



STRUCTURAL BIOLOGY

*Practical NMR
Applications*

QUINCY TENG

Contents

PREFACE	xi
CHAPTER 1. BASIC PRINCIPLES OF NMR	1
1.1. Introduction	1
1.2. Nuclear Spin in a Static Magnetic Field	1
1.2.1. Precession of Nuclear Spins in a Magnetic Field	1
1.2.2. Energy States and Population	3
1.2.3. Bulk Magnetization	5
1.3. Rotating Frame	6
1.4. Bloch Equations	9
1.5. Fourier Transformation and Its Applications in NMR	12
1.5.1. Fourier Transformation and Its Properties Useful for NMR	12
1.5.2. Excitation Bandwidth	14
1.5.3. Quadrature Detection	15
1.6. Nyquist Theorem and Digital Filters	16
1.7. Chemical Shift	17
1.8. Nuclear Coupling	23
1.8.1. Scalar Coupling	23
1.8.2. Spin Systems	26
1.8.3. Dipolar Interaction	27
1.8.4. Residual Dipolar Coupling	28
1.9. Nuclear Overhauser Effect	32
1.10. Relaxation	35
1.10.1. Correlation Time and Spectral Density Function	36
1.10.2. Spin-Lattice Relaxation	36
1.10.3. T_2 Relaxation	39
1.11. Selection of Coherence Transfer Pathways	42
1.12. Approaches to Understanding NMR Experiments	42
1.12.1. Vector Model	43
1.12.2. Product Operator Description of Building Blocks in a Pulse Sequence	44
1.12.2.1. Spin-Echo of Uncoupled Spins	44
1.12.2.2. Spin-Echo of Coupled Spins	45
1.12.2.3. Insensitive Nuclei Enhanced by Polarization Transfer	46
1.12.3. Introduction to Density Matrix	47

Questions	51
Appendix A: Product Operators	52
A1. Uncoupled Spins	52
A2. Two Coupled Spins	54
References	54
CHAPTER 2. INSTRUMENTATION	57
2.1. System Overview	57
2.2. Magnet	57
2.3. Transmitter	60
2.4. Receiver	65
2.5. Probe	68
2.6. Quarter-wavelength Cable	75
2.7. Analog/Digital Converters	76
2.8. Instrument specifications	79
2.9. Test or Measurement Equipment	81
2.9.1. Reflection Bridge	81
2.9.2. Oscilloscope	81
2.9.3. Spectrum Analyzer	84
2.9.4. System Noise Measurement	85
Questions	87
References	88
CHAPTER 3. NMR SAMPLE PREPARATION	89
3.1. Introduction	89
3.2. Expression Systems	90
3.2.1. <i>Escherichia coli</i> Expression Systems	90
3.2.2. Fusion Proteins in the Expression Vectors	91
3.2.3. Optimization of Protein Expression	91
3.3. Overexpression of Isotope-Labeled Proteins	92
3.4. Purification of Isotope-Labeled Proteins	93
3.5. NMR Sample Preparation	94
3.5.1. General Considerations	94
3.5.2. Preparation of Protein–Peptide Complexes	94
3.5.3. Preparation of Protein–Protein Complexes	95
3.5.4. Preparation of Alignment Media for Residual Dipolar Coupling Measurement	96
3.6. Examples of Protocols for Preparing $^{15}\text{N}/^{13}\text{C}$ Labeled Proteins	98
3.6.1. Example 1: Sample Preparation of an LIM Domain Using Protease Cleavage	98
3.6.1.1. Background	98
3.6.1.2. Protein Expression	98
3.6.1.3. Protein Purification and Sample Preparation	98
3.6.2. Example 2: Sample Preparation Using a Denaturation–Renaturation Method	98
3.6.2.1. Background	98

3.6.2.2. Protein Expression	98
3.6.2.3. Protein Purification	99
Questions	99
References	99
CHAPTER 4. PRACTICAL ASPECTS	101
4.1. Tuning the Probe	101
4.2. Shimming and Locking	104
4.3. Instrument Calibrations	106
4.3.1. Calibration of Variable Temperature	106
4.3.2. Calibration of Chemical Shift References	106
4.3.3. Calibration of Transmitter Pulse Length	109
4.3.4. Calibration of Offset Frequencies	110
4.3.4.1. Calibration of Transmitter Offset Frequency	111
4.3.4.2. Calibration of Decoupler Offset Frequency	111
4.3.5. Calibration of Decoupler Pulse Length	112
4.3.6. Calibration of Decoupler Pulse Length with Off-Resonance Null	113
4.4. Selective Excitation with Narrow Band and Off-Resonance Shape Pulses	115
4.5. Composite Pulses	117
4.5.1. Composite Excitation Pulses	117
4.5.2. Composite Pulses for Isotropic Mixing	117
4.5.3. Composite Pulses for Spin Decoupling	118
4.6. Adiabatic Pulses	119
4.7. Pulsed Field Gradients	121
4.8. Solvent Suppression	125
4.8.1. Presaturation	125
4.8.2. Watergate	127
4.8.3. Water-Flip-Back	127
4.8.4. Jump-Return	128
4.9. NMR Data Processing	129
4.9.1. Drift Correction	129
4.9.2. Solvent Suppression Filter	129
4.9.3. Linear Prediction	129
4.9.4. Apodization	130
4.9.5. Zero Filling	132
4.9.6. Phase Correction	133
4.10. Two-Dimensional Experiments	136
4.10.1. The Second Dimension	136
4.10.2. Quadrature Detection in the Indirect Dimension	137
4.10.3. Selection of Coherence Transfer Pathways	138
4.10.4. COSY	140
4.10.5. DQF COSY	141
4.10.6. TOCSY	142
4.10.7. NOESY and ROESY	144
Questions	146
References	147

CHAPTER 5. MULTIDIMENSIONAL HETERONUCLEAR NMR EXPERIMENTS	149
5.1. Two-Dimensional Heteronuclear Experiments	149
5.1.1. HSQC and HMQC	150
5.1.2. HSQC Experiment Setup	152
5.1.3. Sensitivity Enhanced HSQC by PEP	152
5.1.4. Setup of an seHSQC Experiment	154
5.1.5. HMQC	154
5.1.6. IPAP HSQC	156
5.1.7. SQ-TROSY	158
5.2. Overview of Triple-Resonance Experiments	160
5.3. General Procedure of Setup and Data Processing for 3D Experiments	162
5.4. Experiments for Backbone Assignments	163
5.4.1. HNCO and HNCA	164
5.4.1.1. Product Operator Description of the HNCO Experiment	166
5.4.1.2. HNCO Experiment Setup	168
5.4.1.3. HNCA	169
5.4.2. HN(CO)CA	169
5.4.3. HN(CA)CO	171
5.4.4. CBCANH	173
5.4.5. CBCA(CO)NH	176
5.5. Experiments for Side-Chain Assignment	179
5.5.1. HCCH-TOCSY	179
5.6. 3D Isotope-Edited Experiments	184
5.6.1. ^{15}N -HSQC-NOESY	184
5.7. Sequence-Specific Resonance Assignments of Proteins	185
5.7.1. Assignments Using ^{15}N Labeled Proteins	185
5.7.2. Sequence-Specific Assignment Using Doubly Labeled Proteins	186
5.8. Assignment of NOE Cross-Peaks	187
Questions	187
References	188
CHAPTER 6. STUDIES OF SMALL BIOLOGICAL MOLECULES	191
6.1. Ligand-Protein Complexes	191
6.1.1. SAR-by-NMR Method	191
6.1.2. Diffusion Method	195
6.1.3. Transferred NOE	196
6.1.4. Saturation Transfer Difference	198
6.1.5. Isotope-Editing Spectroscopy	200
6.1.6. Isotope-Filtering Spectroscopy	202
6.2. Study of Metabolic Pathways by NMR	204
Questions	208
References	209

CHAPTER 7. PROTEIN STRUCTURE DETERMINATION FROM NMR DATA	211
7.1. Introduction and Historical Overview	211
7.2. NMR Structure Calculation Methods	213
7.2.1. Distance Geometry	214
7.2.2. Restrained Molecular Dynamics	214
7.3. NMR Parameters for Structure Calculation	216
7.3.1. Chemical Shifts	216
7.3.2. <i>J</i> Coupling Constants	217
7.3.3. Nuclear Overhauser Effect (NOE)	218
7.3.4. Residual Dipolar Couplings	220
7.4. Preliminary Secondary Structural Analysis	221
7.5. Tertiary Structure Determination	222
7.5.1. Computational Strategies	222
7.5.2. Illustration of Step-by-Step Structure Calculations Using a Typical XPLOR Protocol	222
7.5.2.1. Preparation of Input Files	222
7.5.2.2. Preparation of Initial Random-Coil Coordinates and Geometric File	224
7.5.2.3. Randomization	224
7.5.2.4. First-Round Structure Calculation—Global Folding	224
7.5.2.5. NOE Violations and Removal of Incorrect Distance Restraints	224
7.5.2.6. Iterative Steps for NOE Analysis and Structure Calculations	225
7.5.3. Criteria of Structural Quality	226
7.5.4. Second-Round Structure Calculation—Structure Refinement	226
7.5.5. Presentation of the NMR Structure	226
7.5.6. Precision of NMR Structures	227
7.5.7. Accuracy of NMR Structures	227
7.6. Protein Complexes	228
7.6.1. Protein–Protein Complexes	228
7.6.2. Protein–Peptide Complexes	228
Questions	229
Appendix B1. Sa.inp—XPLOR Protocol for Protein Structure Calculation	229
Appendix B2. Example of NOE Table	234
Appendix B3. Example of Dihedral Angle Restraint Table	236
Appendix B4. Example of Chemical Shift Table for TALOS	238
Appendix B5. Example of Hydrogen Bond Table	240
Appendix B6. Example of Input File to Generate a Random-Coil Coordinates	240
Appendix B7. Example of Input File to Generate a Geometric PSF File	241
References	241
CHAPTER 8. PROTEIN DYNAMICS	245
8.1. Theory of Spin Relaxation in Proteins	245

8.2.	Experiments for Measurements of Relaxation Parameters	253
8.2.1.	T_1 Measurement	253
8.2.1.1.	Water-Flip-Back Sensitivity-Enhanced T_1 HSQC	253
8.2.1.2.	Experiment Setup and Data Processing	255
8.2.2.	T_2 and $T_{1\rho}$ Measurements	256
8.2.2.1.	Sensitivity-Enhanced HSQC for T_2 and $T_{1\rho}$ Measurements .	256
8.2.2.2.	Experiment Setup and Data Processing	258
8.2.3.	Heteronuclear NOE Measurement	258
8.2.3.1.	Heteronuclear NOE Experiment	258
8.2.3.2.	Experiment Setup and Data Processing	259
8.3.	Relaxation Data Analysis	260
Questions		261
References		261
MULTIPLE CHOICE QUESTIONS		263
ANSWERS TO MULTIPLE CHOICE QUESTIONS		279
NOMENCLATURE AND SYMBOLS		281
INDEX		285