METODOLOGIES FOR METABOLOMICS

Metabolomics, the global characterization of the small molecule complement involved in metabolism, has evolved into a powerful suite of approaches for understanding the global physiological and pathological processes occurring in biological organisms. The diversity of metabolites, the wide range of metabolic pathways, and their divergent biological contexts require a range of methodological strategies and techniques. Methodologies for Metabolomics provides a comprehensive description of the newest methodological approaches in metabolomic research. The most important technologies used to identify and quantify metabolites including nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are highlighted. The integration of these techniques with classical biological methods is also addressed. Furthermore, this book presents statistical and chemometric methods for evaluation of the resultant data. The broad spectrum of topics includes a vast variety of organisms, samples, and diseases, ranging from in vivo metabolomics in humans and animals to in vitro analysis of tissue samples, cultured cells, plants, microbes, and biofluids.

Norbert W. Lutz is Research Professor at the School of Medicine at the University of Aix-Marseille, France. He has previously held positions at the Johns Hopkins University School of Medicine, the German Cancer Research Center, the University of Arizona Cancer Center, and other internationally renowned research institutions. His research is primarily focused on the analysis of metabolic processes in cancer and in neurological diseases. His papers have appeared in Analytical Chemistry, Annals of Neurology, PLoS ONE, International Journal of Cancer, AIDS, Metabolomics, NMR in Biomedicine, and other journals.

Jonathan V. Sweedler is the James R. Eiszner Family Professor of Chemistry and the director of the School of Chemical Sciences at the University of Illinois at Urbana-Champaign, USA. His major research efforts involve developing new approaches for small-volume peptidomics and metabolomics and the application of these technologies to study novel neurochemical pathways. Professor Sweedler has published more than 350 manuscripts, book chapters, and reviews and has presented 350 invited lectures related to research in these areas. He is Editor-in-Chief of Analytical Chemistry.

Ron A. Wevers is head of the laboratory of Genetic Endocrine and Metabolic Diseases of the Radboud University Nijmegen Medical Centre in the Netherlands. He has a chair in Clinical Chemistry of Inborn Errors of Metabolism. Professor Wevers is also a member of the Dutch Health Council. He has co-authored more than 300 peer-reviewed papers and many book chapters and wrote a handbook on body fluid NMR spectroscopy in patients with inborn errors of metabolism.
Methodologies for Metabolomics

EXPERIMENTAL STRATEGIES AND TECHNIQUES

Edited by

Norbert W. Lutz
University of Aix-Marseille, France

Jonathan V. Sweedler
University of Illinois, Urbana-Champaign, USA

Ron A. Wevers
University Medical Centre Nijmegen, the Netherlands
Contents

Contributors  page viii

SECTION 1. BASIC METHODOLOGICAL STRATEGIES IN METABOLOMIC RESEARCH

1 Exploring the Human Metabolome by Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry  3
   David S. Wishart

2 Methodological Requirements for Lipidomics Research  30
   Kui Yang, Michael A. Kiebish, and Richard W. Gross

3 Biological Methods for Metabolic Research  54
   Arancha Cebrián, Laura Menchén, Elsa Sánchez-López, Juan Casado-Vela, Santiago Díaz-Moralli, Marta Cascante, Teresa Gómez del Pulgar, and Juan Carlos Lacal

SECTION 2. METABOLOMIC MASS SPECTROMETRY: EXPERIMENTAL TECHNIQUES AND BIOINFORMATICS

4 Considerations in Sample Preparation, Collection, and Extraction Approaches Applied in Microbial, Plant, and Mammalian Metabolic Profiling  79
   J. William Allwood, Catherine L. Winder, Warwick B. Dunn, and Royston Goodacre

5 Mass Spectrometry–Based Methodologies for Single-Cell Metabolite Detection and Identification  119
   Ann M. Knolhoff, Peter Nemes, Stanislav S. Rubakhin, and Jonathan V. Sweedler

6 Direct Metabolomics from Tissues and Cells: Laser Ablation Electrospray Ionization for Small Molecule and Lipid Characterization  140
   Akos Vertes, Bindesh Shrestha, and Peter Nemes
Contents

7 Bioinformatic Approaches to Processing and Annotation of High-Resolution Mass Spectrometry Data ........................................ 159
Ralf J. M. Weber and Mark R. Viant

8 Approaches for Natural Product Detection and Structural Elucidation Using Mass Spectrometry with High Mass Accuracy .......... 174
Ioanna Ntai and Neil L. Kelleher

9 Metabolomics Using Ion Mobility Mass Spectrometry .................. 185
Kimberly A. Kaplan and Herbert H. Hill, Jr.

10 Metabolomics via Biomedical Mass Spectrometry: From Sampling to Clinical Applications ................................................. 205
Bong Chul Chung and Man Ho Choi

SECTION 3. METABOLOMICS OF BIOFLUIDS: NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AND CHEMOMETRICS

11 Analytical Techniques in Metabolomics Integrating Nuclear Magnetic Resonance Spectroscopy and Chromatography with Mass Spectrometry ....................................................... 227
Ulrich Braumann and Markus Godejohann

12 Chemometric Methods in Nuclear Magnetic Resonance–Based Body Fluid Analysis ......................................................... 244
Ron Wehrens and Udo Engelke

13 Nuclear Magnetic Resonance of Cerebrospinal Fluid: The Neurometabolome ................................................................. 257
Fanny Mochel

14 Nuclear Magnetic Resonance–Based Saliva Metabolomics ............... 271
Hanne Christine Bertram and Morten Rahr Clausen

15 Nuclear Magnetic Resonance Methods for Metabolomic Investigation of Amniotic Fluid ...................................................... 281
Ana M. Gil and Gonçalo Graça

16 Nuclear Magnetic Resonance Analysis and Genetic Metabolic Disease ......................................................................................... 299
Udo Engelke, Angelina Goudswarda, Éva Morava, and Ron A. Wevers

17 Lipid Profiling in Health and Disease .......................................... 317
Christina E. Kostara and Eleni T. Bairaktari
## Contents

### SECTION 4. METABOLOMIC NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY TECHNIQUES FOR BODY TISSUE ANALYSIS

18 **Magnetic Resonance Spectroscopy in Investigating the Cancer Metabolome in Preclinical Model Systems** .................................................. 335
   Marie-France Penet, Zaver M. Bhujwalla, and Kristine Glunde

19 **Phospholipidomics by Phosphorus Nuclear Magnetic Resonance Spectroscopy of Tissue Extracts** .................................................. 377
   Norbert W. Lutz and Patrick J. Cozzone

20 **Carbon-13 Nuclear Magnetic Resonance for Analysis of Metabolic Pathways** ................................................................. 415
   Craig R. Malloy, Elizabeth Maher, Isaac Marin-Valencia, Bruce Mickey, Ralph J. DeBerardinis, and A. Dean Sherry

21 **Hyperpolarized Nuclear Magnetic Resonance Spectroscopy: A New Method for Metabolomic Research** ........................................ 446
   Ralph E. Hurd, Yi-Fen Yen, and Albert Chen

22 **Metabolomic Magnetic Resonance Spectroscopy of Human Tissues: Comparison of In Vivo and High-Resolution Magic Angle Spinning Ex Vivo Techniques** ................................................................. 472
   Geoffrey S. Payne, Yuen-Li Chung, and Martin O. Leach

23 **Reproducible Sample Preparation and Spectrum Acquisition Techniques for Metabolic Profiling of Human Tissues by Proton High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance** ................................................................. 496

24 **Assignment Strategies for Nuclear Magnetic Resonances in Metabolomic Research** ................................................................. 525
   Teresa W.-M. Fan and Andrew N. Lane

### Index

585
Contributors

J. William Allwood
School of Chemistry
Manchester Interdisciplinary Biocentre
University of Manchester
Manchester
United Kingdom

Eleni T. Bairaktari
Laboratory of Clinical Chemistry
Medical School
University of Ioannina
Ioannina
Greece

Jean-Pierre Bellocq
Department of Pathology
University Hospitals of Strasbourg
Strasbourg
France

Malika A. Benahmed
Institut de Chimie
University of Strasbourg
Strasbourg
France

Hanne Christine Bertram
Department of Food Science
Aarhus University
Aarslev
Denmark

Zaver M. Bhujwalla
Division of Cancer Imaging Research
Russell H. Morgan Department of Radiology and Radiological Science

Johns Hopkins University School of Medicine
Baltimore, Maryland
United States

Ulrich Braumann
Bruker BioSpin GmbH
Silberstreifen
Rheinstetten
Germany

Juan Casado-Vela
Instituto de Investigaciones Biomédicas
Consejo Superior de Investigaciones Científicas
Madrid
Spain

Marta Cascante
Department of Biochemistry and Molecular Biology
Facultad de Biología
Universidad de Barcelona
Barcelona
Spain

Arancha Cebrián
Instituto de Investigaciones Biomédicas
Consejo Superior de Investigaciones Científicas
Madrid
Spain

Albert Chen
GE Healthcare
Toronto
Canada
Contributors

Man Ho Choi
Life/Health Division
Korea Institute of Science and Technology
Seoul
Korea

Bong Chul Chung
Life/Health Division
Korea Institute of Science and Technology
Seoul
Korea

Yuen-Li Chung
CRUK and EPSRC Cancer Imaging Centre
Institute of Cancer Research and Royal Marsden Hospital
Sutton, Surrey
United Kingdom

Morten Rahr Clausen
Department of Food Science
Aarhus University
Aarslev
Denmark

Patrick J. Cozzone
Centre de Résonance Magnétique Biologique et Médicale
Faculté de Médecine de la Timone
Université Aix-Marseille
Marseille
France

Ralph J. DeBerardinis
Department of Pediatrics
University of Texas Southwestern Medical Center
Dallas, Texas
United States

Julien Detour
Department of Biophysics and Nuclear Medicine
University Hospitals of Strasbourg
Strasbourg
France

Santiago Díaz-Moralli
Department of Biochemistry and Molecular Biology
Facultad de Biología
Universidad de Barcelona
Barcelona
Spain

Warwick B. Dunn
School of Chemistry
Manchester Interdisciplinary Biocentre
University of Manchester
Manchester
United Kingdom

Karim Elbayed
Institut de Chimie
University of Strasbourg
Strasbourg
France

Udo Engelke
Laboratory of Genetic Endocrine and Metabolic Diseases
Radboud University Nijmegen Medical Centre
Nijmegen
The Netherlands

Teresa W.-M. Fan
Center for Regulatory Environmental Analytical Metabolomics (CREAM)
Department of Chemistry and J. G. Brown Cancer Center
University of Louisville
Louisville, Kentucky
United States

Ana M. Gil
CICECO-Department of Chemistry
University of Aveiro
Campus de Santiago
Aveiro
Portugal

Kristine Glunde
Division of Cancer Imaging Research
Russell H. Morgan Department of Radiology and Radiological Science
Johns Hopkins University School of Medicine
Baltimore, Maryland
United States

Markus Godejohann
Bruker BioSpin GmbH
Silberstreifen
Rheinstetten
Germany
Contributors

Teresa Gómez del Pulgar
Instituto de Investigaciones Biomédicas
Consejo Superior de Investigaciones Científicas
Madrid
Spain

Royston Goodacre
School of Chemistry
Manchester Interdisciplinary Biocentre
University of Manchester
Manchester
United Kingdom

Angelina Goudswaard
Laboratory of Genetic Endocrine and Metabolic Diseases
Radboud University Nijmegen Medical Centre
Nijmegen
The Netherlands

Gonçalo Graça
CICECO-Department of Chemistry
University of Aveiro
Aveiro
Portugal

Richard W. Gross
Department of Medicine
Washington University School of Medicine
St. Louis, Missouri
United States

Herbert H. Hill, Jr.
Department of Chemistry
Washington State University
Pullman, Washington
United States

Ralph E. Hurd
GE Healthcare
Menlo Park, California
United States

Alessio Imperiale
Department of Biophysics and Nuclear Medicine
University Hospitals of Strasbourg
Strasbourg
France

Kimberly A. Kaplan
Department of Chemistry
Washington State University
Pullman, Washington
United States

Neil L. Kelleher
Northwestern University
Department of Chemistry
Chemistry of Life Processes Institute
Evanston, Illinois
United States

Michael A. Kiebish
Department of Medicine
Washington University School of Medicine
St. Louis, Missouri
United States

Ann M. Knolhoff
Department of Chemistry and the Beckman Institute
University of Illinois Urbana, Illinois
United States

Christina E. Kostara
Laboratory of Clinical Chemistry
Medical School
University of Ioannina
Ioannina
Greece

Juan Carlos Lacal
Instituto de Investigaciones Biomédicas
Consejo Superior de Investigaciones Científicas
Madrid
Spain

Andrew N. Lane
J. G. Brown Cancer Center
Center for Regulatory Environmental Analytical Metabolomics (CREAM)
University of Louisville
Louisville, Kentucky
United States

Martin O. Leach
CRUK and EPSRC Cancer Imaging Centre
Institute of Cancer Research and Royal Marsden Hospital
Sutton, Surrey
United Kingdom
Contributors

Norbert W. Lutz
Centre de Résonance Magnétique Biologique et Médicale
Faculté de Médecine de La Timone
Université Aix-Marseille
Marseille
France

Elizabeth Maher
Department of Internal Medicine
University of Texas Southwestern Medical Center
Dallas, Texas
United States

Craig R. Malloy
Advanced Imaging Research Center
University of Texas Southwestern Medical Center
Dallas, Texas
United States

Isaac Marin-Valencia
Department of Pediatrics
University of Texas Southwestern Medical Center
Dallas, Texas
United States

Laura Menchén
Instituto de Investigaciones Biomédicas Consejo Superior de Investigaciones Científicas
Madrid
Spain

Bruce Mickey
Department of Neurological Surgery
University of Texas Southwestern Medical Center
Dallas, Texas
United States

Fanny Mochel
INSERM UMR S975
Hôpital La Salpêtrière
Paris
France

Éva Morava
Department of Pediatrics
Radboud University Nijmegen Medical Centre
Nijmegen
The Netherlands

François-Marie Moussallieh
Institut de Chimie
University of Strasbourg
Strasbourg
France

Izzie J. Namer
Department of Biophysics and Nuclear Medicine
University Hospitals of Strasbourg
Strasbourg
France

Peter Nemes
Department of Chemistry and the Beckman Institute
University of Illinois
Urbana, Illinois
United States

Ioanna Ntai
Department of Chemistry
Northwestern University
Chemistry of Life Processes Institute
Evanston, Illinois
United States

Geoffrey S. Payne
CRUK and EPSRC Cancer Imaging Centre
Institute of Cancer Research and Royal Marsden Hospital
Sutton, Surrey
United Kingdom

Marie-France Penet
Division of Cancer Imaging Research
Russell H. Morgan Department of Radiology and Radiological Science
Johns Hopkins University School of Medicine
Baltimore, Maryland
United States

Martial Piotto
Bruker BioSpin
Wissembourg
France
Contributors

Stanislav S. Rubakhin
Department of Chemistry and the Beckman Institute
University of Illinois
Urbana, Illinois
United States

Elsa Sánchez-López
Instituto de Investigaciones Biomédicas
Consejo Superior de Investigaciones Científicas
Madrid
Spain

A. Dean Sherry
Advanced Imaging Research Center
University of Texas Southwestern Medical Center
Dallas, Texas
United States

Binched Shrestha
Department of Chemistry
George Washington University
Washington, DC
United States

Jonathan V. Sweedler
Department of Chemistry and the Beckman Institute
University of Illinois
Urbana, Illinois
United States

Akos Vertes
Department of Chemistry
George Washington University
Washington, DC
United States

Mark R. Viant
School of Biosciences
University of Birmingham
Birmingham
United Kingdom

Ralf J. M. Weber
School of Biosciences
University of Birmingham
Birmingham
United Kingdom

Ron Wehrens
Biostatistics and Data Management
Fondazione Edmund Mach
Istituto Agrario
San Michele all’Adige
Italy

Ron A. Wevers
Laboratory of Genetic Endocrine and Metabolic Diseases
Radboud University Nijmegen Medical Centre
Nijmegen
The Netherlands

Catherine L. Winder
School of Chemistry
Manchester Interdisciplinary Biocentre
University of Manchester
Manchester
United Kingdom

David S. Wishart
Departments of Computing Science and Biological Sciences
University of Alberta
Edmonton, Alberta
Canada

Kui Yang
Department of Medicine
Washington University School of Medicine
St. Louis, Missouri
United States

Yi-Fen Yen
GE Healthcare
Menlo Park, California
United States