

CONTENTS

Contributors

xi

Preface

xv

Section I

Nuclear Magnetic Resonance Spectroscopy

1. Application of Natural Isotopic Abundance ^1H - ^{13}C - and ^1H - ^{15}N -Correlated Two-Dimensional NMR for Evaluation of the Structure of Protein Therapeutics 3

Luke W. Arbogast, Robert G. Brinson, and John P. Marino

- | | |
|--|----|
| 1. Introduction | 4 |
| 2. Preparation of Protein Therapeutic Samples | 6 |
| 3. Acquisition of 2D Spectra | 9 |
| 4. Processing 2D-Correlated Spectra | 20 |
| 5. Comparative Analysis of 2D-Correlated Spectra | 23 |
| 6. Perspectives | 31 |
| Acknowledgments | 31 |
| References | 32 |

2. A Semiautomated Assignment Protocol for Methyl Group Side Chains in Large Proteins 35

Jonggul Kim, Yingjie Wang, Geoffrey Li, and Gianluigi Veglia

- | | |
|---|----|
| 1. Introduction | 36 |
| 2. Labeling of Side Chain Methyl Groups for Large Proteins | 37 |
| 3. Methyl Labeling Protocol for the cAMP-Dependent Protein Kinase A | 39 |
| 4. Semiautomated Methyl-Group Resonance Assignment Strategies | 44 |
| 5. Semiautomated Assignment Protocol Using FLAMEnGO 2.0 | 46 |
| 6. Conclusions and Perspectives | 54 |
| Acknowledgments | 54 |
| References | 54 |

3. ^{19}F -Site-Specific-Labeled Nucleotides for Nucleic Acid Structural Analysis by NMR 59

Lincoln G. Scott and Mirko Hennig

- | | |
|--|----|
| 1. Introduction | 60 |
| 2. General Enzymatic Synthesis of 2F-ATP, 5F-CTP, and 5F-UTP | 61 |

3. Stability of Modified RNA	64
4. Diagnostics of ^{19}F -Chemical Shifts	66
5. NMR-Assignment Strategies for Fluoro-Modified RNA	68
6. Coupling Measurements in Fluoro-Modified RNA	74
7. Solvent-Induced Isotope Shifts	77
8. Ligand-Induced Chemical Shift Changes	79
9. Conclusion and Remarks	82
Acknowledgments	83
References	83

4. Applying Thymine Isostere 2,4-Difluoro-5-Methylbenzene as a NMR Assignment Tool and Probe of Homopyrimidine/Homopurine Tract Structural Dynamics 89

Robert G. Brinson, Jennifer T. Miller, Jason D. Kahn,

Stuart F.J. Le Grice, and John P. Marino

1. Introduction	90
2. Preparation of dF-Substituted Oligonucleotides for NMR Analysis	93
3. NMR Methods	95
4. dF as an NMR Assignment Tool	96
5. Structural and Dynamic Perturbations Observed by dF Substitution	102
6. Conclusions	108
Acknowledgments	109
References	109

Section II

Scattering Techniques

5. Biomolecular Deuteration for Neutron Structural Biology and Dynamics 113

Michael Haertlein, Martine Moulin, Juliette M. Devos, Valerie Laux,

Orla Dunne, and V. Trevor Forsyth

1. The Motivation for Macromolecular Deuteration Approaches	114
2. Concepts of <i>In Vivo</i> Deuteration	124
3. Levels of Specificity in Deuterium Labeling	140
4. Conclusion and Perspective	146
Acknowledgments	148
References	149

6. Deuterium Labeling Together with Contrast Variation Small-Angle Neutron Scattering Suggests How Skp Captures and Releases Unfolded Outer Membrane Proteins 159

Nathan R. Zaccai, Clifford W. Sandlin, James T. Hoopes, Joseph E. Curtis,
Patrick J. Fleming, Karen G. Fleming, and Susan Krueger

1. Introduction	160
2. SANS from Biological Molecules in Solution	163
3. Materials and Methods	171
4. Results	179
5. Discussion	201
6. Conclusions	204
Acknowledgments	205
References	205

7. Deuteration in Biological Neutron Reflectometry 211

Frank Heinrich

1. Introduction	212
2. Experimental Methods	216
3. Data Analysis	220
4. Conclusion	226
5. Acknowledgments	226
References	227

8. Deuterium Labeling Strategies for Creating Contrast in Structure-Function Studies of Model Bacterial Outer Membranes Using Neutron Reflectometry 231

Anton P. Le Brun, Luke A. Clifton, Stephen A. Holt, Peter J. Holden,
and Jeremy H. Lakey

1. Introduction	232
2. Brief Introduction to Neutron Reflectometry and Data Analysis	234
3. Isolation of Deuterated LPS from Rough Strains of <i>Escherichia coli</i>	239
4. Formation of Rough LPS Monolayers at the Air-Liquid Interface	242
5. Modeling Monolayers of Rc-LPS from Neutron Reflectometry Data	244
6. Forming Asymmetric Bilayers Using Phospholipids and Rough LPS	246
7. Final Remarks	249
Acknowledgments	250
References	250

9. Essential Strategies for Revealing Nanoscale Protein Dynamics by Neutron Spin Echo Spectroscopy **253**

David J.E. Callaway and Zimei Bu

1. Introduction 254
2. Essentials for Determining Nanoscale Protein Internal Motion 255
3. Preparation of Partially Deuterated Protein Samples for NSE Experiments 265
4. Summary 268
- References 268

Section III

Mass Spectrometry

10. Method for the Determination of ^{15}N Incorporation Percentage in Labeled Peptides and Proteins **273**

Eric L. Kilpatrick

1. Introduction 274
2. Materials 275
3. Background 275
4. Trypsin Digestion 276
5. Mass Spectrometry 279
6. Program Description 281
7. Results 285
8. Conclusions 287
- References 288

11. QconCAT: Internal Standard for Protein Quantification **289**

Kerry Bauer Scott, Illarion V. Turko, and Karen W. Phinney

1. Introduction 290
2. QconCAT Design 292
3. Expression and Purification of QconCAT 294
4. Characterization 297
5. Application of QconCAT 300
- References 301

12. Production, Purification, and Characterization of ^{15}N -Labeled DNA Repair Proteins as Internal Standards for Mass Spectrometric Measurements **305**

Prasad T. Reddy, Pawel Jaruga, Bryant C. Nelson, Mark S. Lowenthal, Ann-Sofie Jemth, Olga Loseva, Erdem Coskun, Thomas Helleday, and Miral Dizdaroglu

1. Introduction 306

2. Materials and Methods	310
3. Production and Purification of <i>E. coli</i> ^{15}N -Fpg Protein	311
4. Production and Purification of ^{15}N -hOGG1	314
5. Production and Purification of ^{15}N -hNEIL1	315
6. Production and Purification of ^{15}N -hAPE1	317
7. Production and Purification of ^{15}N -hMTH1	318
8. Mass Spectrometric Analysis of ^{15}N -Labeled Proteins	320
9. Applications to the Measurement of DNA Repair Proteins	321
10. Conclusions	326
Acknowledgments	326
References	327

Section IV

Hydrogen-Deuterium Exchange Mass Spectrometry

13. Hydrogen Exchange Mass Spectrometry	335
Leland Mayne	
1. Introduction	336
2. HX Mechanism	336
3. Experimental Considerations—Exchange Labeling	338
4. Experimental Considerations—MS Measurement	345
5. Mass Spectrometer Instrument Considerations	347
6. Data Analysis	348
7. Conclusion	354
References	355
14. Mapping Protein–Ligand Interactions with Proteolytic Fragmentation, Hydrogen/Deuterium Exchange-Mass Spectrometry	357
Elyssia S. Gallagher and Jeffrey W. Hudgens	
1. Introduction	358
2. H/D Exchange Theory	359
3. The HDX-MS Experiment	368
4. Interpreting HDX-MS Data to Determine Protein–Ligand Interaction Maps	388
Disclaimer	395
References	397
15. Isotope Labeling of Biomolecules: Structural Analysis of Viruses by HDX-MS	405
Miklos Guttman and Kelly K. Lee	
1. Introduction	406
2. Methods	411

3. Example HDX-MS Experiment with Bovine Ribonuclease A	419
Acknowledgments	421
References	421

Author Index	427
Subject Index	453