

Contents

Preface	V
List of Contributors	XI
List of Abbreviations and Chemical Symbols	XV
1 Structural NMR Studies: from One to Multidimensional Frequency Spectra	1
1.1 Introduction.....	1
1.1.1 The Fourier Transform (FT) Revolution	2
1.1.2 Mono-dimensional FT-NMR.....	3
1.2 From 1D to 2D NMR: Scalar Correlation Spectroscopy	5
1.2.1 Sampling Frequency and Sensitivity.....	7
1.2.2 Lineshape and Frequency Discrimination	9
1.2.3 F_1 Quadrature Detection and Absorption Mode.....	12
1.3 Correlation in 2D Spectra.....	15
1.3.1 Quantum Mechanical Description.....	17
1.3.2 Product Operators	19
1.3.2.1 Product Operators Algebra.....	21
1.4 The COSY Experiment	23
1.4.1 Longitudinal Relaxation Effects.....	25
1.5 The Rationale of 2D NMR Strategy	26
1.5.1 Size, Shape and Constituents of Biopolymers and NMR Structural Perspectives.....	27
1.5.2 Appendix.....	28
1.5.2.1 Similarity Theorem.....	28
1.5.2.2 Shift Theorem	28
1.5.2.3 Convolution Theorem.....	29
1.5.2.4 Power Theorem.....	29

2	Frontiers in NMR of Paramagnetic Molecules: ^1H NOE and Related Experiments.....	31
2.1	Introduction.....	31
2.2	The Theory of NOE	32
2.3	Examples of ^1H NOE's in Paramagnetic Molecules	40
2.4	Non-scalar Magnetization Transfer in 2D Experiments	46
2.4.1	NOESY and EXSY Experiments	46
2.4.2	Perspectives in Paramagnetic Systems	51
2.5	Appendix.....	55
3	3D NMR Spectroscopy in High Resolution NMR.....	61
3.1	Introduction.....	61
3.2	Limits of 2D spectroscopy.....	62
3.3	Construction scheme of 3D experiments.....	65
3.4	Classification of peaks in 3D spectra	67
3.5	Information Content of 3D spectra.....	68
3.6	Sensitivity of 3D spectra.....	69
3.7	Practical Aspect of 3D Spectroscopy	71
3.7.1	a) Non-selective Approach	72
3.7.2	b) Selective Approach.....	74
3.8	Extraction of Information.....	76
3.8.1	Sequential assignment.....	77
3.9	Quantitative Analysis	83
3.9.1	a) Cross-relaxation rates.....	84
3.9.2	b) J-coupling constants.....	84
3.10	Conclusion	84
4	Solution Structure Refinement using Complete Relaxation Matrix Analysis of 2D NOE Experiments: DNA Fragments	87
4.1	Introduction.....	87
4.2	Distance Information from 2D NOE Experiments	91
4.2.1	Isolated Spin Pair Approximation (ISPA).....	93
4.2.2	Complete Relaxation Matrix Analysis (CORMA).....	98

4.2.2.1	Direct Calculation of Distances (DIRECT).....	103
4.2.2.2	Structural Refinement using COMATOSE.	106
5	NMR Studies of Proteins, Nucleic Acids and their Interactions.....	113
5.1	Introduction.....	113
5.2	Biomolecular Structures from NMR.....	114
5.2.1	¹ H resonance assignments.....	115
5.2.2	Distance and Dihedral Angle Constraints.....	117
5.2.3	Structure Calculations Based on Geometric Constraints (Distance-geometry, Distance Bounds Driven Dynamics)	118
5.2.4	Structure Refinement Including Energy Terms (Restrained Energy Minimization and Molecular Dynamics)	120
5.3	Iterative Relaxation Matrix Approach (IRMA).....	122
5.3.1	Theory	123
5.3.2	The IRMA Cycle.....	125
5.4	Protein-DNA Interaction	128
5.4.1	Lac repressor headpiece structure.....	128
5.4.2	Lac Headpiece-operator Complexes	130
5.4.3	A Structural Model for the Headpiece-Operator Complex.....	135
6	³¹P and ¹H 2D NMR and NOESY-Distance Restrained Molecular Dynamics Methodologies for Defining Structure and Dynamics of Wild-Type and Mutant Lac Repressor Operators. Sequence-Specific Variations in Double Helical Nucleic Acids.	141
6.1	Introduction.....	141
6.2	Structural Studies of Oligonucleotides by 2D ¹ H NMR.....	142
6.2.1	Restrained Molecular Mechanics Calculations of Duplex Geometries.....	146
6.3	³¹ P NMR of Nucleic Acids; Sequence-Specific Variations in Structure.....	151
6.3.1	¹⁷ O-Labeling methodology for Assigning ³¹ P Signals of Oligonucleotides.....	152
6.3.2	PAC 2D ³¹ P/ ¹ H Heteronuclear Correlated Spectra of Oligonucleotides.....	155

6.3.3	DOC 2D $^{31}\text{P}/^1\text{H}$ Heteronuclear Correlated Spectra of Oligonucleotides.....	159
6.3.4	Variation of ^{31}P Chemical Shifts in Oligonucleotides.....	163
6.3.5	Sequence-Specific Variation in ^{31}P Chemical Shifts, Calladine Rules	164
6.3.6	^{31}P Chemical Shifts and Calladine Rules.....	170
6.3.7	Origin of ^{31}P Chemical Shift Variations and Calladine Rules.....	172
6.3.8	Molecular Mechanics Energy Minimization Calculation of the Sequence-Specific Variation in Deoxyribose Phosphate Backbone Conformation	173
6.3.9	Sequence-Specific Variation of ^{31}P Chemical Shifts and Backbone Torsional Angles.....	175
6.4	Binding of Lac Operator to Lac Repressor Headpiece Protein.....	179
6.5	DNAase I; Significance of the Local Variation in Phosphate Ester Geometry?.....	181
6.6	Conclusions.....	182
7	NMR Studies of Dynamic Processes and Multiple Conformations in Protein-ligand Complexes.....	189
7.1	Introduction.....	189
7.2	Background.....	190
7.3	Dynamic Processes in Protein-Ligand Complexes	191
7.4	Detection of Multiple Conformations.....	195
7.4.1	Group A. Complexes with Pyrimethamine Analogues.....	196
7.4.2	Group B. Complexes with the Substrate Folate.....	198
7.4.3	Group C. Complexes with Analogues of Trimethoprim and NADP ⁺	201