Methods in ENZYMOLOGY

Volume 290

Molecular Chaperones

Edited by

George H. Lorimer

Thomas O. Baldwin

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MOLECULAR CHAPERONES

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OSAMU ASAMI (4), *Toyota Central Research and Development Laboratories, Inc., Nagakute, Aichi 480-11, Japan*

ABDUSSALAM AZEM (22), *Department of Botany, Alexander Silbermann Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel*

KAREN BACOT (19), *Molecular Biology Division, Central Research and Development Department, E. I. duPont de Nemours and Company, Experimental Station, Wilmington, Delaware 19880-0402*

THOMAS O. BALDWIN (1), *Center for Macromolecular Design, Departments of Biochemistry and Biophysics, and Chemistry, Texas A&M University, College Station, Texas 77843-2128*

JAMES C. A. BARDWELL (5), *Department of Biology, University of Michigan, Ann Arbor, Michigan 48109*

JOACHIM BEHLKE (23), *Max Delbrück Center for Molecular Medicine, D-13122 Berlin, Germany*

WOLFGANG BERGMEIER (18), *Institüt für Biophysik and Physikalische Biochemie, Universität Regensburg, 93040 Regensburg, Germany*

SYLVIE BLOND (31), *Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, Chicago, Illinois 60607-7173*

SUCHIRA BOSE (32, 33), *Department of Biochemistry, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, Great Britain*

MICHAEL P. BOVA (30), *Jules Stein Eye Institute, University of California at Los Angeles School of Medicine, Los Angeles, California 90095-7008*

BILL T. BRAZIL (26), *Department of Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7760*

JOHANNES BUCHNER (27, 28, 32, 33), *Institüt für Biophysik und Physikalische Biochemie, Universität Regensburg, 93040 Regensburg, Germany*

HANS BÜGL (33), *Institüt für Biophysik und Physikalische Biochemie, Universität Regensburg, 93040 Regensburg, Germany*

STEVEN G. BURSTON (11), *Department of Genetics, Yale University School of Medicine, New Haven, Connecticut 06510*

S. CHEN (21), *Department of Crystallography, Birkbeck College, London WC1E 7HX, United Kingdom*

MATHIEU CHEVALIER (31), *Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, Chicago, Illinois 60607-7173*

A. CLAY CLARK (8), *Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110-3344*

NICHOLAS J. COWAN (20), *Department of Biochemistry, New York University School of Medicine, New York, New York 10016-6402*

DEBORAH L. DIAMOND (35), *Department of Biochemistry, Washington State University, Pullman, Washington 99164-4660*

RAMONA DICKSON (19), *Molecular Biology Division, Central Research and Development Department, E. I. duPont de Nemours and Company, Experimental Station, Wilmington, Delaware 19880-0402*

LINLIN DING (30), *Jules Stein Eye Institute, University of California at Los Angeles School of Medicine, Los Angeles, California 90095-7008*

YVES DUBAQUIÉ (17), *Basel Abteilung Biochemie, Biozentrum Universite Basel, Basel, Switzerland CH-4056*

MONIKA EHRNSPERGER (28), *Institüt für Biophysik und Physikalische Biochemie, Universität Regensburg, 93040 Regensburg, Germany*

EDWARD EISENSTEIN (9), *Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, Maryland 20850*

ALEXEY N. FEDOROV (1), *Center for Macromolecular Design, Departments of Biochemistry and Biophysics, and Chemistry, Texas A&M University, College Station, Texas 77843-2128*

WAYNE A. FENTON (11), *Department of Genetics, Yale University School of Medicine, New Haven, Connecticut 06510*

MARK T. FISHER (9), *Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas 66160-7421*

CARL FRIEDEN (8), *Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110-3344*

MATTHIAS GAESTEL (28), *Max Delbrück Zentrum für Molekulare Medizin, 13122 Berlin, Germany*

H. F. GILBERT (3), *Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030-3498*

PIERRE GOLOUBINOFF (18, 22), *Department of Botany, Alexander Silbermann Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel*

BORIS M. GOROVITS (25), *Department of Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7760*

HOLGER GRALLERT (27), *Institüt für Biophysik und Physikalische Biochemie, Universität Regensburg, 93040 Regensburg, Germany*

MICHAEL GROSS (24), *Oxford Centre for Molecular Sciences, New Chemistry Laboratory, Oxford OX1 3QT, United Kingdom*

BOYD HARDESTY (2), *Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas 78712*

SIMON J. S. HARDY (35), *Department of Biology, University of York, York YO1 5YW, England*

MASANA HIRAI (4), *Toyota Central Research and Development Laboratories, Inc., Nagakute, Aichi 480-11, Japan*

PAUL M. HOROWITZ (25, 26), *Department of Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7760*

ARTHUR L. HORWICH (11), *Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06510*

JOSEPH HORWITZ (30), *Jules Stein Eye Institute, University of California at Los Angeles School of Medicine, Los Angeles, California 90095-7008*

QING-LING HUANG (30), *Jules Stein Eye Institute, University of California at Los Angeles School of Medicine, Los Angeles, California 90095-7008*

HAJIME ISHIKAWA (16), *Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo 113, Japan*

URSULA JAKOB (27, 32), *Department of Biology, University of Michigan, Ann Arbor, Michigan 48109*

TSUTOMU KAJINO (4), *Toyota Central Research and Development Laboratories, Inc., Nagakute, Aichi 480-11, Japan*

MARTIN KESSEL (18), *Department of Membrane and Ultrastructural Research, Hebrew University-Hadassah Medical School, 91220 Jerusalem, Israel*

LASHAUNDA KING (31), *Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, Chicago, Illinois 60607-7173*

GISELA KRAMER (2), *Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas 78712*

WIESLAW KUDLICKI (2), *Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas 78712*

GARRETT J. LEE (29), *Department of Biochemistry, University of Arizona, Tucson, Arizona 85721-0106*

W. THEODORE LEE (13), *Department of Microbiology, Ohio State University, Columbus, Ohio 43210*

SUSAN LINDQUIST (34), *Howard Hughes Medical Institute and Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637-8049*

GEORGE H. LORIMER (10, 18), *Molecular Biology Division, Central Research and Development Department, E. I. duPont de Nemours and Company, Experimental Station, Wilmington, Delaware 19880-0402*

AUTAR K. MATTOO (7), *Vegetable Laboratory, USDA/ARS, Beltsville Agricultural Research Center (W), Beltsville, Maryland 20705-2350*

CHRISTIAN MAYR (32), *Institüt für Biophysik und Physikalische Biochemie, Universität Regensburg, 93040 Regensburg, Germany*

BRUCE A. MCFADDEN (12), *Department of Biochemistry and Biophysics, Washington State University, Pullman, Washington 99164-4660*

CHIE MIYAZAKI (4), *Toyota Central Research and Development Laboratories, Inc., Nagakute, Aichi 480-11, Japan*

MIZUE MORIOKA (16), *Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo 113, Japan*

NIKHIL D. PHADKE (5), *Department of Biology, University of Michigan, Ann Arbor, Michigan 48109*

FRANZISKA PIRKL (33), *Institüt für Biophysik und Physikalische Biochemie, Universität Regensburg, 93040 Regensburg, Germany*

SHEENA E. RADFORD (24), *Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, United Kingdom*

RAGULAN RAMANATHAN (8), *Center for Mass Spectrometry, Washington University School of Medicine, St. Louis, Missouri 63110-3344*

LINDA L. RANDALL (35), *Department of Biochemistry, Washington State University, Pullman, Washington 99164-4660*

PRASAD REDDY (9), *DNA Technologies Group, Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899*

CAROL V. ROBINSON (24), *Oxford Centre for Molecular Sciences, New Chemistry Laboratory, Oxford OX1 3QT, United Kingdom*

A. M. ROSEMAN (21), *Department of Crystallography, Birkbeck College, London WC1E 7HX, United Kingdom*

SABINE ROSPERT (17), *Basel Abteilung Biochemie, Biozentrum Universite Basel, Basel, Switzerland CH-4056*

HAYS S. RYE (11), *Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06510*

H. R. SAIBIL (21), *Department of Crystallography, Birkbeck College, London WC1E 7HX, United Kingdom*

GOTTFRIED SCHATZ (17), *Basel Abteilung Biochemie, Biozentrum Universite Basel, CH-4056 Basel, Switzerland*

ERIC C. SCHIRMER (34), *Howard Hughes Medical Institute and Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637-8049*

HANS-JOACHIM SCHÖNFELD (23), *Pharmaceutical Research–Infectious Diseases, F. Hoffmann–La Roche Limited, CH-4070 Basel, Switzerland*

ROBERT K. SCOPES (14), *Center for Protein and Enzyme Technology, School of Biochemistry, La Trobe University, Bundoora, Victoria 3083, Australia*

JEFFREY W. SEALE (26), *Monsanto, St. Louis, Missouri 63189*

JOHN J. SIEKIERKA (6), *Discovery Research, The R. W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey 08869-0602*

VIRGINIA F. SMITH (35), *Department of Biochemistry, Washington State University, Pullman, Washington 99164-4660*

F. ROBERT TABITA (13), *Department of Microbiology, Ohio State University, Columbus, Ohio 43210*

HIDEKI TAGUCHI (15), *Research Laboratory of Resources Utilization, R-1, Tokyo Institute of Technology, Yokohama 226, Japan*

MATTHEW J. TODD (10), *Molecular Biology Division, Central Research and Development Department, E. I. duPont de Nemours and Company, Experimental Station, Wilmington, Delaware 19880-0402*

TRACI B. TOPPING (35), *Department of Biochemistry, Washington State University, Pullman, Washington 99164-4660*

JOSE A. TORRES-RUIZ (12), *Department of Biochemistry, Ponce School of Medicine, Ponce, Puerto Rico 00732*

KAYE TRUSCOTT (14), *Center for Protein and Enzyme Technology, School of Biochemistry, La Trobe University, Bundoora, Victoria 3083, Australia*

SHIGEZU UDAKA (4), *Department of Brewing and Fermentation, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156, Japan*

ELIZABETH VIERLING (29), *Department of Biochemistry, University of Arizona, Tucson, Arizona 85721-0106*

PAUL V. VIITANEN (18, 19), *Molecular Biology Division, Central Research and Development Department, E. I. duPont de Nemours and Company, Experimental Station, Wilmington, Delaware 19880-0402*

STEFAN WALKE (28), *Medical Research Council, Laboratory of Molecular Biology, Cambridge CB2 2QH, England*

Gregory M. F. WATSON (13), *Department of Microbiology, Ohio State University, Columbus, Ohio 43210*

TOM WEBB (19), *Molecular Biology Division, Central Research and Development Department, E. I. duPont de Nemours and Company, Experimental Station, Wilmington, Delaware 19880-0402*

TINA WEIKL (33), *Institüt für Biophysik und Physikalische Biochemie, Universität Regensburg, 93040 Regensburg, Germany*

CELESTE WEISS (18, 22), *Department of Botany, Alexander Silbermann Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel*

JONATHAN S. WEISSMAN (11), *Department of Pharmacology, University of California San Francisco School of Medicine, San Francisco, California 94143*

GREGORY WIEDERRECHT (6), *Immunology Research, Merck Research Laboratories, Rahway, New Jersey 07065*

YUKIO YAMADA (4), *Toyota Central Research and Development Laboratories, Inc., Nagakute, Aichi 480-11, Japan*

MASASUKE YOSHIDA (15), *Research Laboratory of Resources Utilization, R-1, Tokyo Institute of Technology, Yokohama 226, Japan*

THOMAS ZANDER (5), *Department of Biology, University of Michigan, Ann Arbor, Michigan 48109*

TONG ZHANG (2), *Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas 78712*

Preface

The chapters contained in this volume represent a collection of methods currently used for the study of assisted folding and assembly of proteins. Much of the literature is published in short articles in journals that do not allow presentation of experimental details, and in this field, as in most, the devil is truly in the details.

Since the pioneering work of Anfinsen and his collaborators and contemporaries, the study of protein folding has been the province of biophysics and biochemistry. The discovery of molecular chaperones and the development of recombinant DNA methods have caused an enormous influx of cell biologists, genetists, and molecular biologists to the field, and, as a result, cultural and language differences often lead to misunderstandings of meaning and interpretation of experimental observations. The laws of thermodynamics pertain *in vivo* as well as *in vitro,* and while refolding of a protein following dilution of urea-containing buffers is hardly biological, the principles learned from such studies are applicable to the understanding of the biological processes as they occur within the living cell. For example, it is well known that to obtain high recovery of soluble protein on refolding from urea or guanidine, it is usually essential to dilute the protein solution into an aqueous buffer so that each molecule may refold effectively in isolation, thereby avoiding assembly of folding intermediates into large aggregates. Likewise, *in vivo,* overexpression of a protein from a plasmid or other vector often leads to aggregation and inclusion body formation as a consequence of the higher than normal steady-state concentration of folding intermediates of that protein.

As with all enzymes, a detailed biochemical description of the mechanism of action of chaperones and folding catalysts requires pure enzyme. Failure to remove bound, contaminating substrate polypeptides from chaperones can and does lead to errors in determination of stoichiometry and other enzymological parameters.

In assembling this volume, one of the major objectives was to solicit contributions from experts knowledgeable in the detailed "personalities" of the chaperones and related components of the protein metabolic machinery. The chemical transformations catalyzed by the chaperones will ultimately be understood in molecular detail. Until then, we hope and believe that the process of unraveling these details will be greatly facilitated through diligent application of the methods presented by the contributors to this volume.

We are deeply indebted to the contributors for their chapters and for their advice, which helped substantially in shaping the final volume. We also appreciate the efforts of the excellent staff of Academic Press, especially Ms. Shirley Light, for their assistance and gentle encouragement.

GEORGE H. LORIMER
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