

Contents

Preface *XXI*

List of Contributors *XXIII*

List of Abbreviations *XXIX*

Part One Introduction 1

1 NMR and its Place in Mechanistic Systems Biology 3

Ivano Bertini, Kathleen S. McGreevy, and Giacomo Parigi

2 Structure of Biomolecules: Fundamentals 7

2.1 Structural Features of Proteins 7

Lucia Banci and Francesca Cantini

2.1.1 Introduction: From Primary to Quaternary Structure 7

2.1.2 Geometrical and Conformational Properties 8

2.1.2.1 Backbone Dihedral Angles 8

2.1.2.2 Side-Chain Dihedral Angles 9

2.1.3 Secondary Structure Elements in Proteins 9

2.1.4 Prediction of Secondary Structure 13

2.1.5 Structural Motifs and Structural Domains – Combination of Secondary Structural Elements and Structural Motifs 13

2.1.6 Types of Folds and their Classification 15

2.1.6.1 Folds of the α Class 15

2.1.6.2 Folds in the β Class 16

2.1.6.3 Folds in the α/β Class 17

2.1.6.4 Folds in the $\alpha + \beta$ Class 17

2.1.7 Tertiary Structure 18

2.1.8 Quaternary Structure 19

2.2 Nucleic Acids 21

Mirko Cevec, Hendrik R.A. Jonker, Senada Nozinovic, Christian Richter, and Harald Schwalbe

2.2.1 Introduction 21

2.2.1.1 Conformations 22

2.2.2 DNA Structure 24

2.2.2.1 B-DNA and Derivatives 24

2.2.2.2 A-DNA 25

2.2.2.3 Z-DNA 25

2.2.2.4 Nonstandard DNA Structures 25

2.2.2.4.1 Circular DNA 25

2.2.2.4.2 Helical Junction 26

2.2.2.4.3 Triple Helix 26

2.2.2.4.4 i-Motif 26

2.2.2.4.5	Quadruplex DNA	26
2.2.3	RNA Structure	27
2.2.3.1	Regular RNA Structure – A-Form Helices	28
2.2.3.2	Mismatches, Bulges, and Unusual Base Pairing	29
2.2.3.3	Reversal and Alteration of Strand Direction: Commonly Observed Loop and Turn Motifs	29
2.2.3.3.1	U-Turn	29
2.2.3.3.2	K-Turn	29
2.2.3.3.3	C-Loop	29
2.2.3.3.4	E-Loop	30
2.2.3.4	Tetraloops and Tetraloop–Receptor Contact	30
2.2.3.5	Higher-Order RNA Tertiary Structure Elements: Coaxial Stacking Motifs	31
2.2.3.6	DNA–RNA Hybrids	31
3	What Can be Learned About the Structure and Dynamics of Biomolecules from NMR	33
3.1	Proteins Studied by NMR	33
	<i>Lucio Ferella, Antonio Rosato, and Paola Turano</i>	
3.1.1	Why NMR Structures?	33
3.1.2	NMR Bundle	37
3.1.3	Protein Dynamics	41
3.1.4	Intermolecular Interactions Involving Proteins	44
3.2	Nucleic Acids Studied by NMR	47
	<i>Janez Plavec</i>	
3.2.1	Structure, Mobility, and Function	47
Part Two	Role of NMR in the Study of the Structure and Dynamics of Biomolecules	51
4	Determination of Protein Structure and Dynamics	53
	<i>Lucio Ferella, Antonio Rosato, and Paola Turano</i>	
4.1	Determination of Protein Structures	53
4.1.1	Resonance Assignment	53
4.2	NMR Restraints	58
4.2.1	Distance Restraints	58
4.2.2	Dihedral Angles	59
4.2.3	Residual Dipolar Couplings	61
4.3	Structure Calculations	65
4.3.1	Traditional	65
4.3.2	Automated NOESY Assignment	69
4.3.3	Energy Refinement of Protein Structures	70
4.3.4	Chemical Shift-Based Approaches for Protein Structure Determination	71
4.4	Validation of Protein Structures	72
4.4.1	Experimental Data	72
4.4.2	Geometric Quality	74
4.5	Protein Dynamics and NMR Observables	76
4.5.1	NMR Observables Affected by Dynamics	76
4.5.2	NMR Experiments to Measure Dynamics and their Interpretation	78
4.6	Protocols	83
4.6.1	Sample Labeling	83
4.6.2	NMR Assignment	83
4.6.3	Manual Collection of Restraints	86
4.6.4	Structure Calculations	87
4.6.5	Structure Refinement	89

4.6.6	Chemical Shift-Based Structure Calculations	90
4.6.7	Structure Validation	90
4.6.8	Protein Dynamics	91
4.7	Troubleshooting	92
4.7.1	Data Collection	92
4.7.2	Structure Calculations	93
	Further Reading	94
5	DNA	97
	<i>Janez Plavec</i>	
5.1	NMR Spectroscopy of DNA	97
5.2	Assessment of the Folding Topology	99
5.3	Resonance Assignment through Sequential and Interstrand Interactions	100
5.4	Pseudorotation of Deoxyribofuranose Rings	104
5.5	Backbone Conformation	105
5.6	Natural Abundance Nucleobase Substitutions	106
5.7	Natural Abundance Heteronuclear Experiments	106
5.8	Site-Specific Low Isotopic Enrichment	107
5.9	Translational Diffusion Coefficients	107
5.10	Determination of Three-Dimensional Structure	107
5.11	Search for Transient Structures	109
5.12	Protocols	110
5.12.1	Sample Preparation and Initial NMR Experiments	110
5.12.2	Stoichiometric Analysis through Translational Diffusion	111
5.12.3	Sequential Assignment	111
5.12.4	Assessment of the Preferred Sugar Pucker	112
5.12.5	Conformations along the Backbone	112
5.12.6	Nucleobase Substitutions and Site-Specific Low $^{13}\text{C}/^{15}\text{N}$ Isotopic Enrichment	112
5.12.7	Topology and Atomic-Detail Three-Dimensional Structure Determination	113
5.13	Example Experiments and Troubleshooting	114
	Further Reading	115
6	RNA	119
	<i>Richard Štefl and Vladimír Sklenář</i>	
6.1	NMR Spectroscopy of RNA	119
6.2	Preparation of RNA Samples for NMR	120
6.2.1	<i>In Vitro</i> Transcription Using T7 RNA Polymerase	120
6.2.2	<i>In Vivo</i> Recombinant RNA Synthesis	120
6.2.3	Chemical Synthesis	120
6.2.4	Segmental Labeling	121
6.3	Probing of the RNA Fold	121
6.4	Assessment of the Spectral Resolution	122
6.5	Strategy for the Resonance Assignment	123
6.5.1	Hydrogen Bond Formation and Base Pair Identification	123
6.5.2	Through-Bond-Type Experiments – Base Spin System Identification	124
6.5.3	Through-Bond-Type Experiments – Sugar Spin System Identification	124
6.5.4	Sequential Connectivities	125
6.6	Collection of Structural Information	126
6.6.1	Collection of Distance-Dependent Structural Restraints	126
6.6.2	Collection of Torsion-Angle-Dependent Structural Restraints	127
6.6.3	Collection of Long-Range Structural Restraints	127
6.7	Structural Calculation of RNA	128
6.8	Assessment of Quality of NMR Structures	129
6.9	Protocols	129

6.9.1	Flowcharts	130
6.9.1.1	Sample Preparation	130
6.9.1.2	Collection of the Experimental Data	130
6.9.1.3	Structure Calculation	131
6.9.2	Protocol for <i>In Vitro</i> Transcription Using Bacteriophage T7 RNA Polymerase	132
6.9.2.1	Template Design	132
6.9.2.2	Transcription Protocol	133
6.9.2.3	Purification of RNA	134
6.9.2.4	Simple Protocol for Bacteriophage T7 RNA Polymerase Preparation	134
6.10	Troubleshooting	135
	Further Reading	135
7	Intrinsically Disordered Proteins	137
	<i>Isabella C. Felli, Roberta Pierattelli, and Peter Tompa</i>	
7.1	Intrinsically Disordered Proteins	137
7.1.1	When the Concept was First Introduced	137
7.1.2	Functions and Functional Advantages Associated with Disorder	138
7.2	Importance of NMR to Study IDPs	140
7.3	Structural and Dynamic Information on IDPs – NMR Observables	141
7.3.1	Reduced Chemical Shift Dispersion and Sequence-Specific Assignment	141
7.3.2	Chemical Shifts and Secondary Structural Propensities	142
7.3.3	Additional Observables and Conformational Averaging	143
7.3.4	Scalar Couplings	143
7.3.5	Residual Dipolar Couplings	143
7.3.6	Solvent Exposure	144
7.3.7	¹⁵ N Relaxation and Heteronuclear NOEs	144
7.3.8	Paramagnetic Relaxation Rate Enhancements	145
7.3.9	Proton–Proton NOEs	145
7.3.10	Relevance of Structural Disorder in <i>In Vivo</i> and In-Cell Studies of IDPs	146
7.4	Protocols	146
7.4.1	Use of Bioinformatic Tools and Databases	146
7.4.2	Use of NMR in the Characterization of IDPs	148
7.5	Troubleshooting	150
7.5.1	Bioinformatics Can Help!	150
7.5.2	Understanding the Function of Unfolded Protein Regions	151
7.5.3	Does <i>In Vitro</i> Reflect <i>In Vivo</i> Behavior?	152
	Further Reading	152
8	Paramagnetic Molecules	155
	<i>Ivano Bertini, Claudio Luchinat, and Giacomo Parigi</i>	
8.1	Paramagnetism-Assisted NMR	155
8.2	Scalar and Dipolar Electron Spin–Nuclear Spin Interactions: Hyperfine Shift	157
8.2.1	Contact Contributions to the Hyperfine Shift	157
8.2.2	Pseudocontact Contributions to the Hyperfine Shift	158
8.3	Scalar and Dipolar Electron Spin–Nuclear Spin Interactions: PRE	159
8.4	Indirect Electron Spin–Nuclear Spin Effects: Paramagnetism-Induced RDCs	161
8.5	Cross-Correlation Between Curie and Dipolar Relaxation	162
8.6	“Good” Metal Ions and “Bad” Metal Ions	163
8.7	Paramagnetism-Based Drug Discovery	164
8.8	Protocols	165
8.8.1	Collecting the Paramagnetism-Based Restraints	165
8.8.2	Protocols to Extract Structural Information from the PCS, PRDC, PRE and PCCR	166

8.8.3	Protocols for Protein–Protein Interactions	167
8.8.4	Protocols for the Analysis of Conformational Freedom in Two-Domain Proteins	168
8.8.5	Example Experiment	169
8.9	Troubleshooting	169
8.9.1	Tips and Tricks to Optimize Signal Detection in Paramagnetic Systems	169
8.9.2	Selection of the Paramagnetic Ion	170
8.9.3	Switching Between Diamagnetic and Paramagnetic Systems	170
	Further Reading	170

Part Three Role of NMR in the Study of the Structure and Dynamics of Biomolecular Interactions 173

9	NMR Methodologies for the Analysis of Protein–Protein Interactions	175
	<i>Tobias Madl and Michael Sattler</i>	
9.1	Introduction	175
9.2	Dynamics and Ligand Binding	176
9.3	General Strategy	177
9.4	Overview of Methods	178
9.4.1	Sample Preparation	178
9.4.2	Structures of Domains/Subunits	179
9.4.3	Interfaces	180
9.4.3.1	Chemical Shift Perturbations (CSPs)	180
9.4.3.2	NOEs	180
9.4.3.3	Cross-Saturation	181
9.4.3.4	Differential Line-Broadening	181
9.4.3.5	Hydrogen Exchange	182
9.4.3.6	Solvent PREs	182
9.4.4	Domain/Subunit Orientation	183
9.4.4.1	NMR Relaxation Data	183
9.4.4.2	Residual Dipolar Couplings (RDCs)	184
9.4.4.3	Paramagnetic Restraints	184
9.4.5	Structure Calculations	185
9.5	Outlook	186
9.6	Protocols for the Analysis of Protein Complexes	186
9.6.1	Spin Labeling and Paramagnetic Tagging	186
9.6.2	Structures of the Individual Domains/Subunits	188
9.6.3	Optimizing Conditions for Structural Studies of the Protein Complex	189
9.6.4	Detection of Dynamics	189
9.6.5	Determining Interaction Interfaces Using Solvent PREs	189
9.6.6	Structure Calculation Approach	191
9.6.7	Example Experiment	193
9.7	Troubleshooting	194
	Further Reading	194
10	Metal-Mediated Interactions	197
	<i>Simone Ciofi-Baffoni</i>	
10.1	Theoretical Background	197
10.2	Protocol for the Structural Determination of a Metal-Mediated Complex	200
10.2.1	Optimization of Experimental Conditions to Obtain the Protein–Protein Complex	200
10.2.2	Titration to Map Protein–Protein Interfaces and Obtain Binding Characteristics	201
10.2.3	Modeling	201
10.2.4	Definition of Metal Coordination	201
10.2.5	Determination of Three-Dimensional Structure	202

10.3	Example Experiment	202
10.4	Troubleshooting	202
	Further Reading	203
11	Protein–Paramagnetic Protein Interactions	205
	<i>Peter H.J. Keizers, Yoshitaka Hiruma, and Marcellus Ubbink</i>	
11.1	Paramagnetic Sources in Protein Complexes	205
11.2	Types of NMR Restraints Obtained from Paramagnetic Centers	206
11.3	Protein Complexes	207
11.3.1	Structures of Protein Complexes	207
11.3.2	Dynamics in Protein Complexes	208
11.3.2.1	Plastocyanin and Cytochrome <i>f</i>	209
11.3.2.2	Cytochrome <i>c</i> and Cytochrome <i>c</i> Peroxidase	209
11.3.2.3	Histidine Phosphocarrier Protein and Enzyme I	209
11.3.2.4	Adrenodoxin and Cytochrome <i>c</i>	210
11.3.2.5	Calmodulin Domain Dynamics	211
11.4	Protocols	211
11.4.1	Protein Titrations to Obtain the K_d and a Binding Map	211
11.4.2	Paramagnetic Tagging and Use of Paramagnets in NMR	212
11.4.3	Ensemble Modeling	214
11.5	Example Experiment	215
11.6	Troubleshooting	215
	Further Reading	217
12	Protein–RNA Interactions	219
	<i>Vijayalaxmi Manoharan, Jose Manuel Pérez-Cañadillas, and Andres Ramos</i>	
12.1	Introduction	219
12.1.1	Post-Transcriptional Regulation and RNA Recognition Domains	219
12.1.2	Protein–RNA Interfaces	219
12.2	NMR Methodology	221
12.2.1	Using NMR to Investigate Protein–RNA Interactions	221
12.2.2	Mapping Interaction Sites	222
12.2.2.1	Chemical Shift Perturbation	222
12.2.2.2	Cross-Saturation	223
12.2.2.3	Paramagnetic Relaxation Enhancement	224
12.2.3	Affinity and Specificity	226
12.2.3.1	Determining Dissociation Constants	226
12.2.3.2	Determining Stoichiometry	226
12.2.3.3	Scaffold-Independent Analysis	227
12.2.4	High-Resolution Structure Determination and Dynamics	227
12.3	Protocols and Troubleshooting	228
12.3.1	Preparation of Protein–RNA Complexes	228
12.3.1.1	Materials and Sample Preparation	229
12.3.1.2	Troubleshooting	229
12.3.2	Protocol and Example Experiment 1: Calculating the Affinity of a Protein–RNA Interaction	230
12.3.2.1	Materials and Sample Preparation	230
12.3.2.2	Troubleshooting	231
12.3.3	Protocol and Example Experiment 2: Paramagnetic Labeling	232
12.3.3.1	Materials and Sample Preparation	233
12.3.3.2	Troubleshooting	233
12.3.4	Protocol and Example Experiment 3: SIA	234
12.3.4.1	Materials and Sample Preparation	234
12.3.4.2	Troubleshooting	235
	Further Reading	235

13	Protein–DNA Interactions	239
	<i>Lidija Kovačič and Rolf Boelens</i>	
13.1	State of the Art	239
13.2	Conclusions and Perspectives	242
13.3	Protocols	243
13.3.1	Sample Preparation	243
13.3.2	NMR Methodology	244
13.3.3	Identification of the Interaction Surfaces	247
13.3.4	Structure Calculations	250
13.4	Troubleshooting	251
	Further Reading	252
Part Four	NMR in Drug Discovery	253
14	High-Throughput Screening and Fragment-Based Design: General Considerations for Lead Discovery and Optimization	255
	<i>Maurizio Pellecchia</i>	
14.1	High-Throughput Screening and Fragment-Based Design	255
14.2	General Aspects of NMR Spectroscopy in Hit Identification and Optimization Processes	257
14.3	Chemical Shift Perturbation as a Screening Method	261
	Further Reading	263
15	Ligand-Observed NMR in Fragment-Based Approaches	265
	<i>Paweł Śledź, Chris Abell, and Alessio Ciulli</i>	
15.1	Ligand-Observed NMR Spectroscopy	265
15.2	On the Transient Binding of Small Molecules to the Protein	266
15.3	Questions Asked by Ligand-Based Fragment Screening	267
15.3.1	Direct Yes/No Binding Experiments	268
15.3.1.1	STD	269
15.3.1.2	WaterLOGSY	270
15.3.1.3	Relaxation-Edited One-Dimensional Experiments	271
15.3.2	Affinity Measurements and Affinity-Oriented Screening	271
15.3.3	Information on the Binding Mode	272
15.4	Summary	274
15.5	Protocols	274
15.5.1	General Aspects of Sample Preparation	274
15.5.2	Preparing Samples	275
15.5.3	Pulse Sequences	276
15.5.4	Automation	276
15.6	Example Experiments	276
15.6.1	One-Dimensional Direct Binding Experiments	276
15.6.2	Competition NMR Screening Experiments	277
15.6.3	Two-Dimensional ILOE Binding Experiments	278
15.7	Troubleshooting	278
15.7.1	Sample Preparation	278
15.7.2	Experimental Setup	280
	Further Readings	280
16	Interactions of Metallodrugs with DNA	283
	<i>Hong-Ke Liu and Peter J. Sadler</i>	
16.1	Metallodrugs and DNA Interactions	283
16.1.1	Metallodrugs	283
16.1.2	General Features of Metallodrug–DNA Interactions	284
16.1.3	NMR Techniques and Metallodrug–DNA Interactions	285
16.2	Coordinative Binding	286
16.3	Groove Binding	289

16.3.1	Major Groove Binding	289
16.3.2	Minor Groove Binding	289
16.4	Intercalation and Insertion	290
16.4.1	Metallo-Intercalators	290
16.4.2	DNA Insertion and Metallo-Inserters	291
16.5	Dual Binding (Coordination and Intercalation)	291
16.5.1	Intercalation by Metallodrug with σ -Bonded Intercalator	291
16.5.2	Intercalation by Metallodrug with π -Bonded Intercalator	292
16.6	Protocols	293
16.6.1	Determination of the Coordinative Binding Sites	293
16.6.2	Detection of Intercalation and Insertion	294
16.6.3	Detection of Metallodrug–DNA Groove-Binding Interactions	294
16.6.4	Example Experiment	294
16.7	Tricks and Troubleshooting	294
	Further Reading	295
17	RNA as a Drug Target	299
	<i>Jan-Peter Ferner, Elke Duchardt Ferner, Jörg Rinnenthal, Janina Buck, Jens Wöhnert, and Harald Schwalbe</i>	
17.1	RNA as a Target for Small Molecules	299
17.2	Chemical Shift Perturbation and Paramagnetic Relaxation Enhancement	301
17.3	Nuclear Overhauser Effect-Based Methods	304
17.4	Fluorine Labeling of RNA	304
17.5	Ligand-Based Methods	305
17.6	Protocols	306
17.6.1	Determination of the Cooperativity Between Mg^{2+} -Binding Sites in RNA by CSP Analysis	306
17.6.2	Ligand-Binding Site Mapping by CSP Tracking	308
17.6.3	Mapping Mg^{2+} -Binding Sites in RNA Using $MnCl_2$ PRE Experiments	308
17.6.4	Ligand-Binding Site Mapping by NOE Methods	308
17.6.5	Mapping Outer Sphere Mg^{2+} -Binding Sites by Co $(NH_3)^{3+}_6$ NOESY Experiments	311
17.7	Troubleshooting	312
	Further Reading	313
18	Fluorine NMR Spectroscopy for Biochemical Screening in Drug Discovery	315
	<i>Claudio Dalvit</i>	
18.1	Enzymatic Inhibition Mechanisms	316
18.2	<i>n</i> -FABS	317
18.2.1	One Substrate and One Enzyme	318
18.2.2	One Substrate and One Enzyme in the Presence of Serum Albumin and/or Enzymes of the Cytochrome P450 Superfamily	318
18.2.3	One Substrate with Multiple Enzymes	319
18.2.4	Multiple Substrates with One Enzyme	320
18.2.5	Multiple Substrates with Multiple Enzymes	320
18.2.6	Application to Functional Genomics	321
18.3	Comparison of <i>n</i> -FABS with Other Biophysical Techniques	322
18.4	Outlook	323
18.5	Protocols	323
18.5.1	Protocol for Setup of the <i>n</i> -FABS Assay	323
18.5.2	Protocol for a Screening Run with <i>n</i> -FABS	324
18.5.3	Protocol for Measuring the IC_{50} of Identified Inhibitors	325
18.5.4	Example Experiment	326

18.6	Troubleshooting	326
	Further Reading	327
19	NMR of Peptides	329
	<i>Johannes G. Beck, Andreas O. Frank, and Horst Kessler</i>	
19.1	Introduction	329
19.2	Resonance Assignment	330
19.3	Stereostructure and Conformational Restraints	330
19.3.1	NOEs and ROEs	331
19.3.2	3J Coupling Constants	332
19.3.3	Residual Dipolar Couplings	332
19.4	Structure Calculation	333
19.5	Importance of Peptide Conformations for Biological Activity	334
19.6	Protocols	334
19.6.1	Resonance Assignment	334
19.6.2	ROESY: Extraction of Accurate Distances	336
19.6.3	3J Couplings: Use in Structure Determination and Useful Experiments	337
19.6.4	Modeling of the Structure	339
19.6.4.1	Distance Geometry	340
19.6.4.2	MD	340
19.7	Troubleshooting	342
	Further Reading	343
Part Five	Solid-State NMR	345
20	Biomolecular Solid-State NMR/Basics	347
	<i>Emeline Barbet-Massin and Guido Pintacuda</i>	
20.1	Introduction	347
20.2	NMR Hamiltonian	347
20.3	Magic Angle Spinning	349
20.4	Cross-Polarization	350
20.5	Heteronuclear 1H Decoupling	350
20.6	Dipolar Recoupling	351
20.7	Recent Progress: New Probes – Ultrafast MAS – High Magnetic Fields	354
20.8	Protocols	355
20.8.1	Hardware Setup	355
20.8.2	Magic Angle Setting	356
20.8.3	Adamantane	356
20.8.3.1	MAS Spinning	356
20.8.3.2	Calibrate the 1H Channel	357
20.8.4	Calibrate the ^{13}C Channel	357
20.8.4.1	Reference the Spectra	358
20.8.5	Cross-Polarization	358
20.8.5.1	Set the Spinning	358
20.8.5.2	Calibrate the 1H Channel	358
20.8.5.3	Setup a Cross-Polarization Experiment	358
20.8.5.4	Calibrate the $^{13}C/^{15}N$ Fields	360
20.8.6	Heteronuclear Decoupling Sequences	360
20.8.7	DCP	360
20.8.8	Recoupling Sequences	361
20.8.9	Example of an Experiment	362
20.9	Troubleshooting	362
20.9.1	Tips to Optimize Signal Acquisition and Sensitivity	362
20.9.2	Narrowing the ^{13}C or ^{15}N Linewidths	363
20.9.3	Recoupling Optimization	363
	Further Reading	364

21	Protein Dynamics in the Solid State	367
	<i>Józef R. Lewandowski and Lyndon Emsley</i>	
21.1	Introduction	367
21.2	Basic Concepts	369
21.3	Coherent versus Incoherent Processes: Decay is not Always Relaxation	370
21.4	Deuterium as a Probe of Dynamics	371
21.5	^{15}N and ^{13}C T_1 – Spin-Lattice Relaxation	372
21.6	Protocols	373
21.6.1	Measuring ^{15}N T_1 Relaxation	373
21.6.2	Measuring ^{13}C T_1 Relaxation	373
21.6.3	Heteronuclear NOE	373
21.6.4	Measuring ^{15}N Dipolar–CSA Cross-Correlated Relaxation	374
21.6.5	Measuring ^{15}N $R_{1\rho}$	374
21.6.6	Measuring Motionally Averaged Secular Interactions	374
21.6.7	Slow Conformational Exchange	374
21.6.8	Identification of Highly Mobile Sites	374
	Further Reading	375
22	Microcrystalline Proteins – An Ideal Benchmark for Methodology Development	377
	<i>W. Trent Franks, Barth-Jan van Rossum, Benjamin Bardiaux, Enrico Ravera, Giacomo Parigi, Claudio Luchinat, and Hartmut Oschkinat</i>	
22.1	Microcrystalline Protein Sample Preparation	377
22.2	Sequential Assignment of Proteins	378
22.3	Structural Restraints	380
22.3.1	Distance Measurements: Cross-Relaxation-Like Transfer	380
22.3.1.1	PDSD/DARR/RAD	380
22.3.1.2	ChhC and NhhC Experiments	381
22.3.1.3	^1H -Detected NOE	381
22.3.2	Distance Measurements: Multiple-Pulse Dipolar Recoupling	382
22.3.2.1	RFDR	382
22.3.2.2	TSAR, PAR, and PAIN	382
22.3.2.3	REDOR	382
22.3.2.4	TEDOR	382
22.3.2.5	Proton Dipolar Recoupling: TMREV and LG-CP	383
22.3.3	Parameters Encoding Torsion Angles	383
22.3.3.1	Chemical Shifts	383
22.3.3.2	Double Dip-Shift Spectroscopy	383
22.3.3.3	CSA–Dipolar Interactions	384
22.4	Paramagnetic Systems	384
22.4.1	PRE	384
22.4.2	PCS	386
22.5	Benchmarking of the Solid-State NMR Structure Determination Methodology: Comparison of Structure Calculation Protocols and Accuracy of Structures	386
22.6	Protocols	389
22.6.1	Three-Dimensional Nanocrystalline Sample Preparation	389
22.6.2	Sequential Assignments of Solid-State NMR Spectra	389
22.6.3	Distance Restraint Assignments with Solid-State NMR	389
22.6.4	Secondary Structure Prediction by TALOS	390
22.6.5	Dipolar Tensor, CSA, and Vector Angle Fitting	390
22.6.6	Use of PCS for Structural Restraints: Structure of the Catalytic Domain of MMP-12 as an Example	390
22.7	Troubleshooting	391
	Further Reading	392

23	Structural Studies of Protein Fibrils by Solid-State NMR	395
	<i>Anja Böckmann and Beat H. Meier</i>	
23.1	Background	395
23.2	NMR Spectra of Fibrils	396
23.3	Outlook	397
23.4	Protocols and Examples	398
23.4.1	Sample Preparation	398
23.4.2	Experiments for the Resonance Assignment	398
23.4.3	Experiments to Obtain Structural Restraints	400
23.4.4	Flexible Elements	401
23.4.5	Structure Calculation in Fibrils	402
23.5	Troubleshooting	404
	Further Reading	405
24	Solid-State NMR on Membrane Proteins: Methods and Applications	407
	<i>A.A. Cukkemane, M. Renault, and M. Baldus</i>	
24.1	Solid-State NMR of Membrane Proteins	407
24.1.1	Magic Angle Spinning	407
24.1.2	Methods for High-Resolution Structural Investigation of Membrane Proteins	408
24.1.3	Investigation of Membrane Protein Topology	409
24.1.4	Sensitivity and Resolution	410
24.2	MAS Applied to Ion Channels and Retinal Proteins	411
24.2.1	Retinal Proteins	411
24.2.2	Chimeric Potassium Channel KcsA-Kv1.3	411
24.3	Protocols	413
24.3.1	Sample Preparation	413
24.3.2	Isotope Labeling	414
24.3.3	Resonance Assignment	415
24.3.4	Collecting NMR Restraints	415
24.3.5	A Real Experiment	416
24.4	Troubleshooting	417
24.4.1	Tips and Tricks in Optimizing Sample Preparation	417
24.4.2	Improving Sensitivity	417
	Further Reading	417
Part Six	Frontiers in NMR Spectroscopy	419
25	Dynamic Nuclear Polarization	421
	<i>Thomas F. Prisner</i>	
25.1	Dynamic Nuclear Polarization at High Magnetic Fields	421
25.2	Theoretical Background	422
25.2.1	Overhauser Effect	423
25.2.2	Solid Effect	425
25.2.3	Three-Spin Cross-Polarization: Cross-Effect	425
25.2.4	Many-Spin Cross-Polarization: Thermal Mixing	426
25.3	Protocols	427
25.3.1	HF Liquid DNP Spectrometers	427
25.3.2	Shuttle DNP	428
25.3.3	SS MAS DNP	428
25.3.4	Low-Temperature Dissolution Polarizer	429
25.4	Example Experiment	429
25.5	Perspectives	430
25.5.1	HF Liquid DNP	430
25.5.2	Shuttle DNP	430
25.5.3	SS MAS DNP	430
25.5.4	Dissolution DNP	431
	Further Reading	431

26	^{13}C Direct Detection NMR	433
	<i>Isabella C. Felli and Roberta Pierattelli</i>	
26.1	^{13}C Direct Detection NMR for Biomolecular Applications	433
26.1.1	^{13}C NMR Properties and Application Areas	433
26.1.2	Problem of Homonuclear Decoupling in the Direct Acquisition Dimension	436
26.1.3	Experiments for High-Resolution NMR	438
26.2	Protocols for Experimental Setup	439
26.3	Troubleshooting	442
	Further Reading	442
27	Speeding Up Multidimensional NMR Data Acquisition	445
	<i>Bernhard Brutscher, Dominique Marion, and Lucio Frydman</i>	
27.1	Multidimensional NMR: Basic Concepts and Features	445
27.1.1	Discrete Data Sampling, Aliasing, and Truncation Artifacts	446
27.1.2	Time Requirements in N -Dimensional NMR	447
27.2	Fast Methods in N -Dimensional NMR	447
27.2.1	Sparse Time-Domain Data Sampling and Processing	447
27.2.1.1	Sampling Scheme and the Point Spread Function	448
27.2.1.2	Sparse Sampling Schemes	448
27.2.1.3	Alternative Data Processing Methods	449
27.2.2	Fast-Pulsing Methods	451
27.2.2.1	Repetition Rate and Experimental Sensitivity	452
27.2.2.2	Longitudinal ^1H Relaxation Enhancement	452
27.2.2.3	Ernst Angle Excitation	453
27.2.2.4	BEST and SOFAST Experiments	453
27.2.3	Single-Scan "Ultrafast" Two-Dimensional NMR	454
27.2.3.1	General Principles	454
27.2.3.2	Spatiotemporal Encoding Process	455
27.2.3.3	Spatiotemporal Decoding: Two-Dimensional NMR in One Scan	455
27.3	Protocols for Fast N -Dimensional NMR and Troubleshooting	457
27.3.1	Setting Up an Appropriate Sampling Grid	457
27.3.1.1	Information-Driven Spectral Aliasing	458
27.3.1.2	Random Sparse Sampling and MaxEnt Processing	458
27.3.2	Practical Considerations for the Setup of Fast-Pulsing Experiments	459
27.3.3	Setting Up a Single-Scan Two-Dimensional Experiment	460
27.3.3.1	Properties of Different Encoding Schemes	460
27.3.3.2	Single-Scan Two-Dimensional NMR Spectrum: Characteristics	461
27.3.4	Fast Two-Dimensional NMR for Sample Quality Screening and Molecular Interaction Studies	462
27.3.5	Real-Time Two-Dimensional NMR Measurements	463
	Further Reading	465
28	Metabolomics	467
	<i>Leonardo Tenori</i>	
28.1	Metabolomics in Systems Biology	467
28.2	NMR and Metabolomics	469
28.3	Data Analysis	471
28.4	Success in the Application of Metabolomics	472
28.5	Protocols	473
28.5.1	Serum Extraction	473
28.5.2	Plasma Extraction	473
28.5.3	Urine Collection	474
28.5.4	Tip on Samples Labeling	474
28.5.5	Sample Preparation for NMR Analysis	474
28.5.5.1	Urine	474

28.5.5.2	Serum/Plasma	475
28.5.6	Buffer Recipes	475
28.5.7	NMR Analysis (Working with a 600-MHz Bruker Spectrometer)	475
28.5.7.1	Urine	475
28.5.7.2	Serum/Plasma	475
28.5.8	Spectral Processing	476
28.5.9	Bucketing	476
28.5.10	Data Mining	476
28.5.11	Example Experiment	477
28.6	Troubleshooting	477
	Further Reading	477
29	In-Cell Protein NMR Spectroscopy	479
	<i>David S. Burz, David Cowburn, Kaushik Dutta, and Alexander Shekhtman</i>	
29.1	Background	479
29.2	Specific Applications	480
29.2.1	Structure Determination	481
29.2.2	Perturbations within the Cell – How In-Cell and <i>In Vitro</i> are Different Environments	481
29.2.3	Specific Protein–Protein Interactions: STINT-NMR	483
29.2.4	Other Protein–Ligand Interactions	484
29.2.5	Post-Translational Modification – In-Cell Biochemistry	484
29.2.5.1	Other In-Cell Biochemical Studies	485
29.2.6	Nucleic Acids In-Cell	485
29.3	Conclusions and Future Directions	485
29.4	Protocols and Example Experiments	486
29.4.1	Experimental Design of Multiple Expression Systems In-Cell	486
29.4.2	Detailed STINT Protocol	487
29.4.2.1	Data Acquisition and Analysis	488
29.4.2.2	Example Experiment	489
29.4.3	Detailed SMILI-NMR Protocol	489
29.4.3.1	Example Experiment	489
29.4.4	Detailed Protocol for Post-Translational Modification	491
29.4.4.1	Example Experiment	491
29.5	Troubleshooting	492
29.5.1	No Signal	492
29.5.2	Weak Signal	493
29.5.3	Signal is Extracellular	493
29.5.3.1	Control Experiments for Protein Leakage/Cell Lysis	493
29.5.3.2	Cell Viability	493
29.5.4	Cell Growth	493
	Further Reading	493
30	Structural Investigation of Cell-Free Expressed Membrane Proteins	497
	<i>Solmaz Sobhanifar, Sina Reckel, Frank Löhr, Frank Bernhard, and Volker Dötsch</i>	
30.1	Introduction	497
30.2	Cell-Free Expression of Membrane Proteins	498
30.3	Cell-Free Expression in Membrane-Mimetic Environments	499
30.4	Strategies for Functional Protein Expression	500
30.5	Cell-Free Approaches for Structural Studies	501
30.6	Cell-Free Labeling Strategies for Backbone Assignment	502
30.7	Structure Determination with Limited Nuclear Overhauser Effect Long-Distance Restraints	502
30.8	Protocols	504
30.8.1	Extract	504
30.8.2	Reaction Devices	505
30.8.3	DNA Template Preparation	506

30.8.4	Different Modes for the Cell-Free Production of Membrane Proteins	506
30.8.5	Example Experiment	507
30.9	Troubleshooting	507
	Further Reading	508

Part Seven Computational Aspects 509

31	Grid Computing	511
	<i>Antonio Rosato</i>	
31.1	Grid Infrastructure	511
31.2	e-NMR Web Platform	512
31.3	Protocols	515
31.3.1	How to Register with the e-NMR/WeNMR VO	515
31.3.2	Performing a CYANA Calculation	516
31.3.3	Refining a Structure with AMBER	516
31.4	Troubleshooting	517
	Further Reading	518
32	Protein-Protein Docking with HADDOCK	521
	<i>Christophe Schmitz, Adrien S.J. Melquiond, Sjoerd J. de Vries, Ezgi Karaca, Marc van Dijk, Panagiotis L. Kastiris, and Alexandre M.J.J. Bonvin</i>	
32.1	Protein-Protein Docking: General Concepts	521
32.1.1	Why Protein-Protein Docking?	521
32.1.2	General Methods for Protein-Protein Docking	522
32.2	Gathering Experimental Information for Data-Driven Docking	522
32.2.1	Chemical Shift Perturbations	523
32.2.2	Cross-Saturation Experiments	524
32.2.3	Hydrogen/Deuterium Exchange	524
32.2.4	Intermolecular NOEs	524
32.2.5	Paramagnetic Relaxation Enhancement	524
32.2.6	Pseudocontact Shift	525
32.2.7	Residual Dipolar Coupling	525
32.2.8	Diffusion Anisotropy	526
32.2.9	Non-NMR Information	526
32.3	How Does HADDOCK Use the Information?	526
32.3.1	Incorporation of Ambiguous Distance Restraints	527
32.3.2	Incorporation of Unambiguous Distance Restraints	527
32.3.3	Incorporation of Shape Restraints	528
32.3.4	Incorporation of Orientation Restraints	528
32.3.5	Symmetry Restraints	528
32.3.6	Additional Docking Mode	528
32.3.7	Overview of a HADDOCK Run	529
32.4	Protocol: A Guided Tour of the HADDOCK Web Interface	530
32.4.1	Prerequisite: Registration	530
32.4.2	Description of the Web Interface	531
32.4.3	Analysis of the Docking Run	533
32.5	Troubleshooting	534
32.5.1	General Considerations	534
32.5.2	Problems Related to the PDB File	534
32.5.3	Problems Encountered During Docking	535
	Further Reading	535
33	Automated Protein Structure Determination Methods	537
	<i>Paul Guerry and Torsten Herrmann</i>	
33.1	NMR Experiment-Driven Protein Modeling	537
33.2	NOE-Based Structure Determination	538
33.2.1	Chemical Shift Ambiguity	539

33.2.2	Ambiguous Distance Restraints	539
33.2.3	Using Intermediate Structures and Elimination of Artifacts	540
33.2.4	Structure Calculation and Energy Refinement	540
33.2.5	Structure Validation	541
33.3	Sequence-Specific Resonance Assignment	541
33.4	NMR Signal Identification	542
33.5	Perspectives	542
33.6	Protocols	543
33.7	Example Structure Determination and Troubleshooting	544
33.7.1	Sequence-Specific Resonance Assignment	545
33.7.2	Amino Acid Sequence, <i>Cis/Trans</i> Isomerization, and Redox State	545
33.7.3	NOE Assignment, Structure Calculation, and Structure Validation	545
	Further Reading	546
34	NMR Structure Determination of Protein–Ligand Complexes	549
	<i>Ulrich Schieborr, Sridhar Sreeramulu, and Harald Schwalbe</i>	
34.1	Protein–Ligand Complex Structure Determination by NMR	549
34.2	Methods for High-Affinity Binders	550
34.3	Methods for Low-Affinity Binders	552
34.4	Protocols and Troubleshooting	558
	Further Reading	561
35	Small Angle X-Ray Scattering/Small Angle Neutron Scattering as Methods Complementary to NMR	563
	<i>M.V. Petoukhov and D.I. Svergun</i>	
35.1	Introduction	563
35.2	Invariants	566
35.3	<i>Ab Initio</i> Shape Determination	567
35.4	Validation of Atomic Models	568
35.5	Rigid-Body Modeling of Quaternary Structure	568
35.6	Equilibrium Mixtures and Flexible Systems	569
35.7	Protocols	570
35.7.1	Validation of High-Resolution Models by Solution Scattering	570
35.7.2	Unrestrained Rigid-Body Modeling of a Complex	571
35.7.3	Rigid-Body Modeling with Contact Restraints from NMR Chemical Shifts	572
35.7.4	Rigid-Body Modeling of a Complex with Orientational Constraints from NMR RDCs	572
35.7.5	Characterization of Flexible Systems	573
35.8	Troubleshooting	573
	Further Reading	574
References		575
Index		609