CONTENTS

Preface ix
Contributors xi

1 Direct Characterization of Protein Complexes by Electrospray Ionization Mass Spectrometry and Ion Mobility Analysis 1
Joseph A. Loo and Catherine S. Kaddis

1.1 Introduction, 2
  1.1.1 Historical Perspective of ESI-MS for Measuring Protein Complexes, 3
  1.1.2 Types of Interactions that Are Probed by ESI-MS, 6
1.2 Critical Aspects of the Experimental Procedure, 8
  1.2.1 Instrumental Parameters, 8
    1.2.1.1 Electrospray Ionization Source, 9
    1.2.1.2 Atmosphere/Vacuum Interface and Pressure, 9
    1.2.1.3 Mass Spectrometry Analyzers, 10
    1.2.1.4 Ion Mobility Analyzers, 12
  1.2.2 Sample Preparation, 15
1.3 Solution Phase Equilibria and Gas Phase Dissociation, 16
  1.3.1 Measuring Solution Dissociation Constants, 16
  1.3.2 Tandem Mass Spectrometry of Protein Complexes, 16
1.4 Conclusions, 18
Acknowledgments, 19
References, 19
2 Softly, Softly—Detection of Protein Complexes by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry

Kevin M. Downard

2.1 Introduction, 25
2.2 First Glimpses and the First-Shot Phenomenon, 28
2.3 Matrix and Solution Criteria to Preserve Protein Complexes, 30
2.4 Laser Fluence, Wavelength, and Ion Extraction, 32
2.5 Preservation of Protein Complexes on Conventional MALDI Targets, 35
2.6 Affinity Targets and Surfaces Coupled to MALDI, 37
2.7 Conclusions, 39

References, 39

3 Probing Protein Interactions Using Hydrogen–Deuterium Exchange Mass Spectrometry

David D. Weis, Suma Kaveti, Yan Wu, and John R. Engen

3.1 Introduction, 46
3.2 Hydrogen Exchange Background, 46
3.3 General HX-MS Method, 47
   3.3.1 Location Information Provided by HX-MS, 49
   3.3.2 Revealing Interactions by Comparison, 50
3.4 Interactions of Proteins, 50
3.5 Examples, 52
   3.5.1 Conformational Changes of Proteins During Binding, 52
   3.5.2 Protein–Protein Interactions, 52
   3.5.3 Protein–Peptide Interactions, 54
   3.5.4 Protein–Small Molecule Interactions, 55
3.6 Conclusions, 57
   Acknowledgements, 57
   References, 57

4 Limited Proteolysis Mass Spectrometry of Protein Complexes

Maria Monti and Piero Pucci

4.1 Introduction, 63
4.2 Limited Proteolysis Analysis, 64
4.3 Experimental Design, 67
4.4 Probing Protein–Protein Interactions, 69
4.5 Probing Protein–Nucleic Acid Interactions, 72
4.6 Probing Protein–Ligand Interactions, 74
5 Chemical Cross-Linking and Mass Spectrometry for Investigation of Protein–Protein Interactions

Andrea Sinz

5.1 Introduction, 84
5.2 Cross-Linking Strategies, 85
  5.2.1 Bottom–Up Approach, 85
  5.2.2 Top–Down Approach, 88
5.3 Functional Groups of Cross-Linking Reagents: Reactivities, 89
  5.3.1 Amine-Reactive Cross-Linkers, 89
  5.3.2 Sulfhydryl-Reactive Cross-Linkers, 91
  5.3.3 Photoreactive Cross-Linkers, 91
5.4 Cross-Linker Design, 92
  5.4.1 Homobifunctional Cross-Linkers, 92
  5.4.2 Heterobifunctional Cross-Linkers, 93
  5.4.3 Zero-Length Cross-Linkers, 93
  5.4.4 Trifunctional Cross-Linkers, 93
5.5 Mass Spectrometric Analysis of Cross-Linked Products, 94
  5.5.1 Bottom–Up Analysis by MALDI-MS, 94
  5.5.2 Bottom–Up Analysis by ESI-MS (LC/MS), 94
  5.5.3 Bottom–Up and Top–Down Analysis by ESI-FTICR-MS, 95
5.6 Identification of Cross-Linked Products, 97
5.7 Computer Software for Data Analysis, 99
5.8 Conclusions and Perspectives, 99

Abbreviations, 100
Acknowledgments, 100
References, 101

6 Genesis and Application of Radical Probe Mass Spectrometry (RP-MS) to Study Protein Interactions

Simin D. Maleknia and Kevin M. Downard

6.1 Genesis of Radical Probe Mass Spectrometry, 110
6.2 The Reactive Residue Side Chains, 111
6.3 Conditions Important to Radical Probe Mass Spectrometry Experiments, 115
6.4 Generation of Radicals on Millisecond Timescales, 117
6.5 Applications of RP-MS to Studies of Protein Interactions, 119
   6.5.1 Intramolecular Interactions, 120
   6.5.2 Intermolecular Interactions: Protein–Peptide and
        Protein–Protein Complexes, 122
6.6 Onset of Oxidative Damage and Its Implications for Protein
    Interactions, 126
6.7 Application of Radical Oxidation to Study Protein
    Assemblies, 128
6.8 Modeling Protein Complexes with Data from
    RP-MS Experiments, 129
6.9 Conclusions, 130
References, 131

Index