

Two-Dimensional NMR Spectroscopy

**Applications For
Chemists and Biochemists**

Edited by
**William R. Croasmun
and Robert M.K. Carlson**



Contents

1. Introduction to Two-Dimensional NMR Methods	1
<i>George A. Gray</i>	
Introduction	1
What Are the Benefits of 2D NMR?	2
Can It Be Painless?	5
How Do I Set Up the 2D NMR Experiment?	5
How Much Sample Do I Need for a 2D NMR Experiment?	6
How Do I Run the 2D NMR Experiment?	6
How and Why Does 2D NMR Work?	10
Preparation	10
Evolution	10
Mixing	10
Detection	11
How Do Pulses Affect Nuclear Spins?	15
The Rotating Frame of Reference	16
The 90° Pulse	17
Precession in the XY Plane	19
Refocusing Pulses	20
Simultaneous A and X 180° Pulses in an AX Coupled System	21
Nonobservable Magnetization and Mixing of Spin States by 90° Pulses	22
Polarization Transfer Pulses	24
The General 2D NMR Experiment	27
Practical Details for the General 2D NMR Experiment	27
Display Modes	29
Data Processing	31
Can I Use What I Already Know About NMR in 2D NMR?	32
Can I Use <i>J</i> -Coupling Information?	33
Data Output in 2D <i>J</i> -NMR	37
Sensitivity in 2D <i>J</i> -NMR	37
Can I Do Selective Experiments on Specific Protons?	38
How Can I Emphasize Long-Range Coupling Information?	38
How Can I Correlate Protons That Are Coupled to Each Other?	40
Data Display and Output	42
How Large a Coupling Is Necessary to Produce a Crosspeak?	44
But What about the Very Small Coupling?	44
How Can I Simplify a Very Crowded COSY Spectrum?	46
Can I Use the Power of ¹³ C to Understand My Proton Spectrum?	47
How Do I Interpret a HECTOR Spectrum?	49

CONTENTS

My Proton Spectrum Is Too Crowded for Homonuclear 2D J-Analysis. Can I Get the Information Some Other Way?	51
Can I Correlate My Carbons with Protons Other Than the Bonded Ones?	52
What about Other Nuclei?	56
Is There Any Way to Relate Three Nuclei at Once?	57
How Can I Learn about Nuclei That Are Not Coupled?	59
The Protons Are Really Peripheral to the Carbon Skeleton. How Can I Determine Directly the Carbon Bonding Framework in My Molecule?	61
My Sample Is Too Small to Use Heteronuclear 2D Shift-Correlation or Double-Quantum Connectivity. Can I Still Get Connectivity Information for Nuclei Other Than Protons?	64
 2. Experimental Aspects of Two-Dimensional NMR	67
<i>William E. Hull</i>	
Introduction: 2D NMR for the Common Man and Woman	67
The Spectrometer	69
Basic Hardware Required for Homonuclear 2D NMR	69
Additional Requirements for Heteronuclear 2D	73
Useful Features for Better Performance and More Sophisticated Applications	75
The Data System	77
Minimal Hardware Requirements	77
Software Requirements	80
Useful Additional Features	84
Spectrometer Calibration and Performance Tests	85
Probehead Tuning	85
Pulse Calibration	87
Sensitivity Tests	90
Phase Shifting	90
The Quadrature Receiver	91
The Sample	93
Optimum Sample Preparation	93
How Much Sample is Needed?	94
Preparatory 1D Experiments	95
Temperature Control and Shimming	95
Pulse Calibration	99
Estimation of Relaxation Parameters	99
A High-Resolution Reference Spectrum	101
Optimizing the Spectral Width for 2D	101

CONTENTS

The Basic 2D Experiment	102
The Time Domains of 2D NMR	102
2D Data Acquisition Parameters	105
Techniques and Parameters for 2D Processing	109
Plotting of 2D Data	121
Quadrature Detection, F_1 Noise, and Artifacts in 2D NMR	122
Quadrature Detection	122
F_1 Noise	128
Artifacts in 2D NMR	132
Common Homonuclear ^1H 2D	140
Two-Pulse Sequences	140
A Three-Pulse Sequence: Shift Correlation via Dipolar Interaction (NOE) or Chemical Exchange	153
Solvent Suppression	162
Common Heteronuclear 2D Experiments	169
Heteronuclear J -resolved 2D NMR	169
Heteronuclear Shift-correlated 2D NMR	178
Shift Correlation with Elimination of Proton-Proton Couplings	185
Heteronuclear Shift Correlation via Dipolar Interactions	188
Setting Up Heteronuclear Correlation Experiments	189
The INADEQUATE Experiment	191
1D INADEQUATE	191
INADEQUATE 2D for J -Couplings	195
INADEQUATE for 2D Shift-Correlation	195
Symmetrical INADEQUATE	197
Compensated INADEQUATE	198
Detection of Long-Range Connectivities	199
Optimized Heteronuclear Polarization Transfer via Long-Range J (COLOC)	199
Local-Remote Discrimination and Observation of $^nJ_{\text{XH}}$	201
Homonuclear Relayed Coherence Transfer	203
Multistep RCT	205
Homonuclear NOESY with RCT	206
Heteronuclear Relayed Coherence Transfer	209
Further Variations and Specialised Techniques	215
Homonuclear COSY, NOESY Variations	215
Homonuclear Correlation with F_1 Decoupling	217
Homonuclear 2D with Multiple-Quantum Filtering (MQF)	218
Multiple-Quantum Spectroscopy	220
“Reverse” Experiments: Heteronuclear Correlation with ^1H Observation	223

CONTENTS

3. Strategies for Applying Combinations of Two-Dimensional NMR Experiments **233**

Michael A. Bernstein

Introduction	233
General	234
Strengths and Limitations of Some 1D NMR Experiments	236
Double Resonance	236
Relaxation Methods	237
Spectral Editing and Multiplicity Sorting	237
Carbon Connectivity Determination	237
A Brief Summary of Some Useful 2D NMR Experiments	238
Scalar Coupling Information	238
Homonuclear Chemical Shift Correlation	239
Heteronuclear Chemical Shift Correlation	242
Cross-Relaxation	244
Strategies for Integration of 2D NMR Experiments	245
Performing Proton Two-Dimensional Experiments—A Pragmatic Approach	246
Strategy 1. ^1H NMR Spectral Assignment Only	247
Strategy 2. ^1H and ^{13}C NMR Spectral Assignment	249
Strategy 3. Oligomer Sequencing and Structure Elucidation	251
Strategy 4. Aromatic Compounds	252
Conclusions	253

4. Two-Dimensional NMR Spectroscopy on the Immunosuppressive Peptide Cyclosporin A **259**

Horst Kessler, Hartmut Oschkinat and Hans-Rudolf Loosli

Introduction	259
General Remarks on Structure Elucidation by NMR Spectroscopy	261
Data Presentation	265
Proton Assignments via Homonuclear Techniques	266
General Considerations	266
Assignment Strategy	267
Homonuclear Correlation Spectroscopy (H,H-COSY)	273
COSY 90/45	275
COSY in the Phase-Sensitive Mode	276
H,H-COSY with Delay	277
Relayed Homonuclear Correlation Spectroscopy: $\text{H-Relayed H,H-COSY}$	278
Proton Homonuclear Double-Quantum Spectroscopy	279

CONTENTS

Sequence Assignment	280
Extraction of Parameters	281
J , δ -Spectroscopy	281
Differences and Sums within COSY Spectra (DISCO)	281
Nuclear Overhauser and Exchange Spectroscopy (NOESY)	285
Carbon, Nitrogen, and Proton Assignment via Heteronuclear Techniques	286
Carbon Assignment	287
The COLOC Technique Applied to the Aliphatic Carbons	289
2D INADEQUATE Technique	292
Comparison of the Techniques Used to Obtain Carbon Connectivities in Peptides	294
Nitrogen Assignments	295
Conclusion	297

5. Internal Motion and Structure of DNA **301**

David R. Kearns

Introduction	301
Proton Relaxation Process in DNA	303
Relaxation in a Two Spin System	304
Comparison of 1D and 2D NOE Relaxation Methods	305
Spectral Densities	308
Internal Motion in DNA	309
DNA Structural Features	313
A Strategy for Structure Determination	316
NMR Studies of Simple-Sequence DNAs	324
The Structure of Poly(dA-dT)·Poly(dA-dT)	325
Structure of Poly(dA)·Poly(dT)	329
Structure of Poly(dI-dC)·Poly(dI-dC)	333
Structure of Poly(dI-dbr ⁵ C)	333
The B- and Z-Forms of Poly(dG-dC)	333
Structure of Poly(dm ⁵ C-dG)	333
The Z-form of d(br ⁵ C-G-br ⁵ C-G-A-T-br ⁵ C-G)	338
Structure of Poly(dNH ₂ A-dT)	340
Conformation of r(CGCGCG) ₂	340
Qualitative 2D NOE Studies of Short DNA Duplexes with Complex Sequences	340
Quantitative Determination of DNA Structures	341
Concluding Remarks	342

6. Application of Two-Dimensional NMR Methods in the Structural Analysis of Oligosaccharides and Other Complex Carbohydrates 349

Janusz Dabrowski

Introduction	349
Identification of Sugar Residues in Oligosaccharides: Mapping Intra- residue Spin Coupled Networks	351
Resolving Overlapping Multiplets with Homonuclear <i>J</i> -Resolved Spectroscopy: A Method of Limited Utility for Oligosaccharides	351
Determining Scalar Coupling Connectivities between Sugar Protons by Homonuclear ^1H - ^1H Shift-Correlated Spectroscopy: A Method of Particular Importance to Oligosaccharides	354
Determination of Oligosaccharide Sequences and Interresidue Linkage Positions	366
2D NOE	366
Using Combinations of 1D and 2D Methods	367
Heteronuclear ^1H - ^{13}C Shift-Correlated Spectroscopy	370
Identification and Analysis of Mixtures of Oligosaccharides	373
Polysaccharides	381
Conclusions	383

7. Steroid Structural Analysis by Two-Dimensional NMR 387

William R. Croasmun and Robert M. K. Carlson

Introduction	387
Connectivity Diagrams for Use with Steroid 2D NMR Data	392
Primary Considerations in Sample-Limited Steroid Studies	394
Field Strength	394
Solvent- and Lanthanide-induced Shifts	397
Pertinent 1D NMR Methods: Difference Spectroscopy and Spin- Lattice Relaxation Studies	399
Difference NOE	399
Partial Relaxation Studies and Relative Spin-Lattice Relaxation Rate Measurements	403
Disentangling Severely Overlapping Steroid ^1H Resonances: Homo- nuclear <i>J</i> -resolved Spectroscopy	404
Unraveling ^1H - ^1H Spin Coupled Networks in Steroids: COSY	412
Identifying Spatially Close but Uncoupled ^1H Nuclei in Steroid Nuclear and Side Chain Systems: NOESY?	417
Carbon Spectra/Heteronuclear Methods Applied to Steroids	418
Generalized 2D NMR Strategies for Steroids	421

8. Applications of Two-Dimensional NMR to the Characterization of Organic Compounds: Relative Configurational Assignment of a Key Synthetic Precursor to Spatol	425
<i>Peter L. Rinaldi</i>	
Introduction	425
Results and Discussion	429
Structure Solution by High-Field NMR	429
Structure Solution by Low-Field NMR	437
Conclusion	443
9. Two-Dimensional NMR Experiments in Natural Products Chemistry: Biological and Geochemical Applications	445
<i>Gary E. Martin</i>	
Introduction	445
Biologically Derived Natural Products—Alkaloids and Terpenes	447
Assignment of the Side Chain Proton Resonances of an Analogue of Secogorgosterol: Power of the COSY Experiment	447
Assignment of the ^{13}C NMR Spectrum of the Cembranoid Diterpene Eunicin	451
Elucidation of the Structure of Plumericin: COSY or Proton Double-Quantum Spectral Methods Used in Combination with Heteronuclear Chemical Shift Correlation	455
Determination of the Structure of Isopteropodine: Heteronuclear Relayed Coherence Transfer (RELAY)	461
Geochemically Derived Materials—Polynuclear Aromatic Thiophenes	469
Specific Difficulties Inherent in the Spectral Assignment of Phenanthro[3,4-b]thiophene and its Homologues	471
Development of an Assignment Strategy	471
^1H and ^{13}C NMR Spectral Assignment	472
Extension of the Chemical Shift Behavior Observed in Phenanthro[3,4-b]thiophene to Benzo[b]phenanthro[4,3-d]thiophene and Phenanthro[4,3-a]dibenzothiophene: The Successes and Failures of the Approach	479
An Overview of the Prospects for Further Developments in the Application of Two-Dimensional NMR Spectroscopy to Complex Problems of Natural-Products Chemistry	490
Modifications of Existing Experiments	491
New Two-Dimensional NMR Experiments	491

CONTENTS

Glossary 497

Index 501