

CONTENTS

Contributors

xiii

Preface

xvii

1. An Easy and Fast Protocol for Affinity Bead-Based Protein Enrichment and Storage of Proteome Samples 1

A. Otto, S. Maaß, F. Bonn, K. Büttner, and D. Becher

1. Introduction 2
2. StrataClean Beads for Protein Enrichment 3
3. General Protocol for the Use of StrataClean Beads in Proteomics 5
4. Application of StrataClean Beads in Protein Sample Preparation and Life Science Research 7
5. Protocol Variations 9
6. Conclusions 12
- References 13

2. Filter-Aided Sample Preparation: The Versatile and Efficient Method for Proteomic Analysis 15

J.R. Wiśniewski

1. Introduction 16
2. The FASP Procedures 16
3. Filter-Aided Sample Preparation Protocols 21
- Acknowledgments 25
- References 25

3. An Overview of Advanced SILAC-Labeling Strategies for Quantitative Proteomics 29

F. Terzi and S. Cambridge

1. Introduction 30
2. Dual Labeling to Exclude Unlabeled Proteins 32
3. Subsaturating Labeling 38
4. 5-Plexed SILAC Labeling 43
5. Conclusion 45
- References 46

4. Label-Free and Standard-Free Absolute Quantitative Proteomics Using the "Total Protein" and "Proteomic Ruler" Approaches	49
J.R. Wiśniewski	50
1. Introduction	50
2. TPA: For Determination of Protein Contents and Concentrations	52
3. Cell Size and Protein Copy Numbers Can Be Assessed by the Proteomic Ruler	53
4. Protein Concentrations and Copy Numbers Provide Different Layers of Information	54
5. Consistency of TPA Values With Biochemical Data	56
6. Normalization and Comparison of Datasets Using DJ-1/PARK7 Titer	57
7. Limitations	58
Acknowledgments	59
References	59
5. Separation of Two Distinct O-Glycoforms of Human IgA1 by Serial Lectin Chromatography Followed by Mass Spectrometry O-Glycan Analysis	61
S. Lehoux and T. Ju	62
1. Introduction	62
2. Methodology	64
3. Discussion	71
Acknowledgment	73
References	73
6. Monitoring Protein Synthesis in <i>Caenorhabditis elegans</i> Using SILAC	77
M. Miyagi	78
1. Introduction	78
2. Principle of SILAC-Based Measurement of Protein Synthesis in <i>C. elegans</i>	79
3. Experimental Procedures	81
4. Conclusion	88
Acknowledgment	89
References	89

7. Identification of Novel Macropinocytosing Human Antibodies by Phage Display and High-Content Analysis 91

K.D. Ha, S.M. Bidlingmaier, Y. Su, N.-K. Lee, and B. Liu

1. Introduction 92
2. HT-HCA Screening of Macropinocytic Antibodies: Overview of the Strategy and Method 92
3. HT-HCA Screening Protocol for Macropinocytic Phage Antibodies 94
4. Conclusion 108
- Acknowledgments 108
- References 108

8. Beyond the Natural Proteome: Nondegenerate Saturation Mutagenesis—Methodologies and Advantages 111

M.M. Ferreira Amaral, L. Frigotto, and A.V. Hine

1. Introduction 112
2. Advantages of Nondegenerate Saturation 114
3. Saturation Mutagenesis Methods 116
4. Screening the Library: Methods, Library Size, and the Importance of Diversity 123
5. Conclusions 129
- Acknowledgments 130
- References 131

9. WGCNA Application to Proteomic and Metabolomic Data Analysis 135

G. Pei, L. Chen, and W. Zhang

1. Introduction 136
2. Overview of WGCNA Protocol 139
3. Application in Proteomic Analysis 141
4. Application in Metabolomics Analysis 151
5. Conclusions 155
- Acknowledgments 156
- Data Availability 156
- References 156

10. A Protocol for Large-Scale Proteomic Analysis of Microdissected Formalin Fixed and Paraffin Embedded Tissue	159
P. Ostasiewicz and J.R. Wiśniewski	
1. Introduction	159
2. FFPE-FASP-SAX Protocol	166
Acknowledgments	172
References	173
11. Body Fluid Degradomics and Characterization of Basic N-Terminome	177
F. Sabino, O. Hermes, and U. auf dem Keller	
1. Introduction	178
2. N-Terminal Enrichment Strategies	182
3. Body Fluid Degradomics by iTRAQ-TAILS	184
4. Validation by Targeted Proteomics and Establishment of Biomarker Assays	194
5. Conclusions	195
Acknowledgments	196
References	196
12. Reannotation of Genomes by Means of Proteomics Data	201
J. Armengaud	
1. Introduction	202
2. Bacterial Proteogenomics: The Methods	206
3. General Protocol	209
4. Conclusion	213
References	214
13. Proteogenomics: Recycling Public Data to Improve Genome Annotations	217
A. McAfee and L.J. Foster	
1. Introduction	218
2. Data	221
3. Databases	223
4. Peptide and Protein Identification	227
5. Proteogenomics Toolkits	228
6. Sequence Validation	235
7. Limitations	237
8. Concluding Remarks	239
References	239

14. Targeted In-Depth Quantification of Signaling Using Label-Free Mass Spectrometry	245
P.R. Cutillas	
1. Introduction	246
2. Phosphoproteomic Analysis as a Means to Quantify Cell Signaling	247
3. Overview of Methodologies for Phosphoproteomics	249
4. The Concepts of TIQUAS	251
5. Obtaining Biological Information From Phosphoproteomics Datasets	257
6. Conclusions	261
References	261
15. Biotin Switch Assays for Quantitation of Reversible Cysteine Oxidation	269
R. Li and J. Kast	
1. Introduction	270
2. Chemicals	274
3. Method	274
4. Application	279
5. Conclusion	281
References	282
16. Application of a Thioredoxin-Trapping Mutant for Analysis of the Cellular Nitrosoproteome	285
M. Benhar	
1. Introduction	286
2. Materials	289
3. Methods	289
4. Concluding Remarks	291
Acknowledgments	293
References	293
17. Combining Click Chemistry-Based Proteomics With Dox-Inducible Gene Expression	295
J. Gebert, M. Schnölzer, U. Warnken, and J. Kopitz	
1. Background	296
2. Combined Experimental Strategy	301
3. Application and Modification	319
4. Conclusion	322
Acknowledgments	322
References	322

18. Analysis of the Proteome of Hair-Cell Stereocilia by Mass Spectrometry	329
J.F. Krey, P.A. Wilmarth, L.L. David, and P.G. Barr-Gillespie	
1. Introduction	330
2. Isolation of Hair Bundles	332
3. Shotgun Mass Spectrometry	337
4. Targeted Mass Spectrometry	345
5. Summary	351
Acknowledgments	352
References	353
19. Proteomic Analysis of Metabolic Responses to Biofuels and Chemicals in Photosynthetic Cyanobacteria	355
T. Sun, L. Chen, and W. Zhang	
1. Introduction	356
2. Current Proteomics Methods Used in Analyzing Metabolic Responses	359
3. Metabolic Responses of Cyanobacteria to Biofuels Revealed by Proteomics	361
4. Metabolic Responses to Chemicals Revealed by Proteomics in Cyanobacteria	368
5. Conclusions	371
Acknowledgments	371
References	372
20. Deep Profiling of Proteome and Phosphoproteome by Isobaric Labeling, Extensive Liquid Chromatography, and Mass Spectrometry	377
B. Bai, H. Tan, V.R. Pagala, A.A. High, V.P. Ichhaporia, L. Hendershot, and J. Peng	
1. Introduction	378
2. Sample Preparation by Harvesting Cells and Tissues	380
3. Protein Extraction, Quality Control Western Blotting, In-Solution Digestion, and Peptide Purification	381
4. TMT Labeling of Peptides	383
5. Offline Basic pH RPLC Fractionation	384
6. TiO ₂ Phosphopeptide Enrichment	386
7. Acidic pH RPLC-MS/MS Analysis	387
8. MS Data Analysis	389
9. Proteomics Results and Selected Validation	390

10. Conclusions and Discussion	392
Acknowledgments	392
References	393
21. Glycoprotein Enrichment Analytical Techniques: Advantages and Disadvantages	397
R. Zhu, L. Zacharias, K.M. Wooding, W. Peng, and Y. Mechref	
1. Proteomics: Posttranslational Modifications	398
2. Challenges Associated With Glycoprotein Analysis	399
3. Enrichment Techniques of Glycopeptides	402
4. High-Performance Liquid Chromatography in Proteomics and Glycoproteomics	406
5. Ion Mobility-Mass Spectrometry	410
6. Capillary Electrophoresis	411
7. Characterization of Intact Glycopeptides	413
8. Characterization of Intact Glycoproteins	415
9. Software Tools for MS/MS-Based Glycopeptide Characterization	417
10. Concluding Remarks and Future Directions	419
Acknowledgments	420
References	420
22. Quantitative Glycomics: A Combined Analytical and Bioinformatics Approach	431
L. Veillon, S. Zhou, and Y. Mechref	
1. Protein Glycosylation	432
2. Sample Handling in Glycomics	434
3. Quantitative Glycomics Platforms	444
Acknowledgments	463
References	463
Author Index	479
Subject Index	517