

CONTENTS

PREFACE	xxiii
FOREWORD	xxv
ACKNOWLEDGEMENTS	xxvii
ABBREVIATIONS.....	xxix
 CHAPTER 1: BASIC CONCEPTS IN NMR SPECTROSCOPY.....	 1
1. HISTORICAL PERSPECTIVES	1
1.1 Quantum Mechanical Model for Spin-1/2 Nuclei	2
1.2 Classical Model	4
1.2.1 Rotating Frame of Reference	5
1.2.2 Strength of RF Pulses	5
1.3 Basic Design of a NMR Spectrometer.....	6
1.4 A Simple Pulse Sequence for NMR Excitation and Detection.....	8
1.5 Fourier Transform	9
1.6 Line Widths	10
1.7 Properties of Spin Operators and Pauli Matrices.....	10
2. NMR SPINS USED IN LIFE SCIENCES	11
3. INTERACTION OF NUCLEAR SPINS AND NMR PARAMETERS	12
3.1 Chemical Shift (δ)	12
3.2 Peak Intensities.....	15
3.3 Nuclear Spin-Spin (Scalar) Coupling (J).....	16
3.4 Hamiltonian for a 2-spin System	17
3.5 Dipolar Coupling (D_{ij})	20
3.6 Quadrupolar Interactions	21
3.7 Electron-Nuclear Interaction	22
4. NMR RELAXATION	23
4.1 Relaxation Rates.....	23
4.2 Molecular Mechanisms Leading to Relaxation	24
4.3 Theoretical Treatment of Relaxation Rates	25
4.4 Correlation Times.....	26
4.5 Dipolar Relaxation due to Several Interacting Spins.....	29
4.6 Field Dependence of Relaxation Rates.....	29
5. CHEMICAL EXCHANGE: DYNAMIC EFFECTS IN NMR SPECTROSCOPY	30
5.1 Conformational Equilibrium.....	30
5.2 Solvent Exchange	32
6. NUCLEAR MAGNETIC DOUBLE RESONANCE.....	33
6.1 Spin Decoupling.....	33
6.2 Heteronuclear and Broad-band Decoupling	34
6.3 Nuclear Overhauser Effect (NOE)	34
7. LINE SHAPES IN NMR.....	37
7.1 Absorptive and Dispersive Signals.....	37

7.2	Characteristics of FT-NMR Signals	38
7.3	Quadrature Phase Detection	39
8.	REFERENCES	40
8.1	History and Early Developments	40
8.2	Theoretical and Physical Aspects of NMR	41
8.3	Chemistry Oriented Books	41
8.4	Specialized Topics	41
CHAPTER 2: INTRODUCTION TO BIOLOGICAL NMR		43
1.	LEVELS OF BIOLOGICAL STRUCTURES	44
2.	NMR AND BIOLOGICAL STRUCTURES	46
3.	DIFFICULTIES IN STUDYING BIOLOGICAL SYSTEMS BY NMR	47
3.1	Sensitivity	47
3.2	Resolution	48
3.3	Assignments	48
3.4	Water Signal	49
3.5	Line Widths	50
3.6	Quantification	50
4.	BIOLOGICAL MACROMOLECULES	50
4.1	Building Blocks of Biological Molecules	51
4.2	Biopolymers	52
4.3	3D Structures of Biological Molecules	53
4.4	Comparison of 3D Structures Obtained from NMR and X-ray	54
5.	NMR IN CELLS AND TISSUES	55
5.1	Cellular Metabolism is the Bridge between Proteomics and Function	55
6.	NMR IN STUDIES OF ORGANS	56
6.1	Historical Development of MRI	56
6.2	Basis of MRI	57
6.3	Comparison of Images from NMR and Other Techniques	57
6.4	Magnetic Resonance Spectroscopy (MRS)	59
7.	BASIC MULTI-PULSE NMR EXPERIMENTS IN BIOLOGICAL SYSTEMS	59
7.1	Signal Averaging and Partial Saturation	59
7.2	Presaturation	61
7.3	Jump and Return (JR) Sequence	61
7.4	Spin-echo (SE)	62
7.4.1	Effect of Inhomogeneity of B_0	62
7.4.2	Behaviour of J-Coupled Systems	63
7.4.3	Application of SE	66
7.5	Carr-Purcell-Meiboom-Gill (CPMG) Sequence	66
7.6	Inversion Recovery Experiment (IR)	66
8.	COMPARISON OF NMR OTHER PHYSICAL TECHNIQUES	67
9.	REFERENCES	68
9.1	Theoretical and Physical Aspects of NMR	68
9.2	Biological Molecules	68
9.3	Biomedical Systems	68

CHAPTER 3: MULTI-DIMENSIONAL NMR	69
1. INTRODUCTION.....	69
1.1 Multi-Dimensional (MD) NMR	69
1.2 General Scheme for 2D NMR	69
1.3 CORrelated SpectroscopY (COSY).....	72
1.4 Advantages of 2D NMR.....	73
2. PRODUCT OPERATOR FORMALISM (POF).....	74
2.1 Product Operators (PO)	75
2.2 Coherences	75
2.3 Observable Coherences	77
2.4 Effect of RF Pulses on Product Operators.....	77
2.5 Evolution of Product Operators under free Hamiltonian.....	78
2.6 Effect of Composite π pulses on Product Operators.....	78
2.7 Evolution of MQ Coherences.....	79
2.8 Evolution of Zero Quantum Coherences.....	79
2.9 Evolution of Two Quantum Coherences	79
3. HOMONUCLEAR CORRELATION SPECTROSCOPY.....	80
3.1 Correlation Spectroscopy (COSY)	80
3.2 Phase Cycling	81
3.2.1 CYCLOPS	82
3.2.2 Axial Peak Suppression	82
3.3 Relayed COSY	83
3.4 TOrtal Correlation SpectroscopY (TOCSY).....	84
3.5 MQ-Filtered COSY	85
3.5.1 Multiple-Quantum Filtering.....	85
3.5.2 Double-Quantum Filtered COSY (2QF-COSY)	86
3.5.3 Three-Quantum Filtered COSY (3QF-COSY)	87
3.5.4 Exclusive COSY	89
3.6 EXSY and NOESY.....	90
3.7 ROTational Nuclear Overhauser Effect SpectroscopY (ROESY).....	91
4. MULTIPLE-QUANTUM (MQ) SPECTROSCOPY	92
4.1 2D Double-Quantum (DQ) Experiment	92
5. HETERONUCLEAR CORRELATION SPECTROSCOPY	94
5.1 Insensitive Nuclei Enhanced by Polarization Transfer (INEPT).....	94
5.2 Reverse INEPT.....	96
5.3 Refocused INEPT	96
5.4 Distortionless Enhanced by Polarization Transfer (DEPT).....	97
5.5 Broadband Decoupling.....	97
5.6 Heteronuclear Single-Quantum Correlation Spectroscopy (HSQC).....	99
5.7 Heteronuclear Multiple-Quantum Correlation Spectroscopy (HMQC)....	101
5.8 Sensitivity Enhanced HSQC.....	101
6. PULSED FIELD GRADIENTS.....	102
6.1 Coherence Pathway Selection using PFG.....	103
6.2 Water Suppression by Gradient Tailored Excitation (WATERGATE).....	104

7. THREE DIMENSIONAL (3D) EXPERIMENTS	105
7.1 3D Double-Resonance Experiments.....	106
7.2 3D NOESY-[$^{13}\text{C}/^{15}\text{N}$ -1H] HSQC	106
7.3 3D TOCSY-[$^{13}\text{C}/^{15}\text{N}$ -1H] HSQC.....	108
7.4 3D Triple Resonance Experiments.....	108
8. TECHNIQUES FOR SOLUTION NMR OF VERY LARGE MOLECULES.....	108
9. REFERENCES.....	110
9.1 Theoretical and Physical Aspects	110
9.2 Review Articles	110
9.3 Three Dimensional NMR	110
10. APPENDIX 3.1	111

CHAPTER 4: BIOMOLECULAR STRUCTURES USING NMR:

GENERAL PRINCIPLES	113
1. INTRODUCTION.....	113
1.1 Conformation of Biological Molecules	114
1.2 Conformational Theory	115
1.3 Conformational Domains of Proteins and Nucleic Acids.....	118
2. ELEMENTS OF MACROMOLECULAR STRUCTURES	119
2.1 Primary Structure	119
2.2 Secondary Structures.....	120
2.3 Tertiary Structures.....	120
2.4 Quaternary Structures.....	120
2.5 Multi-molecular Assemblies.....	121
2.6 Random Coil Structures	121
3. SAMPLE PREPARATION FOR NMR: LABELLING TECHNIQUES.....	121
3.1 Preparation of Samples for NMR	122
3.1.1 Cloning and Expression of Proteins.....	123
3.1.2 Taming Proteins.....	124
3.1.3 Protein Recovery	125
3.1.4 Protein Purification	125
3.1.5 Ion-exchange Chromatography.....	126
3.1.6 Affinity Chromatography	126
3.1.7 Gel Filtration.....	126
3.1.8 Approaches in Structural Genomics	126
3.2 Isotope Labelling.....	127
3.3 Concentration	127
3.4 Quality Assessment and Storage	128
3.5 Synthesis of Nucleic Acids.....	128
3.6 RNA Samples	128
3.7 Purification of Nucleic Acids	129
3.8 ^{13}C and ^{15}N Labelling of Nucleic Acids	129
3.9 In-vitro RNA Transcription and Purification.....	130
4. NMR APPROACH TO STRUCTURAL STUDIES.....	130

4.1	NMR Strategies	131
4.2	General Approach for the Resonance Assignments in Biomolecules.....	131
5.	NMR PARAMETERS FOR STRUCTURAL STUDIES	133
5.1	Chemical Shifts: Magnetic Anisotropy of Chemical Groups	133
5.1.1	Ring Current Effects	134
5.1.2	Changes in Chemical Shifts and Secondary Structures	135
5.2	Chemical Shifts and ^2H Exchange Rates of Hydrogen Bonded Protons	135
5.3	Nuclear Overhauser Effect (NOE)	136
5.3.1	Limitations of NOE	136
5.3.2	Introduction of NOE Constraints	138
5.4	Scalar Coupling Constants (J)	138
5.4.1	Torsion Angle Dependence of ^3J	139
5.4.2	Coupling Constants across Hydrogen Bonds.....	140
5.5	Residual Dipolar Couplings (RDC).....	141
5.5.1	Partial Alignment	142
5.5.2	Methods of Achieving Partial Alignment of Biological Molecules	144
5.5.3	Use of RDC for 3D Structure Determination.....	144
5.5.4	Advantages of RDC	145
5.6	Use of Cross-Correlated Relaxation	145
5.7	Covalent Bond Distances	147
5.8	Stereochemical Assignments.....	148
5.9	Removing Undesirable Restraints	149
6.	PARAMAGNETIC MOLECULES AND REAGENTS	149
6.1	Contact Shifts	150
6.2	Pseudo-Contact Shifts	150
6.3	Relaxation Rates.....	151
6.4	Metallo-Proteins	152
6.5	Lanthanides Shift Reagents	152
6.6	Use of Spin Labels.....	153
7.	FROM NMR PARAMETERS TO STRUCTURES	153
7.1	Starting Structures	153
7.2	Metric Matrix Distance Geometry.....	154
7.3	Variable Target Functions: Torsion Angle Approaches	155
7.4	Molecular Mechanics and Molecular Dynamics Algorithms	155
7.5	Torsion Angle Dynamics (TAD).....	157
7.6	Restraint Energy Minimization	158
7.7	Validation of Final Structures.....	159
7.8	Presenting the Final Structures	160
8.	DATA BANKS FOR STRUCTURES AND NMR OF BIOMOLECULES.....	160
8.1	Protein and Nucleic Acid Data Banks (PDB).....	160
8.2	Biological Magnetic Resonance Data Bank (BioMagResBank; BMRB).....	161
8.3	Applications of Data Banks.....	161
8.4	Where to look for Literature on NMR Structures.....	162
9.	REFERENCES	162
9.1	Further Reading	162
9.2	Books.....	162

CHAPTER 5: PROTEIN NMR: GENERAL PRINCIPLES AND RESONANCE ASSIGNMENTS	163
1. INTRODUCTION.....	163
1.1 Functions of Proteins.....	163
1.2 Conformation and Dynamics of Proteins	164
1.3 History of Protein Structure Determination.....	166
2. ELEMENTS OF PROTEIN STRUCTURES.....	167
2.1 Nomenclature	167
2.2 Backbone and Side-chain Torsion Angles.....	167
2.3 Primary Structure	169
2.4 Conformational Freedom of Peptides	170
2.5 Disulfide Bridges and Proline Rings	172
2.6 Secondary Structures	172
2.6.1 α -helix	173
2.6.2 β -strands and β -sheet.....	173
2.6.3 β -turns.....	175
2.6.4 Collagen.....	175
2.6.5 Poly (L-Proline)	175
2.7 Fibrous Proteins.....	176
2.8 Tertiary Structure: Globular Proteins	176
2.9 Higher Levels of Structural Organization.....	176
3. INTRODUCTION TO NMR OF PROTEINS	177
3.1 History of NMR of Proteins	177
3.2 Current Status	178
3.3 Sample Preparation.....	179
3.4 NMR Approach to Protein Structure	179
3.5 Classification of Chemical Shifts	180
3.6 $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ Chemical Shift Statistics.....	181
4. RESONANCE ASSIGNMENT STRATEGIES	181
4.1 Identification of Networks of J-coupled Spin Systems.....	181
4.1.1 Glycine.....	182
4.1.2 Amino acids Containing Methyl Group(s): Ala, Thr, Val, Ile and Leu	182
4.1.3 Asn, Asp, Cys and Ser	183
4.1.4 His, Phe, Trp and Tyr.....	183
4.1.5 Long Side-chain Residues	184
4.2 Linking Side Chains to the Respective Amide Protons	184
4.3 Sequence Specific Resonance Assignments	184
4.4 Identification of Disulfide Bridges	185
4.5 Stereo-specific Resonance Assignments	186
4.6 Higher Dimensional NMR	186
5. 3D TRIPLE-RESONANCE EXPERIMENTS FOR PROTEINS.....	186

5.1	HNCA.....	187
5.2	HN(CO)CA.....	189
5.3	HNCO.....	190
5.4	HN(CA)CO.....	190
5.5	CBCANH.....	191
5.6	CBCA(CO)NH.....	191
5.7	HN(CA)HA and HN(COCA)HA.....	191
5.8	3D Experiments used for Side-Chain Resonance Assignments in Proteins.....	192
6.	REDUCED DIMENSIONALITY: G-MATRIX FTNMR FOR PROTEINS.....	192
6.1	RD NMR.....	193
6.2	GFT NMR.....	195
7.	AUTOMATED NMR ASSIGNMENTS IN PROTEINS.....	197
7.1	Tracked Automated Assignment in Proteins (TATAPRO).....	198
7.2	Selective Labeling/Unlabeling: Residue Specific Resonance Assignments.....	199
7.3	Side-chain Assignments.....	200
7.4	Automated Chemical Shift Prediction Based on Sequence Homology.....	200
8.	TECHNIQUES FOR STUDYING LARGE PROTEINS.....	201
8.1	Transverse Relaxation Optimized Spectroscopy (TROSY).....	201
8.2	^2H labelling.....	202
9.	PROTONLESS MULTI-DIMENSIONAL NMR.....	203
9.1	2D Protonless NMR.....	204
9.2	The Problem of Large Homonuclear Couplings in Acquisition Dimension.....	204
9.3	3D Protonless NMR.....	207
10.	REFERENCES.....	208
10.1	Historical Foundation of Protein Structure and Function.....	208
10.2	General Books on Proteins.....	208
10.3	Books on Protein NMR.....	209
10.4	Study of Large Proteins.....	209

CHAPTER 6: STRUCTURE, DYNAMICS AND FUNCTION OF PROTEINS..... 211

1.	INTRODUCTION.....	211
2.	CHARACTERIZATION OF SECONDARY STRUCTURE ELEMENTS.....	211
2.1	Chemical Shift Index (CSI).....	211
2.2	Secondary Structure using Chemical Shift and Sequence Homology.....	213
2.3	Nuclear Overhauser Effect (NOE).....	213
2.4	Three bond Scalar-couplings (3J).....	215
2.5	$^1\text{H}^{\text{N}}\text{-}^2\text{H}^{\text{N}}$ Exchange.....	215
3.	OBTAINING THE FINAL STRUCTURES.....	217
3.1	3D Structure Calculation.....	217
3.2	Automated NOESY Assignments.....	218
3.3	Structural Statistics and Quality.....	218

4. PROTEIN DYNAMICS.....	219
4.1 NMR Parameters for Studying Dynamics	220
4.2 Basic Theory.....	220
4.3 Protein Dynamics using NMR.....	221
4.4 Experimental Results.....	222
5. PROTEIN FOLDING AND UNSTRUCTURED PROTEINS	223
5.1 Protein Folding Pathways.....	223
5.2 NMR Methodologies for Studying Unfolded Protein Structures.....	224
5.3 Molten Globules	225
5.4 Unstructured Functional Proteins	225
5.5 Protein Structures under High Pressure	226
6. NMR STUDIES OF PROTEIN STRUCTURE, DYNAMICS AND FOLDING: SOME SPECIFIC EXAMPLES.....	226
6.1 Basic Pancreatic Trypsin Inhibitor (BPTI)	227
6.2 Human Ubiquitin.....	228
6.3 Ribonuclease	228
6.4 Lysozyme	228
6.5 EF-hand Calcium Binding Proteins (CaBP).....	230
6.6 Maleate Synthase G (MSG).....	231
6.7 GroEL-GroES Complex	231
6.8 Membrane Proteins Studied in Solutions	232
7. PARAMAGNETIC PROTEINS	232
7.1 Metal Ions of Interest.....	233
7.2 Heme Iron Proteins.....	234
7.2.1 High-Spin Iron (III)	236
7.2.2 Low Spin Iron (III)	237
7.2.3 High Spin Iron (II)	237
7.2.4 Diamagnetic Iron Heme Proteins.....	238
7.3 Iron-Sulfur Proteins.....	238
7.4 Other Iron Proteins	239
7.5 Other Metal Ions.....	239
8. FUNCTIONAL ASPECTS STUDIED BY NMR.....	240
8.1 pH Titrations.....	240
8.2 Ligand Binding.....	241
8.2.1 Diffusion Studies	242
8.2.2 Mapping of the Binding Site.....	242
8.2.3 Conformation of the Bound Ligand.....	242
8.2.4 Changes in the Structure and Dynamics of the Protein.....	243
8.3 Enzyme Catalysis	243
8.3.1 Dihydrofolate Reductase (DHFR)	244
8.3.2 Triosephosphate Isomerase (TIM).....	244
8.4 Multi-Domain Structures.....	244
9. REFERENCES	244
9.1 Historical Foundation of Protein Structure and Function.....	244
9.2 General Books on Proteins	245
9.3 Books on Protein NMR	245

9.4 Study of Large Proteins	245
9.5 Protein Structure, Dynamics and Folding.....	246
9.6 Paramagnetic Proteins	246
9.7 Protein Binding and Function.....	246
CHAPTER 7: STRUCTURE AND DYNAMICS OF NUCLEIC ACIDS	247
1. INTRODUCTION.....	247
1.1 Chemical Constitution of Nucleic Acids	247
1.2 Biological Role of Nucleic Acids	249
1.3 Historical Background of Nucleic Acid Structures	250
2. ELEMENTS OF STRUCTURE OF NUCLEIC ACIDS	251
2.1 Nomenclature	251
2.2 The Backbone Torsion Angles	253
2.3 Glycosidic Bond Rotation (χ).....	253
2.4 Sugar Pucker: Pseudo-Rotation Angle (P)	253
2.5 Inter-base Hydrogen Bonding	255
3. NMR SPECTROSCOPY OF NUCLEIC ACIDS	256
3.1 Comparison with Protein NMR.....	257
3.2 Classification of Chemical Shifts	258
3.3 Identification of Networks of Coupled Spin-systems	258
3.4 Sequence Specific Resonance Assignment Strategies	261
3.5 Resonance Assignments using ^{13}C and ^{15}N Labelled Nucleic Acids.....	262
4. NMR PARAMETERS IN NUCLEIC ACIDS	264
4.1 Three Bond Coupling Constants (3J)	264
4.2 ^{31}P - ^1H Couplings.....	265
4.3 Scalar Couplings across Hydrogen Bonds.....	265
4.4 Estimation of ^1H - ^1H Distances using NOE.....	266
4.5 Residual Dipolar Couplings (RDC).....	266
4.6 Use of Paramagnetic Labels	267
5. STRUCTURE SIMULATIONS OF NUCLEIC ACIDS	267
5.1 Conformation of Deoxyribose and Ribose Rings	268
5.2 Backbone Torsion Angles	271
5.3 Glycosidic Torsion Angle (χ).....	271
5.4 Hydrogen-bond Constraints for Base-pairs	271
5.5 3D Structure from NMR data	272
6. UNUSUAL DNA STRUCTURES	273
6.1 Sequence Dependent Variations in B-DNA	273
6.2 Mismatch Base-pairs	273
6.3 Hairpin Nucleic Acids	275
6.4 Parallel Stranded (ps) DNA Duplex	276
6.5 Triple Stranded Nucleic Acids	277
6.6 G-Quartet.....	280
6.7 i-motif Tetraplex.....	281

6.8	Cruciform Structure.....	282
6.9	Holliday or Four-way Junction.....	283
7.	POLYMORPHISM IN DNA	283
7.1	Concentration-induced Conformational Transition.....	284
7.2	pH- Induced Polymorphism.....	286
7.3	Effect of Temperature.....	286
7.4	Effect of Metal Ions.....	287
8.	DYNAMICS OF NUCLEIC ACIDS	287
9.	REFERENCES.....	288
9.1	Further Reading.....	288
9.2	Books.....	288
9.3	DNA Structure	289
9.4	Parallel Stranded DNA.....	289
9.5	Triple-Helical Nucleic Acids.....	289
9.6	Isotope Labelling of Nucleic Acids.....	290

CHAPTER 8: CARBOHYDRATES, LIPIDS AND LIPID ASSEMBLIES 291

1.	INTRODUCTION.....	291
2.	CARBOHYDRATES.....	291
2.1	Biological Roles of Carbohydrates.....	292
2.2	Building Blocks of Carbohydrates	293
2.3	Oligosaccharides	293
2.4	General Features of NMR of Carbohydrates	294
2.5	Primary Structures of Carbohydrates.....	297
2.6	NMR Determination of the Conformation of Monosaccharides	297
2.7	Assignments of NMR Spectrum of Carbohydrates	298
2.8	Structure and Dynamics of Polysaccharides.....	299
2.9	Glycoconjugates	300
2.10	Applications in Development of Bacterial Polysaccharides as Vaccines	301
2.11	Recognition Motifs in Polysaccharides	302
2.12	Proteoglycans	302
3.	LIPIDS AND MODEL MEMBRANES	302
3.1	Elements of Biological Membranes	303
3.2	Lipids.....	303
3.3	Conformation of Phospholipids.....	305
3.4	Lipid Vesicles and Bilayer Structures	306
3.5	Membrane Architecture.....	307
4.	NMR STUDIES OF STRUCTURE AND DYNAMICS OF LIPID BILAYERS	308
4.1	Molecular Motions in Model Membranes	308
4.2	Phase Transitions.....	309
4.3	Lipid Polymorphism in Model Membranes.....	311
4.4	^{13}C NMR Studies of Segmental Motions in Alkyl Chains	311
4.5	Molecular Order and ^2H NMR Studies of Membrane Organization	312
4.6	Modulation of Motion and Order of Lipids by Larger Molecules.....	313

4.7 Measurement of Lateral Diffusion using NMR.....	314
5. REFERENCES.....	314
5.1 Further Reading.....	314
5.2 Books and Reviews.....	315
CHAPTER 9: HIGH-RESOLUTION SOLID-STATE NMR.....	317
1. INTRODUCTION.....	317
2. NMR HAMILTONIANS IN SOLID-STATE.....	317
2.1 NMR Hamiltonian in Solid-State.....	318
2.2 Chemical Shift Anisotropy (H_{CSA}).....	320
2.3 Heteronuclear Dipolar Interactions [$H_{het(D)}$].....	323
2.4 Homonuclear Dipolar Interactions [$H_{hom(D)}$].....	324
2.5 Quadrupolar Interaction (H_Q).....	325
2.6 Scalar Coupling [H_J].....	326
3. STRATEGIES FOR OBTAINING HIGH-RESOLUTION NMR SPECTRA IN SOLID-STATE.....	326
3.1 Magic-Angle Spinning (MAS).....	328
3.2 Heteronuclear Dipolar Decoupling (DD).....	329
3.3 Cross Polarization (CP).....	330
3.4 ^{31}P NMR.....	331
3.5 Spectra of Dilute Spin 1/2 Nuclei.....	331
3.6 ^1H NMR.....	332
3.7 Heteronuclear and Homonuclear Correlation Experiments.....	333
3.8 ^2H NMR.....	334
3.9 Measurement of Internuclear Distances in Solid-State: Dipolar Recoupling.....	335
3.10 Reintroduction of CSA.....	336
4. PROTEIN STRUCTURE DETERMINATION USING SOLID-STATE NMR.....	338
4.1 Membrane Proteins.....	338
4.2 Polarisation Inversion at Magic Angle: PISA Wheels.....	340
4.3 Structure Analysis using MAS Solid-State NMR.....	341
4.4 Ion Channel Proteins.....	341
4.5 Bacteriorhodopsin (bR).....	342
4.6 Amyloid Proteins.....	343
4.7 Structure of the Coat Protein in fd Filamentous Bacteriophage Particles.....	345
4.8 Collagen.....	345
4.9 Other Fibrous Proteins.....	347
4.10 Other Systems.....	347
5. FUTURE OF SOLID-STATE NMR IN BIOLOGY.....	347
6. REFERENCES.....	348
6.1 Further Reading.....	348
6.2 Books and Reviews.....	349

CHAPTER 10: BIOMOLECULAR INTERACTIONS AND SUPRAMOLECULAR ASSEMBLIES	351
1. INTRODUCTION.....	351
2. INTERMOLECULAR INTERACTIONS	352
3. NMR APPROACHES TO STUDY BIOMOLECULAR INTERACTIONS.....	352
3.1 Experimental Considerations.....	352
3.2 Use of NOE and RDC	353
3.3 Use of Perturbations in Chemical Shifts (CSPs)	353
3.4 Relaxation Rates.....	354
3.5 Use Paramagnetic Probes	354
3.6 Cross Saturation Method	355
3.7 Strategies for Structure Simulations	356
4. PROTEIN-PROTEIN INTERACTIONS.....	356
5. PROTEIN-DNA INTERACTIONS	359
5.1 Biological Importance	359
5.2 Nature of Protein-Nucleic Acid Interactions	360
5.3 Structure Calculation of Protein-Nucleic Acid Complexes	360
5.4 Examples of Protein-DNA Interactions.....	361
5.4.1 Myocyte Enhancer Factor 2A (MEF2A)-DNA Complex	361
5.4.2 Far-upstream Element-Binding Protein (FBP) Bound to Single-Stranded DNA:.....	363
6. RNA STRUCTURES AND THEIR INTERACTION WITH PROTEINS.....	363
6.1 Structural Motifs in RNA Structures	364
6.2 Introduction to NMR of RNA	364
6.3 Transfer RNA (tRNA) and its Interaction with Aminoacyl-tRNA Synthetase	365
6.4 Ribosomal RNA	368
6.5 Protein-RNA Interactions	369
6.6 NMR Studies of Protein-RNA Interactions	369
6.6.1 JDV Tat-BIV TAR Complex.....	369
6.6.2 HIV-1 Rev peptide-RRE RNA complex	370
6.6.3 Splicing Factor 1-RNA complex	371
7. SUPRAMOLECULAR ASSEMBLIES.....	371
7.1 Bone	371
7.2 Skeletal Muscle	372
7.3 Large DNA and its Complexes.....	372
7.4 Chlorophyll.....	372
8. FUTURE OF NMR STUDIES ON SUPRAMOLECULAR SYSTEMS.....	374
9. REFERENCES.....	374
9.1 Further Reading.....	374
9.2 RNA Structure.....	374
9.3 Protein-Nucleic acid Interactions	375
9.4 Books.....	375

CHAPTER 11: MAGNETIC RESONANCE IMAGING	377
1. INTRODUCTION.....	377
2. PRINCIPLES OF NMR IMAGING.....	377
3. SPATIAL LOCALIZATION OF THE NUCLEUS TO BE IMAGED.....	379
3.1 Slice Selection.....	380
3.2 Sensitive-Point Method.....	381
3.3 Two-Dimensional Fourier Imaging.....	382
3.3.1 Slice Selection.....	383
3.3.2 Phase Encoding.....	383
3.3.3 Frequency Encoding.....	384
3.3.4 Fourier Transform.....	384
3.3.5 Multi-slice Imaging.....	385
3.4 k-Space.....	385
4. PULSE SEQUENCES IN MRI.....	387
4.1 Spin-echo (SE).....	388
4.2 Half Fourier Transform Imaging (HFI).....	389
4.3 Inversion Recovery (IR).....	390
4.4 The Need for Fast Imaging Techniques.....	391
4.5 Gradient Echo (GRE).....	392
4.6 Turbo-Spin-Echo (TSE).....	393
4.7 Single-Shot Techniques: Echo Planar Imaging (EPI).....	395
4.8 Parallel Imaging.....	396
4.9 Three Dimensional Imaging.....	397
4.10 Motion Suppression Techniques.....	397
4.11 Scan Speed.....	397
4.12 Receiver Coils.....	398
4.13 Display.....	398
4.14 Future.....	399
5. TISSUE CONTRAST BASED ON RELAXATION RATES AND ^1H DENSITY.....	399
5.1 Proton Density (PD).....	400
5.2 Relaxation times T_1 and T_2	401
5.3 Achieving Contrast.....	402
5.4 Lipid Signals.....	404
5.5 Susceptibility Effects: Diamagnetic Interfaces.....	404
5.6 Paramagnetic and Ferromagnetic Materials: Contrast Enhancement (CE).....	405
5.7 Other Imaging Parameters.....	406
6. IMAGE CONTRAST BASED ON FLOW, DIFFUSION AND PERFUSION.....	406
6.1 Magnetic Resonance Angiography (MRA).....	407
6.2 Diffusion-weighted Imaging (DWI).....	408
6.3 Perfusion.....	410
6.4 Magnetization Transfer (MT).....	411
7. FUNCTIONAL MRI (fMRI).....	411

7.1	Principle of fMRI	411
7.2	Methodology	412
7.3	Applications of fMRI	413
7.4	Limitations and Future Outlook	415
8.	MRI AS A CLINICAL TOOL	415
8.1	Central Nervous System (CNS).....	415
8.1.1	Ischaemic Stroke	416
8.1.2	Haemorrhage Stroke.....	416
8.1.3	Neurological Diseases	417
8.2	Musculoskeletal System	417
8.3	Heart and Cardiovascular System.....	418
8.4	Breast.....	418
8.5	MRI in Pregnancy	420
8.6	Genitourinary System.....	420
8.7	Gastrointestinal System.....	420
8.8	Prostate Cancer.....	420
8.9	Interventional Imaging	421
8.10	Patient Comfort and Precautions	421
9.	REFERENCES.....	422
9.1	Further Reading	422
9.2	Books and Reviews	422

CHAPTER 12: STUDY OF METABOLISM: CELLS AND TISSUES 423

1.	INTRODUCTION.....	423
1.1	Cell-metabolism is the Bridge between Proteomics and Function	423
1.2	Measurement of Products of Metabolism	424
2.	METABOLIC CYCLES AND PATHWAYS	425
2.1	Glycolysis	426
2.2	Citric Acid (Kreb's) Cycle	427
2.3	Oxidative Phosphorylation	428
2.4	Pentose Phosphate Pathway, Gluconeogenesis and Glycogen Synthesis	428
2.5	Fatty Acid Synthesis and Degradation	429
2.6	Amino Acid Metabolism	429
2.7	Integration and Control.....	429
2.8	Metabolic Profile and Requirements of each Body Organ is Different.....	430
2.9	³¹ P NMR and Metabolism	431
2.10	¹ H NMR Spectroscopy	433
2.11	¹³ C NMR Spectroscopy	434
3.	STUDIES OF BODY FLUIDS	435
3.1	Metabolomic Analysis using Biological Fluids	435
3.2	¹ H and ¹³ C Chemical Shifts of Common Metabolites: Assignments.....	437
3.3	Suppression of Water and Other Undesirable Signals	439
3.4	Multi-dimensional NMR and Quantification.....	440

3.5 Multi-variant Statistical Analysis	440
3.6 Applications of Metabonomics	441
4. CELLULAR NMR SPECTROSCOPY	442
4.1 Technical Aspects of NMR in Cells	443
4.2 Studies on Spermatozoa	444
4.3 Identification of Low Molecular Weight Compounds in Cells	444
4.4 Biochemical Changes during Cell Maturation, Modification and Differentiation	445
4.5 Glycolysis in Cells	446
4.6 Effect of Exogenous Compounds on Metabolism	447
4.7 In-Cell Studies of Macromolecular Structure and Dynamics	447
5. STUDIES OF TISSUES USING SOLID-STATE NMR TECHNIQUES	449
6. REFERENCES	451
6.1 Further Reading	451
6.2 Books and Reviews	452

CHAPTER 13: MRS STUDIES OF METABOLISM IN ANIMALS AND HUMANS..... 453

1. INTRODUCTION.....	453
2. TECHNIQUES FOR DETECTING MR SIGNALS.....	454
2.1 Spectral Localization	454
2.2 Water Suppression	456
2.3 Depth Resolved Surface Coil Spectroscopy (DRESS)	456
2.4 Image Guided Protocols	457
2.5 Image Selected <i>in vivo</i> Spectroscopy (ISIS).....	457
2.6 Stimulated Echo Acquisition Mode Spectroscopy (STEAM)	458
2.7 Point Resolved Spectroscopy (PRESS).....	459
2.8 Comparison of STEAM and PRESS	459
2.9 Multiple Volume Spectroscopy (MVS) or CSI).....	460
2.10 Recent Developments in MRS	461
2.11 Detection of Metabolites using 2D NMR	462
3. UNDERSTANDING THE CHEMISTRY OF BRAIN THROUGH MRS.....	462
3.1 ^1H NMR Spectroscopy	463
3.2 ^{13}C NMR: The Glu, Gln, GABA Cycle	465
3.3 Age and Disease Related Changes in MRS of Brain.....	466
4. MRS OF OTHER ORGANS.....	466
4.1 Muscle	467
4.2 Insulin Regulation of Glycogen Metabolism.....	469
4.3 Breast.....	469
4.4 Prostate.....	471
4.5 Cardiovascular Disorders	472
5. MR IMAGING AND SPECTROSCOPY USING OTHER NUCLEAR SPINS.....	472
5.1 Difficulties in <i>in vivo</i> Spectroscopic Studies using other Spins	473
5.2 ^{19}F	473

5.3	^2H	475
5.4	^7Li	475
5.5	^{23}Na	476
5.6	^3He and ^{129}Xe	476
5.7	Other Nuclear Spins	477
6.	APPLICATIONS OF NMR IN DRUG DEVELOPMENT.....	477
6.1	Stages in Drug Development.....	477
6.2	Target Validation and Receptor Identification	478
6.3	Structural Approach to Drug-design	479
6.4	Drug-Receptor Binding	480
6.5	Lead Identification: Ligands for Hot Spot.....	480
6.6	Second Binding Site: The Linked-Fragment Strategy	482
6.7	Lead Optimization	482
6.8	Pre-clinical Studies: Drug Metabolism using Body Fluids.....	482
6.9	Human Trials	483
6.10	Drug purity: Interfacing NMR with LC and MS	484
6.11	Use of Solid-State NMR for Drug Powders	484
7.	REFERENCES.....	485
7.1	Further Reading	485
7.2	Books and Reviews	486
 CHAPTER 14: STRUCTURE AND METABOLISM OF PLANTS		487
1.	INTRODUCTION.....	487
1.1	Cell Structure of Plants	487
1.2	Biochemistry of Plants	488
1.3	Importance of NMR in Plant Biochemistry	488
2.	UNIQUE METABOLIC PATHWAYS IN PLANTS	489
2.1	Photosynthesis	489
2.2	Nitrogen Metabolism.....	489
2.3	Photorespiration.....	489
2.4	Sulphur Metabolism	490
3.	NMR OF PLANTS: GENERAL FEATURES.....	490
3.1	Handling Whole Plants.....	490
3.2	Tissues and Small Plants	492
3.3	General Features of Plant NMR	492
3.4	Scope of Plant NMR.....	493
3.5	Nuclear Spins used in Plant NMR	493
3.6	Interpretation of Spectra	495
3.7	Compartmentation in Plant Cells	495
4.	PLANT METABOLISM USING NMR	496
4.1	Tissues, Cells and Cell Extracts	496
4.2	Nitrogen Metabolism.....	497
4.3	Amino Acid Metabolism	499
4.4	Photosynthesis	499
4.5	Carbon Metabolism	500

4.6 Sulphur Metabolism	500
4.7 Phosphorus Metabolism	501
4.8 Other ions and Substrates	501
4.9 Paramagnetic Reagents	501
4.10 Effect of Physiological Conditions	502
4.11 Secondary Metabolic Pathways	502
4.12 Plant-Fungal Relations	502
5. METABOLIC FLUXES AND PLANT METABOLOMICS	502
5.1 Analysis of Metabolic Fluxes	503
5.2 Secondary Products of Plant Metabolism	503
5.3 Plant Metabolomics	503
6. IMAGING AND MICROSCOPY IN PLANTS	503
6.1 NMR Microscopy	504
6.2 Studies of Plant Water	504
6.3 Studies of Roots	505
6.4 Stems, Leaves and Flowers	505
6.5 Fruits and Seeds	505
6.6 Chemical Shift Imaging	506
7. SOLID-STATE NMR STUDIES	506
7.1 Studies on Plant Organelles	506
7.2 Solid-State NMR on PS I and PS II	507
7.3 Chlorosomal Bacteriochlorophylls	508
8. REFERENCES	509
8.1 Further Reading	509
8.2 Books and Reviews	510
INDEX	511

A significant number of biologists have been intrigued by the power of NMR to provide an understanding of a molecular problem they have to solve. However, the answers would be answered by NMR, but have no idea how to obtain them. This book is written for (i) those who are already familiar with NMR spectroscopy and (ii) those who are not familiar with NMR and yet do not know how NMR can help to provide them information (qualitative or functional) about proteins, nucleic acids, cells and tissues, membranes and membranes, and other biological materials. We hope that this book builds a number of knowledge transfer between the beginners and the experts as well as applied to all aspects of life sciences.

March 2004

R. V. P. Rao

Chennai, India