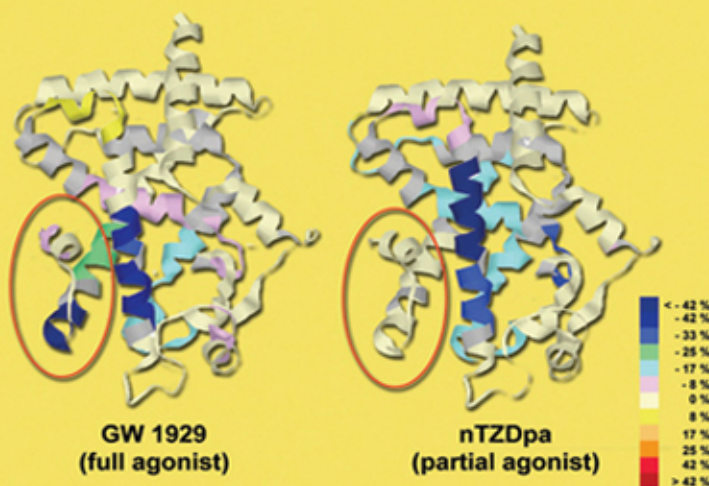


Protein and Peptide Mass Spectrometry in Drug Discovery



Edited by
Michael L. Gross
Guodong Chen
Birendra N. Pramanik

PROTEIN AND PEPTIDE MASS SPECTROMETRY IN DRUG DISCOVERY

PROTEIN AND PEPTIDE MASS SPECTROMETRY IN DRUG DISCOVERY

Edited By

Michael L. Gross

Washington University

Guodong Chen

Bristol-Myers Squibb

Birendra N. Pramanik

Merck Research Laboratories



A JOHN WILEY & SONS, INC., PUBLICATION

Copyright © 2012 by John Wiley & Sons, Inc. All rights reserved

Published by John Wiley & Sons, Inc., Hoboken, New Jersey

Published simultaneously in Canada

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4470, or on the web at www.copyright.com. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, or online at <http://www.wiley.com/go/permission>.

Limit of Liability/Disclaimer of Warranty: While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Neither the publisher nor author shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services or for technical support, please contact our Customer Care Department within the United States at (800) 762-2974, outside the United States at (317) 572-3993 or fax (317) 572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic formats. For more information about Wiley products, visit our web site at www.wiley.com.

Library of Congress Cataloging-in-Publication Data

Protein and peptide mass spectrometry in drug discovery / edited By Michael L.

Gross, Guodong Chen, Birendra N. Pramanik.

p. ; cm.

Includes bibliographical references.

ISBN 978-0-470-25817-0 (cloth)

1. Drug development. 2. Peptides--Spectra. 3. Proteins--Spectra. I.

Gross, Michael L. II. Chen, Guodong, 1966- III. Pramanik, Birendra N., 1944-

[DNLM: 1. Drug Discovery. 2. Mass Spectrometry. 3. Peptides--analysis. 4.

Proteins--analysis. QV 744]

RM301.25.P757 2011

615¹.19--dc23

2011015204

Printed in the United States of America

oBook ISBN: 9781118116555

ePDF ISBN: 9781118116531

ePub ISBN: 9781118116548

eMobi ISBN: 9781118116524

10 9 8 7 6 5 4 3 2 1

CONTENTS

PREFACE	xv
CONTRIBUTORS	xvii
PART I METHODOLOGY	1
1 Ionization Methods in Protein Mass Spectrometry	3
<i>Ismael Cotte-Rodriguez, Yun Zhang, Zhixin Miao, and Hao Chen</i>	
1.1 History of the Development of Protein Mass Spectrometry	4
1.2 Laser-Based Ionization Methods for Proteins	5
1.2.1 Matrix-Assisted Laser Desorption/Ionization (MALDI)	5
1.2.2 Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization (AP-MALDI)	8
1.2.3 Surface-Enhanced Laser Desorption/Ionization (SELDI)	9
1.2.4 Nanostructure-Initiator Mass Spectrometry (NIMS)	11
1.3 Spray-Based Ionization Methods for Proteins	13
1.3.1 Electrospray Ionization (ESI)	13
1.3.2 Sonic Spray Ionization (SSI)	14
1.3.3 Electrosonic Spray Ionization (ESSI)	17
1.4 Ambient Ionization Methods	20
1.4.1 Desorption Electrospray Ionization (DESI)	21
1.4.2 Fused-Droplet Electrospray Ionization (FD-ESI)	24
1.4.3 Electrospray-Assisted Laser Desorption Ionization (ELDI)	27
1.4.4 Matrix-Assisted Laser Desorption Electrospray Ionization (MALDESI)	30
1.5 Conclusions	30
Acknowledgments	30
References	30
2 Ion Activation and Mass Analysis in Protein Mass Spectrometry	43
<i>Cheng Lin and Peter O'Connor</i>	
2.1 Introduction	43
2.1.1 Mass Accuracy	43
2.1.2 Mass Resolving Power	44

2.1.3	Mass Range	44
2.1.4	Scan Speed	45
2.1.5	Tandem MS Analysis	46
2.2	Ion Activation and Tandem MS Analysis	46
2.2.1	Introduction: Fragmentation in Protein MS	46
2.2.2	Collisional Activation Methods	48
2.2.3	Photodissociation	50
2.2.4	Electron-Induced Dissociation	55
2.2.5	Other Radical-Induced Fragmentation Methods	59
2.3	Mass Analyzers	59
2.3.1	Time-of-Flight Mass Analyzer	60
2.3.2	Quadrupole Mass Analyzer and Quadrupole Ion Trap	66
2.3.3	Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer	73
2.3.4	Orbitrap	77
2.3.5	Ion-Mobility Instruments	80
	References	81
3	Target Proteins: Bottom-up and Top-down Proteomics	89
	<i>Michael Boyne and Ron Bose</i>	
3.1	Mass Spectral Approaches to Targeted Protein Identification	89
3.2	Bottom-up Proteomics	90
3.2.1	Peptide Mass Fingerprinting	91
3.2.2	Bottom-up Proteomics Using Tandem MS: GeLC-MS/MS and Shotgun Digests	91
3.2.3	GeLC-MS/MS	93
3.2.4	Shotgun Digest	94
3.3	Top-down Approaches	96
3.4	Next-Generation Approaches	98
	References	99
4	Quantitative Proteomics by Mass Spectrometry	101
	<i>Jacob Galan, Anton Iliuk, and W. Andy Tao</i>	
4.1	Introduction	101
4.2	In-Cell Labeling	105
4.2.1	^{15}N Metabolic Labeling	105
4.2.2	Stable Isotope Labeling by Amino Acid (SILAC)	106
4.3	Quantitation via Isotopic Labeling of Proteins	107
4.3.1	2D PAGE-Based Quantitation	108
4.3.2	Proteolytic Labeling Using ^{18}O Water	109
4.3.3	Quantitative Labeling by Chemical Tagging	110

4.4	Quantitation via Isotopic Labeling on Peptides	112
4.4.1	ICAT	112
4.4.2	iTRAQ	113
4.4.3	SoPIL	113
4.4.4	Absolute Quantitation	114
4.5	Label-Free Quantitation	116
4.6	Conclusions	119
	Acknowledgment	120
	References	120
5	Comparative Proteomics by Direct Tissue Analysis Using Imaging Mass Spectrometry	129
	<i>Michelle L. Reyzer and Richard M. Caprioli</i>	
5.1	Introduction	129
5.2	Conventional Comparative Proteomics	130
5.3	Comparative Proteomics Using Imaging MS	131
5.3.1	Biomarker Discovery: Breast Cancer	131
5.3.2	Biomarker Discovery: Toxicity	133
5.3.3	Correlating Drug and Protein Distributions	134
5.4	Conclusions	136
	Acknowledgments	137
	References	137
6	Peptide and Protein Analysis Using Ion Mobility–Mass Spectrometry	139
	<i>Jeffrey R. Enders, Michal Kliman, Sevugarajan Sundarapandian, and John A. McLean</i>	
6.1	Ion Mobility–Mass Spectrometry: Instrumentation and Separation Selectivity	139
6.1.1	Instrumentation	140
6.1.2	Separation Selectivity in Bioanalyses	145
6.2	Characterizing and Interpreting Peptide and Protein Structures	147
6.2.1	The Motion of Ions within Neutral Gases	147
6.2.2	Considerations for Calculating Collision Cross Sections	148
6.2.3	Computational Approaches for Interpretation of Structure	149
6.3	Applications of IM-MS to Peptide and Protein Characterizations	152
6.3.1	Fundamental Studies of Peptide and Protein Ion Structures	152
6.3.2	Studies in Structural Biology—Protein Complex Characterization	157
6.4	Future Directions	158
6.4.1	Applications	158
6.4.2	Instrumentation	159

Acknowledgments	159
References	160
7 Chemical Footprinting for Determining Protein Properties and Interactions	175
<i>Sandra A. Kerfoot and Michael L. Gross</i>	
7.1 Introduction to Hydrogen–Deuterium Exchange	175
7.1.1 Fundamentals of Hydrogen–Deuterium Amide Exchange in Proteins	176
7.1.2 EX1 and EX2 Rates of HDX	176
7.2 Experimental Procedures	178
7.2.1 Global Hydrogen–Deuterium Exchange	178
7.2.2 HDX at the Peptide Level	179
7.3 Mass Spectrometry-Based HDX in Practice	182
7.3.1 Protein–Ligand Interactions by Automated HDX	182
7.3.2 Solvent Accessibility by HDX and MALDI-TOF Mass Spectrometry	183
7.3.3 High-Throughput Screening of Protein Ligands by SUPREX	184
7.3.4 Functional Labeling and Multiple Proteases	188
7.3.5 PLIMSTEX: Application in Protein–DNA Interactions	188
7.3.6 HDX and Tandem Mass Spectrometry Analysis	191
7.3.7 Optimizing HDX with High Pressure	192
7.4 Protein Footprinting via Free-Radical Oxidation	193
7.4.1 Fenton Chemistry Oxidation	194
7.4.2 Radiolytic Generation of Hydroxyl Radicals	196
7.4.3 Fast Photochemical Oxidation of Proteins (FPOP)	197
7.4.4 SPROX: Stability of Proteins from Rates of Oxidation	198
7.5 Chemical Crosslinking	198
7.5.1 Drawbacks of Crosslinking	199
7.6 Selective and Irreversible Chemical Modification	201
7.6.1 Acetylation of Lysine	202
7.6.2 Thiol Derivatization of Cysteines	203
7.6.3 Footprinting FMO Protein in Photosynthetic Bacteria	203
7.6.4 Potential Pitfalls	205
7.7 Conclusion	205
References	206
8 Microwave Technology to Accelerate Protein Analysis	213
<i>Urooj A. Mirza, Birendra N. Pramanik, and Ajay K. Bose</i>	
8.1 Introduction	213
8.2 Microwave Technology	215

8.2.1	Application of Microwave Irradiation to Akabori Reaction	215
8.2.2	Protein Characterization by Microwave Irradiation and MS	216
8.2.3	Temperature and Microwave Irradiation Effects on the Enzyme in Protein Digestion	217
8.2.4	Use of Microwave Digestion of Proteins from SDS-PAGE Gels	219
8.2.5	Extraction of Intact Proteins from SDS-PAGE Using Microwave Irradiation	219
8.2.6	Application of Microwave-Assisted Proteolysis Using Trypsin-Immobilized Magnetic Silica Microspheres	220
8.2.7	Acid Hydrolysis of Proteins with Microwave Irradiation	221
8.2.8	Do Protein Denature During Microwave Irradiation?	222
8.3	Summary	224
	Acknowledgments	224
	References	224
9	Bioinformatics and Database Searching	231
	<i>Surendra Dasari and David L. Tabb</i>	
9.1	Overview	231
9.2	Introduction to Tandem Mass Spectrometry	231
9.2.1	Protein Sequencing	231
9.2.2	Peptide Fragmentation	232
9.3	Overview of Peptide Identification with Database Searching	234
9.4	MyriMatch-IDPicker Protein Identification Pipeline	235
9.4.1	Raw Data File Formats	235
9.4.2	Protein Sequence Databases	237
9.4.3	MyriMatch Database Search Engine	239
9.4.4	Peptide Identification Reporting	242
9.4.5	Post-processing of Search Results Using IDpicker	243
9.5	Results of a Shotgun Proteomics Study	246
9.6	Improvements to MyriMatch Database Search Engine	248
9.6.1	Parallel Processing	248
9.6.2	Protein Modification Analysis	249
9.7	Applications of MyriMatch-IDPicker Pipeline	250
9.7.1	Characterizing Protein–Protein Interactions	250
9.7.2	Characterizing Yeast Proteome on Diverse Instrument Platforms	250
9.7.3	Characterizing DNA-Protein Crosslinks	250

9.8	Conclusions	251
	Acknowledgments	251
	References	251

PART II Applications 253

10 Mass Spectrometry-Based Screening and Characterization of Protein–Ligand Complexes in Drug Discovery 255

Christine L. Andrews, Michael R. Ziebell, Elliott Nickbarg, and Xianshu Yang

10.1	Introduction	255
10.2	Affinity Selection Mass Spectrometry (AS-MS)	256
10.2.1	Direct Detection of Noncovalent Protein–Ligand Complexes	257
10.2.2	Indirect Detection of Noncovalent Protein–Ligand Complexes	258
10.3	Solution-Based AS-MS as Screening Technologies	258
10.3.1	Automated Ligand Identification System (ALIS)	259
10.3.2	SpeedScreen	263
10.3.3	Ultracentrifugation Coupled to Mass Spectrometry	264
10.3.4	Gel Filtration–MS Platform	264
10.3.5	Frontal Affinity Chromatography–Mass Spectrometry (FAC-MS)	265
10.3.6	Indirect Detection AS-MS	266
10.3.7	Emerging Technology	266
10.4	Gas-Phase Interactions	267
10.4.1	Ion-Mobility Mass Spectrometry (IMS)	269
10.4.2	Hydrogen–Deuterium Exchange (H/DX) (Including SUPREX and PLIMSTEX)	270
10.4.3	Crosslinking (Including Inhibition of Complex Formation)	270
10.5	Enzyme Activity Assays Using MS for Screening or Confirming Drug Candidates	271
10.5.1	MS to Measure Substrate Turnover	272
10.5.2	Multiple Component Measurements	272
10.5.3	Continuous Flow Screening	272
10.5.4	Immobilized Enzyme Reactor (IMER)	273
10.5.5	Application of MALDI to High-Throughput Enzyme Assays	274
10.5.6	Ratiometric Assays Using MALDI	275
10.5.7	Self-assembled Monolayers for MALDI-MS (SAMDI)	275
10.5.8	Desorption/Ionization Process Off of Porous Silicon (DIOS) and Carbon Nanotubes	275

10.5.9	Overcoming Low Serial Throughput by Rapid Chromatography	276
10.5.10	MALDI–Triple Quadrupole Mass Spectrometry (MALDI-3Q)	276
10.6	Conclusions and Future Directions	276
	References	277
11	Utilization of Mass Spectrometry for the Structural Characterization of Biopharmaceutical Protein Products	287
	<i>Amareth Lim and Catherine A. Srebalus Barnes</i>	
11.1	Introduction	287
11.2	MS-Based Approach for the Characterization of Recombinant Therapeutic Proteins	288
11.3	Cell Culture Development	290
11.4	Purification Development	294
11.4.1	Identification of a Pyruvic Acid Modification Covalently Linked at the <i>N</i> -Terminus of a Recombinant IgG4 Fc Fusion Protein	295
11.4.2	Identification of Hinge Region Cleavage in an IgG1 Monoclonal Antibody with Two <i>N</i> -Linked Glycosylation Sites	298
11.5	Formulation Development	300
11.6	Analytical Method Development	304
11.6.1	Utilization of Partial Reduction and LC-MS to Distinguish an IgG4 Monoclonal Antibody Charge Variants That Co-elute in Cation Exchange HPLC	304
11.6.2	Development of an RP-HPLC Method for Monitoring an IgG4 Fc Fusion Protein Post-Translational Modifications	309
11.7	Confirmation of Structure/Product Comparability Assessment	311
11.8	Conclusions	313
	Acknowledgments	315
	References	315
12	Post-translationally Modified Proteins: Glycosylation, Phosphorylation, and Disulfide Bond Formation	321
	<i>Anthony Tsarbopoulos and Fotini N. Bazoti</i>	
12.1	Introduction	321
12.2	Glycosylation	322

12.2.1	MS Detection of Glycoproteins	323
12.2.2	Glycan Identification, Classification, and Heterogeneity	327
12.2.3	Glycoprotein Mapping by LC-ESI and MALDI Tandem MS	329
12.2.4	Glycosylation Site Quantitation	336
12.3	Phosphorylation	338
12.3.1	MS Detection of Phosphorylation	338
12.3.2	Enrichment of Phosphorylated Peptides and Proteins	340
12.3.3	Phosphorylation Site Identification	341
12.3.4	Phosphopeptide Quantitation	346
12.4	Disulfide Bond Detection and Mapping	347
12.4.1	MS Detection	347
12.4.2	Disulfide Mapping	347
12.5	Future Perspectives	350
	Acknowledgments	352
	Abbreviations	353
	References	354
13	Mass Spectrometry of Antigenic Peptides	371
	<i>Henry Rohrs</i>	
13.1	Introduction	371
13.1.1	Brief History of MHC Studies	371
13.1.2	Brief Introduction to Immunobiology	372
13.2	Analysis of Antigenic Peptides	374
13.2.1	MHC Peptide Analysis in Practice—Sample Preparation	376
13.2.2	MHC Peptide Analysis in Practice—HPLC Separation	377
13.2.3	MHC Peptide Analysis in Practice—Mass Spectrometers	377
13.2.4	MHC Peptide Analysis in Practice—Data Analysis	379
13.3	Examples of the Application of Mass Spectrometry to Antigenic Peptide Study	381
13.3.1	Work of D. Hunt	381
13.3.2	Work of E. Unanue	382
13.3.3	Work of H. Rammensee	384
13.3.4	Work of P. Allen	384
13.3.5	Work of P. Thibault	385
13.4	Future Work	385
	Acknowledgments	386
	Abbreviations	387
	References	387

14	Neuropeptidomics	393
	<i>Jonathan V. Sweedler, Fang Xie, and Adriana Bora</i>	
14.1	Introduction	393
14.2	Neuropeptidomics: Characterizing Peptides in the Brain	394
14.3	Sample Preparation for Mass Spectrometry	395
14.3.1	Direct Tissue Profiling	397
14.3.2	Extraction-Based Strategies	399
14.3.3	Collecting Peptide Release	400
14.3.4	Sample Preparation for MSI	403
14.4	Separations	405
14.5	Peptide Characterization via Mass Spectrometry	407
14.5.1	Qualitative Analyses	407
14.5.2	Relative Quantitative Analyses	413
14.5.3	Data Analysis with Bioinformatics	416
14.6	Conclusions	419
14.7	Future Perspectives	419
	Acknowledgments	420
	References	420
15	Mass Spectrometry for the Study of Peptide Drug Metabolism	435
	<i>Patrick J. Rudewicz</i>	
15.1	Introduction	435
15.2	Peptide Drug Metabolism	436
15.3	LC-MS/MS for Metabolite Identification	437
15.4	Quantitative Analysis	439
15.5	Case Study: IL-1 β Protease Inhibitors	440
15.6	Future Directions	445
	References	445
	INDEX	449

PREFACE

For over a decade mass spectrometry (MS) has been one of the most highly utilized analytical technique for analysis of proteins and peptides. This is largely due to continuous refinement of ionization methods, including electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), the improvement of MS instrumentation, and the growth in the data processing. Various niche applications in neuroproteomics and antigenic peptides could have important implications in drug discovery, and these developments are described in two chapters. Furthermore there has been considerable research activity focused on the development of new methodologies for the analysis of proteins and peptides; these methods have exploited ongoing instrumentation improvements and include both bottom-up and top-down protein sequencing. New approaches include those in imaging, ion mobility, and the use of microwave radiation to speed proteolysis, and these new ideas are covered in three chapters in this volume. Accompanying the analytical developments are new techniques for the determinations of protein structure, of their interactions with peptides, proteins, and ligands including drugs, and their folding and unfolding. These techniques are described in detail in this volume.

One of the important and immediate applications in protein and peptide MS is for pharmaceutical analysis throughout each stage of drug development process, ranging from drug discovery to manufacturing. MS-based technologies play critical roles in providing qualitative and quantitative information to characterization of target proteins and protein products for therapeutic use, as illustrated in this volume.

We are delighted to bring together the work of contributors from academe and industry in highlighting current analytical approaches, industry practices, and modern strategies for the characterization of proteins and peptides in drug discovery. Our goal is to present a compilation of the latest methodologies and applications to practitioners of protein and peptide MS with the focus on drug discovery efforts.

We would like to acknowledge the special efforts and patience of all the authors, who have made significant contributions to this book.

MICHAEL L. GROSS
GUODONG CHEN
BIRENDRA N. PRAMANIK

CONTRIBUTORS

Christine L. Andrews, Merck Research Laboratories, Cambridge, MA
Catherine A. Srebalus Barnes, Eli Lilly and Company, Indianapolis, IN
Fotini N. Bazoti, The Goulandris Natural History Museum, Kifissia, Greece
Adriana Bora, University of Illinois, Urbana, IL
Ajay K. Bose (deceased), Stevens Institute of Technology, Hoboken, NJ
Ron Bose, Washington University, St. Louis, MO
Michael Boyne, Washington University, St. Louis, MO
Richard M. Caprioli, Vanderbilt University, Nashville, TN
Guodong Chen, Bristol-Myers Squibb, Princeton, NJ
Hao Chen, Ohio University, Athens, OH
Ismael Cotte-Rodriguez, Procter & Gamble, Loveland, OH
Peter O'Connor, University of Warwick, Coventry, UK
Surendra Dasari, Vanderbilt University Medical Center, Nashville, TN
Jeffrey R. Enders, Vanderbilt University, Nashville, TN
Jacob Galan, Purdue University, West Lafayette, IN
Michael L. Gross, Washington University, St. Louis, MO
Anton Iliuk, Purdue University, West Lafayette, IN
Sandra A. Kerfoot, Seattle Childrens Research Institute, Seattle, WA
Michal Kliman, Vanderbilt University, Nashville, TN
Amareth Lim, Eli Lilly and Company, Indianapolis, IN
Cheng Lin, Boston University, Boston, MA
John A. McLean, Vanderbilt University, Nashville, TN
Zhixin Miao, Ohio University, Athens, OH
Urooj A. Mirza, Merck Research Laboratories, Kenilworth, NJ

Elliott Nickbarg, Merck Research Laboratories, Cambridge, MA

Birendra N. Pramanik, Merck Research Laboratories, Kenilworth, NJ

Michelle L. Reyzer, Vanderbilt University, Nashville, TN

Henry Rohrs, Washington University, St. Louis, MO

Patrick J. Rudewicz, Elan Pharmaceuticals, South San Francisco, CA

Sevugarajan Sundarapandian, Vanderbilt University, Nashville, TN

Jonathan V. Sweedler, University of Illinois, Urbana, IL

David L. Tabb, Vanderbilt University Medical Center, Nashville, TN

W. Andy Tao, Purdue University, West Lafayette, IN

Anthony Tsarbopoulos, University of Patras, Greece

Fang Xie, Pacific Northwest National Laboratory, Richland, WA

Xianshu Yang, Merck Research Laboratories, Cambridge, MA

Yun Zhang, Ohio University, Athens, OH

Michael R. Ziebell, Merck Research Laboratories, Cambridge, MA

METHODOLOGY

Ionization Methods in Protein Mass Spectrometry

ISMAEL COTTE-RODRIGUEZ, YUN ZHANG, ZHIXIN MIAO, and HAO CHEN

Mass spectrometry (MS) has become one of the most powerful and popular modern physical-chemical methods to study the complexities of elemental and molecular processes in nature. The advent of new methods of ion generation, novel mass analyzers, and new tools for data processing has made it possible to analyze almost all chemical entities by MS, ranging from small organic compounds, large biological molecules, to whole living cells/tissues. As proteins fulfill a plethora of biochemical functions within every living organism, equally spectacular efforts and advances have been seen for protein ionization methods. In particular, the invention of matrix-assisted laser desorption ionization (MALDI) [1] and electrospray ionization (ESI) technologies [2,3] allow one to measure protein molecular weights, to determine sequences, and to probe conformations and post-translational modifications of proteins. In addition the mass range of species amenable for MS analysis has been increased immensely, enabling the transfer into the gas phase of ionized noncovalent species with masses well over one million (e.g., a 100 MDa single DNA ion [4]). These advances move MS into the range of intact protein oligomers and functional machineries.

This chapter is an introduction to various ionization methods for proteins. As this is a broad topic with an immense literature coverage including many excellent books [5,6] and reviews [7–17], we will emphasize some types of spray or laser-based protein ionization techniques, including atmospheric pressure MALDI, surface-enhanced laser desorption/ionization (SELDI), nanostructure-initiator MS (NIMS), sonic spray ionization (SSI), electrosonic spray ionization (ESSI), desorption electrospray ionization (DESI), fused-droplet electrospray ionization (FD-ESI), electrospray-assisted laser desorption ionization (ELDI), and matrix-assisted laser desorption electrospray ionization (MALDESI). We begin with the introduction of some historic facts for the

development of protein ionization methods, followed with the description of each method including the ionization principles, strengths, and analytical applications.

1.1 HISTORY OF THE DEVELOPMENT OF PROTEIN MASS SPECTROMETRY

MS originates from nineteen-century physics. The first known mass spectrometer was built by J. J. Thomson in the early 1900s to study and measure the mass (m)-to-charge (z) (m/z) values of the “corpuscles” that make up “positive rays” [18], a type of radiation initially observed by German physicist Eugen Goldstein. Following the seminal work of Thomson, MS underwent countless improvements in instrumentation, ionization methods, and applications. The classical ionization method, electron ionization (EI), was devised by Dempster and improved later by Bleakney [19] and Nier [20], and became a widely used standard for ionization of volatile organic compounds. This ionization technique requires extensive derivatization and evaporation of a nonvolatile analyte into the ion source, and it involves numerous fragmentation and rearrangement reactions.

Applications of MS to peptides (derivatized via acylation) begun in the late 1950s by Biemann [21] and McLafferty [22]. The first methods that allowed analysis of nonderivatized peptides were field desorption (FD) and chemical ionization (CI) developed in the 1960s [23,24]. Ionization by CI is achieved by interaction of its volatile molecules with reagent ions. CI allows ionization without significant degree of ion fragmentation but still requires gas-phase samples. Field desorption was reported by Beckey in 1969 [25], in which electron tunneling triggered by a very high electric field results in ionization of gaseous analyte molecules.

It was plasma desorption (PD) [26] and fast atom bombardment (FAB) [27] that opened the way to protein analysis. PD ionization, invented by R. D. Macfarlane in 1976 [28], a breakthrough in the analysis of solid samples, involves ionization of materials in the solid state by bombardment with ions or neutral atoms formed as a result of the nuclear fission of the Californium isotope ^{252}Cf . In 1982 Sundqvist and coworkers obtained the first spectrum of a protein, insulin (Figure 1.1), using bombardment with a beam of 90 MeV $^{127}\text{I}^{20+}$ ions from a tandem accelerator [26]. Later, FAB involving focusing the sample in liquid matrix with a beam of neutral atoms or molecules, was implemented for the ionization of proteins up to 24 kDa [29]. In 1983 Blakely and Vestal [30] introduced electrospray ionization (ESI) to produce ions from an aqueous solution sprayed directly into a mass spectrometer. Electrospray is a form of atmospheric pressure ionization in MS, transferring ions from the liquid phase to the gas phase for analysis. It was particularly useful in coupling liquid chromatography with mass spectrometry [31].

The breakthrough for large molecule laser desorption ionization came in 1987 when Tanaka combined 30-nm cobalt particles in glycerol with a 337-nm nitrogen laser for ionization and showed that singly charged protein molecular ions up to about 35 kDa can be introduced to a mass spectrometer [32]. During that time, MALDI [15,33], first reported in 1985 by Hillenkamp, Karas, and their colleagues,

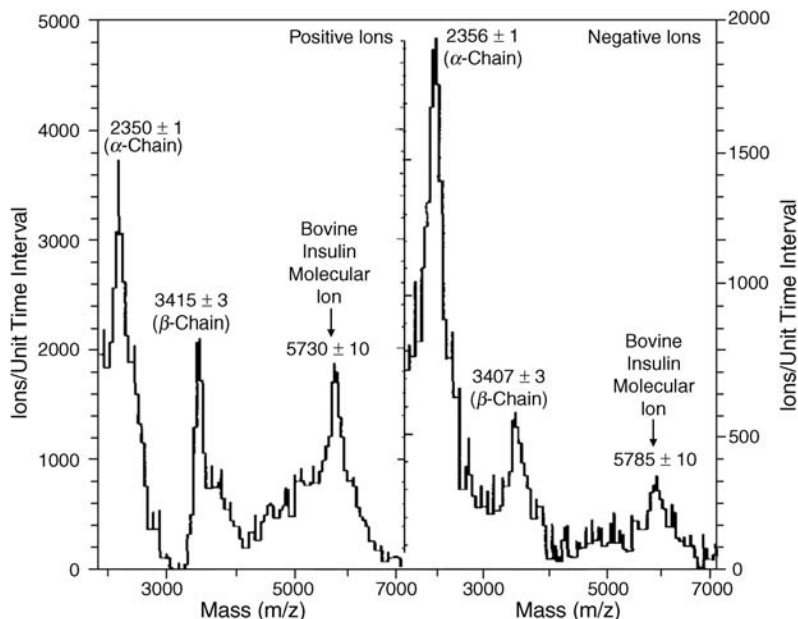


FIGURE 1.1 ^{127}I -PDMS spectra of bovine insulin recorded over a 1.5-h period with a 90-MeV ^{127}I (+20) beam current of 2000 s^{-1} . From [26]. Copyright permission was obtained from ACS.

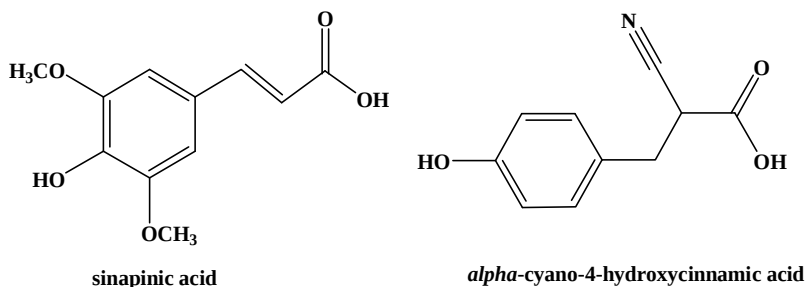
emerged as the culmination of a long series of experiments using desorption ionization (DI). MALDI is a soft ionization technique for the analysis of biomolecules and large organic molecules and has gained wide success in protein analysis, particularly when coupled with time-of-flight (TOF) instruments [34,35].

Another breakthrough occurred in 1984 when Fenn and coworkers used electrospray to ionize biomolecules [2]; the first ESI analyses of biopolymers including proteins were published in 1989 [3]. MALDI and ESI have revolutionized protein mass spectrometry since their invention in 1980s, and they have triggered the explosion in application of mass spectrometry for protein studies [36].

1.2 LASER-BASED IONIZATION METHODS FOR PROTEINS

1.2.1 Matrix-Assisted Laser Desorption/Ionization (MALDI)

Investigations of the wavelength influence in ultraviolet-laser desorption [33] led to invention of ultraviolet-laser matrix-assisted laser desorption ionization (UV-MALDI) between 1984 and 1986 and summarized in a 1987 paper [37]. In 1988 Karas and Hillenkamp reported ultraviolet-laser desorption (UPLD) of bioorganic compounds in the mass range above 10 kDa [1]. As a soft desorption ionization method, MALDI handles thermolabile, nonvolatile organic compounds, especially those with



SCHEME 1.1 Structures of two common MALDI matrices.

high molecular weight and can be successfully used for the analysis of proteins, peptides, glycoproteins, oligosaccharides, and oligonucleotides. Its operation is relatively straightforward, although matrix preparation requires experience and perhaps some artistry.

MALDI is based on the bombardment of sample molecules with laser light, process that allows sample ionization [38]. It requires a specific matrix consisting of small organic compounds (e.g., nicotinic acid) that exhibit a strong resonance absorption at the laser wavelength used. The sample is premixed and diluted with the highly absorbing matrix and allowed to dry on a sample target. A range of compounds is suitable as matrices: sinapinic acid is a common one for protein analysis while *alpha*-cyano-4-hydroxycinnamic acid is often used for peptide analysis (the structures of matrices are shown in Scheme 1.1). This kind of acid serves well as a matrix for MALDI owing to the acid's ability to absorb laser radiation and also to donate protons (H^+) to the analyte of interest. Upon laser irradiation, energy is absorbed by the matrix in a localized region of the surface. As a result an explosive break up of the cocrystallized analyte/matrix sample occurs. The rapid expansion of the vaporized matrix in MALDI leads to the translational excitation of analyte molecules and the release of the analyte molecules from the surface of the condensed phase sample into vacuum. The analyte may be precharged (e.g., exist as a salt), and the intact analyte ion may simply be transferred as an ion from the solid to the vapor state upon laser irradiation of the matrix. Alternatively, a neutral analyte may be ionized through ion–molecule reactions (e.g., proton transfer reaction) occurring in the energized selfedge or interfacial region between the solid and gas phases.

MALDI has remarkable efficiency in producing intact molecular ions (often $[M + H]^+$, $[M + Na]^+$) of large biological compounds. MALDI ionization sensitivity is also extraordinary, and total amounts of sample loaded onto the target surface often are in the picomole to femtomole range. The method has tolerance to buffers and other additives and gives predominantly singly charged ions for large biomolecules [35]. TOF mass analyzers are ideal for use with this ionization technique because they are compatible with high-mass ions and pulsed-ion production [34,35]. TOF analyzers separate ions according to their m/z ratios by measuring the time it takes for ions, accelerated to the same kinetic energy, to travel through a field-free region known as the flight or drift tube. The heavier ions move slower than the lighter ones [6].

An important application of MALDI is chemical imaging, using a technique called matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) [16]. Imaging combines parallel, high-throughput molecular analysis with location-specific information for the characterization of protein distributions directly from thin sections of intact biological tissue [39,40] and offers complementary information to two-dimensional (2D) gel electrophoresis and to shotgun proteomics for investigating proteomic differences. It is covered in Chapter 5 by Reyzer and Caprioli in this volume. Figure 1.2 illustrates the typical experimental process for MALDI imaging. Frozen tissue specimens are sectioned on a cryostat into about 5- to 20- μ m thick sections. The sections are thaw-mounted onto conductive MALDI target plates. Matrix is applied to the sections, depending on the experiment to be performed: droplets (nL to pL) can be deposited in arrays or on discrete morphological areas, or a uniform coating of matrix can be applied to the entire tissue section.

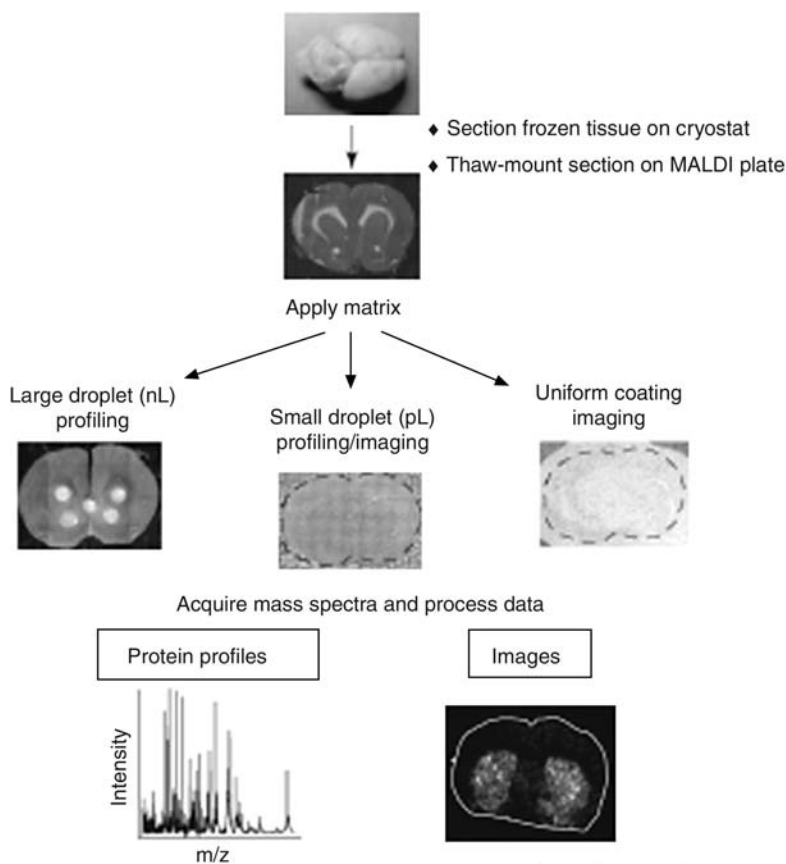


FIGURE 1.2 Typical experimental design for IMS. From [16]. Copyright permission was obtained from Elsevier. (See the color version of this figure in Color Plates section.)

Mass spectra are obtained from each spot or from across the entire tissue section in a defined raster pattern. The acquired spectra can then be examined and processed to form 2D molecule-specific ion images [16]. The power of MALDI-IMS technology is its capability to link reliably protein data with specific cellular regions within the tissue.

MALDI-IMS has been employed as an imaging technology in a wide variety of applications from the analysis of small molecules such as drugs and endogenous metabolites to high molecular weight proteins (e.g., MALDI-IMS of a mouse model of Parkinson's disease revealed a significant decrease in PEP-19 expression levels in the striatum after administration of the drug MPTP [41]).

1.2.2 Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization (AP-MALDI)

Atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) was first described by Laiko et al. [42]. In contrast to conventional vacuum MALDI, AP-MALDI can be operated at atmospheric pressure instead of high vacuum where ions are typically produced at 10 mTorr or less. During the ionization process, the solid-phase target material containing analyte sample and matrix is irradiated with a pulsed laser beam. The matrix absorbs the photon energy and undergoes fast heating and evaporation, which results in the formation of gaseous analyte ions [43]. Because the ionization of AP-MALDI occurs at atmospheric pressure, thermalization of the resulting ions takes place owing to collisions with the ambient gases used in AP-MALDI, accounting for the soft ionization nature of AP-MALDI [42].

The AP-MALDI source makes use of a high voltage potential that is applied between the target tip and the heated inlet transport capillary. The laser is focused onto the surface of the target plate. Ions are desorbed from the angled replaceable target tip and carried by the dry carrier nitrogen gas into a mass spectrometer [44]. The sensitivity of detection for AP-MALDI can be affected by the geometry of the target tip, and its position relative to the inlet orifice, the nitrogen gas flow rate, gas nozzle position, etc. [42]. Furthermore, when a Nd: YAG laser with high laser power rather than a nitrogen laser is used, the signal intensity can be improved [45].

Given that AP-MALDI and conventional vacuum MALDI share common ionization mechanisms, they have many similar features including simplicity of sample preparation and tolerance to interference from salts [42], which can be detrimental for biomolecule analysis. AP-MALDI is an extension of conventional MALDI, but it has some unique characteristics. First, samples are handled at atmospheric pressure. Second, AP-MALDI is a softer ionization technique than vacuum MALDI, which is favored for protein analysis. For example, the heavier peptides/glycopeptides from protein digestion are less likely to fragment by AP rather than vacuum MALDI; thus, more peptides can be detected (Figure 1.3) [42]. Third, AP-MALDI can employ liquid matrices to improve the ionization reproducibility, which otherwise results in source contamination in vacuum MALDI. Fourth, the AP-MALDI ion source is easy to exchange with other atmospheric ionization sources, allowing it to be easily coupled to different mass analyzers (e.g., quadrupole ion trap (QIT) [46], TOF [45],

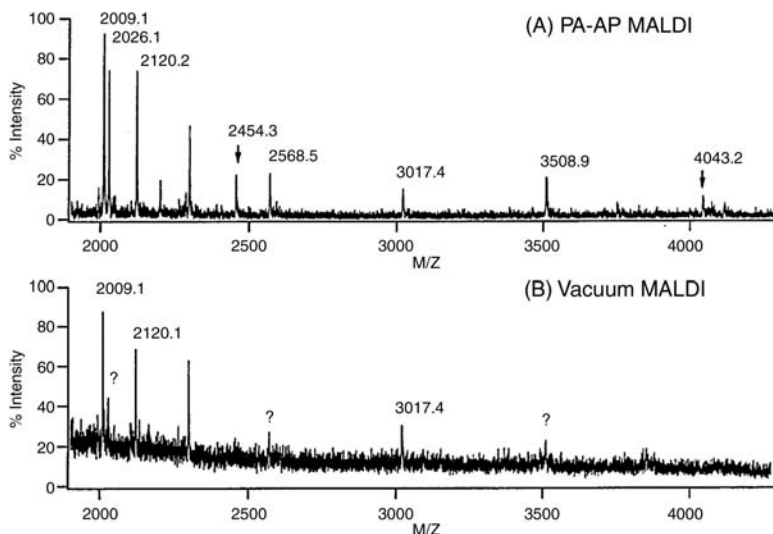


FIGURE 1.3 Partial mass spectra for tryptic digest of bovine fetuin. (A) AP-MALDI spectrum from 1.5-pmol deposition, showing eight identified peaks. (B) Vacuum MALDI spectrum for 1-pmol deposition, showing three identified peaks. From [42]. Copyright permission was obtained from ACS.

and Fourier transform ion cyclotron resonance (FT-ICR) [47]. Fifth, the analyte-matrix cluster ions in AP-MALDI caused by collisional cooling can be observed [43]. Nevertheless, the major disadvantage of AP-MALDI is the ion loss in atmospheric pressure interfaces, giving it lower sensitivity than conventional MALDI [46].

AP-MALDI MS has seen a variety of applications similar to those of conventional vacuum MALDI, including analysis in proteomics and determinations of oligosaccharides, DNA/RNA/PNA, lipids, bacteria, phosphopeptides, small molecules, and synthetic polymers [48]. The convenient and rapid exchange of the AP-MALDI source with other ionization sources and high throughput are attractive features. The major expected application for AP-MALDI is for the analysis of vacuum-incompatible samples like profiling of biological tissue samples, which requires the use of a wide range of liquid matrices at atmospheric pressure [43].

1.2.3 Surface-Enhanced Laser Desorption/Ionization (SELDI)

Surface-enhanced laser desorption/ionization (SELDI) as a prominent form of laser desorption/ionization (LDI) mass spectrometry was first described in 1993 by Hutchens and Yip [49]. It can be classified in three groups: surface-enhanced neat desorption (SEND), surface-enhanced affinity capture (SEAC), and surface-enhanced photolabile attachment and release (SEPAR) [50]. In SEND, analytes even for large molecules can be desorbed and ionized without adding matrix. This occurs because a compound with a chromophore to absorb laser energy is attached to the probe surface via physical adsorption or covalent modification [51]. In SEAC, the

probe surface plays an active role in the extraction, fractionation, cleanup, and/or amplification of the sample of interest. Common are chemical surfaces such as H50 (hydrophobic surface, similar to C_6-C_{12} reverse-phase chromatography materials), CM10 (weak-positive ion exchanger), Q10 (strong anion exchanger), IMAC30 (metal-binding surface), and biochemical surfaces containing antibodies, receptors, enzymes or DNA [50,52]. In SEPAR, an energy-absorbing molecule promotes analyte desorption and ionization, making this approach a hybrid of SECA and SEND [50]. Furthermore SELDI is commercially embodied in Ciphergen's ProteinChip® Array System (Ciphergen Biosystemes, Palo Alto, CA, USA), which simplifies the sample preparation with on-chip binding and detection [50]. SELDI is typically coupled with TOF mass spectrometers and is applied to detect proteins in tissue, urine, blood, and other clinical samples.

SELDI-TOF-MS is the extended form of MALDI-TOF-MS. The differences are the sample preparation and the software tools for interpreting the acquired data. In executing the SELDI process (Figure 1.4) [53], the first step is to select a chromatographic and preactivated ProteinChip array. Next, the protein sample solution is applied and incubated on the spots of the ProteinChip array. Third, by allowing the proteins to interact with the chromatographic array surface, on-spot contaminants and salts of the sample can be washed away to ensure efficient sample cleanup. This binding step to the SELDI surface can be viewed as a separation step, purifying the proteins bound to the surface. Fourth, matrices are added for the formation of a homogeneous layer of cocrystallized target proteins. After that, a laser beam raster is used to irradiate the spot, causing desorption and ionization of the proteins. The laser beam raster can be applied to cover selectively the entire spot surface, affording an output of the entire spot. Finally, multiple spectra are averaged to yield a final spectrum that displays the protein ions. Protein quantification is achieved by the correlation between the signal intensities and analyte concentrations of proteins in the sample.

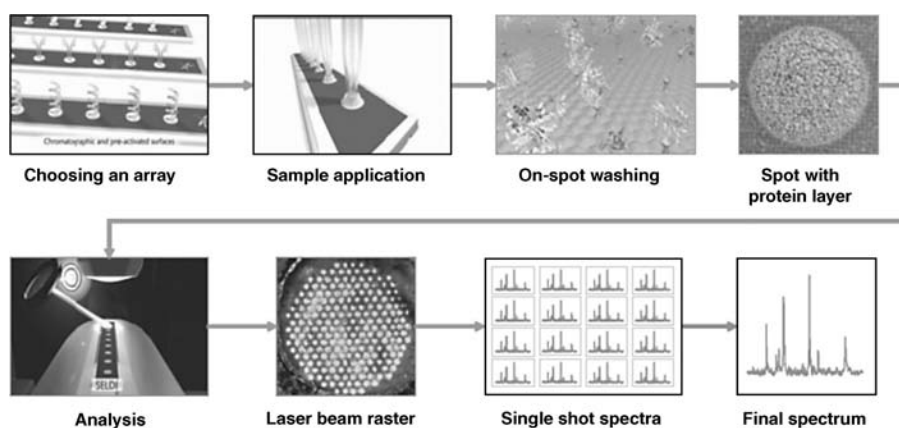


FIGURE 1.4 Experimental steps of SELDI-TOF-MS-based ProteinChip® System. From [53]. Copyright permission was obtained from Nature Publishing Group. (See the color version of this figure in Color Plates section.)