

Contents

List of Contributors *XIII*

Preface *XVII*

Abbreviations *XIX*

1	Introduction to Mass Spectrometry, a Tutorial	1
	<i>Wilfried M.A. Niessen and David Falck</i>	
1.1	Introduction	1
1.2	Figures of Merit	1
1.2.1	Introduction	1
1.2.2	Resolution	2
1.2.3	Mass Accuracy	4
1.2.4	General Data Acquisition in MS	5
1.3	Analyte Ionization	6
1.3.1	Introduction	6
1.3.2	Electrospray Ionization	8
1.3.3	Matrix-Assisted Laser Desorption Ionization	10
1.3.4	Other Ionization Methods	10
1.3.5	Solvent and Sample Compatibility Issues	11
1.4	Mass Analyzer Building Blocks	12
1.4.1	Introduction	12
1.4.2	Quadrupole Mass Analyzer	13
1.4.3	Ion-Trap Mass Analyzer	13
1.4.4	Time-of-Flight Mass Analyzer	15
1.4.5	Fourier Transform Ion Cyclotron Resonance Mass Spectrometer	16
1.4.6	Orbitrap Mass Analyzer	17
1.4.7	Ion Detection	18
1.5	Tandem Mass Spectrometry	18
1.5.1	Introduction: “Tandem-in-Time” and “Tandem-in-Space”	18
1.5.2	Ion Dissociation Techniques	20
1.5.3	Tandem Quadrupole MS–MS Instruments	21
1.5.4	Ion-Trap MS ⁿ Instruments	23
1.5.5	Tandem TOF (TOF–TOF) Instruments	23
1.5.6	Hybrid Instruments (Q–TOF, Q–LIT, IT–TOF)	24

1.5.7	MS–MS and MSn in FT-ICR-MS	26
1.5.8	Orbitrap-Based Hybrid Systems	27
1.5.9	Ion-Mobility Spectrometry–Mass Spectrometry	28
1.6	Data Interpretation and Analytical Strategies	30
1.6.1	Data Acquisition in MS Revisited	30
1.6.2	Quantitative Bioanalysis and Residue Analysis	31
1.6.3	Identification of Small-Molecule “Known Unknowns”	32
1.6.4	Identification of Drug Metabolites	33
1.6.5	Protein Molecular Weight Determination	37
1.6.6	Peptide Fragmentation and Sequencing	38
1.6.7	General Proteomics Strategies: Top-Down, Middle-Down, Bottom-Up	39
1.7	Conclusion and Perspectives	43
	References	43

Part I Direct MS Based Affinity Techniques 55

2	Studying Protein–Protein Interactions by Combining Native Mass Spectrometry and Chemical Cross-Linking	57
	<i>Michal Sharon and Andrea Sinz</i>	
2.1	Introduction	57
2.2	Protein Analysis by Mass Spectrometry	58
2.3	Native MS	59
2.3.1	Instrumentation for High-mass ion Detection	60
2.3.2	Defining the Exact Mass of the Composing Subunits	60
2.3.3	Analyzing the Intact Complex	61
2.4	Chemical Cross-linking MS	64
2.4.1	Types of Cross-linkers	64
2.4.2	MS/MS Cleavable Cross-linkers	66
2.4.3	Data Analysis	68
2.5	Value of Combining Native MS with Chemical Cross-linking MS	68
2.6	Regulating the Giant	69
2.7	Capturing Transient Interactions	70
2.8	An Integrative Approach for Obtaining Low-Resolution Structures of Native Protein Complexes	72
2.9	Future Directions	73
	References	74

3	Native Mass Spectrometry Approaches Using Ion Mobility-Mass Spectrometry	81
	<i>Frederik Lermite, Esther Marie Martin, Albert Konijnenberg, Filip Lemièr, and Frank Sobott</i>	
3.1	Introduction	81
3.2	Sample Preparation	82
3.3	Electrospray Ionization	84

3.4	Mass Analyzers and Tandem MS Approaches	88
3.5	Ion Mobility	90
3.6	Data Processing	95
3.7	Challenges and Future Perspectives	98
	References	102

Part II LC–MS Based with Indirect Assays 109

4	Methodologies for Effect-Directed Analysis: Environmental Applications, Food Analysis, and Drug Discovery	111
	<i>Willem Jonker, Marja Lamoree, Corine J. Houtman, and Jeroen Kool</i>	
4.1	Introduction	111
4.2	Principle of Traditional Effect-Directed Analysis	113
4.3	Sample Preparation	113
4.3.1	Environmental Analysis	113
4.3.1.1	Aqueous Samples	113
4.3.1.2	Biological Samples	116
4.3.1.3	Sediment, Soil, Suspended Matter	120
4.3.2	Food Analysis	121
4.3.2.1	Chromatography-Olfactometry	122
4.3.2.2	Functional Foods	123
4.3.3	Drug Discovery	124
4.3.3.1	Combinatorial Libraries and Natural Extracts	124
4.4	Fractionation for Bioassay Testing	126
4.4.1	Environmental Analysis	126
4.4.2	Food Analysis	130
4.4.3	Drug Discovery	131
4.5	Miscellaneous Approaches	133
4.6	Bioassay Testing	136
4.6.1	Environmental Analysis	136
4.6.2	Food Analysis	140
4.6.3	Drug Discovery	140
4.7	Identification and Confirmation Process	141
4.7.1	Instrumentation	141
4.7.1.1	Gas Chromatography–Mass Spectrometry	141
4.7.1.2	Liquid Chromatography–Mass Spectrometry	142
4.7.2	Data Analysis	143
4.7.2.1	Deconvolution	144
4.7.2.2	Database Spectrum Searching	145
4.7.2.3	Database Searching on Molecular Formula	145
4.7.2.4	In Silico Tools	145
4.7.2.5	Use of Physicochemical Properties	146
4.7.2.6	Applications in Nontarget Screening and EDA	146
4.8	Conclusion and Perspectives	148
	References	149

5	MS Binding Assays 165
	<i>Georg Höfner and Klaus T. Wanner</i>
5.1	Introduction 165
5.2	MS Binding Assays – Strategy 167
5.2.1	Analogies and Differences Compared to Radioligand Binding Assays 167
5.2.2	Fundamental Assay Considerations 169
5.2.3	Fundamental Analytical Considerations 170
5.3	Application of MS Binding Assays 171
5.3.1	MS Binding Assays for the GABA Transporter GAT1 171
5.3.1.1	GABA Transporters 171
5.3.1.2	MS Binding Assays for GAT1 – General Setup 172
5.3.1.3	MS Binding Assays for GAT1 – Speeding up Chromatography 177
5.3.1.4	MS Binding Assays for GAT1 with MALDI-MS/MS for Quantitation 179
5.3.1.5	Library Screening by Means of MS Binding Assays 181
5.3.2	MS Binding Assays for the Serotonin Transporter 183
5.3.2.1	Serotonin Transporter 183
5.3.2.2	MS Binding Assays for SERT – General Setup 184
5.3.3	MS Binding Assays Based on the Quantitation of the Nonbound Marker 187
5.3.3.1	Competitive MS Binding Assays for Dopamine D1 and D2 Receptors 188
5.3.4	Other Examples Following the Concept of MS Binding Assays 189
5.4	Summary and Perspectives 191
	Acknowledgments 192
	References 192
6	Metabolic Profiling Approaches for the Identification of Bioactive Metabolites in Plants 199
	<i>Emily Pipan and Angela I. Calderón</i>
6.1	Introduction to Plant Metabolic Profiling 199
6.2	Sample Collection and Processing 200
6.3	Hyphenated Techniques 203
6.3.1	Liquid Chromatography–Mass Spectrometry 203
6.3.2	Gas Chromatography–Mass Spectrometry 206
6.3.3	Capillary Electrophoresis–Mass Spectrometry 207
6.4	Mass Spectrometry 207
6.4.1	Time of Flight 208
6.4.2	Quadrupole Mass Filter 208
6.4.3	Ion Traps (Orbitrap and Linear Quadrupole (LTQ)) 209
6.4.4	Fourier Transform Mass Spectrometry 210
6.4.5	Ion Mobility Mass Spectrometry 210
6.5	Mass Spectrometric Imaging 210
6.5.1	MALDI-MS 211

6.5.2	SIMS-MS	212
6.5.3	DESI-MS	212
6.5.4	LAESI-MS	213
6.5.5	LDI-MS and Others for Imaging	213
6.6	Data Analysis	214
6.6.1	Data Processing	214
6.6.2	Data Analysis Methods	214
6.6.3	Databases	215
6.7	Future Perspectives	216
	References	216
7	Antivenomics: A Proteomics Tool for Studying the Immunoreactivity of Antivenoms	227
	<i>Juan J. Calvete, José María Gutiérrez, Libia Sanz, Davinia Pla, and Bruno Lomonte</i>	
7.1	Introduction	227
7.2	Challenge of Fighting Human Envenoming by Snakebites	227
7.3	Toolbox for Studying the Immunological Profile of Antivenoms	228
7.4	First-Generation Antivenomics	229
7.5	Snake Venomics	230
7.6	Second-Generation Antivenomics	232
7.7	Concluding Remarks	236
	Acknowledgments	236
	References	236
Part III	Direct Pre- and On-Column Coupled Techniques	241
8	Frontal and Zonal Affinity Chromatography Coupled to Mass Spectrometry	243
	<i>Nagendra S. Singh, Zhenjing Jiang, and Ruin Moaddel</i>	
8.1	Introduction	243
8.2	Frontal Affinity Chromatography	244
8.3	Staircase Method	247
8.4	Simultaneous Frontal Analysis of a Complex Mixture	249
8.5	Multiprotein Stationary Phase	252
8.6	Zonal Chromatography	253
8.7	Nonlinear Chromatography	260
	Acknowledgments	265
	References	265
9	Online Affinity Assessment and Immunoaffinity Sample Pretreatment in Capillary Electrophoresis–Mass Spectrometry	271
	<i>Rob Haselberg and Govert W. Somsen</i>	
9.1	Introduction	271
9.2	Capillary Electrophoresis	272

9.3	Affinity Capillary Electrophoresis	276
9.3.1	Dynamic Equilibrium ACE (Fast Complexation Kinetics)	276
9.3.2	Pre-Equilibrium ACE (Slow Complexation Kinetics)	279
9.3.3	Kinetic ACE (Intermediate Complexation Kinetics)	280
9.4	Immunoaffinity Capillary Electrophoresis	281
9.5	Capillary Electrophoresis–Mass Spectrometry	283
9.5.1	General Requirements for Effective CE–MS Coupling	283
9.5.2	Specific Requirements for ACE–MS and IA-CE-MS	284
9.6	Application of ACE–MS	286
9.7	Applications of IA-CE–MS	292
9.8	Conclusions	295
	References	296
10	Label-Free Biosensor Affinity Analysis Coupled to Mass Spectrometry	299
	<i>David Bonnel, Dora Mehn, and Gerardo R. Marchesini</i>	
10.1	Introduction to MS-Coupled Biosensor Platforms	299
10.2	Strategies for Coupling Label-Free Analysis with Mass Spectrometry	301
10.2.1	On-Chip Approaches	301
10.2.1.1	SPR-MALDI-MS	301
10.2.1.2	SPR-LDI-MS	304
10.2.2	Off-Chip Configurations	305
10.2.2.1	ESI-MS	305
10.2.2.2	In Parallel Approach	305
10.2.3	Chip Capture and Release Chromatography – Electrospray-MS	306
10.3	New Sensor and MS Platforms, Opportunities for Integration	307
10.3.1	Imaging Nanoplasmonics	307
10.3.2	Evanescent Wave Silicon Waveguides	308
10.3.3	New Trends in MS Matrix-Free Ion Sources	309
10.3.4	Tag-Mass	310
10.3.5	Integration	310
	References	310
Part IV	Direct Post Column Coupled Affinity Techniques	317
11	High-Resolution Screening: Post-Column Continuous-Flow Bioassays	319
	<i>David Falck, Wilfried M.A. Niessen, and Jeroen Kool</i>	
11.1	Introduction	319
11.1.1	Variants of On-line Post-Column Assays Using Mass Spectrometry	321
11.1.1.1	Mass Spectrometry as Readout for Biological Assays	323
11.1.1.2	Structure Elucidation by Mass Spectrometry	327
11.1.2	Targets and Analytes	328

11.1.2.1	Targets	328
11.1.2.2	Analytes and Samples	330
11.2	The High-Resolution Screening Platform	330
11.2.1	Separation	330
11.2.2	Flow Splitting	334
11.2.3	Bioassay	336
11.2.4	MS Detection	340
11.3	Data Analysis	342
11.3.1	Differences between HRS and HTS	342
11.3.1.1	Influence of Shorter Incubation Times	342
11.3.1.2	The Assay Signal	344
11.3.1.3	Dilution Calculations	346
11.3.1.4	MS Structure Elucidation	347
11.3.1.5	Structure–Affinity Matching	349
11.3.2	Validation	350
11.4	Conclusions and Perspectives	353
11.4.1	The Relation of On-line Post-Column Assays to Other Formats	353
11.4.2	Trends in High-Resolution Screening	354
11.4.3	Conclusions	357
	References	358
12	Conclusions	365
	<i>Jeroen Kool</i>	
	Index	373

