5499

VOLUME SIX HUNDRED AND FIFTEEN

METHODS IN ENZYMOLOGY

Biological NMR Part B

Edited by

A. JOSHUA WAND

Johnson Research Foundation and the Department of Biochemistry & Biophysics;
Graduate Group in Biochemistry & Molecular Biophysics, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, United States

ACADEMIC PRESS
An imprint of Elsevier

CONTENTS

Contributors *xi*
Preface *xv*

1. Companion Simulations and Modeling to NMR-Based Dynamical Studies of Proteins **1**

Kim A. Sharp

1. Introduction 2
2. The Generalized NMR Order Parameter 4
3. Conformational Entropy and Protein Dynamics 20
4. *J*-couplings 28
5. Residual Dipolar Couplings 30
6. Protein Compressibility 31
7. Molecular Tumbling 33
8. Water Dynamics 36

Acknowledgment 39
References 39

2. Reverse Micelle Encapsulation of Proteins for NMR Spectroscopy **43**

Brian Fuglestad, Bryan S. Marques, Christine Jorge, Nicole E. Kerstetter, Kathleen G. Valentine, and A. Joshua Wand

1. Introduction 44
2. Sample Composition Considerations 50
3. Spectroscopic Considerations 55
4. Method for Screening RM Conditions 56
5. Method for Preparation of RM Solutions in Propane or Ethane 64
6. Benchmarking Encapsulation 70
7. Conclusions and Outlook 71

Acknowledgments 72
References 72

3. Characterizing Protein Hydration Dynamics Using Solution NMR Spectroscopy **77**

Christine Jorge, Bryan S. Marques, Kathleen G. Valentine, and A. Joshua Wand

1. Introduction 78
2. Theoretical and Practical Considerations 79

3. Preparation of Protein Encapsulated RM Samples 85
4. NMR Spectroscopy and Experimental Setup 88
5. Data Collection and Analysis 94
6. Conclusions 98
Acknowledgments 98
References 99

4. Understanding Protein Function Through an Ensemble Description: Characterization of Functional States by ^{19}F NMR 103

Christopher Di Pietrantonio, Aditya Pandey, Jerome Gould, Advait Hasabnis, and Robert Scott Prosser

1. ^{19}F-Reporters That Can Be Biosynthetically Incorporated Into Proteins 105
2. Approaches to Chemical Tagging of Proteins by ^{19}F Reporters 110
3. Improving Delineation of States by ^{19}F NMR 113
4. Distinguishing States by Topology Measurements That Focus on Solvent Exposure and Hydrophobicity 118
5. Relaxation Experiments and Simple Approaches to Delineating States in Fast and Slow Exchange 120
6. Extending Resolution of States by ^{19}F NMR 121
7. Validating ^{19}F NMR Spectroscopy by Computational Methods 123
8. Overview 124
Acknowledgments 124
References 125

5. Overhauser Dynamic Nuclear Polarization for the Study of Hydration Dynamics, Explained 131

John M. Franck and Songi Han

1. Introduction 132
2. Motivation for Studying Hydration Water Dynamics 134
3. Experimental Techniques for Studying Hydration Water Dynamics 136
4. Translational Diffusion Dynamics of Hydration Water Informs on Correlated Properties 140
5. Principle and Benefits of ODNP Relaxometry 141
6. Mechanism of ODNP Relaxometry 147
7. Experimental Protocol for ODNP Measurements 156
8. Common Questions for ODNP Hydration Studies 162
9. Development of the ODNP Technique 164
10. Concluding Remarks 168
Acknowledgments 169
References 169

6. Chemical Exchange **177**

Arthur G. Palmer III and Hans Koss

1. Introduction 178
2. Theory 183
3. Experimental Techniques and Examples 199
4. Conclusion 220

Acknowledgments 221
Appendix A 221
References 227

7. Characterization of Internal Protein Dynamics and Conformational Entropy by NMR Relaxation **237**

Matthew A. Stetz, José A. Caro, Sravya Kotaru, Xuejun Yao, Bryan S. Marques, Kathleen G. Valentine, and A. Joshua Wand

1. Introduction 238
2. NMR Spin Relaxation Methods 239
3. Practical Aspects of Data Collection and Analysis 252
4. The Entropy Meter 271
5. Concluding Remarks 276

Acknowledgments 277
References 277

8. NMR Methods for Characterizing the Basic Side Chains of Proteins: Electrostatic Interactions, Hydrogen Bonds, and Conformational Dynamics **285**

Dan Nguyen, Chuanying Chen, B. Montgomery Pettitt, and Junji Iwahara

1. Introduction 286
2. NMR of Lys and Arg Side Chains 287
3. Conformational Dynamics of Lys/Arg Side Chains 300
4. Ion Pairs and Hydrogen Bonds Involving Lys/Arg Side Chains 312
5. Data Interpretation Facilitated by Molecular Dynamics Simulations 317
6. Concluding Remarks 325

Acknowledgments 325
References 325

9. Solid-State NMR Spectroscopy of RNA **333**

Alexander Marchanka and Teresa Carlomagno

1. Introduction 334
2. Sample Preparation 336

3. Resonance Assignment 339
4. Restraints and Structural Calculations 352
5. Characterization of Protein–RNA Interfaces 362
6. Outlook 365
References 366

10. DNP-Assisted NMR Investigation of Proteins at Endogenous Levels in Cellular Milieu 373

Whitney N. Costello, Yiling Xiao, and Kendra K. Frederick

1. Introduction 374
2. DNP Samples of Proteins at Endogenous Levels in Native Environments 376
3. Purified Amyloid Samples 390
4. Summary and Conclusions 401
Acknowledgments 403
References 403

11. Identification of Unknown Metabolomics Mixture Compounds by Combining NMR, MS, and Cheminformatics 407

Abigail Leggett, Cheng Wang, Da-Wei Li, Arpad Somogyi, Lei Bruschweiler-Li, and Rafael Brüschweiler

1. Introduction 408
2. Sample Preparation 410
3. NMR Experiments 411
4. Analysis of NMR Spectra 412
5. MS Experiments 414
6. Analysis of MS Spectra 416
7. NMR Chemical Shift Prediction of Library Compounds 416
8. Spin System Scoring and Molecular Structural Motif Identification of Chemical Compounds 417
9. Compound Verification: Spiking With Purchased or Synthesized Candidate Compounds 418
10. Conclusions and Outlook 420
Acknowledgments 420
References 421

12. STD NMR as a Technique for Ligand Screening and Structural Studies 423

Samuel Walpole, Serena Monaco, Ridvan Nepravishta, and Jesus Angulo

1. Introduction 424
2. The STD NMR Experiment 426

3. Ligand Screening 436
4. Ligand Epitope Mapping 436
5. Determination of the Dissociation Constant (K_D) 440
6. DEEP-STD NMR 444
7. Summary and Conclusions 449
References 450

13. An ELISA-Based Screening Platform for Ligand–Receptor Discovery 453

Sinem Ozgul, Sventja von Daake, Sumie Kakehi, Davide Sereni, Natalia Denissova, Carlie Hanlon, Yuanpeng Janet Huang, John K. Everett, Cuifeng Yin, Gaetano T. Montelione, and Davide Comoletti

1. Introduction 454
2. Overview of Workflow 456
3. The High-Throughput Screen 458
4. Data Analysis and Laboratory Information Management 466
5. Validation of PPIs and Biophysical Characterization 467
6. PPI ELISA Results 470
7. Comparison With Affinity Chromatography Methods 470
8. Future Prospects 471
Acknowledgments 472
References 472

14. Protein–Small Molecule Interactions by WaterLOGSY 477

Renjie Huang and Ivanhoe K.H. Leung

1. Introduction 478
2. The Basis of WaterLOGSY 479
3. Pulse Sequence of WaterLOGSY 483
4. Basic Setup for Conventional WaterLOGSY Experiments 487
5. Screening by WaterLOGSY 490
6. Binding Constant (K_D) Determination by WaterLOGSY 492
7. The Use of WaterLOGSY to Measure Bound-Ligand Solvent Accessibility 494
8. Conclusion 496
Acknowledgments 496
References 496

15. Applications of Dissolution-DNP for NMR Screening 501

Yaewon Kim and Christian Hilty

1. Introduction 502
2. Methods 504

3. Conclusions 523
Acknowledgment 523
References 523