Biological Magnetic Resonance
Volume 8

Spin Labeling

Theory and Applications

A Continuation Order Plan is available for this series. A continuation order will bring delivery of each new volume immediately upon publication. Volumes are billed only upon actual shipment. For further information please contact the publisher.

Biological Magnetic Resonance
Volume 8

Spin Labeling

Theory and Applications

Edited by

Lawrence J. Berliner

Ohio State University
Columbus, Ohio

and

Jacques Reuben

Hercules Incorporated
Research Center
Wilmington, Delaware

PLENUM PRESS • NEW YORK AND LONDON

The Library of Congress has cataloged the first volume of this series as follows:

Library of Congress Cataloging in Publication Data

Main entry under title:

Biological magnetic resonance:

Includes bibliographies and indexes.

1. Magnetic resonance. 2. Biology—Technique. I. Berliner, Lawrence, J. II. Reuben, Jacques.

QH324.9.M28B56

574.19'285

78-16035

AACR1

Personal computer software intended to supplement Chapter 1 of this volume (Schneider and Freed) will be found on a diskette mounted inside the back cover.

If your diskette is defective in manufacture or has been damaged in transit, it will be replaced at no charge if returned within 30 days of receipt to Managing Editor, Plenum Press, 233 Spring Street, New York, NY 10013.

The publisher makes no warranty of any kind, expressed or implied, with regard to the software reproduced on the diskette or the accompanying documentation. The publisher shall not be liable in any event for incidental or consequential damages or loss in connection with, or arising out of, the furnishing, performance, or use of the software.

ISBN-13:978-1-4612-8060-6 e-ISBN-13:978-1-4613-0743-3
DOI:10.1007/978-1-4613-0743-3

© 1989 Plenum Press, New York
Softcover reprint of the hardcover 1st edition 1989

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

Contributors

Jacob Anglister • Department of Polymer Research, Weizmann Institute of Science, Rehovot 76100, Israel

Barney L. Bales • Department of Physics and Astronomy, California State University, Northridge, California 91330

Albert H. Beth • Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee 37232

P. A. de Jager • Department of Molecular Physics, Agricultural University, 6703 BC Wageningen, The Netherlands

Gareth R. Eaton • University of Denver, Denver, Colorado 80208

Sandra S. Eaton • University of Colorado at Denver, Denver, Colorado 80204

Jim B. Feix • National Biomedical ESR Center, Department of Radiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Jack H. Freed • Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853-1301

Olga H. Hankovszky • Central Laboratory, Chemistry, University of Pécs, H-7643 Pécs, Hungary

M. A. Hemminga • Department of Molecular Physics, Agricultural University, 6703 BC Wageningen, The Netherlands

Kálmán Hideg • Central Laboratory, Chemistry, University of Pécs, H-7643 Pécs, Hungary

James S. Hyde • National Biomedical ESR Center, Department of Radiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

- Derek Marsh** • Department of Spectroscopy, Max Planck Institute for Biophysical Chemistry, D-3400 Göttingen, Federal Republic of Germany
- Ronald P. Mason** • Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709
- Carolyn Mottley** • Department of Chemistry, Luther College, Decorah, Indiana 52101
- Jane H. Park** • Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232
- Bruce H. Robinson** • Department of Chemistry, University of Washington, Seattle, Washington 98195
- David J. Schneider** • Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853-1301. *Present address:* IBM Corporation, Kingston, New York 12401.
- Witold K. Subczynski** • Department of Biophysics, Institute of Molecular Biology, Jagiellonian University, Crakow, Poland
- Wolfgang E. Trommer** • Department of Chemistry, University of Kaiserslautern, Kaiserslautern, Federal Republic of Germany

Preface

We present this special topics volume on an area which has not received thorough coverage for over 12 years. *Spin Labeling: Theory and Applications* represents a complete update on new theoretical aspects and applications of the spin-label method. In the “line-shape theory” sections, we are especially pleased to include an IBM-compatible diskette supplied by David Schneider and Jack Freed which contains fast, accurate, ready-to-use software for slow-motion simulations. Barney Bales discusses inhomogeneous broadening phenomena in detail. Several developments in techniques and interpretation in saturation transfer spectroscopy have appeared since the publication of *Spin Labeling II: Theory and Applications* (L. J. Berliner, ed., Academic Press, 1979). We have included an up-to-date chapter on spin-label applications by M. A. Hemminga and P. A. de Jager. By incorporating ^{15}N and deuterium into nitroxide spin labels, several unique advantages are derived in line-shape analysis. Albert Beth and Bruce Robinson have contributed a detailed chapter on the analysis of these labels in the slow-motion regime while Jane Park and Wolfgang Trommer present the advantages for specific biochemical examples in our “applications” section. Derek Marsh’s contribution on spin-label spectral analysis may be regarded as a summary chapter which touches on several of the detailed spectral analysis methods described in the earlier chapters.

New and specialized applications which focus on electron–electron spin interactions with spin labels are featured in chapters by Jim Hyde and Jim Feix on ELDOR, by Gareth and Sandra Eaton on metal ion–electron spin–spin splittings, and by Jim Hyde and Witold Subczynski on spin-label oximetry. Newer aspects of nitroxide synthesis are discussed in a quite thorough chapter by Kalman Hideg and Olga Hankovsky. Ronald Mason and Carolyn Mottley have provided us with a detailed chapter on the techniques, advantages, and pitfalls of spin trap chemistry. An appendix to this volume contains a comprehensive chapter on the detailed synthesis of ^{15}N perdeuterated spin labels by Jane Park and Wolfgang Trommer. We have retained the recipe format so that the reader can duplicate the syntheses from this book without resorting to the original literature. The marriage of proton NMR, site-specific isotopic labeling, and spin-label paramagnetic relaxation has been eloquently delineated in a problem-oriented chapter on antibody structure by Jacob Anglister.

We are extremely proud of this much-awaited volume. As always, we strive to provide the highest quality volumes for our readers that meet state-of-the-art standards

in both coverage and pedagogical approach. It is always our intention not only to review the literature, but also to teach the principles and criticize the approaches in an unbiased fashion. We sincerely hope to maintain our high standards and to cover new, promising methods as they mature. To this end, comments from our readers would be greatly appreciated.

Lawrence J. Berliner
Jacques Reuben

Contents

Chapter 1

Calculating Slow Motional Magnetic Resonance Spectra: A User's Guide

David J. Schneider and Jack H. Freed

1. Introduction	1
2. General Theoretical Considerations	3
2.1. Terms Included in the Liouville and Diffusion Superoperators	4
2.2. Definitions of Coordinate Systems	5
2.3. Basis Vectors and Scalar Product in Operator Space	7
2.4. Construction of the Spin Hamiltonian	10
2.5. Matrix Elements of the Liouville Superoperator	14
2.6. Construction and Matrix Elements of the Diffusion Superoperator	15
2.7. Components of the Starting Vector	18
2.8. The High-Field Approximation	20
3. Magnetic Resonance Line Shapes and the Complex Symmetric Lanczos Algorithm	21
3.1. The Real Symmetric Lanczos Algorithm	22
3.2. The Complex Symmetric Lanczos Algorithm	25
3.3. The Real Symmetric Conjugate Gradients Algorithm	27
3.4. The Complex Symmetric Conjugate Gradients Algorithm	30
3.5. The Continued-Fraction Representation of the Spectral Function	32
3.6. Convergence of the Sequence of Approximate Spectral Functions	36
4. Computational Considerations	42
4.1. Naming Conventions for Files	44
4.2. Array Dimensions and Common Blocks	44
4.3. The Parameter Input Program: LBL	45

4.4. Spectral Calculations: EPRL and EPRCGL	48
4.5. Calculation of the Spectral Function: TDLL	54
4.6. "Field Sweep" Conjugate-Gradients Calculations: EPRBL and TNLL	55
4.7. Auxiliary Programs: D200 , STVT , MATLST , and VECLST	56
4.8. Porting Programs to Other Machines	57
5. Example Calculations	59
5.1. Model Calculations and General Strategy	60
5.2. Examples from the Literature	65
Appendix: Parameters for Example Calculations	68
<i>References</i>	73

Chapter 2

Inhomogeneously Broadened Spin-Label Spectra

Barney L. Bales

1. Introduction	77
2. Experimental Determination of Hyperfine Coupling Constants...	80
2.1. NMR and ENDOR	80
2.2. ESR Simulation	81
2.3. Solvent Dependence of Hyperfine Coupling Constants.....	88
3. Gaussian Contributions to Spin-Label Line Shapes	88
Example 1	93
4. The Voigt Approximation and a One-Parameter Description of Line Shapes.....	93
Example 2	96
5. Line-Shape Comparisons	97
5.1. Unresolved Hyperfine Multiplets.....	97
5.2. A Universal Nitroxide	98
5.3. Gaussian-Lorentzian Sum Approximations.....	101
6. Correcting the Linewidth of an Inhomogeneously Broadened Line	103
6.1. Known Hyperfine Coupling Constants.....	103
Example 3. Solvent Dependence of ΔH_{pp}^G	103
Example 4. Dependence of ΔH_{pp}^G on Spin-Label Alignment in an Ordered Fluid	104
6.2. Unknown Hyperfine Coupling Constants	105
Example 5	106
6.3. Additional Broadening Method	108
7. The Relationship of Linewidth Ratios to Measured Line-Height Ratios	108
7.1. Rotational Correlation Times.....	108
Example 6	112

7.2. Linewidth Ratios Over a Broader Range	112
Example 7. Measuring Oxygen Concentrations Using Unresolved Spin-Label Spectra	112
8. Accurate Estimate of Relative Doubly-Integrated Spectral Intensities	113
Example 8. A Hypothetical Spin-Label Partitioning Experiment	114
9. Determining Spin-Exchange Frequencies of Spin Labels in Liquids of Low Viscosity	115
10. Deuterated Spin Labels	121
10.1. Gaussian Linewidth of Deuterated Spin Labels	121
10.2. Proton Contamination of a Deuterated Spin Label	122
Example 9	123
Example 10	124
11. Conclusions	124
Appendix	125
References	126

Chapter 3

Saturation Transfer Spectroscopy of Spin Labels: Techniques and Interpretation of Spectra

M. A. Hemminga and P. A. de Jager

1. Introduction	131
2. Basic Principles of Saturation Transfer ESR	132
2.1. The Spin Hamiltonian	132
2.2. ESR Spectra of Immobilized Spin Labels	133
2.3. The Bloch Equations	136
2.4. Qualitative Explanation of ST-ESR	140
2.5. Spectral Displays of ST-ESR	143
2.6. Analysis of ST-ESR Spectra	145
3. Measurements of Saturation Transfer ESR Spectra	148
3.1. The Resonant Cavity	148
3.2. Effect of Sample on Cavity Properties	150
3.3. Phase-Sensitive Detection	152
3.4. Calibration Procedures	154
3.5. Standard Conditions for ST-ESR Spectroscopy	156
4. Analysis of Saturation Transfer ESR Spectra	158
4.1. Isotropic Motion	159
4.2. Anisotropic Motion	166
5. Future Developments	173
References	174

Chapter 4

Nitrogen-15 and Deuterium Substituted Spin Labels for Studies of Very Slow Rotational Motion

Albert H. Beth and Bruce H. Robinson

1. Introduction	179
2. Overview of Rotational Diffusion Models	181
2.1. Definition of Rotational Correlation Times	182
2.2. Isotropic Rotational Diffusion	184
2.3. Anisotropic Rotational Diffusion in an Isotropic Medium	184
2.4. Uniaxial Rotational Diffusion in an Anisotropic Medium	187
3. Sensitivity of cw-ST-EPR Signals to Rotational Motion	188
3.1. Choice of Signal	188
3.2. Sensitivity to Motion	189
3.3. The Case of Isotropic Motion and Anisotropic Magnetic Tensors	195
3.4. Effects of Anisotropic Motion and Anisotropic Tensors	197
3.5. Geometric Considerations for Analyzing Anisotropic Motion	198
4. Analysis of cw-ST-EPR Data	199
4.1. Isotropic Model Systems	199
4.2. Anisotropic Model Systems	200
4.3. Anisotropic Motional Modeling by Computer Simulations	201
4.4. Overview of Theory for Computation of ST-EPR Spectra	202
5. Studies of Isotropic Motion with Nitrogen-15 Spin Labels	202
5.1. V_1 EPR Signals as a Function of τ_r	202
5.2. Dependence of the V'_2 Signal on τ_r	204
6. Effects of Anisotropic Rotational Diffusion on V'_2 Spectra	207
6.1. Sensitivity of V'_2 to Uniaxial Rotation	208
6.2. Effects of Labeling Geometry	210
6.3. Sensitivity of V'_2 to Anisotropic Rotational Diffusion of Axially Symmetric Ellipsoids in an Isotropic Medium	212
6.4. Relationship between Effective Correlation Times and Anisotropic Motion	213
7. Optimization of Sensitivity of V'_2 to Motion	217
7.1. Altering Sensitivity to Motion by Selection of ν_0	217
7.2. Altering Sensitivity to Motion by Selection of ν_m	223
8. Analysis of Overlapping Motional Species	224
8.1. One Fast and One Slow Motional Component	224
8.2. Two or More Slow Motional Components	226
9. Computer Modeling of Nitrogen-14 V'_2 Signals	230
9.1. Isotropic Motion Simulations	230
9.2. Anisotropic Motion Simulations	232
9.3. Signal-to-Noise Ratio and Motional Sensitivity of Nitrogen-15 versus Nitrogen-14 Spin Labels	233

10. Saturation Recovery EPR Studies with Nitrogen-15 Spin Labels	234
10.1. Overview of the Experiment	234
10.2. Spectrometer Variables	235
10.3. Strategies for Extraction of Motional Information	236
10.4. Theory of Saturation Recovery	236
10.5. Pseudosecular Terms	238
10.6. Isotropic Brownian Motion—Secular Terms Only	239
10.7. Results of Calculations of SR Curves for Isotropic Motion	240
10.8. Results of Calculations of SR Curves for Nonaxial Tensors	240
10.9. Effects of Pseudosecular Terms	240
10.10. Pseudosecular Terms using Nitrogen-14	244
10.11. Population Analysis: An Estimate of Amplitudes	245
11. Conclusions	247
<i>References</i>	249

Chapter 5

Experimental Methods in Spin-Label Spectral Analysis

Derek Marsh

1. Introduction	255
2. Inhomogeneous Broadening	256
3. Fast Rotational Motion	259
4. Slow Rotational Motion	262
5. Anisotropic Rotation: Lipids/Membranes	265
6. Spin-Spin Interactions and Lateral Diffusion	269
6.1. Spin-Spin Exchange	270
6.2. Translational Diffusion and Bimolecular Collision Rate	273
6.3. Dipolar Spin-Spin Broadening	274
6.4. Separation of Exchange and Dipole-Dipole Interactions	275
7. Lipid-Protein Interactions	277
7.1. Spectral Subtraction/Addition	278
7.2. Measurements at 35 GHz	280
7.3. Analysis of Lipid-Protein Association	281
7.4. Two-Site Exchange Simulations	286
8. Saturation Transfer ESR	291
8.1. Power and Modulation Calibration and the Effects of Sample Shape, Size, and Dielectric Absorption	291
8.2. Anisotropic Rotation	294
8.3. Integral Method: Multicomponent Spectra	297
8.4. Dispersion Spectra: Difference Spectroscopy	298
<i>References</i>	300

Chapter 6

Electron–Electron Double Resonance

James S. Hyde and Jim B. Feix

1. Introduction	305
1.1. Definitions and Background	305
1.2. Historical Overview	307
2. Rate Equations	310
3. Spin-Label Relaximetry	314
4. Apparatus	317
5. Applications	320
5.1. Lateral Diffusion in Membranes	320
5.2. Studies Utilizing ^{14}N : ^{15}N Spin-Label Pairs	321
5.3. ELDOR in Cells	330
5.4. Comparison with Spin-Exchange Line Broadening	331
5.5. Further Application of ^{14}N : ^{15}N Methodology	331
6. Future Opportunities	333
References	334

Chapter 7

Resolved Electron–Electron Spin–Spin Splittings in EPR Spectra

Gareth R. Eaton and Sandra S. Eaton

1. The Scope of Electron Spin–Spin Interactions	340
2. The Nature of Electron Spin–Spin Interactions	341
2.1. Dipolar Interaction	341
2.2. Exchange Interaction	343
2.3. Hamiltonian for Spin–Spin Interaction	346
2.4. Computational Approaches	348
3. Analogies between Nuclear–Nuclear, Electron–Nuclear, and Electron–Electron Spin–Spin Interactions and Long-Range Electron Transfer	349
4. Spin- $\frac{1}{2}$ –Spin- $\frac{1}{2}$ Interaction	351
4.1. Spin–Spin Splitting	351
4.2. Half-Field Transitions	357
4.3. Geometrical Information	360
5. Spin 1–Spin $\frac{1}{2}$	364
6. Spin $\frac{3}{2}$ –Spin $\frac{1}{2}$	365
7. Spin $\frac{5}{2}$ –Spin $\frac{1}{2}$	366
7.1. Mn(II) Interacting with $S = \frac{1}{2}$	366
7.2. High-Spin Fe(III) Interacting with $S = \frac{1}{2}$	369

8. Spin $\frac{7}{2}$ -Spin $\frac{1}{2}$	371
9. Chemical Properties Revealed via Spin-Spin Interactions	373
9.1. Kinetics of Ligand Exchange for Cu(II)	373
9.2. Coordination Equilibria	373
9.3. Weak Orbital Overlaps	374
10. Spin-Spin Interactions in Biological Systems	376
10.1. Cobalt(II)-Radical Interaction	376
10.2. Mo(V) Interaction with Fe/S Cluster	377
10.3. Iron-Nitroxyl Interaction	378
10.4. Nitroxyl-Nitroxyl Interaction	382
11. Exchange Interaction through Multiatom Linkages	384
12. Quantitative EPR Measurements	389
13. Summary and Prognosis	390
References	391

Chapter 8

Spin-Label Oximetry

James S. Hyde and Witold K. Subczynski

1. Introduction	399
2. Physics	401
2.1. Bimolecular Collisions	401
2.2. Magnetic Interactions	402
2.3. The Absolute T_1 Method	406
2.4. The Absolute T_2 Method	407
3. Experimental Methods	408
3.1. TPX Gas-Exchange Sample Cell	408
3.2. T_1 -Sensitive Methods	413
3.3. T_2 (Linewidth-Sensitive) Methods	416
4. Applications	421
5. Future Opportunities	422
References	422

Chapter 9

Chemistry of Spin-Labeled Amino Acids and Peptides: Some New Mono- and Bifunctionalized Nitroxide Free Radicals

Kálmán Hideg and Olga H. Hankovszky

1. Introduction	427
-----------------------	-----

2. Spin Labeling of Amino Acids and Peptides	429
2.1. Reagents for Labeling at the Amino Terminal	429
2.2. C-Terminal Spin-Labeled Amino Acids and Peptides	440
2.3. Amino Acids and Peptides Labeled in the Side Chain	442
3. Nitroxide Amino Acids	447
3.1. Imidazoliny Nitroxide Amino Acids	447
3.2. Pyrrolidine Nitroxide Amino Acids	448
3.3. Pyrrolinyl Nitroxide Amino Acids	448
3.4. Piperidinyl Nitroxide Amino Acids	448
4. New Mono- and Bifunctionalized Spin Labels	450
4.1. Reactions with 3-Functionalized-3-Pyrroline-1-Oxyl Derivatives	450
4.2. Cross-Linking Spin-Label Reagents	454
4.3. Spin-Labeled Pyrrolidine-1-Oxyl Fatty Acids	463
5. Experimental Procedures for Preparation of Some Pyrroline and Pyrrolidine Nitroxide Spin Labels	466
References	481

Chapter 10

Nitroxide Radical Adducts in Biology: Chemistry, Applications, and Pitfalls

Carolyn Mottley and Ronald P. Mason

1. Introduction	489
2. Halogenated Compounds	491
2.1. Carbon Tetrachloride	491
2.2. Halothane	493
2.3. Others	494
3. Lipid Peroxidation	494
3.1. Linoleic Acid	494
3.2. Methyl Linoleate	495
3.3. Arachidonic Acid	495
3.4. Lipid Peroxyl and Alkoxy Radicals	497
4. Hemoprotein-Organic Hydroperoxides	498
5. Sulfur-Centered Radicals	502
5.1. Sulfur Dioxide, Bisulfite, Sulfite, and Sulfate	502
5.2. Thiols	504
6. Pyridinyl Radicals	507
7. Hydrazines	507
8. Oxygen-Centered Radicals	513
9. Aldehydes	514
9.1. Glyceraldehyde and Other Monosaccharides	514
9.2. Malonaldehyde and Acetylacetone	516

10. Lignin Model Dimers	517
11. 3-Methylindole	519
12. Indole Acetic Acid	519
13. Ethanol	520
14. Tryptophan	521
15. Nitrobenzyl Chlorides	521
16. Azide and Cyanide	522
17. Styrene	522
18. Spin Traps as Enzyme Inhibitors	523
19. Spin Traps as Enzyme Substrates	524
20. <i>In Vivo</i> Spin Trapping and Spin Trapping in Cells	525
20.1. Spin Trapping in Cells	525
20.2. Site-Directed Spin Traps	527
20.3. <i>In Vivo</i> Spin Trapping	528
21. Spin Adduct Identification	529
21.1. Isotope Methods	530
21.2. Isolation and Identification by HPLC, GC, and MS	530
22. Spin Adduct Imposters	531
23. Summary	532
<i>References</i>	532

Chapter 11

Advantages of ^{15}N and Deuterium Spin Probes for Biomedical Electron Paramagnetic Resonance Investigations

Jane H. Park and Wolfgang E. Trommer

1. Introduction	547
2. Maleimide Spin Label (DMSL or ^{15}N ,DMSL): A Covalent Ligand for Sulfhydryl Groups of Proteins	549
2.1. Advantages of Deuterated Maleimide Spin Label (DMSL) for Studies of Proteins	549
2.2. Advantages of Doubly Substituted ^{15}N ,Deuterated Maleimide Spin Label (^{15}N ,DMSL)	552
2.3. Advantages of ^{15}N ,Deuterated Maleimide Spin Label (^{15}N ,DMSL) for Studies of Soluble and Membrane-Bound Enzymes	552
2.4. Future Applications	563
3. Coenzyme Spin Labels: Studies on Coenzyme Binding to Enzymes and Catalytic Mechanisms	563
3.1. Comparison of Spin-Labeled Maleimide Inhibitors (DMSL and ^{15}N ,DMSL) and Coenzyme Analogs (SL-NAD $^{+}$) for EPR Studies of Enzymes	563

3.2.	The Evaluation of Binding of Isotopically Substituted N ⁶ -SL-NAD ⁺ or C8-SL-NAD ⁺ Using Lactic Dehydrogenase	564
3.3.	Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) and SL-NAD ⁺ Binding with Unusual Electron–Electron Dipolar Interactions (Splitting of Peaks)	566
3.4.	Interactions of N ⁶ -(D ₁₇)SL-NAD ⁺ with GAPDH Bound to Band-3 Protein of Erythrocyte Membranes	579
3.5.	Binding of Deuterated and ¹⁵ N,Deuterated-SL-NADH Analogs to β -Hydroxybutyrate Dehydrogenase (BDH)	580
3.6.	Binding of Spin-Labeled Adenine Nucleotides to Dehydrogenases and F ₁ ATPase.....	581
3.7.	Future Applications	582
4.	Advantages of ¹⁵ N, and ¹⁵ N,Deuterated Stearic Acid and Cholestane Spin Labels for Evaluation of Motion of Lipids in Membranes	582
4.1.	Improved Spectral Sensitivity of Rapidly Tumbling ¹⁵ N and ¹⁵ N,Deuterium Substituted Stearic Acid and Cholestane Analogs	583
4.2.	Advantages of ¹⁵ N and ¹⁵ N,Deuterated Stearic Acid Spin Labels for Studies on Binding to Proteins.....	583
4.3.	Improved Spectral Analysis of Lipid Motion in Erythrocyte (RBC) Membranes with ¹⁵ N-5-NS and (¹⁵ N,D ₁₂)-5-NS	587
4.4.	Investigation of Erythrocyte Abnormalities Using ¹⁵ N-5-NS	588
4.5.	Studies of Collision Frequency and Vertical Fluctuations of ¹⁴ N and ¹⁵ N Spin-Labeled Pairs of Fatty Acids in Membranes Using ELDOR Spectroscopy	589
4.6.	Future Applications	591
	<i>References</i>	592

Chapter 12

Magnetic Resonance Study of the Combining Site Structure of a Monoclonal Anti-Spin-Label Antibody

Jacob Anglister

1.	Introduction	597
2.	Amino Acid Composition of the Antibody Combining Site	599
3.	Distance Determination	604
4.	Contributions of Heavy and Light Chains to the Combining Site	607
5.	Determination of Short Distances	608
6.	Conclusion.....	612
	<i>References</i>	612

*Appendix***Approaches to the Chemical Synthesis of ^{15}N and Deuterium Substituted Spin Labels***Jane H. Park and Wolfgang E. Trommer*

1. Introduction	615
2. Synthesis of Deuterated and ^{15}N , Deuterated Maleimide Spin Labels (DMSL and ^{15}N -DMSL)	616
3. Synthesis of Deuterated and ^{15}N , Deuterated Adenine Nucleotide Derivatives	619
3.1. Synthesis of N^6 -(D_{17})SL-AMP (10a) or N^6 -(^{15}N , D_{17})SL-AMP (10b)	619
3.2. Synthesis of C8-(D_{17})SL-AMP (12a) or C8-(^{15}N , D_{17})SL-AMP (12b)	620
3.3. Synthesis of N^6 -SL-ATP (14)	621
3.4. Synthesis of C8-(^{15}N , D_{17})SL-ATP	623
3.5. Synthesis of N^6 - or C8-SL-Analogs of AMPPCP or AMPPNP	624
3.6. Synthesis of a Mixture of 2'- and 3'-(^{15}N , D_{13})SL-ATP (17)	625
3.7. Synthesis of N^6 -SL-ADP and C8-SL-ADP	626
3.8. Synthesis of N^6 -(D_{17})SL- NAD^+ (18a) and N^6 -(^{15}N , D_{17})SL- NAD^+ (18b)	626
3.9. Synthesis of C8-(D_{17})SL- NAD^+ and C8-(^{15}N , D_{17})SL- NAD^+	627
4. Synthesis of ^{15}N and ^{15}N , Deuterated Stearic Acid Spin Labels ...	628
4.1. Synthesis of ^{15}N -5-Doxylstearic Acid Spin Label (26)	628
4.2. Synthesis of ^{15}N -12-Doxylstearic Acid- d_{41} (27)	631
5. Conclusions	632
References	632

Contents of Previous Volumes	635
---	------------

Index	641
--------------------	------------