The Proteomics Protocols Handbook

EDITED BY

John M. Walker

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University of Hertfordshire, Hatfield, UK

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Preface

Recent developments in the field of proteomics have revolutionized the way that proteins, and their contribution to cellular functions, are studied. The subsequent increased understanding of the mechanisms of cellular function and misfunction will have particular impact in the area of medical research, where disease processes will be better understood, many new (protein) therapeutic targets identified, and novel therapeutic agents developed. At the basic research level, phenotype will be explained in terms of cellular mechanisms.

The completion of the sequences of an ever-widening range of genomes—not least of all, the human genome—has provided the molecular biologist with a wealth of data that needs to be analyzed and interpreted. For a variety of reasons (including alternative mRNA splicing, varying translational stop/start sites, frameshifting, and the inability to deduce posttranslational modifications), complete sequences of genomes are insufficient to elucidate the protein components of cells. The focus of attention has therefore turned to directly examining these protein components as the means of understanding cell function, as well as the cellular changes involved in disease states. However, the wealth of gene sequencing data now available has produced a glut of information that challenges the protein chemist to develop new tools to utilize this flood of genomic data.

From the beginning, the cornerstone of proteomics has been the use of two-dimensional gel electrophoresis to compare proteomes of different tissues (for example, normal and diseased tissue) with the subsequent identification of protein differences by the use of mass spectrometry and database searching. These still remain valuable techniques and receive appropriate coverage in this book. However, the term proteomics now encompasses a range of newly developed methodologies for determining the structure and function of a protein. I have therefore included in *The Proteomics Protocols Handbook* a number of novel mass spectrometry and LC-MS techniques, protein array technology, new bioinformatics tools, and the range of techniques central to structural and functional proteomics that are needed to deduce the function of newly discovered protein sequences. The use of these techniques, and no doubt further ones that will be developed in the coming years, will lead to achieving the ultimate goal of proteomics, namely to catalog the identity and function of all proteins in living organisms.

The Proteomics Protocols Handbook should prove a valuable resource for molecular biologists, protein chemists, clinical/medical researchers, structural chemists/biochemists, and microbiologists, as well as those involved in bioinformatics and structural/functional genomics.

John M. Walker

Contributors

Ruedi Aebersold • *Institute for Systems Biology, Seattle, WA*
Nancy Ahnert • *Molecular Probes Inc., Eugene, OR*
Alastair Aitken • *School of Biomedical and Clinical Laboratory Sciences, University of Edinburgh, UK*
Philip C. Andrews • *Michigan Proteome Consortium, University of Michigan, Ann Arbor, MI*
Ron D. Appel • *Swiss Institute of Bioinformatics, University and Geneva University Hospital, Geneva, Switzerland*
Rolf Apweiler • *European Bioinformatics Institute, Cambridge, UK*
Arezou Azarani • *Protogen Consulting, San Jose, CA*
Gary D. Bader • *Computational Biology Center, Memorial Sloan-Kettering Cancer Center, New York, NY*
Volker Badock • *Max Delbrueck Center for Molecular Medicine, Berlin, Germany*
Amos Bairoch • *Swiss Institute of Bioinformatics, University of Geneva, Geneva, Switzerland*
Rashmi Bansal • *Department of Neuroscience, University of Connecticut Medical School, Farmington, CT*
Sarka Beranova-Giorgianni • *Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN*
Svend Birkelund • *Department of Medical Microbiology and Immunology, University of Aarhus, Denmark, Loke Diagnostics ApS, Denmark*
Ewan Birney • *European Bioinformatics Institute, Cambridge, UK*
Gérard Bouchet • *Swiss Institute of Bioinformatics, Geneva, Switzerland*
Joe Boutell • *Discerna Ltd., Cambridge, UK*
Volker Brendel • *Department of Genetics, Development and Cell Biology, Department of Statistics, Iowa State University, Ames, IA*
Henry Brzeski • *Functional Genomics and Proteomics Unit, Windber Research Institute, Windber, PA*
Gerard Cagney • *Department of Medical Research, University of Toronto, Canada*
Emilia Caputo • *Institute of Genetics and Biophysics, Naples, Italy*
Grace Y. J. Chen • *Department of Biological Sciences, Department of Chemistry, National University of Singapore, Republic of Singapore*
Xian Chen • *Biosciences Division, Los Alamos National Laboratory, Los Alamos, NM*
Gunna Christiansen • *Department of Medical Microbiology and Immunology, University of Aarhus, Denmark*
Scott T. Clarke • *Molecular Probes Inc., Eugene, OR*
Lucio Comai • *Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA*
Kelly Cooke • *Institute for Systems Biology, Seattle, WA*
Patrick W. Cooley • *MicroFab Technologies Inc., Plano, TX*
Yohann Couté • *Centre de Genetique Moleculaire et Cellulaire, France*

Brenda Deyarmin • *Clinical Breast Care Project, Windber Research Institute, Windber, PA*
Jean-Jacques Diaz • *Centre de Genetique Moleculaire et Cellulaire, France*
Régis Dieckmann • *Biomedical Proteomics Research Group, Central Clinical Chemistry Laboratory, Geneva University Hospital, Geneva, Switzerland*
Qunfeng Dong • *Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA*
Séverine Duvaud • *Swiss Institute of Bioinformatics, Geneva, Switzerland*
Andrzej Ejchart • *Institute of Biochemistry and Biophysics, Poland*
Darrell L. Ellsworth • *Cardiovascular Disease Research Program and Clinical Breast Care Project, Windber Research Institute, Windber, PA*
Andrew Emili • *Banting and Best Departments of Medical Research, University of Toronto, Canada*
Anton J. Enright • *Computational Biology Center, Memorial Sloan-Kettering Cancer Center, New York, NY*
Narayanan Eswar • *Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, California Institute for Quantitative Biomedical Research, University of California, San Francisco, CA*
Jai Evans • *LECB, CIT, NIH, Frederick, MD*
Ye Fang • *Biochemical Technologies, Science and Technology Division, Corning Incorporated, Corning, NY*
Carlos Fernandez-Patron • *Heart and Stroke Foundation New Investigator, Department of Biochemistry, University of Alberta, Canada*
Ann M. Ferrie • *Biochemical Technologies, Science and Technology Division, Corning Incorporated, Corning, NY*
Tiffany B. Fischer • *Department of Biochemistry and Biophysics, Texas A&M University, TX*
John Flensburg • *Amersham Biosciences AB, GE Healthcare, Uppsala, Sweden*
Michael Fountoulakis • *F. Hoffman-LaRoche Ltd., Center for Medical Genomics, Basel, Switzerland, Foundation for Biomedical Research of the Academy of Athens, Greece*
Elisabeth Gasteiger • *Swiss Institute of Bioinformatics, Geneva, Switzerland*
Alexandre Gattiker • *Swiss Institute of Bioinformatics, Geneva, Switzerland*
Francesco Giorgianni • *Charles B. Stout Neuroscience Mass Spectrometry Laboratory, University of Tennessee Health Science Center, Memphis, TN*
William L. Godfrey • *Molecular Probes, Inc., Eugene, OR*
Erica A. Golemis • *Division of Basic Science, Fox Chase Cancer Center, Philadelphia, PA*
David R. Goodlett • *Institute for Systems Biology, Seattle, WA*
Terrie Goodman • *Molecular Probes Inc., Eugene, OR*
Leo Goodstadt • *MRC Functional Genetics Unit, University of Oxford, Department of Human Anatomy and Genetics, Oxford, UK*
Andrew A. Gooley • *Proteome Systems Ltd., Sydney, Australia*
Franz-Georg Hanisch • *Institute of Biochemistry II and Center for Molecular Medicine Cologne, Cologne, Germany*
Courtenay R. Hart • *Molecular Probes Inc., Eugene, OR*

Setsuko Hashimoto • *Biacore K.K., Tokyo, Japan*
Mingyue He • *Discerna Ltd., Cambridge, UK*
Denis Hochstrasser • *Biomedical Proteomics Research Group, Central Clinical Chemistry Laboratory, Geneva University Hospital, Geneva, Switzerland*
Christine Hoogland • *Swiss Institute of Bioinformatics, Geneva, Switzerland*
Femia G. Hopwood • *Proteome Systems Ltd., Australia*
Sjouke Hoving • *Novartis Institutes for Biomedical Research, Functional Genomics/ Proteome Sciences, Basel, Switzerland*
Li-Shan Hsieh • *Division of New Drug Chemistry 1, Center for Drug Evaluation and Research, Food and Drug Administration, Rockville, MD*
Toshiaki Isobe • *Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University, Tokyo, Japan*
Biju Issac • *Institute of Microbial Technology, Chandigarh, India*
Sujata Iyer • *BD Biosciences, San Jose, CA*
Harren Jhoti • *Astex Technology Ltd., Cambridge, UK*
Bino John • *Computational Biology Center, Memorial Sloan-Kettering Cancer Center, New York, NY*
Janice L. Joss • *Proteome Systems Ltd., Sydney, Australia*
Rachel Karchin • *Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, California Institute for Quantitative Biomedical Research, University of California, San Francisco, CA*
Fang Lai • *Biochemical Technologies, Science and Technology Division, Corning Incorporated, Corning, NY*
Michele Learmonth • *School of Biomedical and Clinical Laboratory Sciences, University of Edinburgh, UK*
Ki-Boom Lee • *The Wistar Institute, Philadelphia, PA*
Richard M. Leimgruber • *Pfizer, Inc., PGRD-World Wide Safety Sciences, St. Louis, MO*
Peter F. Lemkin • *LECB, NCI-Frederick, Frederick, MD*
David W. M. Leung • *School of Biological Sciences, University of Canterbury, New Zealand*
Maria Liminga • *Amersham Biosciences AB, GE Healthcare, Uppsala, Sweden*
Yee-Peng R. Lue • *Department of Biological Sciences, National University of Singapore, Republic of Singapore*
M. S. Madhusudhan • *Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, California Institute for Quantitative Biomedical Research, University of California, San Francisco, CA*
Tadakazu Maeda • *Kitasato University School of Science, Japan*
Michele Magrane • *European Bioinformatics Institute, Cambridge, UK*
Gregory S. Makowski • *Department of Laboratory Medicine, School of Medicine, University of Connecticut Health Center, Farmington, CT*
Cecilia B. Marta • *Department of Neuroscience, University of Connecticut Medical School, Farmington, CT*
Marc A. Marti Renom • *Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, California Institute for Quantitative Biomedical Research, University of California, San Francisco, CA*

Brian M. Martin • *Laboratory of Neurotoxicology, NIMH, National Institutes of Health, Bethesda, MD*
Karen Martin • *Molecular Probes Inc., Eugene, OR*
Maria Jesus Martin • *European Bioinformatics Institute, Cambridge, UK*
Ramy Moharram • *Laboratory of Neurotoxicology, NIMH, National Institutes of Health, Bethesda, MD*
Khaled Mostaguir • *Swiss Institute of Bioinformatics, Geneva, Switzerland*
Nicola Jane Mulder • *European Bioinformatics Institute, Cambridge, UK*
Stefan Müller • *Institute of Biochemistry II and Center for Molecular Medicine Cologne, Cologne, Germany*
Tohru Natsume • *National Institute of Advances Industrial Science and Technology, Biological Information Research Center, Tokyo, Japan*
Sonia Navarro • *Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA*
Joakim Norbeck • *Chalmers Technical University, Göteborg, Sweden*
Claire O'Donovan • *European Bioinformatics Institute, Cambridge, UK*
Masamichi Oh-Ishi • *Kitasato University School of Science, Japan*
Jan van Oostrum • *Novartis Institutes for Biomedical Research, Functional Genomics/Proteome Sciences, Basel, Switzerland*
Albrecht Otto • *Department of Neuroproteomics, Max Delbrueck Center of Molecular Medicine, Berlin, Germany*
Melissa Paczkowski • *Department of Biochemistry and Biophysics, Texas A&M University, TX*
Patricia M. Palagi • *Swiss Institute of Bioinformatics, Geneva University Hospital, Geneva, Switzerland*
Wayne F. Patton • *Molecular Probes Inc., Eugene, OR*
Steven E. Pfeiffer • *Department of Neuroscience, University of Connecticut Medical School, Farmington, CT*
Ursula Pieper • *Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, California Institute for Quantitative Biomedical Research, University of California, San Francisco, CA*
Olivier Poch • *Laboratorie de Biologie et Genomique Structurales, Institut de Genetique et de Biologie Moleculaire et Cellulaire, France*
Julia Poland • *Institut fur Medizinische und Chemische Labordiagnostik, Landeskrankenhaus Klagenfurt, Klagenfurt, Austria*
Chris P. Ponting • *MRC Functional Genetics Unit, University of Oxford, Department of Human Anatomy and Genetics, Oxford, UK*
Manuela Pruess • *European Bioinformatics Institute, Cambridge, UK*
S. Rackovsky • *Department of Biomathematical Sciences, Mt. Sinai School of Medicine, New York, NY*
Thierry Rabilloud • *DBMS/BECP, CEA-Grenoble, France*
Gajendra P. S. Raghava • *Institute of Microbial Technology, Chandigarh, India*
Melinda L. Ramsby • *Department of Medicine, School of Medicine, University of Connecticut Health Center, Farmington, CT*
Diether Recktenwald • *Research Department, BD Biosciences, San Jose, CA*

BURKHARD ROST • *CUBIC, Department of Biochemistry and Molecular Biophysics, Columbia University, Columbia University Center for Computational Biology and Bioinformatics, NorthEast Structural Genomics Consortium, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY*
COLETTE J. RUDD • *BD Biosciences, San Jose, CA*
ROBERT B. RUSSELL • *Structural Bioinformatics EMBL, Heidelberg, Germany*
STEPHEN RUSSELL • *Functional Genomics and Protemics Unit, Windber Research Institute, Windber, PA*
GARY RYMAR • *Michigan Proteome Consortium, University of Michigan, Ann Arbor, MI*
ANDREJ SALI • *Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, California Institute for Quantitative Biomedical Research, University of California, San Francisco, CA*
JEAN-CHARLES SANCHEZ • *Biomedical Proteomics Research Group, Central Clinical Chemistry Laboratory, Geneva University Hospital, Geneva, Switzerland*
BIRTE SCHULENBERG • *Molecular Probes Inc., Eugene, OR*
FRANÇOISE SEILLIER-MOISEIWITSCH • *Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC*
ILYA G. SEREBRIISKII • *Division of Basic Science, Fox Chase Cancer Center, Philadelphia, PA*
MIN-YI SHEN • *Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, California Institute for Quantitative Biomedical Research, University of California, San Francisco, CA*
CRAIG D. SHRIVER • *Clinical Breast Care Project, Walter Reed Army Medical Center, Washington, DC*
PRANAV SINHA • *Institut fur Medizinische und Chemische Labordiagnostik, Landeskrankenhaus Klagenfurt, Klagenfurt, Austria*
LUCY SKRABANEK • *Department of Physiology and Biophysics and Institute for Computational Biomedicine, Weill Medical College of Cornell University, New York, NY*
RICHARD I. SOMIARI • *Functional Genomics and Protemics Unit, ITSI-Biosciences, Johnstown, PA*
STELLA B. SOMIARI • *Windber Research Institute, Windber, PA*
DAVID W. SPEICHER • *The Wistar Institute, Philadelphia, PA*
THOMAS H. STEINBERG • *Molecular Probes, Inc., Eugene, OR*
ANTHONY G. SULLIVAN • *Functional Genomics and Proteomics Unit, Windber Research Institute, Windber, PA; and Thermoelectron Training Institute, West Palm Beach, FL*
LAY-PHENG TAN • *Department of Biological Sciences, National University of Singapore, Republic of Singapore*
CHRISTOPHER M. TAYLOR • *Department of Neuroscience, University of Connecticut Medical School, Farmington, CT*
JULIE D. THOMPSON • *Laboratorie de Biologie et Genomique Structurales, Institut de Genetique et de Biologie Moleculaire et Cellulaire, France*
GREGORY C. THORNWALL • *LECB, SAIC • Frederick, Frederick, MD*

ANNABEL E. TODD • *Department of Biochemistry and Molecular Biology, University College London, London, UK*
ANDREW E. TORDA • *Zentrum für Bioinformatik, University of Hamburg, Hamburg, Germany*
JERRY TSAI • *Department of Biochemistry and Biophysics, Texas A&M University, TX*
PETER UETZ • *Institut für Genetik, Forschungszentrum Karlsruhe, Germany*
BRIAN BERG VANDAHL • *Department of Medical Microbiology and Immunology, University of Aarhus, Denmark, Loke Diagnostics ApS, Denmark*
MLADEN VINKOVIĆ • *Astex Technology Ltd., Cambridge, UK*
SONJA VOORDIJK • *Geneva Bioinformatics S. A., Geneva, Switzerland*
HANS VOSHOL • *Novartis Institutes for Biomedical Research, Functional Genomics/ Proteome Sciences, Basel, Switzerland*
ANGELA K. WALKER • *Michigan Proteome Consortium, University of Michigan, Ann Arbor, MI*
JOHN M. WALKER • *School of Life Sciences, University of Hertfordshire, Hatfield, UK*
DANIEL WALTHER • *Swiss Institute of Bioinformatics, Geneva, Switzerland*
MARC R. WILKINS • *Proteome Systems Ltd., Sydney, Australia*
NICHOLE L. WILSON • *Proteome Systems Ltd., Sydney, Australia*
FRANK A. WITZMANN • *Department of Cellular & Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN*
YOSHIHIDE YAMAGUCHI • *Department of Molecular Neurobiology, Tokyo University of Pharmacy and Life Science, Tokyo, Japan*
SHAO Q. YAO • *Department of Biological Sciences, Department of Chemistry, National University of Singapore, Republic of Singapore*
SU-YIN D. YEO • *Department of Biological Sciences, National University of Singapore, Republic of Singapore*
EUGENE YI • *Institute for Systems Biology, Seattle, WA*
JEFF YON • *Astex Technology Ltd., Cambridge, UK*
MICHAEL F. ZETTEL • *Department of Biochemistry and Biophysics, Texas A&M University, TX*
HUI ZHANG • *Institute for Systems Biology, Seattle, WA*
IGOR ZHUKOV • *Institute of Biochemistry and Biophysics, Warsaw, Poland*
XUN ZUO • *The Wistar Institute, Philadelphia, PA*

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Extraction and Solubilization of Proteins for Proteomic Studies

Richard M. Leimgruber

1. Introduction

For any proteomic study involving various control and experimental specimens, several factors need to be in place. A critical one is the extraction and solubilization of all components, regardless of whether a chromatographic *(1,2)* or two-dimensional (2-D) gel electrophoretic fractionation *(3–6)* is performed prior to analysis of proteins of interest by mass spectrometry of protein digests. All proteins must not only be extracted, but they must also be completely soluble, free from interacting partners (such as protein–RNA/DNA and protein–protein interactions, metabolites, and so on), and, in the case of 2-D gel electrophoresis, they must remain soluble as they approach their isoelectric points. The solubilization process should extract all classes of proteins reproducibly, such that statistically significant quantitative data can be obtained and correlated with experimental perturbations and the resulting biological responses.

To accomplish this task, various approaches have been presented in the literature *(7–11)*, and many solubilization cocktails are now available commercially. However, it should be noted that currently, despite several attempts by multiple groups, there is no single solubilization cocktail that works perfectly for all conditions and samples, due to sample source-related interfering compounds and a high degree of heterogeneity among samples. This heterogeneity can lead to differing protein solubilities. Also, the presence of highly abundant proteins complicates the extraction, solubilization, and analysis of the less abundant species. Extracts from certain plant tissues also present their own set of unique issues *(12)*. In addition to solubilization of all proteins, the solubilization agents used must also be compatible with the subsequent fractionation/analytical method employed. To date, the most efficient solubilization cocktails consist of a mixture of chaotropic agents, a mixture of detergents containing 13–15 carbon long hydrophobic chains, and a reductant *(13–17)*.

It is important to note that the effectiveness of solubilization is not the only factor that affects the quality of the 2-D protein patterns. Gel strip rehydration, sample application method, sample load, electrophoresis conditions, and so on all have an impact on the quality of the 2-D protein fingerprint or pattern.

Recently, there has been a renewed interest in quantitative protein profiling, a process that is critical for an understanding of biological function *(18,19)*. Because the proteome is a very complex, dynamic process that represents events at the functional

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