

Contents

Preface XXI

List of Contributors XXIII

List of Abbreviations XXXI

Part I Perspectives in Proteomics Sample Preparation 1

1 Introduction 3

N. Leigh Anderson

2 General Aspects of Sample Preparation for Comprehensive Proteome Analysis 5

Sven Andrecht and Jörg von Hagen

2.1 The Need for Standards in Proteomics Sample Preparation 5

2.2 Introduction: The Challenge of Crude Proteome Sample Analysis 6

2.3 General Aspects: Parameters which Influence the Sample Preparation Procedure 8

2.3.1 Technical Dependent Aspects for Sample Preparation in Proteomics 9

2.3.2 Sample-Dependent Aspects for Sample Preparation in Proteomics 11

2.3.2.1 Enrichment or Depletion Strategy 11

2.3.2.2 Sample Recovery and Standardization 12

2.3.2.3 Quantification, Internal Standards and Spiking 13

2.3.2.4 Calculating the Amount of Sample for Proteomic Approaches 13

2.3.2.5 Developing Procedures for Different Model Systems:
From Bench to Bedside 15

2.3.2.6 Sample Matrix 15

2.3.2.7 Localization of Target Protein 15

2.3.3 An Example of Subcellular Protein Extraction 17

2.3.3.1 Subcellular Extraction and Monitoring the Redistribution
of Regulatory Proteins 17

2.4	Summary and Perspectives	17
	References	19
3	Proteomics: A Philosophical Perspective	21
	<i>Erich Hämberger</i>	
3.1	Introduction: "In the Beginning was the Word"	21
3.2	The Experiment as a Scientific Method and a Tool of Cognition	23
3.2.1	The Experiment Historically Viewed	23
3.2.2	The Experiment Theoretically Viewed	23
3.2.3	The Entanglement Between Theory and Experiment	25
3.3	The Experiment as a Method (Tool) of Cognition Within the Scope of Biology: The So-Called "Life Sciences"	26
3.4	Proteomics as a Cognition-Theoretical Challenge	30
3.4.1	Cognition-Theoretic Support from Physics	31
3.4.2	The "Pietschmann Axioms" of the Experiment in Biological View	32
3.5	Conclusion	36
	References	37
Part II	Methods	41
4	Mass Spectrometry	43
4.1	A Practical Guideline to Electrospray Ionization Mass Spectrometry for Proteomics Application	43
	<i>Jon Barbour, Sebastian Wiese, Helmut E. Meyer, and Bettina Warscheid</i>	
4.1.1	Introduction	43
4.1.1.1	Electrospray Ionization	43
4.1.1.2	Nano-Electrospray Ionization	45
4.1.1.3	ESI-MS Instrumentation	45
4.1.1.4	Protein Identification Strategies	46
4.1.2	Sample Preparation	47
4.1.2.1	Purification	48
4.1.2.2	Protein Digestion	51
4.1.3	ESI-MS Analysis	52
4.1.3.1	Protein Analysis by ESI-MS	52
4.1.3.2	Peptide Analysis by Nano-HPLC/ESI-MS	53
4.1.4	Application Example of ESI-MS in Proteomics	60
4.1.5	Concluding Remarks	64
4.1.6	Recipes and Methods	64
4.1.6.1	MeOH/Chloroform Protein Precipitation to Remove Salts and Detergents	64
4.1.6.2	Preparation and Washing of a Crude Membrane Pellet	65
4.1.6.3	Proteolytic Digestion and Peptide Extraction	65

4.1.6.4	Off-Line Analysis of Intact Proteins	6/
4.1.6.5	ESI Sample Preparation Checklist	67
	References	68
4.2	Sample Preparation for the Application of MALDI Mass Spectrometry in Proteome Analysis	73
	<i>Andreas Tholey, Matthias Glückmann, Kerstin Seemann, and Michael Karas</i>	
4.2.1	Introduction	73
4.2.2	Sample Preparation for MALDI-Based Protein Identification	75
4.2.2.1	Selection of the MALDI Matrix	75
4.2.3	Sample Preparation	78
4.2.4	LC-MALDI	84
4.2.5	Application Example	85
4.2.5.1	Gel-Based Workflow	85
4.2.5.2	Application Example: LC-MALDI Workflow	87
4.2.6	Summary	88
4.2.7	Perspectives	89
4.2.8	Recipes for Beginners	90
4.2.8.1	Sample Spotting Techniques	90
4.2.8.2	Sample Cleaning Procedures	90
	References	91
4.3	Sample Preparation for Label-Free Proteomic Analyses of Body Fluids by Fourier Transform Ion Cyclotron Mass Spectrometry	95
	<i>Cloud P. Pawletz, Nathan A. Yates, and Ronald C. Hendrickson</i>	
4.3.1	Introduction	95
4.3.2	Perspective	99
4.3.3	Recipe for Beginners	100
4.3.3.1	Step-By-Step Instructions	102
	References	103
4.4	Sample Preparation for Differential Proteome Analysis: Labeling Technologies for Mass Spectrometry	105
	<i>Josef Kellermann</i>	
4.4.1	Introduction	105
4.4.2	Isotopic Labeling of Peptides and/or Proteins	107
4.4.2.1	Stable Isotope Labeling of Proteins in Cell Culture	107
4.4.2.2	Chemical Isotopic Labeling of Peptides or Proteins	108
4.4.2.3	Spiking of Labeled Peptides	111
4.4.3	Summary	111
4.4.4	Perspectives	112
4.4.5	Recipe for Beginners	112
	References	114

4.5	Determining Membrane Protein Localization Within Subcellular Compartments Using Stable Isotope Tagging <i>118</i> <i>Kathryn S. Li Hey, Tom Dunkley, and Paweł Sadowski</i>
4.5.1	Introduction <i>118</i>
4.5.2	Preparation and Treatment of Samples in the Early-Stage LOPIT Protocol <i>120</i>
4.5.2.1	Preparation and Fractionation of Organelles <i>120</i>
4.5.2.2	Carbonate Washing of Fractions to Lyse Organelles, and Removal of Soluble and Peripheral Proteins <i>123</i>
4.5.2.3	iTRAQ Labeling <i>124</i>
4.5.3	Application of LOPIT to Map the Organelle Proteome of <i>Arabidopsis</i> <i>125</i>
4.5.4	Summary <i>126</i>
4.5.5	Recipe for Beginners <i>127</i>
	References <i>127</i>
5	Electrophoresis <i>129</i>
5.1	Sample Preparation for Two-Dimensional Gel Electrophoresis <i>129</i> <i>Walter Weiss and Angelika Corg</i>
5.1.1	Introduction <i>129</i>
5.1.2	General Aspects of Sample Preparation for 2-DGE <i>130</i>
5.1.2.1	Cell Disruption <i>130</i>
5.1.2.2	Sample Clean-Up <i>132</i>
5.1.2.3	Protein Solubilization <i>135</i>
5.1.3	Application Samples <i>137</i>
5.1.3.1	Mammalian Tissues <i>138</i>
5.1.3.2	Microbial Cell Cultures <i>138</i>
5.1.3.3	Plant Cells <i>138</i>
5.1.4	Summary <i>139</i>
5.1.5	Perspective <i>140</i>
5.1.6	Recipes for Beginners <i>141</i>
	References <i>142</i>
5.2	Sample Preparation for Native Electrophoresis <i>144</i> <i>Ilka Wittig and Hermann Schagger</i>
5.2.1	Introduction <i>144</i>
5.2.2	Sample Preparation: General Considerations <i>146</i>
5.2.2.1	Choice of Detergent and Detergent/Protein Ratio <i>146</i>
5.2.2.2	Choice of Ionic Strength and pH for Sample Solubilization <i>147</i>
5.2.2.3	Storage of Biological Membranes <i>148</i>
5.2.2.4	Effects of Adding Coomassie Dye to Sample and/or Cathode Buffer for BNE <i>149</i>
5.2.3	Applications <i>150</i>
5.2.3.1	Solubilization of Bacterial Membranes, Yeast and Mammalian Mitochondria <i>250</i>

5.2.3.2	Homogenization and Solubilization of Mammalian Cells and Tissues	150
5.2.3.3	Recipe for Beginners: Mass Calibration Ladder for BNE	151
5.2.4	Summary and Perspectives	151
	References	152
5.3	Sample Preparation for LC-MS/MS Using Free-Flow Electrophoresis	155
	<i>Mikkel Nissum, Aficmeh Abdolzade-Bavil, Sab'me Kuhfuss, Robert Wildgruber, Gerhard Weber, and Christoph Eckerskorn</i>	
5.3.1	Introduction	155
5.3.2	The Problems of Sample Preparation: The Pros and Cons	157
5.3.2.1	Separation	157
5.3.2.2	Extraction	158
5.3.2.3	Media Composition	158
5.3.3	Application Example	159
5.3.3.1	Reagents	159
5.3.3.2	Sample Preparation for FFE	160
5.3.3.3	FFE Separation of Peptides	160
5.3.3.4	RPLC-MS/MS Analysis	161
5.3.3.5	Data Processing	161
5.3.4	Summary	161
5.3.5	Perspective	165
5.3.6	Recipe for Beginners	166
5.3.6.1	FFE Set-Up Procedure	166
5.3.6.2	Pre-Experimental Quality Control (QC)	167
5.3.6.3	Experiment	168
	References	168
5.4	Sample Preparation for Capillary Electrophoresis	171
	<i>Ross Burn and David Perrett</i>	
5.4.1	Introduction	171
5.4.2	Sample Preparation	173
5.4.2.1	Sample Collection and Storage	174
5.4.2.2	Sample preparation for CE	174
5.4.2.3	Sample Concentration	175
5.4.2.4	Off-Line Preconcentration	275
5.4.2.5	On-Line Preconcentration	175
5.4.2.6	Desalting	176
5.4.2.7	Analyte Modification	176
5.4.3	Background Electrolyte	277
5.4.4	Capillary Preparation	178
5.4.4.1	Capillary Dimensions	178
5.4.4.2	Capillary Conditioning	178
5.4.4.3	Capillary Coating	179
5.4.5	Summary	179

5.4.6	Perspective	179
5.4.7	Recipe for Beginners	180
5.4.7.1	Method 1: Analysis of Human Serum/Plasma by C2E	180
5.4.7.2	Method 2: Analysis of Tryptic Digests by CZE	282
5.4.7.3	Method 3: Analysis of Proteomes by CIEF	183
	References	185
6	Optical Methods	187
6.1	High-Throughput Proteomics: Spinning Disc Interferometry (SDI)	187
	<i>Patricia Espinoza Vallejos, Greg Lawrence, David Nolte, Fred Regnier, and Joerg Schreiber</i>	
6.1.1	Proteomics as a Tool for Health Assessment	287
6.1.2	Translational Proteomics	188
6.1.3	The Principles of Spinning Disc Interferometry	189
6.1.3.1	The Spinning Disc	289
6.1.3.2	Why Spin?	191
6.1.3.3	In-line Quadrature	292
6.1.3.4	Scaling Mass Sensitivity	194
6.1.4	The Spinning Disc as a High-Throughput Immunological Assay Platform	296
6.1.4.1	Immunological Assays Using a Disc Array Format	297
6.1.4.2	Assay Formats	298
6.1.4.3	Assay Protocols	299
6.1.5	Types of Assay that Fit the Spinning Disc	200
6.1.5.1	Assay Structure	200
6.1.5.2	Assay Development Kit (ADK)	202
6.1.6	Assay and Sample Processing	202
6.1.6.1	High-Throughput System	201
6.1.6.2	The ADK	203
6.1.7	Conclusions	205
	References	206
6.2	Optical Proteomics on Cell Arrays	208
	<i>Andreas Qrod and Philippe Bastiaens</i>	
6.2.1	Introduction	208
6.2.2	A Description of the Problem with Regards to Sample Preparation	222
6.2.2.1	General Remarks	222
6.2.2.2	Cell Line Selection	222
6.2.2.3	Sample Preparation	222
6.2.2.4	Choice of Transfection Reagent	222
6.2.2.5	Nucleic Acid Preparation	223
6.2.2.6	Sample Scale: How Many Duplicates are Required?	223
6.2.2.7	Choice of Microarrayer/Microspotting System (Spotter)	223
6.2.2.8	Layout Design (Spotting Pattern)	224

6.2.3	Summary	215
6.2.4	Perspectives	235
6.2.5	Sample Preparation: Short Protocol	215
6.2.5.1	Recommended Equipment and Consumables	215
6.2.5.2	Preparation of the Source Plate	216
6.2.5.3	Spotting	217
6.2.5.4	Cell Culture and Experiment	217
	References	218
6.3	Sample Preparation by Laser Microdissection and Catapulting for Proteome Analysis	219
	<i>Karin Schütze, Andrea Buchstaller, Yilmaz Niyaz, Christian Melle, Cünther Ernst, Kerstin David, Thorsten Schlomm, and Ferdinand von Eggeling</i>	
6.3.1	Introduction: Laser Microdissection and Functional Proteomic Research	219
6.3.2	The Relevance of Pure Starting Material for Proteomics	219
6.3.3	Examples of Combined LMPC and Proteomic Analyses	220
6.3.3.1	LMPC and Preeclampsia	220
6.3.3.2	LMPC and Renal Cell Carcinoma	221
6.3.3.3	LMPC and Hepatocellular Carcinoma	221
6.3.3.4	LMPC and Brain Disorders	221
6.3.3.5	LMPC and Plant Biology	221
6.3.4	LMPC Adapted for Proteomic Applications	222
6.3.5	LMPC Combined with SELDI-TOF MS: A Promising Approach for Patient-Specific Analyses	224
6.3.6	Correlation of Gene and Protein Expression: The Best Data Capture for Comprehensive Diagnosis	228
6.3.7	Recipe for Beginners	228
6.3.7.1	Patients and Specimens	228
6.3.7.2	Laser Microdissection of Tissue Sections	230
6.3.7.3	ProteinChip Array Preparation and Analysis	230
6.3.8	Summary and Outlook	231
	References	232
6.4	Sample Preparation for Flow Cytometry	234
	<i>Derek C. Davies</i>	
6.4.1	Introduction	234
6.4.2	Sample Preparation for Flow Cytometry	236
6.4.2.1	Preparation from Cells in Suspension	236
6.4.2.2	Preparation from Adherent Cells	237
6.4.2.3	Preparation from Solid Tissue	237
6.4.2.4	General Considerations	237
6.4.3	Identification of Relevant Cells	238
6.4.4	Cell Sorting	238
6.4.4.1	Cells and Samples	238
6.4.4.2	Cytometer Considerations	239

6.4.5	Application Example	240
6.4.6	Summary	242
6.4.7	Perspectives	242
6.4.8	Recipes for Beginners	242
6.4.8.1	Cultured Suspension Cells	242
6.4.8.2	Adherent Cells	242
6.4.8.3	Solid Tissue	243
	References	243
7	Chromatography	245
7.1	Sample Preparation for HPLC-Based Proteome Analysis	245
	<i>Egidijus Machtejevas and Klaus K. linder</i>	
7.1.1	Introduction	245
7.1.2	Problems Related to Direct Sample Injection in HPLC	246
7.1.3	Trial and Error Selection of the Sample Preparation Method	247
7.1.4	Classical Approaches	249
7.1.5	Specific Approaches Applied to Sample Clean-Up in Proteomics	250
7.1.5.1	Miniaturized Extraction Techniques	250
7.1.5.2	Most Abundant Component Depletion	250
7.1.5.3	Affinity-Enrichment Approaches	251
7.1.6	On-line Sample Clean-Up Approaches	252
7.1.7	<i>IX.I</i> Restricted Access Technology	254
7.1.8	Application Example: The Case Study	259
7.1.9	Conclusion and Perspectives	260
	References	262
7.2	Sample Preparation for Two-Dimensional Phosphopeptide Mapping and Phosphoamino Acid Analysis	265
	<i>Anamarija Kruljac-Letunic and Andree Blaukat</i>	
7.2.1	Introduction	265
7.2.2	Important Aspects in Sample Preparation Procedures	265
7.2.3	Application Example	267
7.2.4	Summary	267
7.2.5	Perspective	269
7.2.6	Recipe for Beginners	269
7.2.6.1	2-D Phosphopeptide Mapping Procedure	269
7.2.6.2	Phosphoamino Acid Analysis Procedure	271
	References	271
8	Structural Proteomics	273
8.1	Exploring Protein-Ligand Interactions by Solution NMR	273
	<i>Rudolf Hartmann, Thomas Stangler, Bernd W.Konig, and Dieter Willbold</i>	
8.1.1	Introduction	273
8.1.2	Localization of Interaction Sites by Chemical Shift Perturbation (CSP) Mapping	274

8.1.3	Saturation Transfer Difference Spectroscopy	276
8.1.4	Ligand Screening by NMR	278
	References	2,79
8.2	Sample Preparation for Crystallography	281
	<i>Djordje Musil</i>	
8.2.1	Introduction	281
8.2.2	Use of Recombinant Proteins in Crystallization	282
8.2.3	Protein Solubility and Crystallization	284
8.2.4	Protein Crystallization	286
8.2.5	Practical Examples	291
	References	292
9	Interaction Analysis	295
9.1	Sample Preparation for Protein Complex Analysis by the Tandem Affinity Purification (TAP) Method	295
	<i>Bertrand Séraphim and Andrzej Dziembowski</i>	
9.1.1	Introduction	295
9.1.2	The Problem with Regards to Sample Preparation: The Pros and the Cons	296
9.1.3	Application Example	300
9.1.4	Summary	301
9.1.5	Perspective	301
9.1.6	Recipe for Beginners	301
	References	302
9.2	Exploring Membrane Proteomes	303
	<i>Filippa Stenberg and Daniel O. Daley</i>	
9.2.1	Introduction	303
9.2.2	Defining Membrane Proteomes	303
9.2.3	Separation of Membrane Proteomes	304
9.2 A	Experimental Identification of Membrane Proteins	307
9.2.5	Mapping Membrane Interactomes	307
9.2.6	Structural Analysis of Membrane Proteomes	308
9.2.7	Summary and Perspective	309
9.2.8	Recipe for Beginners	311
9.2.8.1	Sample Preparation	311
9.2.8.2	BN-PAGE	311
9.2.8.3	SDS-PAGE	312
	References	312
10	Post-Translational Modifications	317
10.1	Sample Preparation for Phosphoproteome Analysis	317
	<i>René P. Zahedi and Albert Sickmann</i>	
10.1.1	Introduction	317
10.1.2	General Sample Preparation	317

10.1.3	Reduction of Sample Complexity	318
10.1.3.1	Gel Electrophoresis	318
10.1.3.2	Isoelectric Focusing	318
10.1.4	Methods for Phosphopeptide/Protein Enrichment	319
10.1.4.1	Immunoprecipitation	319
10.1.4.2	Immobilized Metal Ion Affinity Chromatography (IMAC)	319
10.1.4.3	Metal Oxides	320
10.1.4.4	Cation-Exchange Chromatography	321
10.1.4.5	Derivatization Approaches	322
10.1.5	Summary	323
10.1.6	Perspective	324
10.1.7	Recipe for Beginners: IMAC	324
	References	325
10.2	Sample Preparation for Analysis of Post-Translational Modifications: Glycosylation	328
	<i>David S. Selby, Martin R. Larsen, Miren J. Omaetxebarria, and Peter Roepstorff</i>	
10.2.1	Introduction	328
10.2.2	Advantages and Disadvantages of Different Sample Preparation Methods	331
10.2.3	Example Applications of Enrichment Methods	334
10.2.3.1	ZIC-HILIC Microcolumns for Preparation of N-Linked Glycan-Containing Samples	334
10.2.3.2	Titanium Dioxide Microcolumns for Enrichment of Sialic Acid-Containing Glycopeptides and Glycosylphosphatidylinositol Lipid-Anchored Peptides	336
10.2.4	Summary	337
10.2.5	Perspective	338
10.2.6	Recipe for Beginners: Enrichment of Glycopeptides with a HILIC Microcolumn	338
10.2.6.1	Materials	338
10.2.6.2	Procedure: Purification of Glycopeptides	339
10.2.6.3	Procedure: Deglycosylation of N-Linked Glycopeptides	339
	References	340
11	Species-Dependent Proteomics	343
11.1	Sample Preparation and Data Processing in Plant Proteomics	343
	<i>Katja Baerenfaller, Wilhelm Gruisse, and Sacha Baginsky</i>	
11.1.1	Introduction	343
11.1.2	Plant-Specific Considerations in Proteomics	344
11.1.2.1	Cell Walls	344
11.1.2.2	Plastids	344
11.1.2.3	Protein Extraction from Plant Tissue	345
11.1.2.4	Extraction from Recalcitrant and Resistant Tissue	345

11.1.2.5	Dynamic Range Limitations	346
11.1.2.6	Proteomics in As-Yel Unsequenced Organisms	346
11.1.3	Sample Preparation Protocols	347
11.13.1	Ceil Wail Protein Extraction	348
11.13.2	Plastic! Isolation	349
11.1.3.3	Protein Extraction with TCA/ Acetone	350
11.1.3.4	Phenol Extraction	351
11.1.3.5	Serial Extraction	351
11.1.3.6	Extraction from Recalcitrant and Resistant Tissue	352
11.1.3.7	Extraction and Fractionation with Polyethylene Glycol (PEG)	353
11.1.3.8	Stages Following Protein Extraction	353
11.1.4	MS/MS Data Processing for Unsequenced Organisms	354
11.1.5	Concluding Remarks	355
	References	356
11.2	Sample Preparation for MudPIT with Bacterial Protein Samples	358
	<i>Ansgar Poetsch and Dirk Wolters</i>	
11.2.1	Introduction	358
11.2.2	The MudPIT Technology	359
11.2.3	Membrane Proteins and MudPIT	361
11.2.4	Quantitative MudPIT	363
11.2.5	Limitations of MudPIT	364
11.2.6	Pitfalls of MudPIT	365
11.2.7	Summary	365
11.2.8	Perspective	365
11.2.9	Recipe for Beginners: MudPIT: Soluble and Membrane Proteins	366
	References	368
11.3	Sample Preparation for the Cell-Wall Proteome Analysis of Yeast and Fungi	371
	<i>Kai Sohn, Ekkehard Hitler, and Steffen Rupp</i>	
11.3.1	Introduction	371
11.3.2	Description of the Problem with Regards to Sample Preparation	372
11.3.3	Application Example	373
11.3.4	Summary	375
11.3.5	Perspective	376
11.3.6	Recipe for Beginners	376
113.6.1	Cultures	376
113.6.2	Preparation of Soluble Cell-Surface Proteins	376
113.6.3	Preparation of Peptides from Covalently Linked Cell-Wall Proteins	377
	References	378
12	The Human Proteosome	379
12.1	Clinical Proteomics: Sample Preparation and Standardization	379
	<i>Gerd Schmitz and Carsten Gnewuch</i>	
12.1.1	Introduction	379

12.1.2	The Preanalytical Phase: Sample Preparation, Standardization, and Quality Management	380
12.1.2.1	Standardization of the (Pre)-Analytical Process	381
12.1.3	Proteomics in Body Fluids	382
12.1.3.1	Techniques for Proteomic Analysis	382
12.1.3.2	Applications	383
12.1.3.3	Preparation of Clinical Samples for Fluidic Proteomics	386
12.1.4	Cellular Proteomics (Cytomics)	389
12.1.4.1	Sample Preparation and Standardization for Clinical Cytomics	389
12.1.4.2	Tissue Arrays	390
12.1.4.3	Bead-Based Immunoassays for Protein Analysis	391
12.1.4.4	Preparative Methods	391
12.1.4.5	Clinical Applications in Cytomics	399
12.1.5	Conclusion	404
	References	405
12.2	Stern Cell Proteomics	412
	<i>Regina Ebert, Gabriele Möller, Jerzy Adamski, and Franz Jakob</i>	
12.2.1	Introduction	412
12.2.2	Stem Cell Niches	413
12.2.3	Why Study Proteomes in Stem Cells?	413
12.2.4	Technical Challenges and Problems	414
12.2.4.1	Stem Cell Preparation	414
12.2.4.2	Cultivation	415
12.2.4.3	Treatment	415
12.2.4.4	Whole-Cell Proteome	415
12.2.4.5	Secretory Proteome	425
12.2.5	Recipes for Beginners	417
12.2.5.1	Whole-Cell Lysate	417
12.2.5.2	Secretory Proteome Procedure	418
12.2.5.3	Labeling with ^{35}S	418
12.2.5.4	Ethanol Precipitation	419
12.2.5.5	TCA Precipitation	419
	References	419
13	Bioinformatics	423
13.1	Bioinformatics Support for Mass Spectrometric Quality Control	423
	<i>Knut Reinert, Tim Conrad, and Oliver Kohlbacher</i>	
13.1.1	Introduction	423
13.1.2	Problem description	423
13.1.2.1	Signal Processing Pitfalls	424
13.1.2.2	Map Quality Control	425
13.1.2.3	Statistical Validation Results	425
13.1.3	Quality Assessment for One-Dimensional (1-D) MS Data	426
13.1.3.1	Filter	427

13.1.4	Application Example; Absolute Quantification of an Unknown Peptide Content	428
13.1.5	Summary	429
13.1.6	Perspective	430
13.1.7	Recipe for Beginners	430
13.1.7.1	Acquiring the Raw Data	430
13.1.7.2	Preprocessing the Data	431
13.1.7.3	Analyzing the Preprocessed Data	431
	References	431
13.2	Use of Physico-Chemical Properties in Peptide and Protein Identification	433
	<i>Anastasia K. Yocum, Peter J. Ulitz, and Philip C. Andrews</i>	
13.2.1	Introduction	433
13.2.2	Isoelectric Point	434
13.2.3	Ion-Exchange Chromatography	436
13.2.4	Reversed-Phase Chromatography	438
13.2.5	Mass Accuracy	442
13.2.6	Summary	443
	References	444
	Index	449