamburg-Harburg, Hamburg 21073, Germany*

JAMES R. BROWN (7), *Department of Bioinformatics, SmithKline Beecham Pharmaceuticals, Collegeville, Pennsylvania 19426-0989*

SUSAN G. CADY (22), *Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905*

WALTER CALLEN (13, 15, 22, 33), *Diversa Corporation, San Diego, California 92121-7032*

LARA S. CHANG (27, 32), *Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905*

STEPHEN T. CHANG (16), *MedImmune, Inc., Gaithersburg, Maryland 20878*

SWAPNIL R. CHHABRA (13, 14), *Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905*

MARIA CIARAMELLA (11), *Institute of Protein Biochemistry and Enzymology, CNR, 80125 Naples, Italy*

PEDRO M. COUTINHO (10), *Centre for Biological and Chemical Engineering, Instituto Superior Técnico, 1049-001 Lisboa, Portugal*

ROY M. DANIEL (19), *Department of Biological Sciences, Waikato University School of Science and Technology, Hamilton, New Zealand*

EDWARD F. DELONG (1), *Monterey Bay Aquarium Research Institute, Moss Landing, California 95039*

WILLEM M. DE VOS (21, 24, 25), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands, and Wageningen Centre for Food Sciences, Wageningen NL-670 AM, The Netherlands*

MARCEL DIJKGRAAF (25), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

JOCELYNE DIRUGGIERO (7), *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, Maryland 21202*

DIANNE M. DUNN (7), *Department of Human Genetics, University of Utah, Salt Lake City, Utah 84112*

JONATHAN A. EISEN (9), *The Institute for Genomic Research, Rockville, Maryland 20850*

JAMES G. FERRY (28), *Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, Pennsylvania 16802*

CLAIRE M. FRASER (9), *The Institute for Genomic Research, Rockville, Maryland 20850*

SHINSUKE FUJIWARA (20), *Department of Biotechnology, Graduate School of Engineering, Osaka University, Osaka 565-0871, Japan*

TOSHIAKI FUKUI (20), *Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 606-8501, Japan*

MOUSUMI GHOSH (30), *Department of Biochemistry, West Virginia University, Morgantown, West Virginia 26505*

MORELAND D. GIBBS (19), *Department of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia*

DAVID E. GRAHAM (5), *Department of Biochemistry, Virginia Polytechnical Institute & State University, Blacksburg, Virginia 24061-0308*

AMY M. GRUNDEN (30), *Department of Microbiology, North Carolina State University, Raleigh, North Carolina 27695*

VALERIE J. HARWOOD (31), *Department of Biology, University of South Florida, Tampa, Florida 33620*

BERNARD HENRISSAT (10), *Architecture et Fonction des Macromolécules Biologiques, CNRS-IFR1, Marseille, Cedex 20, France*

PAULA M. HICKS (27, 32), *Cargill Biotechnology Research, Excelsior, Minnesota 55331*

ROBERT HUBER (2), *Lehrstuhl für Mikrobiologie und Archaeenzentrum, Universität Regensburg, Regensburg D-93053, Germany*

TADAYUKI IMANAKA (20, 29), *Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 606-8501, Japan*

THIJS KAPER (21, 24), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

YUTAKA KAWARABAYASI (6), *Biotechnology Center, National Institute of Technology and Evaluation, Tokyo 151-0066, Japan*

ROBERT M. KELLY (4, 12, 13, 14, 15, 16, 22, 27, 32, 33), *Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27606*

SERVÉ W. M. KENGEN (21, 24), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

LEON D. KLUSKENS (21, 25), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

NIKOS KYRPIDES (5), *Integrated Genomics, Inc., Chicago, Illinois 60612*

DAVID LAM (13, 14, 15), *Diversa Corporation, San Diego, California 92121-7032*

JOYCE H. G. LEBBINK (21, 24), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

WOLFGANG LIEBL (15, 18), *Institut für Mikrobiologie und Genetik, Georg-August-Universität, Göttingen D-37077, Germany*

J. A. LITTLECHILD (26), *Schools of Chemistry and Biological Sciences, University of Exeter, Exeter EX4 4QD, United Kingdom*

DENNIS L. MAEDER (7), *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, Maryland 21202*

ERIC J. MATHUR (13, 14, 15, 22, 33), *Diversa Corporation, San Diego, California 92121-7032*

ANGELI LAL MENON (3), *Department of Biochemistry and Molecular Biology, Center for Metalloenzyme Studies, University of Georgia, Athens, Georgia 30602*

Edward S. MILLER, JR. (15), *DuPont Central Research and Development Experimental Station, Wilmington, Delaware 19880-0328*

MARCO MORACCI (11), *Institute of Protein Biochemistry and Enzymology, CNR, 80125 Naples, Italy*

MASAAKI MORIKAWA (29), *Department of Material and Life Science, Graduate School of Engineering, Osaka University, Osaka 565-0871, Japan*

DANIEL D. MORRIS (19), *Department of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia*

KAREN E. NELSON (9), *The Institute for Genomic Research, Rockville, Maryland 20850*

ROSS OVERBEEK (5), *Integrated Genomics, Inc., Chicago, Illinois 60612*

KIMBERLEY N. PARKER (13, 14, 15, 16), *DuPont Chemical Research and Development Experimental Station, Wilmington, Delaware 19880-0328*

MARYBETH A. PYSZ (4), *Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27606*

KRISTINA D. RINKER (4), *Department of Chemical Engineering, Colorado State University, Fort Collins, Colorado 80523-1370*

FRANK T. ROBB (7), *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, Maryland 21202*

MOSE ROSSI (11), *Institute of Protein Biochemistry and Enzymology, CNR, Naples 80125, Italy, and Dipartimento di Chimica Organica e Biologica, Universita di Napoli, Naples 80134, Italy*

ALEXEI SAVCHENKO (23), *Banting and Best Department of Medical Research, C. H. Best Institute, Toronto, Ontario M5G1L6, Canada*

HAROLD J. SCHREIER (31), *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, Maryland 21202*

GERRIT J. SCHUT (3), *Department of Biochemistry and Molecular Biology, Center for Metalloenzyme Studies, University of Georgia, Athens, Georgia 30602*

A. C. SEHGAL (33), *Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27606*

KEITH R. SHOCKLEY (4), *Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27606*

JAY M. SHORT (13, 15, 22, 33), *Diversa Corporation, San Diego, California 92121-7032*

ROLAND J. SIEZEN (25), *NIZO Food Research, Ede NL-6701 BA, The Netherlands*

M. R. SINGLETON (26), *Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, United Kingdom*

MARJORY A. SNEAD (13, 14, 15, 22), *Diversa Corporation, San Diego, California 92121-7032*

DINLAKA SRIPRAPUNDH (12), *Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824-1319*

KARL O. STETTER (2), *Lehrstuhl für Mikrobiologie und Archaeenzentrum, Universität Regensburg, Regensburg D-93053, Germany*

MARK D. STUMP (7), *Department of Human Genetics, University of Utah, Salt Lake City, Utah 84112*

RONALD V. SWANSON (8), *Department of Molecular Biology, Syrrx, Inc., San Diego, California 92121*

DION R. THOMPSON (19), *Department of Biological Sciences, Waikato University School of Science and Technology, Hamilton, New Zealand*

ANDREAS M. UHL (19), *Department of Biological Sciences, Waikato University School of Science and Technology, Hamilton, New Zealand*

JOHN VAN DER OOST (21, 24, 25), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

JOHAN F. T. VAN LIESHOUT (21), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

MARC F. J. M. VERHAGEN (3), *Allergan, Inc., Irvine, California 92612*

CORNÉ H. VERHEES (21), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

CLAIRE VIEILLE (12, 23), *Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824-1319*

WILFRIED G. B. VOORHORST (25), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

DON E. WARD (21), *Laboratory of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, The Netherlands*

ROBERT B. WEISS (7), *Department of Human Genetics, University of Utah, Salt Lake City, Utah 84112*

WILLIAM B. WHITMAN (5), *Department of Microbiology, University of Georgia, Athens, Georgia 30602-2605*

RAYMOND K. YEH (7), *Department of Human Genetics, University of Utah, Salt Lake City, Utah 84112*

J. GREGORY ZEIKUS (12, 23), *Michigan Biotechnology Institute International, Lansing, Michigan 48909*

Preface

More than thirty years ago, the pioneering work of Thomas Brock of the University of Wisconsin on the microbiology of hot springs in Yellowstone National Park alerted the scientific community to the existence of microorganisms with optimal growth temperatures of 70°C and even higher. In the early 1980s, the known thermal limits of life were expanded by the seminal work of Karl Stetter and colleagues at the University of Regensburg, who isolated from a marine volcanic vent the first microorganisms that could grow at, and even above, the normal boiling point of water. Subsequent work by Stetter and several other groups have led to the discovery in a variety of geothermal biotopes of more than twenty different genera that can grow optimally at or above 80°C. Such organisms are now termed *hyperthermophiles.*

Initial efforts to explore the enzymology of hyperthermophiles were impeded by the difficulty of culturing the organisms on a scale large enough to allow the purification of specific proteins in sufficient quantities for characterization. This often meant processing hundreds of liters of nearly boiling fermentation media under anaerobic conditions. In addition, relatively low biomass yields were typically obtained. Nevertheless, the first "hyperthermophilic enzymes" were purified in the late 1980s. It was demonstrated that they are, indeed, extremely stable at high temperatures, that this is an intrinsic property, and that they exhibit no or very low activity at temperatures below the growth conditions of the organism from which they were obtained. At that time it was difficult to imagine how quickly the tools of molecular biology would make such a dramatic impact on the world of hyperthermophiles. In fact, it was unexpected that the recombinant forms of hyperthermophilic enzymes would, to a large extent, correctly achieve their active conformation in mesophilic hosts grown some 70°C below the enzyme's source organism's normal growth temperature. This approach provided a much-needed alternative to large-scale hyperthermophile cultivation. With the ever-expanding list of genomes from hyperthermophiles that have been or are being sequenced, molecular biology provides universal access to a treasure chest of known and putative proteins endowed with unprecedented levels of thermostability.

In Volumes 330, 331, and 334 of *Methods in Enzymology,* a set of protocols has been assembled that for the first time describe the methods involved in studying the biochemistry and biophysics of enzymes and proteins from hyperthermophilic microorganisms. As is evident from the various chapters, hyperthermophilic counterparts to a range of previously stud-

ied but less thermostable enzymes exist. In addition, the volumes include descriptions of many novel enzymes that were first identified and, in most cases, are still limited to, hyperthermophilic organisms. Also included in these volumes are genomic analyses from selected hyperthermophiles that provide some perspective on what remains to be investigated in terms of hyperthermophilic enzymology. Specific chapters address the basis for extreme levels of thermostability and special considerations that must be taken into account in defining experimentally the biochemical and biophysical features of hyperthermophilic enzymes.

There are many individuals whose pioneering efforts laid the basis for the work discussed in these volumes. None was more important than the late Holger Jannasch of Woods Hole Oceanographic Institute. His innovation and inspiration opened a new field of microbiology in deep-sea hydrothermal vents and provided the research world access to a biotope of great scientific and technological promise. Holger will be remembered in many ways, and it is a fitting tribute that the first genome of a hyperthermophile to be sequenced should bear his name: *Methanocaldococcus jannaschii.* We wish to recognize Holger's pioneering efforts by dedicating these volumes to him.

MICHAEL W. W. ADAMS
ROBERT M. KELLY

METHODS IN ENZYMOLOGY

VOLUME I. Preparation and Assay of Enzymes
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME II. Preparation and Assay of Enzymes
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME III. Preparation and Assay of Substrates
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME IV. Special Techniques for the Enzymologist
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME V. Preparation and Assay of Enzymes
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME VI. Preparation and Assay of Enzymes (*Continued*)
Preparation and Assay of Substrates
Special Techniques
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME VII. Cumulative Subject Index
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME VIII. Complex Carbohydrates
Edited by ELIZABETH F. NEUFELD AND VICTOR GINSBURG

VOLUME IX. Carbohydrate Metabolism
Edited by WILLIS A. WOOD

VOLUME X. Oxidation and Phosphorylation
Edited by RONALD W. ESTABROOK AND MAYNARD E. PULLMAN

VOLUME XI. Enzyme Structure
Edited by C. H. W. HIRS

VOLUME XII. Nucleic Acids (Parts A and B)
Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XIII. Citric Acid Cycle
Edited by J. M. LOWENSTEIN

VOLUME XIV. Lipids
Edited by J. M. LOWENSTEIN

VOLUME XV. Steroids and Terpenoids
Edited by RAYMOND B. CLAYTON

VOLUME XVI. Fast Reactions
Edited by KENNETH KUSTIN

VOLUME XVII. Metabolism of Amino Acids and Amines (Parts A and B)
Edited by HERBERT TABOR AND CELIA WHITE TABOR

VOLUME XVIII. Vitamins and Coenzymes (Parts A, B, and C)
Edited by DONALD B. MCCORMICK AND LEMUEL D. WRIGHT

VOLUME XIX. Proteolytic Enzymes
Edited by GERTRUDE E. PERLMANN AND LASZLO LORAND

VOLUME XX. Nucleic Acids and Protein Synthesis (Part C)
Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME XXI. Nucleic Acids (Part D)
Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XXII. Enzyme Purification and Related Techniques
Edited by WILLIAM B. JAKOBY

VOLUME XXIII. Photosynthesis (Part A)
Edited by ANTHONY SAN PIETRO

VOLUME XXIV. Photosynthesis and Nitrogen Fixation (Part B)
Edited by ANTHONY SAN PIETRO

VOLUME XXV. Enzyme Structure (Part B)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVI. Enzyme Structure (Part C)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVII. Enzyme Structure (Part D)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVIII. Complex Carbohydrates (Part B)
Edited by VICTOR GINSBURG

VOLUME XXIX. Nucleic Acids and Protein Synthesis (Part E)
Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XXX. Nucleic Acids and Protein Synthesis (Part F)
Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME XXXI. Biomembranes (Part A)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME XXXII. Biomembranes (Part B)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME XXXIII. Cumulative Subject Index Volumes I–XXX
Edited by MARTHA G. DENNIS AND EDWARD A. DENNIS

VOLUME XXXIV. Affinity Techniques (Enzyme Purification: Part B)
Edited by WILLIAM B. JAKOBY AND MEIR WILCHEK

VOLUME XXXV. Lipids (Part B)
Edited by JOHN M. LOWENSTEIN

VOLUME XXXVI. Hormone Action (Part A: Steroid Hormones)
Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XXXVII. Hormone Action (Part B: Peptide Hormones)
Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XXXVIII. Hormone Action (Part C: Cyclic Nucleotides)
Edited by JOEL G. HARDMAN AND BERT W. O'MALLEY

VOLUME XXXIX. Hormone Action (Part D: Isolated Cells, Tissues, and Organ Systems)
Edited by JOEL G. HARDMAN AND BERT W. O'MALLEY

VOLUME XL. Hormone Action (Part E: Nuclear Structure and Function)
Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XLI. Carbohydrate Metabolism (Part B)
Edited by W. A. WOOD

VOLUME XLII. Carbohydrate Metabolism (Part C)
Edited by W. A. WOOD

VOLUME XLIII. Antibiotics
Edited by JOHN H. HASH

VOLUME XLIV. Immobilized Enzymes
Edited by KLAUS MOSBACH

VOLUME XLV. Proteolytic Enzymes (Part B)
Edited by LASZLO LORAND

VOLUME XLVI. Affinity Labeling
Edited by WILLIAM B. JAKOBY AND MEIR WILCHEK

VOLUME XLVII. Enzyme Structure (Part E)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XLVIII. Enzyme Structure (Part F)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XLIX. Enzyme Structure (Part G)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME L. Complex Carbohydrates (Part C)
Edited by VICTOR GINSBURG

VOLUME LI. Purine and Pyrimidine Nucleotide Metabolism
Edited by PATRICIA A. HOFFEE AND MARY ELLEN JONES

VOLUME LII. Biomembranes (Part C: Biological Oxidations)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LIII. Biomembranes (Part D: Biological Oxidations)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LIV. Biomembranes (Part E: Biological Oxidations)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LV. Biomembranes (Part F: Bioenergetics)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LVI. Biomembranes (Part G: Bioenergetics)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LVII. Bioluminescence and Chemiluminescence
Edited by MARLENE A. DELUCA

VOLUME LVIII. Cell Culture
Edited by WILLIAM B. JAKOBY AND IRA PASTAN

VOLUME LIX. Nucleic Acids and Protein Synthesis (Part G)
Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME LX. Nucleic Acids and Protein Synthesis (Part H)
Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME 61. Enzyme Structure (Part H)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME 62. Vitamins and Coenzymes (Part D)
Edited by DONALD B. MCCORMICK AND LEMUEL D. WRIGHT

VOLUME 63. Enzyme Kinetics and Mechanism (Part A: Initial Rate and Inhibitor Methods)
Edited by DANIEL L. PURICH

VOLUME 64. Enzyme Kinetics and Mechanism (Part B: Isotopic Probes and Complex Enzyme Systems)
Edited by DANIEL L. PURICH

VOLUME 65. Nucleic Acids (Part I)
Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME 66. Vitamins and Coenzymes (Part E)
Edited by DONALD B. MCCORMICK AND LEMUEL D. WRIGHT

VOLUME 67. Vitamins and Coenzymes (Part F)
Edited by DONALD B. MCCORMICK AND LEMUEL D. WRIGHT

VOLUME 68. Recombinant DNA
Edited by RAY WU

VOLUME 69. Photosynthesis and Nitrogen Fixation (Part C)
Edited by ANTHONY SAN PIETRO

VOLUME 70. Immunochemical Techniques (Part A)
Edited by HELEN VAN VUNAKIS AND JOHN J. LANGONE

VOLUME 71. Lipids (Part C)
Edited by JOHN M. LOWENSTEIN

VOLUME 72. Lipids (Part D)
Edited by JOHN M. LOWENSTEIN

VOLUME 73. Immunochemical Techniques (Part B)
Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 74. Immunochemical Techniques (Part C)
Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 75. Cumulative Subject Index Volumes XXXI, XXXII, XXXIV–LX
Edited by EDWARD A. DENNIS AND MARTHA G. DENNIS

VOLUME 76. Hemoglobins
Edited by ERALDO ANTONINI, LUIGI ROSSI-BERNARDI, AND EMILIA CHIANCONE

VOLUME 77. Detoxication and Drug Metabolism
Edited by WILLIAM B. JAKOBY

VOLUME 78. Interferons (Part A)
Edited by SIDNEY PESTKA

VOLUME 79. Interferons (Part B)
Edited by SIDNEY PESTKA

VOLUME 80. Proteolytic Enzymes (Part C)
Edited by LASZLO LORAND

VOLUME 81. Biomembranes (Part H: Visual Pigments and Purple Membranes, I)
Edited by LESTER PACKER

VOLUME 82. Structural and Contractile Proteins (Part A: Extracellular Matrix)
Edited by LEON W. CUNNINGHAM AND DIXIE W. FREDERIKSEN

VOLUME 83. Complex Carbohydrates (Part D)
Edited by VICTOR GINSBURG

VOLUME 84. Immunochemical Techniques (Part D: Selected Immunoassays)
Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 85. Structural and Contractile Proteins (Part B: The Contractile Apparatus and the Cytoskeleton)
Edited by DIXIE W. FREDERIKSEN AND LEON W. CUNNINGHAM

VOLUME 86. Prostaglandins and Arachidonate Metabolites
Edited by WILLIAM E. M. LANDS AND WILLIAM L. SMITH

VOLUME 87. Enzyme Kinetics and Mechanism (Part C: Intermediates, Stereochemistry, and Rate Studies)
Edited by DANIEL L. PURICH

VOLUME 88. Biomembranes (Part I: Visual Pigments and Purple Membranes, II)
Edited by LESTER PACKER

VOLUME 89. Carbohydrate Metabolism (Part D)
Edited by WILLIS A. WOOD

VOLUME 90. Carbohydrate Metabolism (Part E)
Edited by WILLIS A. WOOD

VOLUME 91. Enzyme Structure (Part I)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME 92. Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)
Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 93. Immunochemical Techniques (Part F: Conventional Antibodies, Fc Receptors, and Cytotoxicity)
Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 94. Polyamines
Edited by HERBERT TABOR AND CELIA WHITE TABOR

VOLUME 95. Cumulative Subject Index Volumes 61–74, 76–80
Edited by EDWARD A. DENNIS AND MARTHA G. DENNIS

VOLUME 96. Biomembranes [Part J: Membrane Biogenesis: Assembly and Targeting (General Methods; Eukaryotes)]
Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 97. Biomembranes [Part K: Membrane Biogenesis: Assembly and Targeting (Prokaryotes, Mitochondria, and Chloroplasts)]
Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 98. Biomembranes (Part L: Membrane Biogenesis: Processing and Recycling)
Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 99. Hormone Action (Part F: Protein Kinases)
Edited by JACKIE D. CORBIN AND JOEL G. HARDMAN

VOLUME 100. Recombinant DNA (Part B)
Edited by RAY WU, LAWRENCE GROSSMAN, AND KIVIE MOLDAVE

VOLUME 101. Recombinant DNA (Part C)
Edited by RAY WU, LAWRENCE GROSSMAN, AND KIVIE MOLDAVE

VOLUME 102. Hormone Action (Part G: Calmodulin and Calcium-Binding Proteins)
Edited by ANTHONY R. MEANS AND BERT W. O'MALLEY

VOLUME 103. Hormone Action (Part H: Neuroendocrine Peptides)
Edited by P. MICHAEL CONN

VOLUME 104. Enzyme Purification and Related Techniques (Part C)
Edited by WILLIAM B. JAKOBY

VOLUME 105. Oxygen Radicals in Biological Systems
Edited by LESTER PACKER

VOLUME 106. Posttranslational Modifications (Part A)
Edited by FINN WOLD AND KIVIE MOLDAVE

VOLUME 107. Posttranslational Modifications (Part B)
Edited by FINN WOLD AND KIVIE MOLDAVE