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Second Edition

David Neuhaus
Michael P. Williamson

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PREFACE

When we completed the first edition of this book, just over 10 years ago, we did not expect to undertake rewriting it for a long time to come. It is certainly true that the theory behind the NOE has altered little in the last 10 years (as reflected by the fact that Chapters 1, 2, 3, and 6 are largely unchanged). However, there has continued to be a very rapid growth in the range of applications, particularly in the macromolecular area.

To a large extent, this has dictated the changes we have made to the book. The biggest change from the first edition is the inclusion of a completely new chapter (Chapter 12), which deals with the way NOE enhancements are used to calculate structures of biomolecules. Chapter 13, which discusses applications of the NOE to calculations of macromolecular structures, has been largely rewritten. Much the same is true for Chapter 4, which deals with the kinetics of the NOE; although the theory is largely the same, we have now placed much more emphasis on transient NOE experiments (such as 2D and gradient-selected experiments), which have seen a major increase in use over the last 10 years.

Interest in internal motions in macromolecules was the main driving force behind the changes we have made to Chapter 5 (which also includes a new section on NOE enhancements involving water, and a major update on the transferred NOE), and a large number of new approaches have been incorporated into Chapters 7 (particularly gradient enhanced experiments) and 8 (network editing). Chapter 9 (“Other Developments”) remains the home for “miscellany” and contains a lot of new material, much of it concerned with the new experiments that became available in the wake of isotope labeling.

We have not significantly changed the content of the chapters dealing with applications to small molecules (Chapters 10 and 11), partly because neither of us is still active in that field, but more importantly because we feel the examples we gave in the first edition still represent a balanced selection that demonstrates coherently what is usefully possible. It is true that some types of structural problem are now more frequently solved by other means (e.g., positional isomers on aromatic rings might now be more frequently distinguished using long-range $^{13}C-^{1}H$ correlation than by using the NOE), but we felt illustration of the utility of such NOE experiments was still important.

Overall, we have thus broadened the scope of the first edition somewhat, feeling that to exclude (for example) applications of the NOE to relaxation measurements, because it does not relate directly to structure or conformation, was unnecessarily restrictive.

One of the aims we set ourselves in writing this second edition was to make the book shorter. The most casual glance will reveal that we have achieved the opposite of success in this regard. The reason, unfortunately, is simple: as George Bernard Shaw (or was it Blaise Pascal?) is supposed to have said, “I am sorry to have written you a long letter, but I did not have time to write you a short one.” For this and other reasons, we feel it is safe to predict that there will be no third edition.

David Neuhaus
Michael P. Williamson

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PREFACE TO THE FIRST EDITION

In the past ten years a quiet revolution in the applications of NMR to organic chemistry and biochemistry has occurred. One of the main changes has been a great increase in the use of the nuclear Overhauser effect (NOE) to solve structural and conformational problems, which in turn was largely due to experimental developments such as the advent of NOE difference spectroscopy and the two-dimensional NOE experiment (NOESY). In this book we have tried to bring chemists and biochemists who are not NMR specialists up to date with these trends. The book aims to provide readers with sufficient background information to enable them to apply the NOE successfully within their own work, and also assess critically other papers in which the NOE is used to solve structural problems.

Because the NOE is transmitted directly through space, it is uniquely well suited to revealing the spatial arrangements of nuclei within molecules, but its interpretation is particularly vulnerable to the overapplication of inappropriate equations. For this reason, we have tried above all to emphasize a clear understanding of the underlying concepts, since only through an awareness of these can the most useful NOE experiments be devised and interpreted. Unavoidably, we have had to use a certain amount of mathematics to do this, but it is kept to a minimum, consistent with a clear explanation. Perhaps contrary to first appearances, most of the expressions in this book require no more than elementary mathematical skills.

As the title implies, our treatment is deliberately restricted to those aspects of the NOE that are relevant to the study of stereochemistry and conformation; topics such as dipolar interactions in solids, and the use of the NOE to study relaxation phenomena, kinetics, or exchange, have therefore been omitted.

The book is organized into three parts, dealing with theory, experimental practice, and an illustrative overview of applications of the NOE to problems in chemistry and biochemistry.

SI units have been used throughout, as far as possible. This results in occasional numerical factors in some expressions (e.g., the factor $\mu_0/4\pi$, which appears in expressions relating relaxation rates to internuclear distances).

David Neuhaus
Michael P. Williamson

SYMBOLS, ABBREVIATIONS, AND UNITS

Throughout, subscript letters are used to denote spin states and transitions; thus, N_α is the number of molecules in spin state α, and R_I is the spin−lattice relaxation rate of spin I. Superscript letters are used to denote different molecules or conformations; thus, S^{a} is the signal from spin S in molecules with conformation a, and R_S^{b} is the spin−lattice relaxation rate of spin S in molecules with conformation b. Vector quantities are written in bold.

ADC	analog-to-digital converter
AQ	acquisition time
$\gamma\mathbf{B}/2\pi$	field strength in Hz
$\gamma\mathbf{B}$	field strength in rad s^{-1}
$\mathbf{B}_0$	applied magnetic field (tesla)
$\mathbf{B}_1$	irradiating magnetic field (tesla)
CW	continuous wave
$d_{AB}(i, j)$	distance between proton A in residue i and proton B in residue j in a peptide or protein
D	dwell time in t_2 (s)
1D	one-dimensional
2D	two-dimensional
3D	three-dimensional
dB	decibels
DG	distance geometry
DPFGSE	double pulsed field gradient spin echo
DQF-COSY	double-quantum filtered 2D correlation spectroscopy
$f_I\{S\}$	fractional NOE enhancement of I on saturating S

FID	free induction decay
FT	Fourier transform
$g(\tau)$	correlation function, usually $\exp(-\tau/\tau_c)$
GOESY	gradient enhanced NOE spectroscopy
h	Planck's constant = 6.626×10^{-34} Js
$\hbar$	Planck's constant divided by $2\pi = 1.055 \times 10^{-34}$ Js
HMQC	heteronuclear multiple quantum correlation (also coherence)
HSQC	heteronuclear single quantum correlation (also coherence)
I	nuclear spin quantum number, *or* general symbol for a nucleus; in expressions concerning NOE experiments, I generally represents the nucleus at which the enhancement is measured.
I_z	longitudinal component of I magnetization
I_z^0	equilibrium value of I_z
IN	t_1 increment in a 2D experiment (s)
ISPA	isolated spin pair approximation
J	spin–spin coupling constant (Hz)
$J(\omega)$	spectral density function, usually $2\tau_c/(1 + \omega^2\tau_c^2)$
k	Boltzmann's constant = 1.38×10^{-23} J K^{-1}
$\mathbf{M}$	macroscopic magnetization vector (J T^{-1})
M_z	z component of $\mathbf{M}$
M_{xy}	transverse component of $\mathbf{M}$
MAS	magic angle spinning
N_i	number of molecules (population) in state i
n_i	population deviation from equilibrium of state i, namely $N_i - N_i^0$
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect correlation spectroscopy
R_I^{DD}	dipolar contribution to the spin–lattice relaxation rate of I, defined as $W_{0IS} + 2W_{1I} + W_{2IS} + \Sigma_x (W_{0IX} + W_{2IX})$ (cf. Eqs. 2.31 and 3.8) (s^{-1})
$R_I \equiv R_{1I}$	longitudinal relaxation rate of $I = R_I^{\mathrm{DD}} + \rho_I^*$, roughly equal to the inverse of the selective T_1 value (cf. Section 2.3.1) (s^{-1})
R_2	transverse relaxation rate (s^{-1})
r	internuclear distance
r_{Tropp}	effective internuclear distance sensed by the NOE in the presence of rapid internal motion; most commonly used to denote the effective distance to a methyl group (cf. Section 5.5.2)
RF	radiofrequency
rmsd	root mean squared deviation (or difference); usually implies root mean squared *atomic* deviation when comparing structures
ROE	rotating frame NOE; also known as CAMELSPIN
ROESY	rotating frame 2D NOE spectroscopy
S	general symbol for a nucleus; in expressions concerning NOE experiments, S generally represents the nucleus which is saturated (S can also represent the electron spin quantum number).

	Also, generalized order parameter for describing the geometrical extent of internal motions relative to the molecular frame (cf. Section 5.4)
S^2	square of the generalised order parameter (cf. Section 5.4)
S^{ang}	angular order parameter (cf. Section 12.4.2)
S_z	longitudinal component of S magnetization
S_z^0	equilibrium value of S_z
SPT	selective population transfer
SW_1	spectral width in F_1 of a multidimensional experiment (Hz)
SW_2	spectral width in F_2 of a multidimensional experiment (Hz)
SW_3	spectral width in F_3 of a multidimensional experiment (Hz)
t_1	incremented time in 2D experiments; first incremented time in multidimensional experiments
$t_{1\,\text{max}}$	maximum time reached by t_1 in the last increment of a 2D or multidimensional experiment (s)
t_2	detection period in 2D experiments; second incremented time in multidimensional experiments
$t_{2\,\text{max}}$	maximum time reached by t_2 (s)
t_3	detection period in 3D experiments
$t_{3\,\text{max}}$	maximum time reached by t_3 (s)
T	temperature (K)
T_1	normally used to mean $T_1^{\text{nonselective}}$ (see below)
$T_1^{\text{nonselective}}$	nonselective longitudinal relaxation time, obtained by following the course of longitudinal relaxation after a nonselective pulse; approximately given by $(R_I + \sigma_{IS} + \Sigma_X\ \sigma_{IX})^{-1}$ (s) (cf. Section 2.3.2)
$T_1^{\text{selective}}$	selective longitudinal relaxation time, obtained by following the course of longitudinal relaxation after a selective pulse; approximately equal to R_I^{-1} (s)
$T_{1\rho}$	spin–lattice relaxation time in the rotating frame (s)
T_2	spin–spin, or transverse, relaxation time (s)
$T_{2\rho}$	spin–spin relaxation time in the rotating frame (s)
T_2^*	decay constant for free precession, which is shorter than T_2 because of inhomogeneous broadening (s)
$T_{2\rho}^*$	decay constant for free precession in the rotating frame (s)
t_{D}	relaxation delay (s)
TMS	tetramethylsilane
TOCSY	total correlation spectroscopy
TOE	truncated driven NOE
TPPI	time-proportional phase incrementation
TRNOE	transferred NOE
VDW	van der Waals
VT	variable temperature
W	transition probability (s^{-1})

X	general symbol for a nucleus; in expressions concerning NOE experiments in this book, X represents all nuclei other than I and S (when not italicized, X can also refer to a non-hydrogen nucleus)
X_z	longitudinal component of X magnetization
X_z^0	equilibrium value of X_z
α, β	spin states for a spin 1/2 nucleus
γ	gyromagnetic ratio (rad T^{-1} s^{-1})
η	viscosity (cP)
η_{max}	maximum enhancement attainable in a two-spin system at a given value of τ_c and ω
μ	magnetic dipole moment (J T^{-1})
μ_0	permeability constant in a vacuum = $4\pi \times 10^{-7}$ kg m s^{-2} A^{-2}
ν	precession rate (Hz)
ν_0	reference frequency of rotating frame (Hz)
ρ_{IS}	direct dipole–dipole relaxation rate between I and S, defined by $\rho_{IS} = W_{0IS} + 2W_{1I} + W_{2IS}$ [Eq. 2.30] (s^{-1})
ρ^*	external spin–lattice relaxation rate (s^{-1})
ρ_1	alternative notation for ρ_{IS}, useful when comparing the NOE with the ROE
ρ_2	direct relaxation rate in the rotating frame (s^{-1})
σ_I	chemical shift tensor for nucleus I (cf. Sections 5.4 and 6.3)
σ_{IS}	cross-relaxation rate between I and S, defined by $\sigma_{IS} = W_{2IS} - W_{0IS}$ (Eq. 2.29) (s^{-1})
σ_1	alternative notation for σ_{IS}, useful when comparing the NOE with the ROE
σ_2	cross-relaxation rate in the rotating frame (s^{-1})
τ_c	rotational correlation time (for overall molecular tumbling) (s)
τ_e	correlation time for an internal motion (cf. Section 5.4) (s)
τ_m	mixing time in a 2D or multidimensional NOE experiment (s)
τ	buildup time in kinetic NOE experiment; also general symbol for a fixed delay other than τ_m (s)
ω_0	Larmor precession frequency due to $\mathbf{B}_0$ (rad s^{-1})
ω_1	Larmor precession frequency due to $\mathbf{B}_1$ (rad s^{-1})
$\{\ \}$	denotes irradiated spin
$\langle\ \rangle$	denotes average or expectation value *or* conformational average
$\langle\vert$, $\vert\rangle$	Dirac notation for a wave function (ket) and its complex conjugate (bra), respectively

INTRODUCTION

The fundamentals of the NOE were described very early in the history of NMR, in a classic paper by Solomon published in 1955.[1] This paper included the first experimental demonstration of the NOE. Solomon's work followed studies on nuclear spin relaxation by Bloembergen, Purcell, and Pound,[2] and Overhauser's original prediction that saturation of electrons in a metal would produce a large polarization of the metal nuclear spins.[3,4]

Solomon's work then lay almost dormant for some years until the advent of double resonance techniques[5,6] led to the more widespread availability of spectrometers having a decoupler. Papers appeared by Kaiser on distance estimation using the intermolecular ^{1}H{^{1}H} NOE,[7] and by Lauterbur on signal enhancement in ^{13}C spectra.[8]

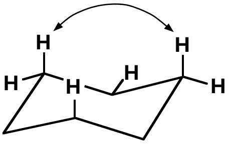
The first paper to demonstrate the power of the NOE in structural problems was by Anet and Bourn in 1965.[9] The next few years saw a surge in the number of papers reporting applications of the NOE, especially after publication of the book by Noggle and Schirmer.[10] On the CW spectrometers of the day, these experiments involved comparison of integrals to measure steady-state NOE enhancements.

Noggle and Schirmer's book surveyed the field as it stood in 1971, and for some years there was little that needed to be added. There were two major advances in the decade or so following 1971. The first was the application of the NOE to large molecules. In 1972, Balaram et al.[11] observed a negative NOE enhancement between a protein and a peptide binding to it, and they showed that negative enhancements are a general property of large molecules. Further applications to large molecules soon revealed the problem of spin diffusion, and the truncated driven NOE (TOE) experiment was suggested by Wagner and Wüthrich[12] as a means of getting around this difficulty.

The second major advance was the introduction of the two-dimensional NOE experiment, largely by Ernst's group. The first use of the experiment to measure NOE enhancements (as opposed to chemical exchange) was published in 1980,[13,14] and the experiment has come to play a central role in work with biological macromolecules. This area has burgeoned still further with the arrival in the late 1980s of isotopically labeled proteins and the many multidimensional NOE experiments that can be applied to them.

Both these advances could not have had the impact they did without developments in instrumental techniques. The first FT NMR spectrometers became available around 1970, and brought much improved sensitivity. To observe small NOE enhancements, one needs not only sensitivity but also instrumental stability, and this was provided by superconducting magnets, which arrived at the end of the 1970s. The third in this trio of instrumental advances was the advent of dedicated computers, which could control the cycling of decoupler frequencies and gating. A direct result of these changes was the NOE difference experiment, which emerged at about this time. A much more recent development is the use of pulsed field gradients, which have found applications in almost all parts of NMR. Gradients provide little benefit for 2D NOESY experiments, but promise to revolutionize 1D measurements.[15]

Although the theory of the NOE as applied to organic molecules has scarcely changed since 1971, its application has increased dramatically. Using the techniques available in 1971, enhancements smaller than 5% could be seen only with difficulty. Now it is not particularly difficult to detect enhancements of less than 1%, or even 0.1% using newer gradient assisted experiments. Hall and Sanders[16] point out that the maximum possible size of NOE enhancement seen between 1,3 diaxial protons in a cyclohexane chair is about 3%. This size of enhancement was just below the threshold of detection in the CW era, but it is well within modern instrumental capabilities.



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