Contents

List of Figures xvii
List of Tables xxvi

1. NMR SPECTROSCOPY 1
   1.1 Introduction to NMR Spectroscopy 2
   1.2 One Dimensional NMR Spectroscopy 3
      1.2.1 Classical Description of NMR Spectroscopy 3
      1.2.2 Nuclear Spin Transitions 3
   1.3 Detection of Nuclear Spin Transitions 7
      1.3.1 Continuous Wave NMR 7
      1.3.2 Pulsed NMR 8
      1.3.3 Summary of the Process of Acquiring a One Dimensional Spectrum 15
   1.4 Phenomenological Description of Relaxation 16
      1.4.1 Relaxation and the Evolution of Magnetization 18
   1.5 Chemical Shielding 19
   1.6 Characteristic ^{1}H, ^{13}C and ^{15}N Chemical Shifts 21
      1.6.1 Effect of Electronic Structure on Chemical Shifts 21
      1.6.2 Ring Current Effects 23
      1.6.3 Effects of Local Environment on Chemical Shifts 25
      1.6.4 Use of Chemical Shifts in Resonance Assignments 25
      1.6.5 Chemical Shift Dispersion & Multi-dimensional NMR 26
   1.7 Exercises 26
   1.8 Solutions 26

2. PRACTICAL ASPECTS OF ACQUIRING NMR SPECTRA 29
   2.1 Components of an NMR Spectrometer 29
      2.1.1 Magnet 29

2.1.2 Computer 31
2.1.3 Probe 31
2.1.4 Pre-amplifier Module 32
2.1.5 The Field-frequency Lock 33
2.1.6 Shim System 34
2.1.7 Transmitter & Pulse Generation 34
2.1.8 Receiver 36
2.2 Acquiring a Spectrum 38
2.2.1 Sample Preparation 38
2.2.2 Beginning the Experiment 39
2.2.3 Temperature Measurement 39
2.2.4 Shimming 40
2.2.5 Tuning and Matching the Probe 41
2.2.6 Adjusting the Transmitter 42
2.2.7 Calibration of the 90° Pulse Length 46
2.2.8 Setting the Sweepwidth: Dwell Times and Filters 48
2.2.9 Setting the Receiver Gain 53
2.2.10 Spectral Resolution and Acquisition Time of the FID 54
2.3 Experimental 1D-pulse Sequence: Pulse and Receiver Phase 57
2.3.1 Phase Cycle 58
2.3.2 Phase Cycle and Artifact Suppression 61
2.4 Exercises 63
2.5 Solutions 64

3. INTRODUCTION TO SIGNAL PROCESSING 65
3.1 Removal of DC Offset 66
3.2 Increasing Resolution by Extending the FID 66
3.2.1 Increasing Resolution by Zero-filling 67
3.2.2 Increasing Resolution by Linear Prediction (LP) 69
3.3 Removal of Truncation Artifacts: Apodization 74
3.3.1 Effect of Apodization on Resolution and Noise 74
3.3.2 Using LP & Apodization to Increase Resolution 77
3.4 Solvent Suppression 78
3.5 Spectral Artifacts Due to Intensity Errors 79
3.5.1 Errors from the Digital Fourier Transform 79
3.5.2 Effect of Distorted and Missing Points 80
3.5.3 Delayed Acquisition 82
3.6 Phasing of the Spectrum 82

3.6.1 Origin of Phase Shifts 83
3.6.2 Applying Phase Corrections 85
3.7 Chemical Shift Referencing 86
3.8 Exercises 87
3.9 Solutions 87

4. QUANTUM MECHANICAL DESCRIPTION OF NMR 89
4.1 Schrödinger Equation 89
4.1.1 Vector Spaces and Properties of Wavefunctions 90
4.1.2 Particle in a Box 92
4.2 Expectation Values 93
4.3 Dirac Notation 94
4.3.1 Wavefunctions in Dirac Notation 94
4.3.2 Scalar Product in Dirac Notation 96
4.3.3 Operators in Dirac Notation 96
4.3.4 Expectation Values in Dirac Notation 96
4.4 Hermitian Operators 97
4.4.1 Determining Eigenvalues 97
4.5 Additional Properties of Operators 100
4.5.1 Commuting Observables 100
4.5.2 Time Evolution of Observables 100
4.5.3 Trace of an Operator 100
4.5.4 Exponential Operator 101
4.5.5 Unitary Operators 101
4.5.6 Exponential Hermitian Operators 101
4.6 Hamiltonian and Angular Momentum Operators for a Spin-1/2 Particle 102
4.7 Rotations 105
4.7.1 Rotation Groups 105
4.7.2 Rotation Operators 106
4.7.3 Rotations of Wave Functions and Operators 109
4.8 Exercises 112
4.9 Solutions 112

5. QUANTUM MECHANICAL DESCRIPTION OF A ONE PULSE EXPERIMENT 113
5.1 Preparation: Evolution of the System Under B_o 114
5.2 Excitation: Effect of Application of B_1 116
5.2.1 The Resonance Condition 118

5.3 Detection: Evolution of the System Under B_o 120

6. THE DENSITY MATRIX & PRODUCT OPERATORS 121
6.1 Introduction to the Density Matrix 122
6.1.1 Calculation of Expectation Values From ρ 123
6.1.2 Density Matrix for a Statistical Mixture 123
6.2 One-pulse Experiment: Density Matrix Description 126
6.2.1 Effect of Pulses on the Density matrix 127
6.3 Product Operators 129
6.3.1 Transformation Properties of Product Operators 130
6.3.2 Description of the One-pulse Experiment 131
6.3.3 Evaluation of Composite Pulses 132
6.4 Exercises 133
6.5 Solutions 133

7. SCALAR COUPLING 135
7.1 Introduction to Scalar Coupling 135
7.2 Basis of Scalar Coupling 136
7.2.1 Coupling to Multiple Spins 138
7.3 Quantum Mechanical Description 140
7.3.1 Analysis of an AX System 140
7.3.2 Analysis of an AB System 142
7.4 Decoupling 145
7.4.1 Experimental Implementation of Decoupling 145
7.4.2 Decoupling Methods 146
7.4.3 Performance of Decoupling Schemes 148
7.5 Exercises 150
7.6 Solutions 150

8. COUPLED SPINS: DENSITY MATRIX AND PRODUCT OPERATOR FORMALISM 153
8.1 Density Matrix for Two Coupled Spins 153
8.2 Product Operator Representation of the Density Matrix 155
8.2.1 Detectable Elements of ρ 156
8.3 Density Matrix Treatment of a One-pulse Experiment 159
8.4 Manipulation of Two-spin Product Operators 162
8.5 Transformations of Two-spin Product Operators 164
8.6 Product Operator Treatment of a One-pulse Experiment 165

9. TWO DIMENSIONAL HOMONUCLEAR J-CORRELATED SPECTROSCOPY 169
9.1 Multi-dimensional Experiments 170
9.1.1 Elements of Multi-dimensional NMR Experiments 171
9.1.2 Generation of Multi-dimensional NMR Spectra 172
9.2 Homonuclear J-correlated Spectra 173
9.2.1 COSY Experiment 173
9.3 Double Quantum Filtered COSY (DQF-COSY) 182
9.3.1 Product Operator Treatment of the DQF-COSY Experiment 182
9.4 Effect of Passive Coupling on COSY Crosspeaks 185
9.5 Scalar Correlation by Isotropic Mixing: TOCSY 187
9.5.1 Analysis of TOCSY Pulse Sequence 188
9.5.2 Isotropic Mixing Schemes 191
9.5.3 Time Dependence of Magnetization Transfer by Isotropic Mixing 192
9.6 Exercises 194
9.7 Solutions 195

10. TWO DIMENSIONAL HETERONUCLEAR J-CORRELATED SPECTROSCOPY 197
10.1 Introduction 197
10.2 Two Dimensional Heteronuclear NMR Experiments 198
10.2.1 HMQC Experiment 199
10.2.2 HSQC Experiment 204
10.2.3 Refocused-HSQC Experiment 207
10.2.4 Comparison of HMQC, HSQC, and Refocused-HSQC Experiments 209
10.2.5 Sensitivity in 2D-Heteronuclear Experiments 209
10.2.6 Behavior of XH_2 Systems in HSQC-type Experiments 210

11. COHERENCE EDITING: PULSED-FIELD GRADIENTS AND PHASE CYCLING 213
11.1 Principals of Coherence Selection 214
11.1.1 Spherical Basis Set 214
11.1.2 Coherence Changes in NMR Experiments 216
11.1.3 Coherence Pathways 218
11.2 Phase Encoding With Pulsed-Field Gradients 218
11.2.1 Gradient Coils 218

11.2.2 Effect of Coherence Levels on Gradient Induced Phase Changes 220
11.2.3 Coherence Selection by Gradients in Heteronuclear NMR Experiments 222
11.3 Coherence Selection Using Phase Cycling 225
11.3.1 Coherence Changes Induced by RF-Pulses 226
11.3.2 Selection of Coherence Pathways 229
11.3.3 Phase Cycling in the HMQC Pulse Sequence 233
11.4 Exercises 235
11.5 Solutions 235

12. QUADRATURE DETECTION IN MULTI-DIMENSIONAL NMR SPECTROSCOPY 239
12.1 Quadrature Detection Using TPPI 240
12.2 Hypercomplex Method of Quadrature Detection 242
12.2.1 States-TPPI - Removal of Axial Peaks 243
12.3 Sensitivity Enhancement 245
12.4 Echo-AntiEcho Quadrature Detection: N-P Selection 247
12.4.1 Absorption Mode Lineshapes with N-P Selection 247

13. RESONANCE ASSIGNMENTS: HOMONUCLEAR METHODS 251
13.1 Overview of the Assignment Process 251
13.2 Homonuclear Methods of Assignment 254
13.3 ^{15}N Separated Homonuclear Techniques 256
13.3.1 2D ^{15}N HSQC Experiment 259
13.3.2 3D ^{15}N Separated TOCSY Experiment 259
13.3.3 The HNHA Experiment - Identifying H_α Protons 262
13.3.4 The HNHB Experiment- Identifying H_β Protons 265
13.3.5 Establishing Spin-system Connectivities with Dipolar Coupling 267
13.4 Exercises 272
13.5 Solutions 273

14. RESONANCE ASSIGNMENTS: HETERONUCLEAR METHODS 277
14.1 Mainchain Assignments 278
14.1.1 Strategy 278
14.1.2 Methods for Mainchain Assignments 279
14.2 Description of Triple-resonance Experiments 282
14.2.1 HNCO Experiment 282
14.2.2 HNCA Experiment 290
14.3 Selective Excitation and Decoupling of ^{13}C 294

14.3.1 Selective 90° Pulses 294
14.3.2 Selective 180° Pulses 297
14.3.3 Selective Decoupling: SEDUCE 298
14.3.4 Frequency Shifted Pulses 299
14.4 Sidechain Assignments 300
14.4.1 Triple-resonance Methods for Sidechain Assignments 301
14.4.2 The HCCH Experiment 302
14.5 Exercises 308
14.6 Solutions 310

15. PRACTICAL ASPECTS OF N-DIMENSIONAL DATA ACQUISITION AND PROCESSING 313
15.1 Sample Preparation 313
15.1.1 NMR Sample Tubes 313
15.1.2 Sample Requirements 313
15.2 Solvent Considerations - Water Suppression 315
15.2.1 Amide Exchange Rates 315
15.2.2 Solvent Suppression 316
15.3 Instrument Configuration 324
15.3.1 Probe Tuning 324
15.4 Calibration of Pulses 326
15.4.1 Proton Pulses 326
15.4.2 Heteronuclear Pulses 326
15.5 T_1, T_2 and Experimental Parameters 328
15.5.1 Fundamentals of Nuclear Spin Relaxation 328
15.5.2 Effect of Molecular Weight and Magnetic Field Strength on T_1 and T_2 330
15.5.3 Effect of Temperature on T_2 332
15.5.4 Relaxation Interference: TROSY 332
15.5.5 Determination of T_1 and T_2 337
15.6 Acquisition of Multi-Dimensional Spectra 338
15.6.1 Setting Polarization Transfer Delays 338
15.6.2 Defining the Directly Detected Dimension: t_3 339
15.6.3 Defining Indirectly Detected Dimensions 340
15.7 Processing 3-Dimensional Data 346
15.7.1 Data Structure 346
15.7.2 Defining the Spectral Matrix 346
15.7.3 Data Processing 348
15.7.4 Processing the Directly Detected Domain 348

15.7.5 Variation in Processing 349
15.7.6 Useful Manipulations of the Free Induction Decay 351
16. DIPOLAR COUPLING 353
16.1 Introduction 353
16.1.1 Energy of Interaction 353
16.1.2 Effect of Isotropic Tumbling on Dipolar Coupling 356
16.1.3 Effect of Anisotropic Tumbling 357
16.2 Measurement of Inter-proton Distances 358
16.2.1 NOESY Experiment 360
16.2.2 Crosspeak Intensity in the NOESY Experiment 363
16.2.3 Effect of Molecular Weight on the Intensity of NOESY Crosspeaks 364
16.2.4 Experimental Determination of Inter-proton Distances 366
16.3 Residual Dipolar Coupling (RDC) 368
16.3.1 Generating Partial Alignment of Macromolecules 369
16.3.2 Theory of Dipolar Coupling 371
16.3.3 Measurement of Residual Dipolar Couplings 375
16.3.4 Estimation of the Alignment Tensor 380
17. PROTEIN STRUCTURE DETERMINATION 383
17.1 Energy Functions 385
17.1.1 Experimental Data 385
17.1.2 Covalent and Non-covalent Interactions 391
17.2 Energy Minimization and Simulated Annealing 392
17.2.1 Energy Minimization 393
17.2.2 Simulated Annealing 393
17.3 Generation of Starting Structures 395
17.3.1 Random Coordinates 395
17.3.2 Distance Geometry 395
17.3.3 Refinement 397
17.4 Illustrative Example of Protein Structure Determination 399
18. EXCHANGE PROCESSES 403
18.1 Introduction 403
18.2 Chemical Exchange 404
18.3 General Theory of Chemical Exchange 407
18.3.1 Fast Exchange Limit 409
18.3.2 Slow Exchange Limit 410
18.3.3 Intermediate Time Scales 410
18.4 Measurement of Chemical Exchange 411

18.4.1 Very Slow Exchange:$k_{ex} << \Delta\nu$ 411
18.4.2 Slow Exchange: $k_{ex} < \Delta\nu$ 413
18.4.3 Slow to Intermediate Exchange: $k_{ex} \approx \Delta\nu$ 414
18.4.4 Fast Exchange: $k_{ex} > \Delta\nu$ 414
18.4.5 Measurement of Exchange Using CPMG Methods 419
18.5 Distinguishing Fast from Slow Exchange 425
18.5.1 Effect of Temperature 425
18.5.2 Magnetic Field Dependence 426
18.6 Ligand Binding Kinetics 427
18.6.1 Slow Exchange 428
18.6.2 Intermediate Exchange 429
18.6.3 Fast Exchange 429
18.7 Exercises 430
18.8 Solutions 430
19. NUCLEAR SPIN RELAXATION AND MOLECULAR DYNAMICS 431
19.1 Introduction 431
19.1.1 Relaxation of Excited States 432
19.2 Time Dependent Field Fluctuations 434
19.2.1 Chemical Shift Anisotropy 434
19.2.2 Dipolar Coupling 437
19.2.3 Frequency Components from Molecular Rotation 438
19.3 Spin-lattice (T_1) and Spin-spin (T_2) Relaxation 442
19.3.1 Spin-lattice Relaxation 442
19.3.2 Spin-lattice Relaxation of Like Spins 445
19.3.3 Spin-lattice Relaxation of Unlike Spins 445
19.3.4 Spin-spin Relaxation 446
19.3.5 Heteronuclear NOE 447
19.4 Motion and the Spectral Density Function 448
19.4.1 Random Isotropic Motion 448
19.4.2 Anisotropic Motion - Non-spherical Protein 448
19.4.3 Constrained Internal Motion 449
19.4.4 Combining Internal and External Motion 451
19.5 Effect of Internal Motion on Relaxation 451
19.5.1 Anisotropic Rotational Diffusion 454
19.6 Measurement and Analysis of Relaxation Data 455
19.6.1 Pulse Sequences 455
19.6.2 Measuring Heteronuclear T_1 457

19.6.3 Measuring Heteronuclear T_2 459
19.7 Data Analysis and Model Fitting 463
19.7.1 Defining Rotational Diffusion 463
19.7.2 Determining Internal Rotation 466
19.7.3 Systematic Errors in Model Fitting 467
19.8 Statistical Tests 468
19.8.1 χ^2 Test for Goodness-of-fit 468
19.8.2 Test for Inclusion of Additional Parameters 470
19.8.3 Alternative Methods of Model Selection 472
19.8.4 Error Propagation 472
19.9 Exercises 473
19.10 Solutions 474

Appendices 475
A Fourier Transforms 475
A.1 Fourier Series 475
A.2 Non-periodic Functions - The Fourier Transform 476
A.2.1 Examples of Fourier Transforms 477
A.2.2 Linearity 481
A.2.3 Convolutions: Fourier Transform of Products of Two Functions 481
B Complex Variables, Scalars, Vectors, and Tensors 485
B.1 Complex Numbers 485
B.2 Representation of Signals with Complex Numbers 486
B.3 Scalars, Vectors, and Tensors 487
B.3.1 Scalars 487
B.3.2 Vectors 487
B.3.3 Tensors 488
C Solving Simultaneous Differential Equations: Laplace Transforms 491
C.1 Laplace Transforms 491
C.1.1 Example Calculation 492
C.1.2 Application to Chemical Exchange 493
C.1.3 Application to Spin-lattice Relaxation 494
C.1.4 Spin-lattice Relaxation of Two Different Spins 495
D Building Blocks of Pulse Sequences 497
D.1 Product operators 497
D.1.1 Pulses 497
D.1.2 Evolution by J-coupling 497
D.1.3 Evolution by Chemical Shift 498

D.2 Common Elements of Pulse Sequences 498
D.2.1 INEPT Polarization Transfer 498
D.2.2 HMQC Polarization Transfer 499
D.2.3 Constant Time Evolution 499
D.2.4 Constant Time Evolution with J-coupling 500
D.2.5 Sequential Chemical Shift & J-coupling Evolution 501
D.2.6 Semi-constant Time Evolution of Chemical Shift & J-Coupling 501

References 505

Index 519