Doctoral Thesis

On the extraction of exact distances and internal motions measured by NOEs in biomolecules

Author(s):
Leitz, Dominik Christoph

Publication Date:
2011

Permanent Link:
https://doi.org/10.3929/ethz-a-006930257

Rights / License:
In Copyright - Non-Commercial Use Permitted
On the extraction of exact distances and internal motions measured by NOEs in biomolecules

D I S S E R T A T I O N

for the degree of

DOCTOR OF SCIENCES

submitted to

ETH ZÜRICH

presented by

DOMINIK CHRISTOPH LEITZ

Dipl. Phys. ETH

born September 29th 1982

citizen of Singen, Germany

accepted on the recommendation of

Prof. Roland Riek, examiner

Prof. Matthias Ernst, co-examiner

Prof. Gunnar Jeschke, co-examiner

MMXI

Walter Moers
Acknowledgements

After my studies in physics I had to make a decision what I want to do with a diploma thesis in quantum optics. Thanks to a professor in the department of physical chemistry I decided to apply in the new group of Professor R. Riek in the field of Nuclear Magnetic Resonance. Having not much clue about this topic, Roland managed to spark my interest in NMR and proposed a lot of interesting possible research topics. So I started in his new group which was the begin of an exciting PhD time.

During these four years I met a lot a people whom I’d like to thank at this place for their different support both on a scientific as well as on a personal level.

First I’d like to thank Professor Roland Riek for fruitful and passional discussion and support. Whenever I stepped into his office, he was full of new ideas and helpful input. My gratitude goes also to Dr. Beat Vögeli who supported me whenever I had problems or questions - you’ve been a great office mate.

Form the very beginning I shared my good an bad times with Alice and Cedric. We had up and downs together but overall you’ve offered me great support.

When I started in Roland’s group there have been only him, me and one postdoc. Jason managed to build up the infrastructure and was around whenever I had a question - regardless of IT support, chemistry or biology.

A special thank goes to Matthias Ernst, who invested a lot of time and passion in the 4th order theory. I owe it to you and your script that I understood NMR much better, which I hopefully could show in the fruitful and interesting discussions we had.

A group is only as good as it’s secretary and without Kristina, life would have been much more painful. Thank you for your organizational skills and for the always warm welcome in your office.
During the years the group grew, as a lot of people joined the BioNMR project. I want to thank all of them, in detail Caro (for nice coffee breaks and taking my moments of rage not too seriously), Christos (for cheering up Cedric), Dean (for honey and whisky), Diego (for organizing the Lab), Julien (for an interesting discussion about the IS System), Lei (for some real Chinese experiences), Michael (for sharing the last bar tender shift), Marielle (for always trusting my chemical knowledge not to blow up the lab), Nadja (for lending me your clean hood), Riccardo (for several security instructions), Sasha (for good old Russian -not only NMR- knowledge), Silvia (for never giving up) and Vivian (for being the best fairy godmother ever seen).

For a good work - live balance I had several groups aiding me with not getting bored in my free time. For two years the Union of the Assistants at the Chemical Laboratories of ETH Zürich (VAC) and especially the board members there made Thursday mornings much more enjoyable. From this time a wonderful friendship grew and it was a real honor to be part of the legendary Ladies Night. To my beloved angles Andrea, Gisela and Stefanie: I will always be your Charlie.

But also the actresses and actors from the group akitiv helped me to make the time in Zurich unforgettable. Four years with four plays took a lot of my free time but it was worth. Thank you all for cheerful rehearsals, successful shows and a wonderful experience. Especially I’d like to thank Anastasia, Gerd and Tobias for the nice tradition of Gadt - evenings and a lot of great discussions with some lovely glasses of vine. And also the friendship with Jan grew from this acting time. Thanks for being a good friend and around whenever I needed you. I will miss our Thursday Lunches.

What would life be without friends who are around to share time with you. Hereby I’d like to thank Kerstin (for three awesome years in the Eisfeldstrasse), Christian (simply for agonizing me), Martin (for great cooking times), Chriggi (for some nice
paper chases), Andreas (for sailing, driving and rewarding my food), Olaf (for sharing almost the best Port with me), Sabine (for a lovely year), Sarah (for reminisce about good, old times) and all the rest I missed here, for remembering Zurich as a home (and for the courage not to be offended as they are not named personally).

Last but surly not least I’d like to thank my family and especially my parents. You supported me wherever you could. I’d had a childhood full of love. Without your help I wouldn’t be where I am now. And many thanks go to my brother Gregor. Although it wasn’t always easy with me you are a real big brother for me. Thanks for sharing house and car with me. But also my aunt Doris and her dog Aiko for nice dinners and lovely Rommé matches. And finally I wanted to thank all the rest of my family. You will always be unforgettable.
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zusammenfassung</strong></td>
<td>xv</td>
</tr>
<tr>
<td><strong>Summary</strong></td>
<td>xvii</td>
</tr>
<tr>
<td><strong>I Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>II Relaxation Theory</strong></td>
<td>7</td>
</tr>
<tr>
<td>1 Introduction</td>
<td>8</td>
</tr>
<tr>
<td>2 Master Equation of Relaxation</td>
<td>10</td>
</tr>
<tr>
<td>3 Two-Spin Solution</td>
<td>13</td>
</tr>
<tr>
<td>4 Higher Order Terms</td>
<td>16</td>
</tr>
<tr>
<td>5 Internal Motions</td>
<td>18</td>
</tr>
<tr>
<td>5.1 Modeling internal motion</td>
<td>20</td>
</tr>
<tr>
<td>5.1.1 Decorrelation assumption</td>
<td>21</td>
</tr>
<tr>
<td>5.1.2 Jump Model</td>
<td>23</td>
</tr>
<tr>
<td>5.1.3 &quot;Diffusion in a cone&quot; model</td>
<td>31</td>
</tr>
<tr>
<td>**III Protocol for and problems in the determination of effective</td>
<td>35</td>
</tr>
<tr>
<td>distances**</td>
<td></td>
</tr>
<tr>
<td>6 Introduction</td>
<td>36</td>
</tr>
<tr>
<td>7 Protocol for the extraction of exact distances from NOESY experiments</td>
<td>38</td>
</tr>
</tbody>
</table>
7.1 NOESY pulse sequence ........................................ 38
7.2 Measuring the correlation time $\tau_c$ ......................... 42
7.3 Extraction of the auto-relaxation rate constants .......... 45
7.4 Extraction of the cross-relaxation rate constants ........ 46

8 Problems in the determination of exact distances from NOESY experiments 48
8.1 Determination of the correlation time ......................... 50
8.2 The translation of NOE rate constants to average distances 53
8.3 Spin diffusion in a multiple spin system ....................... 60
8.4 Higher order contributions to the classical description of spin diffusion . 63
  8.4.1 Introduction .................................................. 63
  8.4.2 Solution of the Liouville-von-Neuman equation to fourth order 64
  8.4.3 The cumulant expansion ..................................... 65
  8.4.4 Master equation of fourth order under a dipole-dipole Hamiltonian 69
  8.4.5 Simulations of a two step process in the presence of three spins
       for small rate constants ....................................... 80
  8.4.6 Simulations of a two step process in the presence of three spins
       for fast processes ............................................. 85
  8.4.7 Discussion ..................................................... 88

IV Application of exact NOEs (eNOE) to the model protein
ubiquitin .......................................................... 91
9 Ubiquitin as a model system for the extraction of exact distances 92
  9.1 Sample preparation ............................................. 93
10 Exact distances and internal dynamics of perdeuterated ubiquitin from
    NOE buildups .................................................... 94
  10.1 Introduction .................................................... 94
10.2 Methods .......................................................... 95
10.3 Comparison between exact distances extracted from NOEs and distances obtained from the X-ray structure ........................................... 95
10.4 Order parameters for ubiquitin and the Internal Motion encoded in them 98
10.5 Conclusion ........................................................... 100

11 Temperature-dependence of $^1$H$_N$ - $^1$H$_N$ distances in ubiquitin as studied by exact measurements of NOEs

11.1 Introduction .......................................................... 102
11.2 Methods ............................................................. 103
   11.2.1 NMR and data analysis ...................................... 103
   11.2.2 CD spectroscopy .............................................. 104
   11.2.3 MD simulations .............................................. 104
   11.2.4 Temperature normalized cross-relaxation rate constants ......... 105
11.3 Temperature-dependent $^1$H$_N$ - $^1$H$_N$ NOE rate constants of ubiquitin . . . 105
11.4 Comparison between measured NOEs and those calculated from various structural models .......................................................... 108
11.5 Temperature-dependent $^1$H$_N$ - $^1$H$_N$ distance in ubiquitin .................. 109
11.6 Comparison of the $^1$H$_N$ - $^1$H$_N$ NOE rate constants to other temperature-sensitive probes ...................................................... 115
11.7 Unfolding pathway of ubiquitin ..................................... 121
11.8 Conclusion ............................................................. 122

12 The influence of Binding on the Distances Studied by Measurements of Ubiquitin/UBM2 complexes

12.1 Introduction .......................................................... 124
List of Figures

3.1 Time dependance of the longitudinal magnetization of a two spin system 15
5.1 Three spin jump system with different transition rate constants . . . . . 27
7.1 NOESY pulse sequence used for the measurement of exact distances . . 39
7.2 Schemes of the pulse sequences and real data points used for measuring
the rotational correlation time . . . . . . . . . . . . . . . . . . . . . . . . . . 44
7.3 Experimental NOE buildup curves for pairs of residues in various sec-
ondary structures . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 47
8.1 Flowsheet for the extraction of exact distances from a series of NOESY
spectra . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 49
8.2 Concentration dependence of the correlation time . . . . . . . . . . . . 51
8.3 Correlation times for ubiquitin at different temperatures compared with
literature values . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 53
8.4 Cartoon illustrating the extraction of the order parameter of the NOE
from local order parameters . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 54
8.5 Relationship between calculated order parameters and local order para-
meters . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 56
8.6 Comparison of fast order parameters derived with the simulations and
the NMR bundle 2k39 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 59
8.7 The influence of fast motion on the NOE for different sterical conformation........................................ 60
8.8 Spin-diffusion effects on fitting of the NOE buildups .......................................................... 61
8.9 Three spin relaxation simulations for a linear three jump model assuming correlated and uncorrelated motion ................................................................. 83
8.10 Three spin relaxation simulations for a static linear three jump model and the correlated system compared to the fast limit average .......... 84
8.11 Two spin relaxation simulations for a linear two jump model optimized for a correlation time of $\tau_c = 100$ ns .................. 87

9.1 Secondary structure and Amino Acid sequence of Ubiquitin ............ 93

10.1 Comparison between distances derived from the X-ray structure and effective distances derived from experimental data. .................. 97

10.2 Order parameters depicted on the ribbon representation of ubiquitin ... 100

11.1 $\tau_c$- corrected normalized intensities of a representative cross peaks versus the mixing time for the three temperatures .................... 107
11.2 Relative temperature - dependent changes of NOE rate constants $\sigma^*$ .... 111
11.3 NOE - derived effective distances $r_{\text{eff}}$ between two $^1\text{H}_\text{N}$ atoms in ubiquitin measured for three temperatures .................. 112
11.4 Temperature-dependent changes of the NOE-derived order parameters in ubiquitin. ...................................................... 114
11.5 Temperature-dependent $^1\text{H}_\text{N}$ chemical $\delta(^1\text{H})$ shifts versus $\tau_c-$ corrected NOE rate constants $\sigma^*$ ............................... 118
11.6 Correlation plots between differences in chemical shifts of $^{13}\text{C}_\alpha$ and $^{13}\text{C}_\beta$ versus the corresponding $\tau_c$- corrected NOE $\sigma^*$ ........................ 119
11.7 Scalar coupling constants across hydrogen bonds versus $\tau_c$ - corrected

NOE rate constants $\sigma^*$ ........................................ 120

11.8 CD spectra of ubiquitin at the three temperature used for this study . . . 121

12.1 TROSY spectra at different UBM2 concentrations . . . . . . . . . . 125

12.2 Experimental NOE buildup curves for pairs of $^1$H-$^1$H residues in ubiquitin 127

12.3 Correlation plot and bar plot for the binding studies of ubiquitin with

UBM2 ................................................................. 129

A1.1 The integration of the correlation function following Goldman [1] . . . 156

B1.1 Source code for calculation of fast internal motion . . . . . . . . . . 171

B1.2 Source code for calculation of spin diffusion in NOE buildups . . . . . 173
**List of Tables**

5.1  Overview of the spectral density function and relaxation rate constants obtained for the jump model .............................................. 30

8.1  Rotational correlation times $\tau_c$ of ubiquitin at four different temperatures 52
8.2  Mean values with standard deviations for the fast order parameters . . 58

10.1 Slopes $s$ and Pearson's correlation coefficient $r$ between effective experimental distances and those extracted from the X-ray structure. . . . . . . 97
10.2 Slopes $s$ and Pearson's correlation coefficient $r$ between experimental cross-relaxation rate constants and rigid ones back-calculated from the X-ray structure. ........................................... 99

11.1 Slopes $s$ and Pearson's correlation coefficient $r$ between experimental and predicted cross-relaxation rate constants $\sigma$ for ubiquitin for all three temperatures .............................................................. 108
11.2 Temperature-dependent changes of $^1\text{H}_N$-$^1\text{H}_N$ distances within the $\alpha -$ helix of ubiquitin derived from NOE measurements. .................... 115

A.1  Complete set of eigenoperators of the unperturbed Zeeman-Hamiltonian 160
A.2  Double Commutators for dipole-dipole relaxation .......................... 161
A.3  Expectation values for dipole-dipole auto- and cross-interaction ....... 161
Abbreviations

COSY .................................................. COrrelation SpectroscopY
TOCSY ............................................... TOTal Correlation SpectroscopY
TROSY ........................................... Transverse Relaxation Optimized SpectroscopY
NOESY ................................... Nuclear Overhauser Effect SpectroscopY
HMQC ........................................ Heteronuclear Multiple-Quantum Correlation spectroscopy

\[ \hbar = 1.055 \cdot 10^{-34} \text{ Js} \] .............................. Planck constant

\[ \mu_0 = 4\pi \cdot 10^{-7} \frac{\text{Vs}}{\text{Am}} \] .............................. Magnetic constant

\[ \gamma_H = 267.5 \frac{\text{MHz}}{\text{T}} \] .............................. Gyromagnetic ratio of the proton

\[ \gamma_N = -27.1 \frac{\text{MHz}}{\text{T}} \] .............................. Gyromagnetic ratio of nitrogen

\[ \sigma_{zz} \] .............................. zz component of the chemical-shift tensor

\[ \omega_0 \] .............................. Lamour frequency of the individual spin

\[ \tau_c \] .............................. Rotational correlation time

\[ D = \frac{1}{6\tau_c} \] .............................. Diffusion constant of the molecule

\[ \sigma, \tilde{\sigma} \] .............................. Density matrix

\[ \mathcal{H} \] .............................. Hamilton operator

\[ \hat{\cdot} \] .............................. Operator in the Liouville space

\[ J(\omega) \] .............................. Spectral density function

\[ C(t) \] .............................. Correlation function

\[ S^2 \] .............................. Order parameter

\[ \rho, \rho_i \] .............................. Auto - relaxation rate constant of spin \( i \)

\[ \sigma_{ij} \] .............................. Cross - relaxation rate constant between spin \( i \) and spin \( j \)

\[ Y^l_m(\theta, \varphi) \] .............................. Spherical harmonic function of degree \( l \) and order \( m \)

\[ P^l_m(\cos \theta) \] .............................. Associated Legendre polynomials of degree \( l \) and order \( m \)
\langle \cdot \rangle \quad \text{Boltzmann ensemble average}

T_1 \quad \text{Longitudinal relaxation time}

T_2 \quad \text{Transversal relaxation time}

T_{1\rho} \quad \text{Rotating frame relaxation time}

Arg \quad \text{Arginine (A)}

Cys \quad \text{Cysteine (C)}

His \quad \text{Histidine (H)}

Ubq \quad \text{Ubiquitin}

UBM \quad \text{Ubiquitin-binding molecule}

CD \quad \text{Circular dichroism}

GB3 \quad \text{Protein G (B3 domain)}
Zusammenfassung


Um diese konformationelle Änderung zu analysieren, benötigt man somit die genaue Kenntnis der internen Bewegungen des Proteins. Ziel dieser Arbeit ist es daher, eine neue Methode zu beschreiben, um Distanzen zwischen Atomen in einem Protein zu messen und daraus Informationen über die innere Bewegung zu erhalten.

Um eine Beziehung zwischen Distanzen und interner Bewegung zu finden, erfolgt zuerst eine kurze Einführung in die Relaxationstheorie der Kernspinresonanzspektroskopie (NMR), um die Grundlagen für den nuklearen Overhausereffekt (NOE) zu schaffen. Dieser bildet die Kernidee in der vorgestellten Methode zur exakten Bestimmung von zeitlich und ensemble gemittelten Distanzen sowie der Extraktion von Bewegung aus diesen.
Summary

The internal motion of proteins is closely related to their function. This motions are attributed to different conformational states which can be influenced by interactions with other proteins. This enables their functions as switches or signal transmitters.

To study such conformational changes it is therefore necessary to know the internal motion of a protein. The aim of this work is to establish a method for measuring exact distances between atoms in a protein in order to extract information about internal motions.

To find a relation between distances and motions, a short introduction to the theory of relaxation in nuclear magnetic resonance (NMR) is given in the first part, in order to revise the basic knowledge on the nuclear Overhauser effect (NOE). The NOE is the core phenomenon for the presented method on the measurement of time and ensemble averaged exact distances and the extraction of motion out of them.
In the second part, a protocol for the measurement of NOEs is presented. Afterwards, problems of converting NOEs into exact distances and solutions to overcome them are discussed. In the third part, a practical application of the method presented is given. NOESY spectra of the model protein ubiquitin were measured. The extracted distances were compared to different data sets from literature. To use the potential of dynamical informations stored in the NOE, measurements were performed at three different temperatures as well as in presence of a binding partner for the lowest temperature. From the intensities of the diagonal peaks and the NOE cross-peaks relaxation rates could be determined which were translated into distances. In conclusion, the presented method of quantitative NOE rates can be used to highlight small conformational and dynamical changes (i) upon variations of the temperature and (ii) in the presence of a binding partner.
Part I

Introduction
When Otto Stern and Walther Gerlach [2] discovered the quantisation of the magnetic momentum of silver atoms in 1922, they might have guessed that they changed the understanding of nature on a quite fundamental level. But they could never have seen that they paved the way for a wide range of practical applications beyond the borders of physics towards chemistry, biology and medicine like structure determination of molecules or magnetic resonance tomography.

Anyhow it was a long way towards these modern applications of the spin quantity. An important leap was taken by Felix Bloch and Edward Mills Purcell in the late 1940s. They discovered, independent of each other, the nuclear magnetic moment and were awarded the Nobel prize in 1952 "for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith" [3, 4].

But 20 more years had to pass until the modern concepts of Fourier Transformed Spectroscopy replaced the continuous wave method used before. Due to the efforts of Richard Ernst [5] and Kurt Wüthrich [6] Nuclear Magnetic Resonance (NMR) has become a powerful tool in the structure determination of molecules in chemistry and biology.

Now, almost 90 years and four Nobel prices later, some people claim that the field of NMR has reached its limit and is fated to die. But the strength of NMR of having for almost each atom within a molecule a probe (i.e. the nuclear spin) is unprecedented and hence NMR will continue to be one of the major techniques for the observation of quantities on an atomic level. This includes further technical developments, although most of the basic concepts of nuclear magnetic resonance are established. One of the major advantages of NMR, when for example compared to other tools useful in the determination of structures (for example X-ray crystallography), is that NMR can capture motions. Therefore it can be used to observe chemical reactions, diffusion processes or
internal motions within the molecule. Especially for biomolecules, this fact has come into focus since several years.

There are three main types of motions in proteins: the overall motion, the inner motion and the rearrangement of the protein three-dimensional structure. The overall motion describes the translational or rotational motion of the whole protein through space. The inner motion derives from movements of each individual atom as a result of the energy stored in the system, such as the vibrational motions of a carbon-carbon bond in the backbone of a protein. The changes in conformation are due to a structural rearrangement, and they are of main interest in biology since they are often necessary for a protein to accomplish its function. As an example, the sodium-potassium channel is a membrane protein responsible for the active transport of sodium ions from the citosol to the extracellular space and of potassium ions from the extra- to the intracellular space. This process is crucial to maintain cell volume, to generate the resting potential in neurons and is implied in signal transduction [7]. To exert these functions, the protein has to undergo major conformational changes in order to bind the ions on one side, transport, and finally release them to the opposite site. These conformational changes can be described in a motional picture.

This example illustrates the requirement of methods that are sensitive to both structure and motion, such as NMR. And as claimed, the knowledge of such internal motion is crucial for the understanding of the function of a protein. We will focus in the following on large biomolecules to be studied in solution and try to combine the two advantages of NMR - to measure distances and detect motion - in one experiment, the NOESY experiment.

The elucidation of the complete dynamics of a biomolecule proves to be difficult. A step towards this aim was done in 1953 by Albert Warner Overhauser, when he discovered
the polarization transfer between electrons and the nuclei in a metal [8] which is based on a dipole-dipole interaction. The transfer rate constant is proportional to the inverse sixth power of the distance between the two dipoles. Hence, this effect can be used to measure through space distances and since it is a time-averaged probe, it is sensitive to internal motions, too. Although data derived from the Nuclear Overhauser Effect (NOE) are nowadays the most important restraints for the structure determination of biomolecules in solution, they are usually only used qualitatively as highlighted in the following. The common procedure for 3D structure determination by NMR includes first the sequential assignment that can be obtained by triple-resonance experiments in $^{15}$N, $^{13}$C labelled biomolecules [9] or by recording COSY (COrrelation SpectroscopY) or TOCSY (TOtal Correlation SpectroscopY) experiments [10] for unlabeled systems. Once, for each NMR resonance a nuclear spin has been assigned a NOESY spectrum is recorded. The intensities of the cross-peaks therein can be converted to a maximum distance. This conversion is not trivial as the complete set of nuclear spins have be be taken into account. The best approach is the to use a full relaxation matrix analysis [11] where the matrix elements (e.g. the auto- and cross-relaxation rates) are fitted to the measured intensities. This approach takes spin diffusion effects into account and enables therefore longer mixing times for a better signal-to-noise ratio. The distances derived thereof are finally used as a restraint in structure calculation programs. The first one (called MEDUSA) was presented in 1991 and used a search algorithm for the determination of multiple conformations of biomolecules [12]. The program calculated low-energy structures to fulfill all experimental constraints including NOE distance constraints. The resulting bundle represented the system much better than a single average structure. Nowadays, a lot more programs for structure calculation, such as CYANA [13] or XPLOR-NIH [14], are in use.
But the potential of extracting motional effects from NOE data was not used so far. In routine NMR spectroscopy, local information on motion with time scales up to nanoseconds (fast motion) is obtained for $^{15}\text{N}-^1\text{H}$ bond vectors from measurements of the backbone nitrogen transverse and longitudinal relaxation times and heteronuclear [$^{15}\text{N}-^1\text{H}$] NOEs [15]. Relaxation dispersion NMR experiments accurately sample the time scale on which very slow (i.e. ms-s range) local motions take place [16]. The measurement of residual dipolar couplings (RDCs) allows the sampling of bond orientations over a time scale up to milliseconds [17], [18]. Recently, these local experimental restraints in combination with molecular dynamic simulation or structure prediction software were used towards a comprehensive dynamic picture of a biomolecule. Furthermore, cross-correlated relaxation NMR can be used to study correlated motion in proteins [19], [20]. We recently proposed an alternative approach towards the aim of determining the entire structure and motion of a protein, which is also the essence of the thesis presented. We demonstrated that the measurement of NOE rate constants [10, 21] between amide protons in perdeuterated as well as protonated ubiquitin and GB3 enables the determination of time-averaged $^1\text{H}_N^{-1}\text{H}_N$ distances up to 5 Å with high accuracy and precision. Since the NOE-derived distance is a time-averaged parameter covering both fast and slow motions (faster or slower than the rotational correlation time of the molecule, respectively), the potential of NOEs to provide information on fast and slow motion is evident although the relationship between NOEs, dynamics and structure might be complex. Due to the abundance of protons in a protein, this collection of exact NOEs may lead to a rather complete map of the internal motion of a biomolecule.

In this thesis we will give a brief introduction into the relaxation theory for the determination of the auto- and cross-relaxation rate constant for dipole-dipole couplings called NOE. Afterwards, a detailed discussion about the problems of the extraction of exact distances from cross-relaxation rate constants measured by NMR is given, including the
determination of the overall correlation time $\tau_c$ of a biomolecule, the influence of fast internal motion and spin diffusion on the NOE. At the end of this part, a discussion about the limits of the semi-classical description of relaxation phenomena as well as a fourth order expansion of the solution of the Liouville - von Neumann equation is presented. In the last part of the thesis, the presented protocol and approach for the extraction of exact average distances from NOE buildup rate constants is applied to ubiquitin. From NOEs, temperature dependent distance changes in ubiquitin and distance changes upon binding of a protein partner are extracted and compared to common structural models as well as distances derived with other techniques. In the Appendix a more detailed derivation of the master equation of relaxation is given. Further, the software programs used for the calculation of the influence of spin diffusion and fast internal motion to the NOE are explained.
Part II

Relaxation Theory
1 Introduction

The state of a spin system in an ensemble can be described by a density matrix $\sigma$, whose time evolution is given by the Liouville-von Neumann equation [22]. A simple approach to calculate this time dependency is to derive a formal Picard-Lindelöf expansion up to n-th order [23]. If after a time $t$ the density matrix does not differ much from its original value at $t = 0$, the expansion can be truncated after the second order. In general, such an expansion up to second order is a good description for a one step process where energy (in form of a quantum mechanical Hamiltonian) is transferred in a single step from one state of the density matrix to another. The Redfield theory [24] to describe relaxation phenomena in (nuclear) magnetic resonance is such a second order approximation. Performing the second order expansion makes it necessary to calculate all occurring commutators and to derive the transition rate constants describing the mixing of the different states in the density operator. Solving the differential equation using the derived expressions for the transition yields the time dependence of the density operator. This differential equation has a formal exponential solution. For short evolution times a Taylor expansion of the exponential function up to first order gives a good approximation to describe the one step transfer process between the two states involved [25] resulting in a linearly increasing function. For longer mixing times higher order processes (such as two step processes) get important and a straight solution is only derived by evaluation of the whole exponent which is similar to a solution of the differential equations. For more than two spins a closed solution can in general not be obtained. In
this part we first derive an expression for the master equation of relaxation. Afterwards, several models to describe internal motions of the proteins are presented.
2 Master Equation of Relaxation

In this chapter only an overview of the most important steps in the derivation of a so-called master equation of relaxation is given. For a more detailed description see chapter A in the Appendix.

Starting from the Liouville-von Neumann equation

\[
\frac{d\sigma}{dt} = -\frac{i}{\hbar}[\mathcal{H}, \sigma]
\] (2.1)

one can calculate the evolution of the density matrix under a Hamiltonian modulated by a random fluctuation of the form \( \mathcal{H} = \mathcal{H}_0 + \mathcal{H}(t) \). Here, a brief overview on the central steps and assumptions is given for the derivation of a so-called master equation of relaxation (following [1]).

1. First one has to change into the interaction frame to eliminate the effect of the Zeeman Hamiltonian and make an ensemble average.

2. A solution of the Liouville-von Neumann equation is given by its formal Picard-Lindelöf equation.

3. The first order of the expansion is zero due to the zero average of the stochastic process modulating the Hamiltonian. The second order term describes the auto-
and cross-relaxation rate constants. If the mixing time $t$ is short enough so that the density matrix does not change much from its initial value at $t = 0$, higher order terms can be neglected. For spin flip rate constants in the order of seconds - as given for NOE transfers - this assumption is quite reasonable.

4. As the system has a finite temperature, it will relax into an equilibrium state unequal to zero. Taking this into account one has to subtract this equilibrium value from the original density matrix.

5. The Hamiltonian can be decomposed into a spin part and a random function of time. Changing back into the laboratory frame by finding a basis of eigenoperators of $\mathcal{H}_0$ one assumes that each spin operator oscillates at one frequency. As the spin part is time independent, one can define a correlation function as the time averaged product of the two random time functions at different time points.

At this point the Liouville-von Neumann equation can be written as

\[
\dot{\hat{\sigma}}(t) - \hat{\sigma}(0) \propto \sum_{m=-l}^{l} \sum_{p,p'} [V_p, [V^\dagger_{p'}, \hat{\sigma}(0)]] \\
\int_0^t \int_0^{t_1} C_m(t_1 - t_2) e^{i(\omega_{p,t_1} - \omega_{p',t_2})} dt_2 dt_1
\]

(2.2)

where $V^{p,p'}$ label the spin operators oscillating at frequencies $\omega_{p,p'}$ and $C_m(t_1 - t_2)$ is the correlation function.

6. In the rotating wave approximation (where $(\omega_p - \omega_{p'}) t \gg 1$) only terms with $p = p'$ have to be taken into account. The correlation function can than be integrated within the new variables $t_1$ and $\tau = t_1 - t_2$ under the assumption that the time scale on which the correlation function decays is much smaller than the relaxation time used in the experiment. This is called the Redfield limit. The
characteristic time scale for the decay of the correlation function is called the correlation time $\tau_c$. Due to this assumption one can replace the integration limit $t \to \infty$. The integral
\[
\int_0^{\infty} C^m(\tau)e^{i\omega \tau}d\tau := J^m(\omega)
\]
is then the Fourier transform of the correlation function and named spectral density.

7. As the difference $\tilde{\sigma}(t) - \tilde{\sigma}(0)$ is small due to the first assumption, one can write $\frac{\tilde{\sigma}(t) - \tilde{\sigma}(0)}{t} \approx \frac{\tilde{\sigma}(t)}{dt}$ and finally finds the master equation of relaxation in the laboratory frame of the form
\[
\frac{d}{dt}\tilde{\sigma} \propto \sum_{m=-l}^{l} \sum_p [V_p, [V_p^\dagger, \tilde{\sigma}(0)]], J^m(\omega)
\]
3 Two-Spin Solution

The density matrix of a two-spin $1/2$ system is given by the Pauli matrixes. In the NOESY experiment (see chapter 7) one observes the relaxation of the longitudinal components labeled $I_z$ and $S_z$. In the following we will assume, that the relaxation is only caused by dipole-dipole interaction of the spins. Other relaxation mechanism can be attributed to chemical shift anisotropy for example. The master equation of relaxation (also called the Solomon equation [26]) for a two spin system under a dipole-dipole Hamiltonian is then given by

$$\frac{d}{dt}\begin{pmatrix} I_z \\ S_z \end{pmatrix} = -\begin{pmatrix} \rho_I & \sigma_{IS} \\ \sigma_{IS} & \rho_S \end{pmatrix} \cdot \begin{pmatrix} I_z \\ S_z \end{pmatrix} \tag{3.1}$$

where $\rho_x = \frac{1}{T_x}$ is the inverse of the longitudinal relaxation time of the spin $x \in \{I, S\}$ and $\sigma_{IS}$ is the cross-relaxation rate constant. The matrix elements can now be calculated using $\rho_I = \text{Tr} \left\{ I_z \hat{R} I_z \right\}$, $\rho_S = \text{Tr} \left\{ S_z \hat{R} S_z \right\}$ and $\sigma_{IS} = \text{Tr} \left\{ S_z \hat{R} I_z \right\}$ where $\hat{R}$ is the super operator which can be extracted from eq. (2.4). After calculating all occurring commutators and the final trace, one gets for the auto- and cross-relaxation rate constants under a pure dipole-dipole Hamiltonian (for a detailed derivation see again
\[ \rho_I = \frac{\gamma_I^2 \gamma_S^2 \mu_0^2}{(4\pi)^2} \frac{1}{4} \{ J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S) \} \]  
\[ \rho_S = \frac{\gamma_I^2 \gamma_S^2 \mu_0^2}{(4\pi)^2} \frac{1}{4} \{ J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I + \omega_S) \} \]  
\[ \sigma_{IS} = -\frac{\gamma_I^2 \gamma_S^2 \mu_0^2}{(4\pi)^2} \frac{1}{4} \{ J(\omega_I - \omega_S) - 6J(\omega_I + \omega_S) \} \]

The master equation of relaxation for a two-spin system can be solved analytically. By diagonalizing the matrix defined in eq. (3.1) one finds:

\[ I_z(t) = \frac{1}{2} \cdot (I_0^z + S_0^z) \cdot e^{\lambda_1 t} + \frac{1}{2} \cdot (I_0^z - S_0^z) \cdot e^{\lambda_2 t} \]  
\[ S_z(t) = \frac{1}{2} \cdot (I_0^z + S_0^z) \cdot e^{\lambda_1 t} - \frac{1}{2} \cdot (I_0^z - S_0^z) \cdot e^{\lambda_2 t} \]

where \( \lambda_{1,2} = -\frac{1}{2} \left[ (\rho_I + \rho_S) \pm \sqrt{(\rho_I - \rho_S)^2 + 4\sigma_{IS}^2} \right] \). Starting on spin \( I \) with only \( I_z \) magnetization present at \( t = 0 \), one can simulate a two-spin relaxation process using the formulas:

\[ \frac{I_z(t)}{I_0^z} = \frac{1}{2} \left( e^{\lambda_1 t} + e^{\lambda_2 t} \right) \]  
\[ = \cosh \left( \frac{1}{2} \sqrt{(\rho_I - \rho_S)^2 + 4\sigma_{IS}^2} \cdot t \right) \cdot e^{-\frac{1}{2}(\rho_I+\rho_S)t} \]

which is the decay of the diagonal peak and

\[ \frac{S_z(t)}{I_0^z} = \frac{1}{2} \left( e^{\lambda_1 t} - e^{\lambda_2 t} \right) \]  
\[ = \sinh \left( \frac{1}{2} \sqrt{(\rho_I - \rho_S)^2 + 4\sigma_{IS}^2} \cdot t \right) \cdot e^{-\frac{1}{2}(\rho_I+\rho_S)t} \]

representing the build-up of the magnetization on the \( S \) spin.
Figure 3.1: Time dependence of the longitudinal magnetization of a two spin system assuming pure dipole-dipole interaction. The red line is the auto-relaxation of the starting spin, while the green line is the normalized intensity of the second spin over time. The formulas derived above labels the two curves.
4 Higher Order Terms

An isolated two-spin system is very unlikely in a protein. Only for perdeuterated proteins (where only the amid attached protons remains) or spins in a helix, where next neighbor spins are close in space and all other atoms are too far apart to have a major contribution to the cross-peak intensity, a two spin system is a good approximation for the system. Hence, a better description would be a three-spin system. The master equation of relaxation (also called the general Solomon equation [25, 26]), is given by

$$ \frac{d}{dt} \begin{pmatrix} I_{1z} \\ I_{2z} \\ I_{3z} \end{pmatrix} = - \begin{pmatrix} \rho_1 & \sigma_{12} & \sigma_{13} \\ \sigma_{12} & \rho_2 & \sigma_{23} \\ \sigma_{13} & \sigma_{23} & \rho_2 \end{pmatrix} \cdot \begin{pmatrix} I_{1z} \\ I_{2z} \\ I_{3z} \end{pmatrix} \Leftrightarrow \frac{d\sigma(t)}{dt} = -\hat{R} \cdot \sigma(t) \quad (4.1) $$

where the auto- and cross-relaxation rate constants $\rho_i$ and $\sigma_{ij}$, respectively, can be calculated as shown above. This system of coupled differential equations can not be solved analytically any more. As the matrix elements of $\hat{R}$ are time independent, a formal solution is of the form

$$ \sigma(t) = e^{-\hat{R}t} \cdot \sigma(0). \quad (4.2) $$
Under the assumption that $\rho_1 \approx \rho_2 \approx \rho_3$, one can decomposed the matrix $\hat{K}$ into a diagonal and a non-diagonal part (as $[\hat{\rho}, \hat{\sigma}] \approx 0$) resulting in

$$e^{-\hat{R} \cdot t} \approx e^{-\hat{\rho} \cdot t} \cdot e^{-\hat{\sigma} \cdot t}$$

$$= \begin{pmatrix} e^{-\rho_1 \cdot t} & 0 & 0 \\ 0 & e^{-\rho_2 \cdot t} & 0 \\ 0 & 0 & e^{-\rho_3 \cdot t} \end{pmatrix} \cdot \left[ 1 - \hat{\sigma} \cdot t + \frac{1}{2} \hat{\sigma}^2 \cdot t^2 + \ldots \right].$$

The first term in the formal solution given in eq. (4.3) is the auto-relaxation rate constant while the second term is the known cross-relaxation rate constant, as for short times eq. (3.8) can be expanded to $\sinh(x \cdot t) \approx x \cdot t$. The third term which gets dominant for longer mixing times $t$ is called the spin diffusion contribution where magnetization is transferred from one spin to another via an intermediate one.

While such a three spin approach is rather sufficient to describe most or all phenomena occurring in a NOESY experiment, a numerical solution of the Solomon equation for more spins is also possible using the so called "full matrix approach", where the master equation of relaxation is solved for the entire spin system [11]. But for this approach it is assumed, that the initial magnetization is uniform for all spins. In a 2D NOESY this condition is in general fulfilled. But for measuring time reasons, the interscan delay in a 3D NOESY (as used for the experiments presented in this thesis) is too short and not all spins will be back to equilibrium. Hence, a full matrix approach is not usable for the analysis presented in this thesis.
5 Internal Motions

The aim of this work is to extract internal motions from exact distances calculated from cross-relaxation rate constants. So far, we derived a relation between the cross-relaxation rate constant and the spectral density function. To extract distances from the cross-relaxation rate constant determined in eq. (3.4 - 3.3) one needs a detailed expression for the density function. First, a general discussion about these function is given, followed by expressions for the distance dependance in the presence of fast and slow motion when compared to the overall rotational correlation time $\tau_c$ of the molecule.

As the density function is the Fourier transform of the correlation function one has to find an expression for this function. Under the assumption of isotropic molecular tumbling $J(\omega)$ is given by [27]

$$J(\omega) = \left(S_{\text{HH}}^{\text{fast}}\right)^2 \frac{\tau_c}{1 + (\tau_c \omega)^2} + \left(S_{\text{HH}}^{\text{rigid}}\right)^2 \frac{1}{r_{\text{HH}}^6} \left\langle \frac{1}{r_{\text{HH}}^6} \right\rangle - \left(S_{\text{HH}}^{\text{fast}}\right)^2 \frac{\tau_{\text{tot}}}{1 + (\tau_{\text{tot}} \omega)^2}$$

with

$$\frac{1}{\tau_{\text{tot}}} = \frac{1}{\tau_c} + \frac{1}{\tau_{\text{int}}}$$

where $\tau_c$ is the rotational correlation time of the molecule and $\tau_{\text{int}}$ is the correlation time for internal motion. The squared brackets denote a Boltzmann ensemble average. The order parameter for fast internal motion $\left(S_{\text{HH}}^{\text{fast}}\right)^2$ used in this work depends on the polar
and azimuthal angles s (i.e. $\vartheta_{\text{mol} \text{HH}}$ and $\varphi_{\text{mol} \text{HH}}$, respectively) as well as the distances:

$$
(S_{\text{HH}})^2 = \left( \frac{r_{\text{rigid} \text{HH}}^6}{\sigma_{\text{HH}}^2} \right)^\frac{2}{5} \sum_{q=-2}^{2} \left\{ \frac{Y_{2q} \left( \vartheta_{\text{mol} \text{HH}}, \varphi_{\text{mol} \text{HH}} \right)}{(r_{\text{HH}}^3)^q} \right\}^2.
$$

(5.3)

An experimentally accessible order parameter is defined by the ratio of the experimentally derived cross-relaxation rate constant and the one expected for a rigid molecule:

$$
(S_{\text{HH}})^2 = \frac{\sigma_{\text{exp} \text{HH}}}{\sigma_{\text{rigid} \text{HH}}}.
$$

(5.4)

For internal motion much faster than nanoseconds ($\tau_{\text{int}} \ll \tau_c$) under the assumption $J(0) \gg J(\omega)$ (which holds true at $\omega = 2\pi \cdot 700$ MHz), $(S_{\text{HH}})^2$ reduces to the order parameter of fast motion as defined in eq. (5.3)

$$
(S_{\text{HH}})^2 = (S_{\text{fast} \text{HH}})^2.
$$

(5.5)

The order parameter for fast motion introduced above can be approximately decomposed into an angular component $(S_{\text{HH}}^\text{fast \ text{HH}})^2 \leq 1$ and a radial $(S_{\text{HH}}^\text{fast \ text{HH}})^2 \geq 1$ one.

The definition of the radial order parameter is different from the one defined in [27] (where it is the ratio of the fast and slow radial distance averaging), with the advantage that it is an absolute quantity rather than a relative one. Hence, depending on the exact nature of the fast internal motions, $(S_{\text{HH}}^\text{fast \ text{HH}})^2$ can be smaller or larger than 1. For motion much slower than the molecular tumbling ($\tau_{\text{int}} \gg \tau_c$), $(S_{\text{HH}})^2$ becomes independent of angular coordinates

$$
(S_{\text{HH}})^2 = \left( \frac{r_{\text{rigid} \text{HH}}^6}{\sigma_{\text{HH}}^2} \right)^\frac{2}{5} \left\langle \frac{1}{r^6} \right\rangle.
$$

(5.6)

The simplest and most common way to extract a distance is to use eq. (5.1) under the assumption of a rigid molecule. The distance has to be replaced by an effective one.
\( r_{HH}^{\text{eff}} \) into which all motional effects are absorbed:

\[
\sigma_{HH} = \left( \frac{\mu_0}{4\pi} \right)^2 \gamma^4 h^2 \frac{1}{40\pi^2 (r_{HH}^{\text{eff}})^6} J^{\text{rigid}}(0)
\]  

(5.7)

Here we assumed equal spins, e.g. \( \gamma_I = \gamma_S = \gamma \) (see eq.(3.3)). The effective distances can be expressed with the distances in a rigid molecule and the order parameter \( (S_{HH})^2 \) as follows from eq. (5.4):

\[
r_{HH}^{\text{eff}} = \frac{r_{HH}^{\text{rigid}}}{\sqrt{(S_{HH})^2}}
\]

(5.8)

If only fast motion is present, the effective distance \( r_{HH}^{\text{eff}} \) is

\[
r_{HH}^{\text{eff, fast}} = 6 \left( \frac{4\pi}{5} \sum_{q=-2}^{2} \left\langle \frac{Y_{2q}(\vartheta_{\text{mol}}, \varphi_{\text{mol}}, r_{HH})^2}{(r_{HH})^3} \right\rangle \right)^{-1}
\]

(5.9)

The expression for the effective distance \( r_{HH}^{\text{eff}} \) if only slow motion is present is much simpler:

\[
r_{HH}^{\text{eff, slow}} = 6 \left\langle \frac{1}{r_{HH}^{\text{slow}}} \right\rangle
\]

(5.10)

### 5.1 Modeling internal motion

The description of motion in a protein is quite complex. To determine a so called "master equation" one tries therefore to model these motions. As the internal motion has both, an angular as well as a distance dependency, it is challenging to take both effects into account and hence they are separated into two models. The "diffusion in a cone" model
which takes only angular variations into account and the "Tropp N-jump model"
[29], where the nuclei can jump between different distances with respect to each other.
In general, the "master equations" for these models can only be solved analytically in
the limit of fast or slow internal motion compared to the overall tumbling time. But for
a linearly aligned two or three spin system, a general analytical solution can be derived
for the Tropp model. In the following, the N site jump model is solved first in general
within the limits described above and finally for a linearly aligned two and three spin
system for all time scales yielding a continuous solution. Afterward, the "diffusion in a
cone" model, which is used later one for simulations of fast internal motion, is presented
but only within the limits of slow and fast internal motion.

5.1.1 Decorrelation assumption

Starting from the correlation function in the laboratory frame (indicated by a superscript
L) given in chapter 1 and defined in the Appendix (chapter A)

\[ C_m(t) = \frac{4\pi}{5} \left( \frac{Y_{2,m}(\vartheta^L(t), \varphi^L(t))Y_{2,m}^*(\vartheta^L(0), \varphi^L(0))}{r^3(t)r^3(0)} \right) \]

(5.11)

one can change into the molecular frame (superscript M) by using the Wigner rotation
matrices [30] that accounts for the global rotational motion, obtaining

\[ C_m(t) = \frac{4\pi}{5} \sum_{n,n'=-2}^2 \left( \frac{D_{2n}^{2n'}(\Omega_{LM}(0))D_{2n'}^{2n}(\Omega_{LM}(t))}{r^3(0)r^3(t)} \right) \langle Y_{2,n}(\vartheta^M(0), \varphi^M(0))Y_{2,n'}(\vartheta^M(t), \varphi^M(t)) \rangle \]

(5.12)

where \( \Omega = (\vartheta, \varphi) \). Here, \( \vartheta^L(t) \) and \( \varphi^L(t) \) denote the time-dependent polar angles in the
laboratory frame, \( \vartheta^M(t) \) and \( \varphi^M(t) \) are the polar angles in the molecular frame, \( D_{mn} \)
are the Wigner rotation matrices and \( \Omega_{LM}(t) \) represents the three Euler angles that relate
the laboratory frame to the molecular frame at time $t$. With the time scale separation
one assumes that the intra-molecular motion is uncorrelated with the global rotational
diffusion. This is equal to the fact that the modulation of the overall correlation time
does not depend on the internal motion. The correlation function can then be factorized
into contributions from global motions and from intra-molecular motions, and is given
by the product of the two correlation functions

$$C^m(t) = C^m_{\text{global}}(t)C^m_{\text{ij,local}}(t)$$

$$= \sum_{n,n'= -2}^{2} \left\{ D^{2*}_{nn'}(0) D^{2}_{nn'}(t) \right\} \cdot$$

$$\left\{ \frac{4\pi}{5} \left\langle Y^*_{2,n}(\vartheta^M(0), \varphi^M(0)) Y_{2,n'}(\vartheta^M(t), \varphi^M(t)) \right\rangle \left( \frac{r(0)^3 r(t)^3}{r(0)^3 r(t)^3} \right) \right\}$$

$$= \frac{4\pi}{(5)^2} e^{-6Dt} \sum_{n=-2}^{2} \left\{ \frac{Y^*_{2,n}(\vartheta^M(0), \varphi^M(0)) Y_{2,n}(\vartheta^M(t), \varphi^M(t))}{(r(0)^3 r(t)^3)} \right\}$$

(5.13)

where

$$C^m_{\text{global}}(t) = \frac{4\pi}{5^2} e^{-6Dt}$$

(5.14)

due to the relation for the conditional probability and the orthogonality of the Wigner
rotation matrices ([31] eq. (130) sqq. and Appendix). Here, $D = \frac{1}{6\tau_c}$ ($\tau_c$ is the rotational
correlation time) is the diffusion coefficient for isotropic rotational Brownian motion
using the rigid spherical top for the description of the master equation of relaxation. If
no internal motion would be present, which means, that the molecule structure is rigid,
the spectral density function would be the Fourier transform of the rigid correlation
function and is given by

$$J^m(\omega) = \frac{2}{5r^6} \frac{\tau_c}{1 + (\omega \tau_c)^2}$$

(5.15)
and the auto- and cross-relaxation rate constant (eq. (3.2) and eq. (3.4)) finally results in

\[
\rho_I = \frac{1}{10} \frac{\gamma^4 h^2 \mu_0^2}{(4\pi)^2} \left\{ \frac{\tau_c}{1 + \omega_0 \tau_c} + 6 \frac{\tau_c}{1 + (2\omega_0 \tau_c)^2} \right\} \tag{5.16}
\]

\[
\sigma_{1S} = -\frac{1}{10} \frac{\gamma^4 h^2 \mu_0^2}{(4\pi)^2} \left\{ \frac{\tau_c}{1 + \omega_0 \tau_c} - 6 \frac{\tau_c}{1 + (2\omega_0 \tau_c)^2} \right\} \tag{5.17}
\]

### 5.1.2 Jump Model

In the N-Tropp model [29], the internal motion is described by N discrete transitions between conformational states that are superimposed on the global rotational motion. The master equation for the internal motion for the Tropp N-site jump model is given by

\[
\frac{d}{dt} P_a(t) = \sum_{b=1}^{N} A_{ab} P_b(t) \tag{5.18}
\]

where \( (A_{ab}) \) is a matrix element of the transition rate matrix and \( P_b(t) \) is the population of the \( b \)th conformation at time \( t \). With the knowledge of the conditional probability of the Jump model the correlation function can be expressed by

\[
C_m(t) = C_{global}^m(t) \sum_{n=-2}^{2} \frac{\langle Y_{2,n}^*(\theta_{ab}(0), \varphi_{ab}(0)) Y_{2,n}(\theta_{ab}(t), \varphi_{ab}(t)) \rangle}{r_{ab}(0)^3 r_{ab}(t)^3} \tag{5.19}
\]

\[
= C_{global}^m(t) \sum_{n=-2}^{2} \sum_{a,b=1}^{N} c_{a,b}^q \frac{Y_{2,n}^*(\theta_{ab}(a), \varphi_{ab}(a)) Y_{2,n}(\theta_{ab}(b), \varphi_{ab}(b))}{(r_{ab}(a)^3 r_{ab}(b)^3)} \sum_{q=1}^{N} c_{a,b}^q e^{\lambda_q t}
\]

where \( \lambda_a \) are the eigenvalues of \( A \), \( \lambda_1 = 0 \) and \( c_{a,b}^q \) are the eigenfunctions derived by diagonalization of \( A \) (see [31]) and

\[
C_{global}^m(t) = \left( \frac{4\pi}{5} \right) \frac{1}{5} e^{-6Dt} \tag{5.20}
\]
is the overall correlation function.

The conditional probability of finding the relaxation vector in the state b at time t, if it was in the initial state a at time 0, is represented by

\[ P(b, t|a, 0) = \sum_{q=1}^{N} c_{aq}^q e^{\lambda_q t} \]  \hspace{1cm} (5.21)

and the a priori probability is \( P_a = c_{aa}^1 \). The spectral density is then given by calculating the Fourier transform of the correlation function

\[ J^m(\omega) = \frac{4\pi}{5^2} \sum_{n=-2}^{2} \sum_{a,b=1}^{N} c_{ab}^1 Y_{2,n} (\varphi_{ab}(a), \varphi_{ab}(b)) Y_{2,n} (\varphi_{ab}(b), \varphi_{ab}(b)) \cdot \sum_{q=1}^{N} 2c_{ab}^q \tau_q (5.22) \]

where \( \tau_q' = \frac{\tau_q}{1+(\omega \tau_q)^2} \) and the correlation time is given by, \( \frac{1}{\tau_q} = 6D - \lambda_q \).

**Slow internal motion** \(|\lambda_q| \ll D\)

In the presence of slow intramolecular motion, which is restricted to translational variations of the distance \( r \) over time (i.e. \( \varphi^{M}_{ab}(t) = \text{const.} \), \( \varphi^{M}_{ab}(t) = \text{const.} \)), the spectral density is then given by (setting \( \tau_{q=1} = \tau_c \))

\[ J^m(\omega) = \frac{2}{5} \left\langle \frac{1}{r^6} \right\rangle \cdot \tau_{c'} = \sum_{a=1}^{N} \frac{P_a}{5r^6(a)} \tau_{c'} \]  \hspace{1cm} (5.23)

with \( \tau_{c'} = \frac{\tau_c}{1+(\omega \tau_c)^2} \).
Fast internal motion ($|\lambda_k| \gg D$)

In the presence of fast internal motion, expression eq. (5.22) reduces to

$$J^m(\omega) = \frac{4\pi}{5^2} \frac{2 \tau_{c'}}{r^3} \left| \sum_{n=-2}^{2} \sum_{a=1}^{N} P_a Y_{2,n}(\vartheta^M(a), \varphi^M(a)) \frac{r^3(a)}{r^3(a)} \right|^2$$  \hspace{1cm} (5.24)

where $\tau_{c'} = \frac{\tau_c}{1+(\omega \tau_c)^2}$. The key quantity in this expression is the squared modulus of the weighted average $\frac{Y_{2,n}(\vartheta^M(a), \varphi^M(a))}{r^3(a)}$ over the N conformational states. If the intramolecular motion is restricted to translational variations of the distance $r$ over time (i.e. $\vartheta^M_a(t) =$ const., $\varphi^M_a =$ const.) the angular dependence averages out and following expression is obtained:

$$J^m(\omega) = \frac{2}{5} \left\langle \frac{1}{r^3} \right\rangle^2 \tau_{c'} = \frac{2}{5} \sum_{a=1}^{N} \frac{P_a}{(r^3(a))^2} \tau_{c'}$$  \hspace{1cm} (5.25)

Two and three site jump models for all time scales

In most descriptions of motion it is often assumed (as we did before) that the internal motions are much faster (or slower) than the rotational correlation time of the molecule and therefore more simple equations of relaxation processes can be used. However, even if the internal motion is 10 times faster than the rotational correlation time of the molecule, this assumption appears to be not correct (as we shall see) and hence a more comprehensive description of motion and therefore for the NMR probes measured is required. This is also important if theoretical derived equations are compared with simulations as will be done later (see chapter 8.4.5).

It is the attempt of this paragraph to establish such a rigorous description for the two and three site jump models. Let us start first with the two site jump model:
For a two site jump model the transition matrix is given by
\[
A = k_A \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix}
\]
with the eigenvalues \( \lambda_{1,2} = \{-2k_A, 0\} \).

The eigenvector-matrix and its inverse can be calculated to
\[
R = \begin{pmatrix} -1 & 1 \\ 1 & 1 \end{pmatrix}
\] and
\[
R^{-1} = \frac{1}{2} \begin{pmatrix} -1 & 1 \\ 1 & 1 \end{pmatrix}
\]

The correlation function under the dipole-dipole Hamiltonian is
\[
C^m(t) = \sum_{n=-2}^{2} \sum_{i,j=1}^{2} c_{ij}^l \cdot \frac{Y_{2,n}^*(\Phi_i^M)Y_{2,n}(\Phi_j^M)}{r_i^3 r_j^3} \cdot \sum_{k=1}^{2} c_{ij}^k e^{\lambda_k t} \quad (5.26)
\]
where \( c_{ij}^k = R_{jk} R^{-1}_{ki} \). Using the relation \( \sum_{m=-2}^{2} Y_{2,m}^*(\Phi_1)Y_{2,m}(\Phi_2) = \sqrt{\frac{5}{4\pi}} \cdot Y_{2,0}(\vartheta, 0) \) one gets
\[
C^m(t) = \frac{1}{4} \cdot \left( \left( \frac{1}{r_{11}^0} + \frac{1}{r_{22}^0} \right) \cdot \left( 1 + e^{-2k_A t} \right) \right.
\]
\[
+ \left. \frac{(3\cos^2 \vartheta - 1)}{r_1^3 r_2^3} \left( 1 - e^{-2k_A t} \right) \right) \quad (5.27)
\]

The simulated model of a linear alignment with \( \vartheta = 0 \) yields (for the Redfield limit) after Fourier transformation for the density function (with \( \tau_{\text{tot}}^{-1} = \tau_{\text{c}}^{-1} + \tau_{\text{int}}^{-1} \))
\[
J^m(\omega) = \frac{1}{4} \cdot \left( \left( \frac{1}{r_{11}^0} + \frac{1}{r_{22}^0} \right) \cdot \left( \frac{\tau_c}{1 + (\omega\tau_c)^2} + \frac{\tau_{\text{tot}}}{1 + (\omega\tau_{\text{tot}})^2} \right) \right.
\]
\[
+ \left. \frac{2}{r_1^3 r_2^3} \left( \frac{\tau_c}{1 + (\omega\tau_c)^2} - \frac{\tau_{\text{tot}}}{1 + (\omega\tau_{\text{tot}})^2} \right) \right) \quad (5.28)
\]
Under the assumption of fast internal motion $\tau_{\text{int}} \ll \tau_c \iff \tau_{\text{tot}} \approx 0$, which is called the fast motion limit, eq. (5.28) simplifies to

$$J^m(\omega) = \frac{1}{4} \left( \frac{1}{r_{11}^0} + \frac{2}{r_{12}^3} + \frac{1}{r_{22}^0} \right) \cdot \left( \frac{\tau_c}{1 + (\omega \tau_c)^2} \right)$$  \hspace{1cm} (5.29)

### Three site jump model

We assume in this section that the sites are equal to nuclear spins which can populate three different distances relative to each other which are denoted by A, B and C. The spins can jump between these distances with equal probability.

For this three spin system we first write down the transition equations as shown in Figure 5.1.2.

Figure 5.1: On the left site, the differential equations for the population changes of the three states A, B and C are given, where $P_A$, $P_B$ and $P_C$ labels the populations of the three states A, B and C. The probability of populating the middle state B is twice as high as for the other two (see text). On the right side, the possible jumps between the two distances for each spin pair (e.g. $I_1$, $I_2$ and $I_2$, $I_3$) is shown, resulting in the states A, B and C.

The letters A, B and C labels the possible states of the three spin system. The underlying system assumes a direct energy transfer between state A and C and also an indirect one.
via the intermediate state B. This transfer is modulated by jumps between two distances, $r_{11}$ and $r_{12}$ for the distance between spin one and two and $r_{21}$ and $r_{22}$ for the distance between spin two and three, respectively, where two distances should be equal, e.g. $r_{12} = r_{21}$ and $r_{11} = r_{22}$. Assuming this, the effective distances for the indirect transfer between spin one and three are $2r_{11}$ (represented by state A), $2r_{12}$ (represented by state C) and $r_{12} + r_{11}$ (represented by state B), where the population of the state B is twice as big as the ones of the other two. The transition rate matrix extracted from the differential equations from above is given by

$$
A = k_A \begin{pmatrix} -3 & 1 & 1 \\ 2 & -2 & 2 \\ 1 & 1 & -3 \end{pmatrix}
$$

with the eigenvalues $\lambda_{1,2,3} = \{0, -4k_A, -4k_A\}$

The eigenvector matrix and the inverse can be calculated to

$$
R = \begin{pmatrix} 1 & 2 & 1 \\ -1 & 1 & 0 \\ -1 & 0 & 1 \end{pmatrix}
$$

and

$$
R^{-1} = \frac{1}{4} \begin{pmatrix} 1 & -1 & -2 \\ 1 & -1 & 2 \\ 1 & 3 & -2 \end{pmatrix}
$$

The density function is then given in accordance to eq. (5.28)

$$
J^m(\omega) = \frac{1}{16} \cdot \left\{ \left( \frac{1}{r_{11}^6} + \frac{1}{r_{33}^6} \right) \cdot \left( \frac{\tau_c}{1 + (\omega \tau_c)^2} + 3 \frac{\tau_{\text{tot}}}{1 + (\omega \tau_{\text{tot}})^2} \right) \right. \\
+ 4 \frac{1}{r_{22}^6} \cdot \left( \frac{\tau_c}{1 + (\omega \tau_c)^2} + \frac{\tau_{\text{tot}}}{1 + (\omega \tau_{\text{tot}})^2} \right) \\
+ 2 \left( \frac{2}{r_{12}^3} + \frac{2}{r_{23}^3} + \frac{1}{r_{13}^3} \right) \cdot \left( \frac{\tau_c}{1 + (\omega \tau_c)^2} - \frac{\tau_{\text{tot}}}{1 + (\omega \tau_{\text{tot}})^2} \right) \right\}
$$

(5.30)

In presence of fast motion where $\tau_{\text{int}} \ll \tau_c \Leftrightarrow \tau_{\text{tot}} \approx 0$, the spectral density function
can be simplified

\[ J^m(\omega) = \frac{1}{16} \cdot \left( \frac{\tau_c}{1 + (\omega \tau_c)^2} \right) \cdot \left\{ \left( \frac{1}{r_{11}^6} + \frac{4}{r_{22}^6} + \frac{1}{r_{33}^6} \right) + \left( \frac{4}{r_{12}^3 r_{21}^3} + \frac{4}{r_{23}^3 r_{32}^3} + \frac{2}{r_{13}^3 r_{31}^3} \right) \right\} \quad (5.31) \]

For a better overview, the results derived in this chapter are tabled in the following:
Table 5.1: Listed are the results for the different models discussed in section 5.1.2. In accordance to eq. (3.2) - (3.4),

<table>
<thead>
<tr>
<th>Jump Model</th>
<th>$J^m(\omega)$</th>
<th>$\sigma = -\frac{\gamma^4\tilde{d}_0^2}{(4\pi)^2} \frac{1}{4} {J^m(0) - 6J^m(2\omega_0)}$</th>
<th>$\rho = -\frac{\gamma^4\tilde{d}_0^2}{(4\pi)^2} \frac{1}{4} {J^m(0) + 3J^m(\omega_0) + 6J^m(2\omega_0)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>slow internal motion</td>
<td>$J^m(\omega) = \frac{2}{5} \left{ \frac{1}{\omega} \right} \cdot \tau_c' \tau_c = \frac{\tau_c}{1 + (\omega \tau_c)^2}$</td>
<td>$\sigma = -\frac{\gamma^4\tilde{d}_0^2}{10(4\pi)^2} \left{ \frac{1}{\omega} \right} \left{ \frac{\tau_c}{1 + (2\omega \tau_c)^2} \right}$</td>
<td>$\rho = -\frac{\gamma^4\tilde{d}_0^2}{10(4\pi)^2} \left{ \frac{1}{\omega} \right} \left{ \frac{\tau_c}{1 + (2\omega \tau_c)^2} \right}$</td>
</tr>
<tr>
<td>fast internal motion</td>
<td>$J^m(\omega) = \frac{2}{5} \left{ \frac{1}{\omega^3} \right} \cdot \tau_c' \tau_c = \frac{\tau_c}{1 + (\omega \tau_c)^2}$</td>
<td>$\sigma = -\frac{\gamma^4\tilde{d}_0^2}{10(4\pi)^2} \left{ \frac{1}{\omega} \right} \left{ \frac{\tau_c}{1 + (2\omega \tau_c)^2} \right}$</td>
<td>$\rho = -\frac{\gamma^4\tilde{d}_0^2}{10(4\pi)^2} \left{ \frac{1}{\omega} \right} \left{ \frac{\tau_c}{1 + (2\omega \tau_c)^2} \right}$</td>
</tr>
<tr>
<td>2 site jump model</td>
<td>$J^m(\omega) = \frac{1}{4} \left( \left( \frac{1}{\omega \tau_2} + \frac{1}{\omega \tau_1} \right) \left( \frac{1}{\omega \tau_1} + \frac{1}{\omega \tau_0} \right) \right) \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right) + \frac{2}{\omega \tau_0} \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right)$</td>
<td>$\sigma = -\frac{\gamma^4\tilde{d}_0^2}{4(4\pi)^2} \left( \left( \frac{1}{\omega \tau_2} + \frac{1}{\omega \tau_1} \right) \left( \frac{1}{\omega \tau_1} + \frac{1}{\omega \tau_0} \right) \right) \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right) + \frac{2}{\omega \tau_0} \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right)$</td>
<td>$\rho = -\frac{\gamma^4\tilde{d}_0^2}{4(4\pi)^2} \left( \left( \frac{1}{\omega \tau_2} + \frac{1}{\omega \tau_1} \right) \left( \frac{1}{\omega \tau_1} + \frac{1}{\omega \tau_0} \right) \right) \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right) + \frac{2}{\omega \tau_0} \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right)$</td>
</tr>
<tr>
<td>3 site jump model</td>
<td>$J^m(\omega) = \frac{1}{16} \left( \left( \frac{1}{\omega \tau_3} + \frac{1}{\omega \tau_2} + \frac{1}{\omega \tau_1} + \frac{1}{\omega \tau_0} \right) \right) \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right) + \frac{1}{\omega \tau_0} \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right)$</td>
<td>$\sigma = -\frac{\gamma^4\tilde{d}_0^2}{4(4\pi)^2} \left( \left( \frac{1}{\omega \tau_3} + \frac{1}{\omega \tau_2} + \frac{1}{\omega \tau_1} + \frac{1}{\omega \tau_0} \right) \right) \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right) + \frac{1}{\omega \tau_0} \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right)$</td>
<td>$\rho = -\frac{\gamma^4\tilde{d}_0^2}{4(4\pi)^2} \left( \left( \frac{1}{\omega \tau_3} + \frac{1}{\omega \tau_2} + \frac{1}{\omega \tau_1} + \frac{1}{\omega \tau_0} \right) \right) \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right) + \frac{1}{\omega \tau_0} \left( \frac{1}{\omega \tau_0} + \frac{1}{\omega \tau_0} \right)$</td>
</tr>
</tbody>
</table>
5.1.3 "Diffusion in a cone" model

The "diffusion in a cone" model describes the motion of the internuclear magnetization vector between two nuclei. This motion is restricted to diffusion in a cone of fixed radius \( r_{ij} = \text{const.} \) and with a semi-angle (opening angle) \( \beta \). The master equation of internal relaxation is given by [28]:

\[
\frac{\partial}{\partial t} W(\Omega, t) = -D_W \left( \frac{1}{\sin \vartheta} \frac{\partial}{\partial \vartheta} \left( \sin \vartheta \frac{\partial}{\partial \vartheta} \right) + \frac{1}{\sin^2 \vartheta} \frac{\partial^2}{\partial \varphi^2} \right) W(\Omega, t)
\]

where \( D_W \) is the diffusion coefficient, \( W(\Omega, t) \) is the probability to find the vector for time \( t \) at orientation \( \Omega = (\vartheta, \varphi) \) (with respect to the cone axis which is parallel to the external magnetic field). As we will handle with the associated Legendre functions in this subsection, we will name the probabilities introduced above by \( W(\Omega, t) \) to avoid confusion. The distance vector is constant and therefore the Laplace operator \( \vec{L}_\Omega^2 \) is given in spherical tensor notation [32]. To ensure the restriction of the motion to the diffusion in a cone with opening angle \( \beta \), one has to define a boundary condition at \( \vartheta = \beta \) of the form \( \frac{\partial}{\partial \vartheta} W(\Omega, t) \big|_{\vartheta=\beta} = 0 \). The solution of the angular part (\( \sim \vec{L}_\Omega^2 \)) are the well known spherical harmonics [32] and the eigenvalues and the eigenvectors of the master equation (eq. (5.32)) are given as:

\[
W_l^m(\Omega, t) = Y_l^m(\vartheta, \varphi) e^{-D_W(l+1)t}
\]

\[
= \sqrt{\frac{(2l + 1)}{4\pi} \frac{(l-m)!}{(l+m)!}} P_l^m(\cos \vartheta) e^{im\varphi} e^{-D_W(l+1)t}
\]
with \(0 \leq m \leq l\). The \(P^m_l(\cos \vartheta)\) are the associated Legendre functions. The a priori probability is given in accordance to the boundary condition as

\[
W(\Omega_0) = \begin{cases} 
\frac{2}{(1-\cos \beta)} & 0 \leq \vartheta \leq \beta \\
0 & \text{otherwise}
\end{cases} \quad (5.34)
\]

The correlation function (with \(C^m_{\text{global}}(t) = C^m_{\text{global}}(t) \cdot r^{-6}\) as \(r_{ij}(t) = r_{ij} = \text{const.}\)) is given in accordance to eq. (5.13) by

\[
C^m_{ij}(t) = C^m_{\text{global}}(t) \sum_{n=-2}^{2} \langle \hat{Y}^*_{2,n}(\Omega(0)) \hat{Y}_{2,n}(\Omega(t)) \rangle \quad (5.35)
\]

If we assume a diffusion in a cone model, we have to calculate the correlation function between the magnetization vector \(\mu\) at two different times. Using the addition theorem for spherical harmonics \([33]\) one gets

\[
C^m(t) = C^m_{\text{global}}(t) \cdot \sum_{n=-2}^{2} \langle \hat{Y}^*_{2,n}(\Omega(0)) \hat{Y}_{2,n}(\Omega(t)) \rangle \\
= \frac{4\pi}{5^{2/6}} e^{-6Dt} \cdot \frac{5}{4\pi} \langle P_2(\cos \vartheta) \rangle \\
= \frac{1}{5^{2/6}} e^{-6Dt} \cdot \langle P_2(\vec{\mu}(0) \cdot \vec{\mu}(t)) \rangle \quad (5.36)
\]

where \(\vec{\mu}\) is the unit vector of the magnetization at time \(t\). This equation can be written as \([34]\)

\[
C^m(t) = \frac{1}{5^{2/6}} e^{-6Dt} \sum_{i=1}^{\infty} A_i e^{-\frac{E_i}{\sigma_i} t} \quad (5.37)
\]

where \(A_i\) and \(\sigma_i\) are constants which depends on \(\beta\). This expression has no analytical representation but can be approximated like it’s done in \([34]\) and \([31]\). For the spectral
density function, one results in
\[ J^m(\omega) = \frac{2}{5r^6} \sum_{i=1}^{\infty} A_i \int_{-\infty}^{\infty} e^{-\left(6D + \frac{D_W}{\tau_i}\right)t^2} dt \]

or
\[ J^m(\omega) = \frac{2}{5r^6} \sum_{i=1}^{\infty} A_i \frac{\tau_i}{1 + (\omega \tau_i)^2} \]

where \( \tau_i = \frac{1}{6D + \frac{D_W}{\tau_i}} \).

As before two different scenarios are discussed.

**Slow internal motion (\( D_W \ll D \))**

The case \( D_W \ll D \) is equivalent to the case \( t \to 0 \), so we can set \( e^{-\left(6D + l(l+1)D_W\right)t} \approx e^{-6Dt} \) and replace \( W(\Omega, t | \Omega_0, 0) \) by \( W(\Omega_0, 0 | \Omega_0, 0) \). After Fourier transformation one results for the spectral density function:

\[ J^m(\omega) = \frac{2}{5r^6} \tau_i \]

where \( \tau_i = \frac{1}{6D + \frac{D_W}{\tau_i}} \) and \( \tau_c = \frac{1}{6D} \). This is similar to the result for a rigid molecule (e.g. (5.15)) for \( D_W = 0 \).

**Fast internal motion (\( D_W \gg D \))**

The general model discussed here is symmetric with respect to the z-axis. The case \( D_W \gg D \) is equivalent to the case \( t \to \infty \) so we can replace \( W(\Omega, t | \Omega_0, 0) \) by \( \lim_{t \to \infty} W(\Omega, t | \Omega_0, 0) = \frac{1}{2\pi(1-\cos \beta)} \). For the spectral density function one obtains after
a Fourier transformation

\[ J^m(\omega) = \frac{2}{5r^6\tau} \left( \frac{1}{2} \cos \beta (1 + \cos \beta) \right)^2 \tag{5.41} \]

where \( \tau = \frac{\tau_c}{1+(\omega\tau_c)^2} \) and \( \tau_c = \frac{1}{6D} \).

**Order parameter for the diffusion in a cone model**

As we shall see in part III, we will simulate the effect of fast internal motion of the \(^1\text{H} - ^{15}\text{N}\) vector in an amino acid backbone residue on the \(\text{H}_N - \text{H}_N\) NOE. To do this, it appears to be advantageous to introduce an order parameter following the idea by Lipari and Szabo \[15\] because the correlation function (eq. (5.37)) can not be further expressed analytically. For the diffusion in a cone model Kinosita et al. \[34\] modeled a mono-exponential decay of the correlation function which gives the same values for \(t = 0\) and \(t \rightarrow \infty\) and has the same defined integral value as the correlation function of eq. (5.37) but otherwise a different form:

\[ C^m(t) = \frac{1}{5r^6} e^{-6Dt} \left( S_W^2 + (1 - S_W^2) \cdot e^{-D_s(\sigma)} \right) \tag{5.42} \]

where \( \langle \sigma \rangle = \sum_{i \neq \infty} A_i \sigma_i \). The order parameter for the wobbling in a cone model \(S_W\) can be defined as

\[ S_W = \left( \frac{1}{2} \cos(\beta) \cdot (1 + \cos(\beta)) \right)^2 \tag{5.43} \]

From the order parameter one can therefore calculate the semi-angle \(\beta\) of the cone as follows:

\[ \beta = -\frac{1}{2} + \sqrt{\frac{1}{4} + 2\sqrt{S_W}} \tag{5.44} \]
Part III

Protocol for and problems in the determination of effective distances
6 Introduction

Albert W. Overhauser discovered in 1953 a new method for the polarization of nuclei in metals. He showed that the saturation of the conduction electrons in a metal leads to a polarization of the nuclei [8]. This effect is nowadays called the nuclear Overhauser effect (NOE). Two years later, Solomon applied this effect to pairs of spins under dipole-dipole interaction and observed this phenomena in anhydrous hydrofluoric acid [26]. Nowadays, the NOE is used in structure determination for spacial confinement [35]. But although the NOE is dependent on the inverse sixth power of the distance between the two spins observed (as shown in the previous chapter (eq. (5.7))), the cross relaxation rate constant obtained from the interaction is in NMR structure determination not transferred into exact average distances, but only used semi-quantitatively for upper distance constraints. Recently, we reinvestigated this poor translation from NOE to distances with the aim to use the NOE quantitatively for a simultaneous structure and dynamics determination. This could be possible because beside exact distance determination from the NOE rate constants, information on internal motion are also extractable from the data measured [36, 37] as implied by eq. (5.4) or eq. (5.8), respectively. In principle, the NOE is dependent of both, slow and fast motion in a rather complex way (see eq. (5.1)). While, on the one hand this may result in a rather complete map of internal motion due to the abundance of protons in a protein and consequently thousands of NOEs, on the other hand the correspondence between motion and NOE may be too
complex to be resolved. However, as we shall see in this chapter, the influence of fast motion on the NOE is rather small and only slow motion has to be taken into account. Overall, we aim to sketch the principle approach for the measurement of cross-relaxation rate constants and the problems by calculating exact distances from these rate constants. The pulse sequence, the determination of the correlation time and the programs for the intensity fitting are presented. The influence of spin diffusion and fast internal motion on the NOE is discussed. Finally higher order contributions are calculated.
7 Protocol for the extraction of exact distances from NOESY experiments

In this chapter the general treatment for the extraction of the cross-relaxation rate constants from the NOESY spectra as well as the calculation of the effective distances are presented.

7.1 NOESY pulse sequence

This chapter was taken from [36] and kindly provided by B. Vögeli

The first step is to record a series of NOESY spectra. To increase the number of evaluable peaks, a 3D $^{15}$N or $^{13}$C resolved [$^1$H,$^1$H] NOESY experiment or a $^{15}$N, $^{13}$C contained [$^1$H,$^1$H] NOESY experiment are recommended and used in the following, although these experiments have a major disadvantage when compared to the 2D [$^1$H,$^1$H] NOESY experiment as we shall see. The pulse sequence for the $^{15}$N resolved [$^1$H,$^1$H] NOESY experiment is shown in Figure 7.1.
Figure 7.1: 3D $^{15}$N-resolved HMQC-NOESY experiment [36] used for the measurement of for the extraction of exact distances. The radio frequency pulses on $^1$H, $^{15}$N and $^{13}$C were applied at 4.7, 116 and 110 ppm, respectively. The narrow and wide black bars indicate non-selective 90° and 180° pulses. The curved shapes on the $^1$H line represent Gaussian-shaped selective 90° pulses truncated at the 5% level on the water resonance with a length of 1.0 ms. Pulsed field gradients along the z-axis are shown on the line marked PFG. All gradients have a smoothed square shape with a length of 1.0 ms. The individual gradient strengths are 44% for G1, 70% for G2 and 80% for G3 with a maximal strength of 53 G/cm. Quadrature detection is obtained by phases $\phi_1$ in the $t_2$ ($^1$H) dimension, and by $\phi_3$ in the $t_1$ ($^{15}$N) dimension, respectively, both cycled according to the States-TPPI method [38]. 512 complex points were recorded for the direct ($^1$H), 256 and 22 for the indirect $t_2$ ($^1$H) and $t_1$ ($^{15}$N) dimensions with $t_{1,\text{max}} = 24$ ms and $t_{2,\text{max}} = 22.4$ ms, respectively. $\frac{1}{2J} = 5.4$ ms. $^{15}$N was decoupled during acquisition using the WALTZ-16 sequence [39]. All radio frequency pulses are applied with phase x, unless a different phase is indicated in the Figure: $\phi_1 = \{x, x, -x, -x\}$, $\phi_2 = \{x, -x\}$, $\phi_{\text{rec}} = \{-x, x, x, -x\}$. The mixing time $t = \tau_{\text{mix}}$ is fixed but has to be changed in order to detect the build-ups over time of the cross-peaks.
For the determination of the NOE rate constants, a series of NOESY spectra with different mixing times $t = \tau_{\text{mix}}$ are required. The mixing time selected are crucial and must be adjusted for each protein using the knowledge of the overall correlation time of the molecule which has to be determined anyhow (see next section). As a benchmark, in the experiments presented in part 11 for double labelled ($[^{13}\text{C},^{15}\text{N}]$) ubiquitin mixing times of $\tau_{\text{mix}} = [10, 15, 20, 25$ and $30] \text{ ms}$ were used while for triple labelled ($[^{2}\text{H},^{13}\text{C},^{15}\text{N}]$) ubiquitin longer mixing times could be used as less relaxation pathways are active. Here, the deuteration level was as follows: H$_N$ 0 %, H$_\alpha$ 99 % and for all other carbon bound protons 95 %. Mixing times of $\tau_{\text{mix}} = [30, 60, 90$ and $120] \text{ ms}$ turned out to be appropriate. One has to keep in mind that for higher temperatures the correlation time gets shorter which can be countered by using longer $\tau_{\text{mix}}$ following eq. (3.8) and eq. (5.17), which shows the proportionality between the NOE rate constant $\sigma$, $\tau_c$ and $t = \tau_{\text{mix}}$, respectively.

In the pulse sequence presented in Figure 7.1, the HMQC element is placed before the NOESY element. This is advantageous, as the relaxation during this element is the same for the diagonal and all corresponding NOESY cross-peaks, since they share the same magnetization pathway. The detected intensities of the $^1\text{H}$ signals in the spectrum is composed out of each individual element, resulting in

$$I_{ij}^{\text{det}}(\tau_{\text{mix}}) = \alpha_i^{\text{rec}} \cdot I_{ii}^{\text{init}} \cdot T_{ii}^{\text{HMQC}} \cdot T_{ij}^{\text{NOESY}}(\tau_{\text{mix}}) \cdot T_{jj}^{\text{WG}} \quad (7.1)$$

Here, $\alpha_i^{\text{rec}} \leq 1$ accounts for the part of the magnetization that has recovered during the interscan delay. $T_{ii}^{\text{HMQC}}$ denotes the loss of magnetization during the HMQC element, $T_{ij}^{\text{NOESY}}(\tau_{\text{mix}})$ describes the magnetization transfer from spin $i$ to spin $j$ (and is given by eq. (3.7) for auto-relaxation or eq. (3.8) for cross-relaxation during the mixing time $t = \tau_{\text{mix}}$ and $T_{jj}^{\text{WG}}$ describes the loss of magnetization during the Watergate element [40] before acquisition. Slight $^1\text{H}_j$ dependence during $T_{jj}^{\text{WG}}$ can be neglected by the
assumption that it is identical for all $j$. In case this assumption is invalid it would be reflected in the experimental error. Note that if the peak intensity is determined by the maximum height the nonuniform line width translates into an additional correction term that can be absorbed by $T_{jj}^{WG}$. The term of interest is $T_{ij}^{NOESY}(\tau_{mix})$ which can now be extracted by the normalization of the cross-peak intensities ($i \neq j$) by the diagonal peak intensity ($i = j$) at $t = \tau_{mix} = 0$:

$$T_{ij}^{NOESY}(\tau_{mix}) = \frac{I_{ij}^{det}(\tau_{mix})}{I_{ii}^{det}(\tau_{mix})} \quad (7.2)$$

where the initial diagonal peak intensity at $t = \tau_{mix} = 0$ must be either measured or is back-predicted as shown below. Note, this approach allows the interscan delay to be reduced without compromising on the extraction of the NOE and hence experimental time can be saved.

**HXCQ-NOESY versus NOESY-HXCQ**

In the 3D $^{15}$N or $^{13}$C resolved [$^{1}$H,$^{1}$H] NOESY experiment presented above, the HXQC element is placed before the NOESY (for the pulse sequence shown in Figure 7.1 a HMQC is used; in principle, also a HSQC would be possible). As mentioned, during the WATERGATE element a slight $^{1}$H$_{j}$ dependence might be present, resulting in a experimental error. Furthermore, if the pulse sequence is applied to a protonated sample, the WATERGATE element suppresses in part the $^{1}$H$_{\alpha}$ signals. Therefore, a frequency dependance for theses peaks would arise and the assumption that $T_{jj}^{WG}$ in eq. (7.1) is equal for all protons is no longer valid. By changing the order of the elements in the sequence, this problem can be avoided. Placing the HMQC element after the NOESY, the water signal can be suppressed right before the acquisition by the use of trim pulses and/or gradient coherence selection [41]. The detected intensity of the $^{1}$H signal in the
spectrum for this case is given in accordance to eq. (7.1)

\[ I_{ij}^{\text{det}} (\tau_{\text{mix}}) = \alpha_i^{\text{rec}} \cdot I_{i}^{\text{init}} \cdot T_{ij}^{\text{NOESY}} (\tau_{\text{mix}}) \cdot T_{ii}^{\text{HMOC}} \tag{7.3} \]

For the extraction of the NOESY element \( T_{ij}^{\text{NOESY}} = T_{ji}^{\text{NOESY}} \) both cross- and diagonal-peak intensities are needed as the relaxation during the HMQC element is not identical any more. The term of interest can be therefore calculated as

\[ T_{ij}^{\text{NOESY}} (\tau_{\text{mix}}) = T_{ji}^{\text{NOESY}} (\tau_{\text{mix}}) = \sqrt{\frac{I_{ij}^{\text{det}} (\tau_{\text{mix}}) I_{ji}^{\text{det}} (\tau_{\text{mix}})}{I_{ii}^{\text{det}} (0) I_{jj}^{\text{det}} (0)}}. \tag{7.4} \]

Here \( I_{ij}(t) \) denotes the cross-peak intensity at time \( t \) if \( i \neq j \) and the diagonal - peak intensity at time \( t = 0 \) if \( i = j \). Note that this approach could also be used for HXQC-NOESY experiments, if the assumption of an offset-independency of the \(^1H_j\) during the WATERGATE element is no longer valid. One major disadvantage is that if one cross-peak is missing no value can be obtained or a larger error has to be tolerated. Furthermore, no random error can be estimated with this method.

### 7.2 Measuring the correlation time \( \tau_c \)

As one needs the correct relaxation time to calculate exact effective distances (see chapter and eq. (5.17), it is recommended to measure 2D \( T_1 \) and \( T_1 \rho \) TROSY experiments for different relaxation times, where the magnetization is transfered to the \(^{15}\text{N} \) nuclei to measure the decoupled isolated 2 spin system (see Figure 7.2). The decay of each peak in the spectrum is fitted exponentially to get the relaxation decays \( T_1 \) and \( T_1 \rho \). The correlation time can be extracted from the ratio of the longitudinal and transversal relaxation time \( \frac{T_1}{T_2} \). For this purpose, one has to translate \( T_1 \rho \) into \( T_2 \). This is done as
follows:

\[
\frac{1}{T_{1\rho}} = \frac{1}{T_1} \cos^2 (\vartheta) + \frac{1}{T_2} \sin^2 (\vartheta)
\]

(7.5)

where \(\tan \vartheta = \frac{\gamma_iB_1}{\Omega}\). \(B_1\) is the strength of the spin-lock field in Hz and \(\Omega\) is the difference between the frequency of the individual peak in the spectrum and the carrier frequency (e.g. the offset of the peak). The longitudinal relaxation time \(T_1\) is the inverse of the auto - relaxation rate constant \(\rho\). But, in general, this rate constant is not given only by eq. (5.16) but an additional term to account for chemical shift anisotropy (CSA) has to be added (as we only considered dipole-dipole interaction before). The complete expressions for \(\Gamma_{I_s,I_s} = \frac{1}{T_1}\) as well as \(\frac{1}{T_2}\) are given by (see e.g. [42]):

\[
\frac{1}{T_1} = \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_i^2 \gamma_S^2 \hbar^2}{r_{IS}^6} \left\{ J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S) \right\} + \frac{3}{4} (\omega_I \sigma_{zz})^2 J(\omega_I) \\
\frac{1}{T_2} = \frac{1}{8} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_i^2 \gamma_S^2 \hbar^2}{r_{IS}^6} \left\{ 4J(0) + J(\omega_I - \omega_S) + 3J(\omega_I) \\
+ 3J(\omega_S) + 6J(\omega_I + \omega_S) \right\} + \frac{1}{2} (\omega_I \sigma_{zz,i})^2 J(0) + \frac{3}{8} (\omega_I \sigma_{zz,i})^2 J(\omega_I) \\
+ \frac{3}{8} (\omega_S \sigma_{zz,j})^2 J(\omega_S) + \frac{3}{16} (\omega_S \sigma_{zz,j}) (\omega_S \sigma_{zz,s}) J(\omega_I)
\]

(7.6)

For this equation one has to use for the TROSY based experiment presented here for \(\gamma_i = \gamma_H\), \(\gamma_j = \gamma_N\), \(r_{ij} = r_{NH} = 1.02\ \text{Å}\), \(\sigma_{zz,H} = -5 \cdot \frac{2}{3}\ \text{ppm}\) [43], \(\sigma_{zz,N} = -165 \cdot \frac{2}{3}\ \text{ppm}\) [44] and for \(\omega_N\) and \(\omega_H\) the spectrometer specific frequencies. For the determination of the overall correlation time, a rigid structure of the molecule is assumed. Putting everything together yields the rotational correlation time \(\tau_c\) from the ratio of the longitudinal and transversal relaxation times \(T_1\) and \(T_2\). Both times are needed to cancel out the influence of the internal order parameter \(S_{loc}^2\).
Figure 7.2: Schemes of the pulse sequences and real data points for measuring $T_1$ and $T_{1\rho}$ to determine the rotational correlation time. In both sequences, the magnetization is transferred via an INEPT element [45] from the $^1H$ spin to the $^{15}N$ spin. The back transfer is done using a ST2-PT element [46]. The relaxation time is labelled with $\tau_{rel}$ which is equal to the x-axis in the right diagrams.

(A) The 2D pulse sequence for measuring $T_1$ is shown on the left side. Next to the sequence, a typical decay of a peak in the spectrum is shown. The decay is obtained by increasing the number of loops. As one can see, a single exponential decay fits the data nearly perfect.

(B) The scheme for the measurement of $T_{1\rho}$ is shown. Changing into the rotating frame results in the formula given above the pulse sequence By changing the duration time of the spin look, a decay of the peaks in the spectrum can be observed. On the right side such a decay is shown. Again, a single exponential decay fits the data well.
7.3 Extraction of the auto-relaxation rate constants

Together with the TROSY spectrum derived for the determination of the rotational correlation time and triple resonance experiments, one can assign the 3D $^{15}$N-resolved HMQC-NOESY. After assigning the spectrum with the longest mixing time (as the cross-peaks are most prominent there) one can extract the intensities of the diagonal as well as the cross-peaks. One easy way is to use the program nmrPipe [47], which fits a Lorentzian to each peak in the spectrum resulting in a peak height and a volume. The intensities in each individual spectrum can then be easily determined using a simple C++ program. The output is a text file containing the assignment, the peak intensity in the assigned spectrum and the intensity of each peak in every spectrum considered normalized to the assigned one. Although a better approach would be the integration of the peak, we use here the intensity at the maximum of the Lorentzian because this approach results in our hands in less artifacts attributed to peak overlap and base line distortion at the diagonal.

These intensities are then transferred to a mathematical program such as MATLAB. Before the cross-peaks can be analyzed, the auto-relaxation rate constant for each peak as well as the initial intensity at zero time must be determined. Therefore eq. (3.7) is used assuming a single exponential decay of the form

$$I_z(t) = I_0 \cdot e^{-\rho t}$$  \hspace{2cm} (7.7)

as for short mixing times the first order expansion of eq. (3.7) is $\cosh \approx 1$. The output is a list with the initial magnetization for each residue (e.g. $I_0$) and the cross-relaxation rate constant $\rho$. 

45
7.4 Extraction of the cross-relaxation rate constants

The cross-peak intensities are red out of the list and normalized to the corresponding initial corresponding diagonal-peak intensity extrapolated to \( t = 0 \) and fitted with a two-spin approach in accordance to eq. (3.8) as shown in Figure (7.3) using the derived individual auto-relaxation rate constants \( \rho \). Note, that the auto-relaxation rate constants of both spins have to be present as indicated by eq. (3.8).

From the fits of this formula to the data versus the different mixing times, cross-relaxation rate constants \( \sigma \) are determined. The output is therefore a list of the two-spin buildup rate constants for each spin pair. As shown in Figure (7.3), experimental NOE buildup curves for pairs of residues (i.e. shown are buildups for \( A \rightarrow B \) as well as \( B \rightarrow A \)) are not equal. The presence of two buildup curves per spin pair enabled on the one hand an improvement in accuracy of the cross-relaxation rate constants by averaging. On the other hand, an experimental error could be determined that includes simplifications made as discussed above (e.g. slight \(^1\)H\(_j\) dependence during \( T_{jj}^{WG} \) eq. (7.1)).
Figure 7.3: Experimental NOE buildup curves for pairs of $^1H_N-^1H_N$ residues (i.e. shown are buildups for A $\rightarrow$ B as well as B $\rightarrow$ A) in various secondary structures: along $\beta$ strands (“Sequential Beta Sheet”), across $\beta$ strands (designated by “Across Beta Sheet”) and sequential and non-sequential within the helix and at the end of the helix and in loops. The x-axis shows the mixing time ([30, 60, 90, 200] ms for 284 K, [30, 60, 90, 120] ms for 307 K and [60, 90, 120, 150] ms for 326 K), whereas the normalized intensities (with respect to the interpolated diagonal peak intensity at zero mixing time) are plotted on the y-axis. Experimentally measured values are indicated by circles (blue: 284 K, green: 307 K and red: 326 K), while the fit of the data using the exact two spin solution ([25, 36]) is illustrated by the black lines (without corrections for spin diffusion). Only for non-sequential NOEs in the $\alpha$-helix (i.e. Peak No. 33/35) a poor fit is obtained because of strong spin diffusion (see chapter 8.3).
8 Problems in the determination of exact distances from NOESY experiments

In the last chapter, a protocol for the extraction of cross-relaxation rate constants out of a series of NOESY experiments is given, including the determination of the overall correlation time. The procedure is explained again in a flowsheet below (Figure 8.1): The rotational correlation time $\tau_c$ of the protein is determined by $T_1$ and $T_{1\rho}$ measurements (of the $^{15}$N spins) following established procedures (see right column). To extract the NOE rate constants, the auto-relaxation rate constants of both spins and the initial magnetization at time $t = 0$ of the reference spin must be determined. With this values, the NOE rate constants from the cross-peaks on both sides of the diagonal can be determined using a two spin approach (see left side). Although this procedure is straightforward, several problems may arise, including in particular the omnipresent spin diffusion as well as the translation from eNOEs to distances because this relationship is in principle dependent on the motion present in the system as discussed in chapter 5. In the following, we will discuss these issues in details.
Figure 8.1: Schematic overview for the protocol introduced and explained in chapter 7. On the left side, the procedure for the determination of the cross-relaxation rate constant $\sigma$ is shown. First one has to fit the relaxation decay of the diagonal peaks to obtain the auto-relaxation rate constant $\rho$ and the initial magnetization $I_0$ for each residue. Finally, the buildup of the diagonal peak can be fitted, using a 2-spin approach (eq. (3.8)). On the right hand side of the figure, the extraction of the correlation time $\tau_c$ is shown. Out of the 2D TROSY spectra, the decay of each spin can be fitted and from the ratio of the longitudinal and transversal relaxation time $\tau_c$ can be extracted.
8.1 Determination of the correlation time

The extraction of order parameters from the cross-relaxation rate constants requires an accurate determination of $\tau_c$ as an error in the overall correlation time has a linear impact on the $(S_{HH})^2$ order parameter (see e.g. table 5.1).

We measured the correlation time for ubiquitin conventionally by $T_1/T_1\rho$ $^{15}$N-relaxation measurements. The method was validated by the application to a GB3 sample, for which $\tau_c$ deviated less than 1% from literature values [48]. As demonstrated in Figure 8.2, the concentration of the different samples has a direct impact on the correlation time. As the concentration itself was not measured but only adjusted vague, the deviations for GB3 at 4 mM sample concentration might be related to this issue. However, a clear trend for an influence of concentration on the overall tumbling of the molecule can be observed.
Figure 8.2: Plot of the correlation times $\tau_c$ derived from our different measurements and literature values at 298 K versus concentration.
The blue circles indicate the correlation times measured for GB3 via $T_1/T_1\rho$ $^{15}$N-relaxation measurements while the red ones were measured for ubiquitin. The straight lines show the literature values taken from [48] for GB3 and [49] for ubiquitin.

For ubiquitin, another validation for the determined $\tau_c$ was performed with an alternative approach. The cross-correlated relaxation between chemical shift anisotropy (CSA) of $^{15}$N and $^{15}$N-$^1$H dipolar interaction was measured. Although the correlation time $\tau_c$ cannot be extracted accurately with this method due to uncertainties in the CSA tensor, it is instructive to compare the cross-correlated relaxation rate constants $\eta$ for each of the three temperatures and correlate them with the temperature-dependent viscosity of water. Since there is an excellent temperature-sensitive correlation between the $\tau_c$ de-
termined by $^{15}$N-relaxation measurements, the cross-correlated relaxation rate constants and the viscosity, the obtained $\tau_c$ appears to be accurate. Figure 8.3 shows the correlation times derived from the $T_1/T_{1\varphi}$ $^{15}$N-relaxation measurements versus the temperature with comparison to literature values ([50, 51]). The black lines represent the calculated $\tau_c$ based on the temperature-dependent changes of the viscosity of the water, as implied by the Stokes-Einstein relationship [10]. From this relationship the correlation time at any temperature can be calculated setting the measured $\tau_c$ at 298 K or 300 K as a reference. As shown in Figure 8.3, all correlation times follow the same temperature dependence. The offset in the curves is attributed to differences in concentration and buffer conditions used: while [50] and [51] have relatively low sample concentrations with 2.3 mM and 1 mM respectively, the sample used here is higher concentrated (4.3 mM) and close to the solubility limit of ubiquitin. For the correlation times we obtained the values listed in Table 8.1.

<table>
<thead>
<tr>
<th>$T$ [K]</th>
<th>$\tau_c$ [ns]</th>
</tr>
</thead>
<tbody>
<tr>
<td>284</td>
<td>7.72</td>
</tr>
<tr>
<td>298</td>
<td>5.20</td>
</tr>
<tr>
<td>307</td>
<td>4.32</td>
</tr>
<tr>
<td>326</td>
<td>3.02</td>
</tr>
</tbody>
</table>

Table 8.1: Rotational correlation times $\tau_c$ of ubiquitin at four different temperatures obtained from $T_1/T_{1\varphi}$ $^{15}$N-relaxation measurements. [52, 53].
Figure 8.3: Plot of the correlation times $\tau_c$ derived from our different measurements and literature values versus temperature. The orange circles indicate the correlation times measured in this study via $T_1/T_{1e}$ $^{15}$N-relaxation while the violet and the turquoise ones, reported in [50] and [51], were measured with conventional $T_1/T_2$ relaxation experiments. The pink circles indicate the correlation times derived from cross-correlated relaxation measurements (TROSY/Anti-TROSY). The black lines follow the calculated $\tau_c$ based on the temperature-dependent viscosity changes of water with 298 K (upper line) or 300 K (lower line) as a reference temperature.

### 8.2 The translation of NOE rate constants to average distances

As described in chapter 5, the cross-relaxation rate constant has a complex relationship to the distance if motion is present. For slow internal motion (compared to the overall tumbling), the NOE depends on the average of the inverse sixth power of the distance
In the presence of fast motion however, the relationship is much more complex because both, angular as well as distance fluctuations, must be taken into account as revealed in eqs. (5.3) and (5.9). Hence, the extraction of true distances in presence of motion appears to be difficult. However, as we shall see in the following, the presence of fast motion does not change the NOE significantly (in most cases in a protein backbone) and can therefore be neglected to a first approximation.

Figure 8.4: Cartoon illustrating the extraction of the order parameter \( (S_{\text{fast}})^2 \) of the NOE from local order parameters \( S_{\text{HN}}^2 \) of \( ^{15}\text{N} - ^1\text{H}_N \) dipoles derived from \( ^{15}\text{N} \) relaxation measurements. First, the local order parameter \( S_{\text{HN}}^2 \) is translated into a \( ^1\text{H}_N \) Gaussian distribution within a cone as shown on the left panel of the Figure. Second, as illustrated by the middle and right panels, \( (S_{\text{HH}}^{\text{fast}})^2 \) is determined by ensemble averaging, following eq. (5.3).

\( ^1\text{H}_N - ^1\text{H}_N \) NOE order parameters based on the NMR structure (pdb code 1Ubq) were simulated for ubiquitin following the idea illustrated in Figure 8.4. We assumed that both \( ^1\text{H}_N - ^{15}\text{N} \) spin pairs undergo only fast motion that can be determined by conventional \( ^{15}\text{N} \) relaxation measurements and are described by the local Lipari-Szabo order parameters \( S_{\text{HN}}^2 \) [15]. From these local order parameters, a standard deviation \( \sigma \) for a 2-dimensional Gaussian distribution of opening angles \( \vartheta \) was calculated. Next, 5000
vector orientations were generated using a cut-off-angle $\beta$ for the distribution calculated from the local order parameters under the assumption of a "diffusion in a cone" model [31] (see subsection 5.1.3), sampling the polar angle $\vartheta$ in $5^\circ$ steps from zero to $\beta$ (see Fig. 8.4) and the azimuthal angles in $20^\circ$ steps from zero to $360^\circ$. Then, the order parameter of the NOE between the two $^1\text{H}_N$ spins of interest was calculated following eq. (5.3). This procedure describes the entire rotational and translational fast motion between the two $^1\text{H}_N$ spins under the assumption that no correlated fast motion is present. Applying this approach to the $^1\text{H}_N$ spins of ubiquitin with the use of published relaxation data kindly provided by Nico Tjandra [50], a correlation plot between the local $S_{\text{HN}}^2$ and $(S_{\text{HH}}^{\text{fast}})^2$ is obtained (Figure 8.5). Note, the latter order parameter can be larger or smaller than 1 depending on the type of motion, while in a rigid structure it would be equal to 1.
Figure 8.5: Relationship between calculated order parameters $\left( S_{\text{fast}}^{\text{HH}} \right)^2$ describing the influence of fast motion on the NOE and local order parameters $S_{\text{NH}}^2$ of $^1$H$_N$-$^1$H$_{15}$N spin vectors of relevance measured at 288 K [50]. No local order parameters were available for residues 13, 19, 24, 25, 31, 37, 38, 53, 67 and 72.

(A) Product of the local order parameters of ubiquitin determined experimentally [50] versus the corresponding fast NOE order parameter. Consecutive residues in α helices (Alpha), β sheets (Beta) and loops (Loop) are represented by open circles and non-consecutive residues by filled circles, respectively. For large and small values of $\left( S_{\text{fast}}^{\text{HH}} \right)^2$ the residues involved are labelled. Note, the relationship shown is only true for a temperature of 288 K.

(B) Surface plot showing $\left( S_{\text{fast}}^{\text{HH}} \right)^2$ versus the product of two corresponding $S_{\text{NH}}^2$ (having identical values) and the distance between the two cones in the interval from 2 Å to 5 Å. The two conformational scenarios, facing of the cones (top surface) or opposing each other (bottom surface), are illustrated. Facing yields an order parameter smaller than 1 while opposing larger than 1.

It is evident from Figure 8.5 A, that the fast motion of the individual $^1$H$_N$ does only slightly influence the corresponding NOE. This appears to be true irrespectively of the secondary structure the spins are located in (Table 8.2). Only for low values of $S_{\text{HN}}^2$, which are usually only observed in flexible regions of a protein such as the C-terminus of ubiquitin (i.e. residues 73-76), the influence of fast motion on the NOE might become relevant. For example as shown in Figure 8.5, a product of two order parameters
of $S_{\text{HN},x}^2 \cdot S_{\text{HN},y}^2 = 0.2$ modifies the corresponding NOE by only 10%. This finding can be rationalized as follows: While fast radial motion enhances the NOE by the $\langle r^{-3} \rangle^2$ dependence, the angular part of the motion results in a decrease of the NOE. Hence, the effect of fast motion is largely canceled. Inspection of Figure 8.5 A shows that for spin pairs that have individual local order parameters both above 0.6 (i.e. $S_{\text{HN},x}^2 \cdot S_{\text{HN},y}^2 > 0.36$) the remaining influence of the fast motion is a truncation of the NOE in the order of less than 5%. This yields less than a 0.83% uncertainty in the distance, which is well within the experimental error of the measurements.

These observations of the ubiquitin backbone (Fig. 8.5 A) can be generalized as demonstrated in Fig. 8.5 B. Two conformational scenarios, facing or opposing of the two $^{15}\text{N} - ^1\text{H}$ cones, are illustrated (other scenarios are shown in Figure 8.7). If the two cones are facing each other, which is typically observed across a $\beta-$sheet, the order parameter $\left(S_{\text{fast}HH}^2\right)^2$ is smaller than 1 and hence, the corresponding NOE is quenched. In the other scenario, typically observed at consecutive positions within a $\beta-$strand, the NOE is enhanced with a $\left(S_{\text{fast}HH}^2\right)^2 > 1$. These effects are more pronounced for small distances $r$. Overall however, the influence for common scenarios with local order parameters $S_{\text{NH}}^2 > 0.5$ is small (Table 8.2). In other words, NOEs between spin systems with a product of local order parameters $S_{\text{HN},x}^2 \cdot S_{\text{HN},y}^2 > 0.25$ are insensitive to fast motion. In such cases the NOE appears to follow approximately eq. (5.10) and is therefore only dependent on slow motions. However, if correlated motion of the whole spin system is present, $\left(S_{\text{HH}}^2\right)^2$ is accordingly reduced due to its angular motion. To get insight into the effect of partially correlated motion, $\left(S_{\text{fast}HH}^2\right)^2$ for the RDC-derived NMR bundle 2k39 [54] was determined (Table 8.2, Figure 8.6 B). Since the bundle includes both slow as well as fast motion, a pure fast motional averaging yields an overestimation of the fast $^1\text{H}_N - ^1\text{H}_N$ order parameters as evidenced by lower $S_{\text{HN}}^2$ when compared to reference [50]. As a consequence, the $\left(S_{\text{fast}HH}^2\right)^2$ derived from the bundle can be regarded as an upper limit for fast motion present in the system. Nonetheless, as shown in Table 8.2,
these fast order parameters \( (S_{HH}^{\text{fast}})^2 \) are in average very similar to the ones derived from the simulations and the ones presented in Figure 8.5. In summary, all the calculations and simulations indicate that there is only little influence of fast motion to the order parameters \( (S_{HH})^2 \).

<table>
<thead>
<tr>
<th>( (S_{HH}^{\text{fast}})^2 )</th>
<th>( \beta ) – Strands</th>
<th>( \alpha ) – Helix</th>
</tr>
</thead>
<tbody>
<tr>
<td>2k39</td>
<td>Figure 8.5</td>
<td>2k39</td>
</tr>
<tr>
<td>mean value</td>
<td>0.99 ± 0.09</td>
<td>1.00 ± 0.02</td>
</tr>
</tbody>
</table>

Table 8.2: Mean values with standard deviations for the fast order parameters \( (S_{HH}^{\text{fast}})^2 \) derived from the NMR bundle 2k39 [54] and the simulations discussed above and presented in Figure 8.5.
Figure 8.6:
(A) The order parameters derived from the NMR bundle 2k39 [54] (red circles) are compared to the simulated ones presented in this study (blue circles). The red circles give an upper limit for the influence of fast motion as the averaging over the bundle assumed only fast motion (see B). The most prominent outliers are labeled. Some of them, especially at the C-terminus of ubiquitin and in the first loop, are related to low $^{1}H_N-^{15}N$ local order parameters in the NMR bundle 2k39. For example the local order parameter for residue 9 is 0.61 extracted from the bundle compared to 0.75 as presented in [50] - resulting in a product $S^{2}_{HN,x} \cdot S^{2}_{HN,y} = 0.31$ compared to $S^{2}_{HN,x} \cdot S^{2}_{HN,y} = 0.49$.
(B) The fast motion $^{1}H_N-^{15}N$ order parameters derived from the NMR bundle 2k39 are plotted versus the corresponding order parameters presented in reference [50]. Generally, the 2k39 order parameters are similar to or smaller than the ones measured by [50], which is attributed to the fact that also slow motion is present in the 2k39 bundle which however could not be excluded for the simulations of the $S^{2}_{HN}$ as well as the $(S^{fast}_{HH})^{2}$ order parameters.
Figure 8.7: Relationship between calculated order parameters \( \left( S_{\text{HH}}^{\text{fast}} \right)^2 \) describing the influence of fast motion on the NOE and local order parameters \( S_{\text{NH}}^2 \) for different sterical conformations.

A: The polar angle between the two cones axes is 90° while the azimuthal angle is 0°
B: The polar angle between the two cones axes is 90° while the azimuthal angle is 90°
C: The polar angle between the two cones axes is 0°. The two cone axes are parallel to each other - the distance between the two protons is the same as between the two nitrogen atoms.

### 8.3 Spin diffusion in a multiple spin system

In addition to small errors in the experimental errors in the extraction of the NOE build-up rate constants (chapter 7.4) and in the determination of the rotation correlation time \( \tau_c \) (chapter 8.1), a potential source of errors is the spin diffusion present in a multiple spin system. Within this effect, one has to distinguish between two different potential problems: (i) The principle presence of spin diffusion that has to be accounted for, and
within this (ii) the correction of spin diffusion using an inaccurate structural model and (iii) the principle problem that spin diffusion might not accurately be described by the Redfield theory (chapter 8.4).

Let us consider first the presence of spin diffusion per se. If short mixing times are selected, most of the buildup curves for different secondary structure elements normally indicate a good fit quality. Only for next but one neighbours in the $\alpha$-helix the fit is poor (see Figure 7.3). These NOE transfers have a higher order (in addition to the 2-spin solution) contribution due to spin diffusion, preventing a satisfying two-spin fit. Figure 8.8 shows the spin-diffusion corrected intensities as calculated from the program DOMINO [55] (for a detailed description see Appendix) for the model protein Ubiquitin. The corrected intensities yield a much better fit as the uncorrected ones.

Figure 8.8: Spin-diffusion effects on fitting of the NOE buildups. The graph shows on the left hand side the fit of the spin diffusion corrected intensities for the cross peak between residue 33 and residue 35 of the model protein Ubiquitin. On the right hand side the uncorrected intensities extracted from the NOESY experiments are plotted versus the mixing time.
However, even if spin diffusion pathways are taken into account, the inaccuracy of available structures (and with it inaccurate distances) needed for the calculation may lead to poor predictions for the correction factors. As described by [36, 55], spin diffusion pathways are calculated by the software routine DOMINO using atomic-resolution structures such as those obtained from NMR or X-ray methods, both of which may be an inaccurate representation of the protein structure. For example, although an X-ray structure at 1.8 Å resolution is available (pdb code 1Ubq [56]) the distance between $^1\text{H}_N$ of residue 34 and $^1\text{H}_N$ of residue 35 is 1.94 Å and therefore contradicts the averaged distances of 2.48 Å obtained from the high-resolution NMR structure (pdb code 1D3Z [57]). Although this disparity has no direct influence in determining the exact average distance by the cross-relaxation rate constants $\sigma$ between $^1\text{H}_N$ of residue 34 and $^1\text{H}_N$ of residue 35, it has an impact on the distance between $^1\text{H}_N$ of residue 33 and $^1\text{H}_N$ of residue 35 because of the strong spin diffusion correction mediated by $^1\text{H}_N$ of residue 34. The correction for the NOE based on the distance obtained from the X-ray structure is 57 %, whereas the correction for the spin diffusion derived from the product of the relevant NOEs measured experimentally is only 25 % (data not shown). The overestimation of 32 % alternatively yields an apparent effective distance of 4.25 Å instead of 3.87 Å (for 307 K e.g.) as obtained from the the NMR structure 1D3Z.

Because the presented example is by far the largest error found, this potential source of error in determination of an exact effective distance from NOEs appears to be small because of the inverse relationship to the power of six between NOE rate constant and distance. Nonetheless, this source of error could be eliminated by the use of a full relaxation matrix refinement [11], a method that is currently established in our group.

One of the major problems here is the fact, that the initial magnetization is not the same at the beginning of each experiment. The interscan delay is too short to establish the Boltzmann equilibrium in the sample. Hence, each spin starts with a different initial magnetization disabling the assumption of a unity matrix at the beginning. Therefore, a
single spectrum is no longer sufficient in this approach and at least two spectrums must be recorded - one to determine the starting magnetization matrix and one to obtain the auto- and cross-relaxation rate constants.

8.4 Higher order contributions to the classical description of spin diffusion

8.4.1 Introduction

Calculating the magnetization transfer for a three spin system with the procedure explained in the introduction (chapter 1) would result in a second order expansion of the Liouville-von Neumann equation. But this procedure is, in principle, not sufficient to describe higher-order processes, as possible multi-spin correlations are neglected. To take three-spin correlations into account, the Liouville-von Neumann equation has to be expanded up to fourth order. Similarly, to describe correlations between the motional modes of n spins, an expansion up to n-th order would be required.

The aim of this chapter is to show, that an expansion up to second order is in general insufficient to describe multiple step transfers but adequate in NMR spectroscopy. As we shall see the fourth order treatment discloses, that if a correlation such as concerted motion between the two transfer steps is present in the system, a second order approximation of the second order approximation is insufficient to describe the system adequately. The aim of this work is to show that an expansion up to second order is in general insufficient to describe multiple step transfers but adequate in NMR spectroscopy. As we shall see, the fourth order treatment discloses, that if a correlation such as concerted motion between the two transfer steps is present in the system, a second
order approximation of the second order approximation is insufficient to describe the system adequately. With other words, while in the absence of a correlated process a simple second order expansion is sufficient to describe the anticipated two step process, for all other cases a fourth order expansion is in principle necessary to describe the entire system accurately. In addition, three spin simulations using the software package GAMMA [58] were performed as well. The comparison between the fourth order approximation presented here and its counterpart of a second order approximation reveals that in principle the fourth order cumulant is sensitive to concerted motion. However, the application of this general calculation to the relaxation theory in NMR spectroscopy reveals that the NMR relevant measures are far to slow for the fourth order cumulant to be unequal to 0 resulting in the conclusion, that semi-classical relaxation theory of second order is sufficient to describe one, two or n step processes in NMR on all time scales.

8.4.2 Solution of the Liouville-von-Neuman equation to fourth order

We want to solve the Liouville-von-Neuman equation for a spin system within a lattice at a finite temperature under a Hamiltonian $H^p(t)$ modulated by a stochastic rate process with zero average. To isolate the effect of the stochastic perturbation, an interaction representation is used where $H(t) = H^p(t)$. This is the only restriction to our system at the moment. We revisit the Liouville-von-Neuman equation using the approach by Goldmann [1]:

$$\frac{d\sigma(t)}{dt} = -\frac{i}{\hbar} [H^p(t), \sigma(t)]$$

(8.1)

Formal integration results in

$$\sigma(t) = \sigma(0) - \frac{i}{\hbar} \int_0^t [H^p(t'), \sigma(t')] dt'$$

(8.2)
Insert eq. (8.2) into eq. (8.1) (as an iterative process) and using the fact, that the spin-lattice coupling has a zero average as well as replace \( \sigma(t) \rightarrow \sigma(t) - \sigma_{eq} := \tilde{\sigma}(t) \) due to the finite lattice temperature, the following equation is obtained:

\[
\sigma(t) \approx \hbar \int_{0}^{t} \int_{0}^{\tilde{t}} \langle [H^P(t'), [H^P(t''), \tilde{\sigma}(0)]] \rangle dt' dt'' + \hbar \int_{0}^{t} \int_{0}^{\tilde{t}} \int_{0}^{\tilde{t}'} \int_{0}^{\tilde{t}''} \langle [H^P(t'), [H^P(t''), [H^P(t'''), [H^P(t''''), \tilde{\sigma}(0)]]]] \rangle dt''' dt'''' dt'' dt'.
\]

(8.3)

The averaging is necessary to take into account that \( H^P(t) \) is a random function in time. This is a fourth order approximation of the solution of a Liouville-von Neuman equation. The first order expansion can be neglected due to the zero average of the Hamiltonian \( H^P(t) \) as claimed above. The third order can in general not be neglected. However, assuming a dipole-dipole Hamiltonian, the third order term vanishes as no relaxation pathway for longitudinal relaxation is present. To calculate the density matrix under a certain Hamiltonian the formalism of the cumulant expansion, first applied to correlated processes by Freed [59], is used.

### 8.4.3 The cumulant expansion

To study the fourth order expansion with four commutators derived above (eq. 8.3), one has to establish a "master equation" up to fourth order, which governs the relaxation of the spin-density matrix in the flexibly narrowed regions. For a better understanding, we want to introduce in the following the so called cumulant expansion [59]. With this approach, we can rewrite the equations derived above in a more compact form. Furthermore, an easier interpretation of the occurring terms is possible therewith. Following the description by Freed [59], the Liouville-von-Neuman equation (eq. (8.1)) has an
integral solution of the form

\[ \tilde{\sigma}(t) = \tilde{\sigma}(0) + \left(-\frac{i}{\hbar}\right) \int_{0}^{t} \langle \mathcal{H}_p(t_1) \times \tilde{\sigma}(t_1) \rangle \, dt_1 \]  

(8.4)

where we used the shortcut \( a \times b = [a, b] \) (compare with eq. (8.2)). The exact solution can be written as a Neumann series

\[ \tilde{\sigma}(t) = \left(\sum_{n=0}^{\infty} \frac{1}{n!} \left(-\frac{i}{\hbar}\right)^n \int_{0}^{t} \ldots \int_{0}^{t} T \langle \mathcal{H}_p(t_1) \times \ldots \times \mathcal{H}_p(t_n) \times \rangle \, dt_n \ldots dt_1 \right) \tilde{\sigma}(0) \]  

(8.5)

which can formally be expressed as

\[ \tilde{\sigma}(t) = \left\langle e^{-\frac{i}{\hbar} \int_{0}^{t} \mathcal{H}_p(t') \times dt'} \right\rangle \tilde{\sigma}(0) \]  

(8.6)

and \( T \) indicates a time ordered product. To obtain the "master equation" of relaxation, the basic idea of the cumulant method is to find a solution for the equation \( \frac{d\tilde{\sigma}(t)}{dt} = \frac{dK(t)}{dt} \tilde{\sigma}(t) \) which is of the form

\[ \left\langle e^{-\frac{i}{\hbar} \int_{0}^{t} \mathcal{H}_p(t') \times dt'} \right\rangle = e^{K(t)} \]  

(8.7)

where \( K(t) = \sum_{n=1}^{\infty} K_n(t) \)
with $K_n$ being the $n$th cumulant. The cumulants of interest (for an expansion up to 4th order) have the following expressions:

$$K_2 = -\frac{1}{\hbar^2} \int_0^t \int_0^{t_1} \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_2) \times \rangle \, dt_2 \, dt_1$$

$$K_4 = \frac{1}{\hbar^4} \int_0^t \int_0^{t_1} \int_0^{t_2} \int_0^{t_3} \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_2) \times \mathcal{H}^p(t_3) \times \mathcal{H}^p(t_4) \times \rangle \, dt_4 \, dt_3 \, dt_2 \, dt_1 - \frac{1}{\hbar^4} \int_0^t \int_0^{t_1} \int_0^{t_2} \int_0^{t_3} \{ \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_2) \times \mathcal{H}^p(t_3) \times \mathcal{H}^p(t_4) \times \rangle - \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_2) \times \rangle \langle \mathcal{H}^p(t_3) \times \mathcal{H}^p(t_4) \times \rangle - \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_3) \times \rangle \langle \mathcal{H}^p(t_2) \times \mathcal{H}^p(t_4) \times \rangle - \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_4) \times \rangle \langle \mathcal{H}^p(t_2) \times \mathcal{H}^p(t_3) \times \rangle \} \, dt_4 \, dt_3 \, dt_2 \, dt_1$$

(8.8)

An important property of $K_n(t)$ with $n > 2$ is, that if any one of the random variables contained in it is uncorrelated with the others, the cumulant is zero [59]. This is nicely illustrated in a two step process for which in absence of concerted motion $\mathcal{H}^p(t_i)$ for $i = 1, 2$ is uncorrelated with the Hamiltonians for $i = 3, 4$. Hence, in absence of concerted motion, the quadruple averaged term in $K_4$ can be separately averaged over pairs of Hamiltonians resulting in

$$K_4 = \frac{1}{\hbar^4} \int_0^t \int_0^{t_1} \int_0^{t_2} \int_0^{t_3} \{ \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_2) \times \rangle \langle \mathcal{H}^p(t_3) \times \mathcal{H}^p(t_4) \times \rangle - \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_2) \times \rangle \langle \mathcal{H}^p(t_3) \times \mathcal{H}^p(t_4) \times \rangle - \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_3) \times \rangle \langle \mathcal{H}^p(t_2) \times \mathcal{H}^p(t_4) \times \rangle - \langle \mathcal{H}^p(t_1) \times \mathcal{H}^p(t_4) \times \rangle \langle \mathcal{H}^p(t_2) \times \mathcal{H}^p(t_3) \times \rangle \} \, dt_4 \, dt_3 \, dt_2 \, dt_1$$

(8.9)

The third and fourth terms are 0 (because $\mathcal{H}^p(t_{1,2})$ is uncorrelated with $\mathcal{H}^p(t_{3,4})$ - and therefore the correlation function is zero) and concomitantly $K_4(t) = 0$. This has the consequence that only the term $K_2(t)$ is necessary to describe the system. How-
ever, $K_2(t)$ is equivalent to the second-order approximation of the relaxation theory and therefore the Redfield theory describes correctly the evolution of the density matrix. In contrast, in presence of concerted motion $K_4(t)$ may not be zero and hence a fourth order approximation of the relaxation theory might be necessary. Concentrating on a practical example, we want to study the dipole-dipole Hamiltonian $\mathcal{H}_p(t)$. This one contains only a secular perturbation and the non-secular second term can be neglected [1] obtaining the following simplified equation (as an expansion up to fourth order which is equal to the sum of $K_2$ and $K_4$):

$$\tilde{\sigma}(t) - \tilde{\sigma}(0) = \left\{ -\frac{1}{\hbar^2} \int_0^t \int_0^{t_1} \langle \mathcal{H}_p(t_1) \times \mathcal{H}_p(t_2) \rangle \, dt_2 dt_1 ight. \right.$$  

$$+ \frac{1}{\hbar^4} \int_0^t \int_0^{t_1} \int_0^{t_2} \int_0^{t_3} \langle \mathcal{H}_p(t_3) \times \mathcal{H}_p(t_2) \times \mathcal{H}_p(t_3) \times \mathcal{H}_p(t_4) \times \rangle \, dt_4 dt_3 dt_2 dt_1$$  

$$\left. - \frac{1}{\hbar^4} \int_0^t \int_0^{t_1} \langle \mathcal{H}_p(t_1) \times \mathcal{H}_p(t_2) \rangle \, dt_2 dt_1 \langle \mathcal{H}_p(t_3) \times \mathcal{H}_p(t_4) \times \rangle \, dt_4 dt_3 \right\} \cdot \tilde{\sigma}(0)$$  

Note that for a fourth order expansion the Neumann series (eq. (8.4)) is truncated after the fourth term and $\tilde{\sigma}(t)$ can be replaced by $\tilde{\sigma}(0)$. A correct solution would be obtained by keeping the time dependence of $\tilde{\sigma}$.

Matrix representation

As the equation $\frac{d\tilde{\sigma}(t)}{dt} = \frac{dK(t)}{dt} \tilde{\sigma}(t)$ is defined in the Liouville space, the density operator is represented by a vector, while $\frac{dK(t)}{dt}$ is a matrix. In the following, we will call the diagonal elements of this matrix auto-relaxation rate constants while the off-diagonal elements are called cross-relaxation rate constants.

The master equation of relaxation of a three spin system (aka Solomon equation) can be rewritten under the following assumptions (i) for reasons of simplicity there is no direct interaction (i.e. cross relaxation rate constant) between spin $I_1$ and $I_3$ (ii) the auto- and cross-relaxation rate constant terms are described sufficiently well by a second order

68
expansion of the exponential function and (iii) a fourth order semi-classical expansion is sufficient to describe the two step process (i.e. spin diffusion).

For a description of a two step process between $I_1$ and $I_3$ via $I_2$ in presence of a fast rate process (faster than the rotational correlation time of the molecule) we can therefore design a relaxation matrix of the form:

$$\frac{d}{dt} \begin{bmatrix} I_{1z} \\ I_{2z} \\ I_{3z} \end{bmatrix} = - \begin{bmatrix} \Gamma_{11} & \Gamma_{12} & \Gamma_{123} \\ \Gamma_{12} & \Gamma_{22} & \Gamma_{23} \\ \Gamma_{123} & \Gamma_{23} & \Gamma_{33} \end{bmatrix} \begin{bmatrix} I_{1z} \\ I_{2z} \\ I_{3z} \end{bmatrix}$$

$$\iff \dot{I}_z = -\mathcal{R}I_z \quad (8.11)$$

It is now requested to derive the matrix elements from the formal solution up to fourth order of the Liouville-von-Neuman equation (8.10).

### 8.4.4 Master equation of fourth order under a dipole-dipole Hamiltonian

To describe the master equation of relaxation up to fourth order, a Hamiltonian in spherical tensor notation, that considers only dipole-dipole interactions, is used. Therefore one has to consider the scalar product of two tensors which can be represented by a sum of products between time-invariant spin operators $T^l_m$ and time-dependent space tensor components

$$A_{lm}(\Omega_{ij}) = -\sqrt{6} \cdot \frac{\mu_0}{4\pi} \gamma_i \gamma_j \hbar^2 \sqrt{\frac{4\pi}{2l+1}} \frac{Y_{lm}(\theta(t), \varphi(t))}{r_{ij}^3} := B^{ij}_{2l} F^l_t(\Omega(t)) \quad l \leq 2 \quad (8.12)$$

that account for the relaxation-inducing stochastic process. Here, we defined $B^{ij}_{2l} = -\sqrt{6} \cdot \frac{\mu_0}{4\pi} \gamma_i \gamma_j \hbar^2 \sqrt{\frac{4\pi}{2l+1}}$ and $F^l_t(\Omega(t)) = \frac{Y_{lm}(\theta(t), \varphi(t))}{r_{ij}^3(t)}$. The dipole-dipole Hamiltonian is
then given by
\[ \mathcal{H}^p(t) = \sum_{i<j} \sum_{l=0}^{2} \sum_{m=-l}^{l} (-1)^m B_{2}^{ij} T_{m}^{d} \tilde{F}_{l}^{m} (\Omega_{ij}(t)) \] (8.13)

where the indices \( i \) and \( j \) label the spin interaction between spin \( i \) and \( j \). (Note: the number of spins is in principle arbitrary. However, in the following a three spin system is considered).

By back transformation into the laboratory frame and expanding the spin operators in the basis of eigenoperators \( V_p \) with coefficients \( v_p \) of the Hamiltonian (e.g. \( e^{i \mathcal{H} t} T_{m}^{d} e^{-i \mathcal{H} t} \propto V_p e^{i \omega_p t} \) where \( \omega_p \) are the eigenfrequencies of the Hamiltonian) one results in

\[ \tilde{\sigma}(t) - \tilde{\sigma}(0) \approx \sum_{i<j} \left( Z_{2}^{ij} \right)^2 \sum_{m=-l}^{l} \sum_{p} \left| v_p^m \right|^2 \left[ V_p, [V_p^\dagger, \tilde{\sigma}(0)] \right] \int_0^t \int_0^{t_1} C^m(t_2 - t_1) e^{i \omega_p (t_2 - t_1)} dt_2 dt_1 \]

\[ + \sum_{m=-l}^{l} \sum_{n=-k}^{k} \sum_{p,q} \left| v_p^m \right|^2 \left| v_q^n \right|^2 \left[ V_p, [V_q^\dagger, [V_q^\dagger, \tilde{\sigma}(0)]] \right] \frac{1}{2} \sum_{j<k} \sum_{i<j} \left( Z_{2}^{ij} \right)^2 \left( Z_{2}^{jk} \right)^2 \int_0^t \int_0^{t_1} \int_0^{t_2} \int_0^{t_3} C^{mn}(t_1, t_2, t_3, t_4) \cdot e^{i \omega_p (t_2 - t_1)} e^{i \omega_q (t_3 - t_1)} e^{i \omega_q (t_4 - t_2)} dt_4 dt_3 dt_2 dt_1 \] (8.14)

The indices \( m \) and \( n \) label the magnetic quantum number of the spins while \( Z_{2}^{ij} = \frac{1}{\hbar} B_{2}^{ij} = -\sqrt{6} \cdot \frac{m}{4\pi} \gamma_i \gamma_j \hbar \). In principle, the decomposition of the Hamiltonian would result in a sum over \( p \) and \( p' \). But for both limits discussed below, the oscillating terms \( \sim \left[ V_p, [V_p^\dagger, \tilde{\sigma}(0)] \right] \cdot e^{i (\omega_p - \omega_p') t} \) can be neglected as the assumption \( \omega_p - \omega_p' \cdot t \gg 1 \) is fulfilled in all time regimes discussed. The two correlation functions are defined by

\[ C^{m'm'}(t - t_1) = \left\langle F_l^{m'}(\Omega_{ij}(t)) F_l^{m's}(\Omega_{ij}(t_1)) \right\rangle \]

\[ = C^{m'm'}(\tau) = \left\langle F_l^{m'}(\Omega_{ij}(\tau)) F_l^{m's}(\Omega_{ij}(0)) \right\rangle \] (8.15)
and

\[
C_{m'm''m'''} = \left\langle F^m_t(\Omega_{ij}(t_1)) F^{m'*}_t(\Omega_{ij}(t_2)) F^{m''}_t(\Omega_{jk}(t_3)) F^{m'''}(\Omega_{jk}(t_4)) \right\rangle \\
\approx C_{m'm''m'''}(\tau_2, \tau_4) = \left\langle F^m_t(\Omega_{ij}(\tau_2 + \tau_3)) F^{m'*}_t(\Omega_{ij}(\tau_3)) F^{m''}_t(\Omega_{jk}(\tau_4)) F^{m'''}(\Omega_{jk}(0)) \right\rangle
\]

(8.16)

with \( t_4 = 0, \tau_4 = t_3 - t_4, \tau_3 = t_2 - t_4 \) and \( \tau_2 = t_1 - t_2 \) and \( \tau_2 + \tau_3 \geq \tau_3 \geq \tau_4 \geq 0 \).

The advantage of this definition is, that the first transfer happens during time \( \tau_4 \) while the second transfer happens during time \( \tau_2 \).

The correlation function of second order \( C_{m'm'}(t, t_1) = C_{m'm'}(t - t_1, 0) := C_{m'm'}(\tau) \) of eq. (8.15) is only dependent of a time \( \tau \) describing the time difference between the two random functions \( F^m_t(t) \) and \( F^{m'}_{t}(t_1) \). In the case of the correlation function of fourth order, \( C_{m'm''m'''m'''} \) is dependent on \( F^m_t(t_1), F^{m'}_{t_2}, F^{m''}_{t_3} \) and \( F^{m'''}_{t_4} \) and the transformation of the time dependence is more complex.

Correlation functions of nth order are well known from the field of quantum field theory (see for example [60]). There, the order of a correlation function is related to the number of occurring field operators which are related to the number of particles involved. A
correlation function of the form

\[
\int_0^t \int_0^{t_1} \langle \mathcal{H}^P(t_1) \times \mathcal{H}^P(t_2) \times \rangle \, dt_2 dt_1 \tag{8.17}
\]

is therefore comparable associated with a two particle process, where one state gets destroyed and another one created. This is similar to the one step process of NOE during which one spin flips back to equilibrium (gets destroyed) and the other spin flips accordingly (gets created). During this procedure there is only one symmetry of time invariance and hence one integral can be calculated - as shown in Appendix A.

Here, we want to try to understand the next higher term of the form

\[
\int_0^t \int_0^{t_1} \int_0^{t_2} \int_0^{t_3} \langle \mathcal{H}^P(t_1) \times \mathcal{H}^P(t_2) \times \mathcal{H}^P(t_3) \times \mathcal{H}^P(t_4) \times \rangle \, dt_4 dt_3 dt_2 dt_1 \tag{8.18}
\]

Since this correlation function consists of four Hamiltonians, it is related to a three or four particle process in quantum field theory (as a three particle process would only handle with real particles this schema is more related to our problem). There is still only one symmetry, the time invariance, to solve one integral. But we have to find another symmetry to get rid of a second integral. This is only possible if something is known about the process of interest which is a two step process

\[I_3 \rightarrow I_2 \rightarrow I_1\]

First, the Hamiltonian of interest has the form

\[
\mathcal{H}^P(t) = \sum_{i<j} \sum_{m=-l}^{l} (-1)^m B^{ij}_m T^{l,ij}_m F^{m}_l (\Omega_{ij}(t)) \tag{8.19}
\]

For a three spin system there are only two interaction terms, assuming that spins 1 and 3 are too far apart to have an important contribution. Hence, the correlation function of

72
fourth order is given by

\[
\left\langle \left( F^m_{12}(t_1) + F^m_{23}(t_1) \right) \cdot \left( F^{m'}_{12}(t_2) + F^{m'}_{23}(t_2) \right) \cdot \left( F^{m''}_{12}(t_3) + F^{m''}_{23}(t_3) \right) \cdot \left( F^{m'''}_{12}(t_4) + F^{m'''}_{23}(t_4) \right) \right\rangle = \left\langle F^m_{12}(t_1) F^{m'}_{12}(t_2) F^{m''}_{23}(t_3) F^{m'''}_{23}(t_4) \right\rangle
\]

(8.20)

as we can choose for times \( t \leq t_2 \) \( F^m_{23}(t) = 0 \) because there is only interest in interactions between the first and the second spin. As a consequence, the only correlation left (which is the correlation for the spin diffusion process) is the one above (eq. 8.20). The random time functions \( F^m(\Omega(t)) \) and \( F^{m'}(\Omega(t)) \) as well as \( F^{m''}(\Omega(t)) \) and \( F^{m'''}(\Omega(t)) \) are statistically dependent - otherwise the ensemble average would be zero - so we have to set \( m = m' \) and \( m'' = m''' = n \) (in terms of correlation an autocorrelation between the time functions \( F^m(\Omega(t)) \) and \( F^{m'}(\Omega(t)) \) as well as \( F^{m''}(\Omega(t)) \) and \( F^{m'''}(\Omega(t)) \) and a cross correlation between \( F^{m'}(\Omega(t)) \) and \( F^{m'''}(\Omega(t)) \) is assumed respectively). Therefore it’s wrong to set all \( m \) equal.

The exchange of energy via dipole-dipole coupling can be observed in NMR in the NOESY experiment. The signal occurs from a spin flip, where the energy from one spin is transferred to another one via dipole-dipole coupling. The transition probability between the two states \( i \) and \( j \) is given in accordance to Fermi’s golden rule [32] by the square of the absolute value of the interaction \( V \) (e.g. \( P_{i \rightarrow j} \propto |\langle i | V | j \rangle|^2 \)) and can be calculated using the anisotropy for the dipole-dipole Hamiltonian in spherical tensor notation which is \( \frac{\delta}{2\pi} = -2\frac{\mu_0}{4\pi} \frac{\gamma_i \gamma_j h}{r_{ij}^3} \). Using \( r = 3 \text{ Å} \) for NMR and \( r = 15 \text{ Å} \) for EPR one results in

\[
\text{NMR} \quad \frac{\delta}{2\pi} = 8.9 \text{ kHz} \\
\text{EPR} \quad \frac{\delta}{2\pi} = 31 \text{ MHz}
\]

73
The coupling for NMR is much lower than the one for EPR. This reflects the fact, that relaxation in NMR is quit slow as a spin flip is very unlikely. In the following, we want to distinguish between the two scenarios of (i) small anisotropy, e.g. the molecule tumbling of the molecule is much faster than a typical inverse spin flip rate constant and (ii) high anisotropy, e.g. the spin flip rate constant is in the order of the molecular tumbling.

**Master equation of fourth order under a slow rate process**

Let us consider first the case for which the process of interest is slower than the correlation time of the molecule. The process is so slow that the correlation is lost before the second step of the process happens. With other words with \( \tau_3 \gg \tau_4 \) the first step of the process decouples from the second one enabling individual averaging between the two substeps of the two step process. Following this argument, eq. (8.16) can be replaced by eq. (8.21):

\[
C^{m'm'm'm'}(\tau_2, \tau_3, \tau_4) = \left\langle F^m(\Omega_{ij}(\tau_2 + \tau_3))F^{m'*}(\Omega_{ij}(\tau_3))F^{m''}(\Omega_{jk}(\tau_4))F^{m''*}(\Omega_{jk}(0)) \right\rangle \\
= \left\langle F^m(\Omega_{ij}(\tau_2 + \tau_3))F^{m'*}(\Omega_{ij}(\tau_3)) \right\rangle \left\langle F^m(\Omega_{jk}(\tau_4))F^{m''*}(\Omega_{jk}(0)) \right\rangle \\
= C^m(\tau_2) \cdot C^m(\tau_4)
\]

The consequence is that there is no correlation between the Hamiltonians at the given times yielding a cumulant to the fourth order of zero \( (K_4 = 0) \). In the example of interest (here spin diffusion in liquid NMR) it is evident that the process of a spin flip is very slow (i.e. in the regime of seconds) when compared to the rotational correlation time \( \tau_c \) of the molecule (i.e. in the regime of ns), which is due to the small anisotropy of the dipole-dipole Hamiltonian. Hence, spin diffusion does not require the relaxation theory of the fourth order and the Redfield theory describes adequately such slow (multi-step)
Master equation of fourth order under a fast rate process

In the case of very fast processes the argumentation is different. Since the process is very fast it can be assumed that $\tau_3 = \tau_4$ while during $\tau_4$ the first transfer step of the process happens and during $\tau_2 + \tau_3 = \tau_2 + \tau_4$ the second step. Following this Ansatz, eq. (8.16) can be replaced by the following equation (in accordance to eq. (8.20))

$$C^{m'm''m'''} = \langle F_m^{l'}(\Omega_{ij}(t_1))F_{m'}^{l''}(\Omega_{ij}(t_2))F_{m''}^{l'''}(\Omega_{jk}(t_3))F_{m'''}^{l'''}(\Omega_{jk}(t_4)) \rangle$$ (8.22)

However, before we can continue to solve eq. (8.14) towards a master equation of relaxation, we have to revisit the described approach of solving the Liouville - von Neumann equation (eq. 8.1 onward), because with the presence of a fast rate process some of the assumptions made above, in particular the so called Redfield limit, are no longer valid. In details, the Redfield limit requests a slow rate process (compared to the overall correlation time) in order to (i) satisfy a second order approximation of the Liouville - von Neumann equation, to (ii) decouple the spin part from the spatial part of the Hamiltonian in deriving the master equation of relaxation, and to (iii) integrate the correlation function up to infinity. The first assumption to be revisited is that due to a slow evolving density matrix a second order approximation of the Liouville - von Neumann equation is sufficient to describe the processes of interest. Since due to the requested fast rate process also the density matrix will evolve fast (eq. (8.10)), a second order description of the Liouville-von Neumann equation is here not adequate anymore. Obviously, a fourth order approximation thereof – the interest of the presented study - is more appropriate. Hence, the fourth order approximation of the Liouville - von Neumann equation should be able to describe fast rate processes within a short evolution
time \( t \). The request of a fast rate process also limits the permission (point (ii) above) to decompose the Hamiltonian into a spin part that is time insensitive and a a random function of time where one assumes, that each spin operator oscillates at one frequency (eq. (8.13)). Because this decomposition appears to be necessary for an analytical solution of the Liouville-von Neumann equation, it is still assumed here, taking into account that oscillations between the spin operators and the random function of time are not adequately described for long times \( t \). This description limits thus the following solution of the Liouville - von Neumann equation to relatively short times \( t \), as demonstrated below in the comparision between simulations and analytical equations (Fig. 8.11). In particular, it will miss the oscillation of the observable at longer times \( t \). Finally, because of the fast rate process, the correlation function can not be integrated straight forward to infinity (as shown in Appendix A), which is usually done because it allows the use of a Fourier transformation. With this discussion in mind, the finding of a solution of the Liouville-von Neumann equation is continued by solving one integral of eq. (8.10) with the procedure explained in Appendix A and defining the functional of the spectral density function and the time as

\[
G^m(\omega, t) = \int_0^t (t - \tau) C^m(\tau) e^{i\omega \tau} d\tau
\]

\[
= t \cdot J(\omega, t) - \int_0^t \tau \cdot C^m(\tau) e^{i\omega \tau} d\tau
\]

(8.23)

where \( J(\omega, t) := \int_0^t C^m(\tau) e^{i\omega \tau} d\tau \) is a generalized spectral density function. The fourth order integral from eq. (8.10) results in

\[
G^m(\omega_p, \omega_q, t) = \int_0^t \int_0^t (t - \tau)^2 C^{mn}(\tau_2, \tau_4) e^{i\omega_p \tau_2} e^{i\omega_q \tau_4} d\tau_2 d\tau_4,
\]

(8.24)

Note, that this functionals as well as the modified spectral density functions are time dependent. In Appendix D is a short discussion about theses functionals, including the
influence of the different boundary conditions. Now, one can rewrite eq. (8.14):

\[
\tilde{\sigma}(t) - \tilde{\sigma}(0) = - \sum_{i<j} \left( Z_{ij}^2 \right)^2 \sum_{m=-l}^{l} \sum_{p} |v_{p}^{m}|^2 [V_{p}, [V_{p}^\dagger, \tilde{\sigma}(0)]] \cdot \frac{1}{2} G^m(\omega_p, t)
\]

\[
+ \sum_{j<k} \sum_{i<j} \left( Z_{ij}^2 \right)^2 \left( Z_{jk}^2 \right)^2 \sum_{m=-l}^{l} \sum_{n=-k}^{k} \sum_{p,q} |v_{p}^{m}|^2 |v_{q}^{m}|^2 [V_{p}, [V_{p}^\dagger, [V_{q}^\dagger, \tilde{\sigma}(0)]]]
\]

\[
\cdot \frac{1}{12} \left( G^{mn}(\omega_p, \omega_q, t) - G^m(\omega_p, t) \cdot G^m(\omega_q, t) \right) \quad (8.25)
\]

Eq. (8.25) is a formal solution for the fourth order expansion of the density operator. However, in the second order case the differential equation, which is called the ”master equation” of relaxation, is often used as a starting point. Hence, from eq. (8.25), the master equation of relaxation up to fourth order is formulated.

\[
\frac{d\tilde{\sigma}(t)}{dt} = - \sum_{i<j} \left( Z_{ij}^2 \right)^2 \sum_{m=-l}^{l} \sum_{p} |v_{p}^{m}|^2 [V_{p}, [V_{p}^\dagger, \tilde{\sigma}(0)]] \frac{1}{2} \frac{d}{dt} G^m(\omega_p, t)
\]

\[
+ \sum_{j<k} \sum_{i<j} \left( Z_{ij}^2 \right)^2 \left( Z_{jk}^2 \right)^2 \sum_{m=-l}^{l} \sum_{n=-k}^{k} \sum_{p,q} |v_{p}^{m}|^2 |v_{q}^{m}|^2 [V_{p}, [V_{p}^\dagger, [V_{q}^\dagger, \tilde{\sigma}(0)]]]
\]

\[
\cdot \frac{1}{24} \frac{d}{dt} \left( G^{mn}(\omega_p, \omega_q, t) - G^m(\omega_p, t) \cdot G^m(\omega_q, t) \right) \quad (8.26)
\]

where the time ordering is already taken into account.

Integration of the Correlation function

The functionals (eq. (8.24)) can not be integrated without an additional assumption: It is in the following assumed that the correlation function shows an exponential decay where the characteristic time is given by \(\tau_c\). In absence of internal motion, the correlation
function is then described by

\[ C_{\text{global}}(t) = \frac{4\pi}{5^2} e^{-t/\tau_c} \]  

(8.27)

which enables the integration of

\[
\frac{d}{dt} G^m(\omega, t) = \frac{d}{dt} \int_0^t (t - \tau) C^m(\tau) e^{i\omega \tau} d\tau \\
= \frac{4\pi}{5^2} \frac{\tau_c}{(1 + \omega^2 \tau_c^2)} \left( 1 - \cos(\omega t) \cdot e^{-\frac{t}{\tau_c}} + \omega \tau_c \sin(\omega t) \cdot e^{-\frac{t}{\tau_c}} \right)
\]  

(8.28)

To indicate that this approach is reasonable, one can show that for \( \omega = 0 \) and times \( t \gg \tau_c \), eq. (8.28) results to the known spectral density function (see e.g. [31], \( \frac{d}{dt} G^m(\omega = 0, t \gg \tau_c) \approx J^m(0) = \frac{4\pi}{5^2} \tau_c \)) which is proportional to \( \tau_c \) as expected for the Redfield limit.

If internal motion is present, a model to describe the motion is in addition required for further calculations. The model of motion used in the following is the two – and three-site jump models (see Appendix G), which enable the integration of eq. (8.28). Doing so, the following expression is obtained:

\[(G^m(\omega, t))' = \frac{d}{dt} G^m(\omega, t) = \frac{1}{4\pi} \cdot \frac{1}{16} \cdot \left\{ \left( \frac{1}{r_1^2} + \frac{1}{r_3^2} \right) \cdot (\tau_x + 3\tau_y) + \frac{4}{r_2^2} \cdot (\tau_x + \tau_y) \right\}
\]

(8.29)

where

\[ \tau_{x,y} = \frac{\tau_j \left( 1 - \cos \omega t \cdot e^{-\frac{t}{\tau_j}} + \omega \tau_c \sin \omega t \cdot e^{-\frac{t}{\tau_c}} \right)}{1 + (\omega \tau_j)^2} \text{ with } \tau_j \in \{\tau_c, \tau_{int}\} \]

Again, to indicate that this approach is reasonable, we show that for the Redfield-limit at times \( t \gg \tau_c, \tau_{int} \) and \( \omega = 0 \) the derivative of the functional is proportional to \( \tau_c \)
and can be expressed by the known spectral density function as $\tau_{x,y}(\omega = 0, t \gg \tau_j) = \frac{\tau_j}{1 + (\omega \tau_j)^2}$.

To derive the matrix elements in eq. (8.11), equation (8.26) allows to treat further the second and fourth order terms:

$$\frac{d}{dt} \tilde{\sigma}(t)_{2\text{nd order}} = -\frac{1}{2} \sum_{i<j} (Z_{ij}^2)^2 \sum_{m=-l}^{l} \sum_{p} |v_p^m|^2 [V_p, [V^\dagger_p, \tilde{\sigma}(0)]] (G_{mn}^m(\omega, t))'$$

After calculating all occurring commutators, the cross-relaxation coefficient $\Gamma_{12}$ is

$$\Gamma_{12} = -\frac{1}{h^2} \frac{\gamma_1^2 \gamma_2^2 \mu_1 \mu_2}{(4\pi)^2} \left\{ \frac{2}{24} G_{mn}^m(\omega_p, t) - \frac{2}{4} G_{mn}^m(\omega_p, t) \right\}$$

$$= -\frac{\gamma_1^2 \gamma_2^2 \mu_1 \mu_2}{(4\pi)^2} \left\{ 6 G_{mn}^m(\omega_p, t) \right\} = \frac{d}{dt} \Delta_{12}$$

which looks similar to the known cross-relaxation rate constant term ([23],[25]) if $(G_{mn}^m(\omega, t))'$ is replaced by the spectral density function $J_m^m(\omega)$, which is the time insensitive counterpart of $(G_{mn}^m(\omega, t))'$.

Next, the second term of eq. (8.26)

$$\frac{d}{dt} \tilde{\sigma}(t)_{4\text{th order}} = \sum_{j<k} \sum_{i<j} (Z_{ij}^2)^2 \sum_{m=-l}^{l} \sum_{n=-k}^{k} |v_p^m|^2 |v_q^n|^2 [V_p, [V^\dagger_q, [V_p^\dagger, [V_q^\dagger, \tilde{\sigma}(0)]]]]$$

$$= \frac{1}{2} \left[ \frac{1}{4} \frac{d}{dt} (G_{mn}^{pp}(\omega_p, \omega_p, t) - G_{mn}^m(\omega_p, t) \cdot G_{nm}^m(\omega_q, t)) \right]$$

$$:= \frac{d}{dt} (\Delta_{123} - \Delta_{12} \Delta_{23})$$

is treated.

The Hamiltonian for our three spin system is the sum of the two Hamiltonians (e.g. $\mathcal{H}_p(t) = \mathcal{H}_{12}^p(t) + \mathcal{H}_{23}^p(t)$) if we assume no direct energy transfer from spin $I_1$ to spin $I_3$ (see above). After calculating all double commutators we end up with a cross-
relaxation coefficient of fourth order between spin $I_1$ and $I_3$ of the form

$$\Gamma_{123} = \frac{6^2 (\mu_0 h)^4}{4\pi} \frac{\gamma_1 \gamma_2 \gamma_3}{288} \frac{1}{4} \left\{ G'_{123}(\omega_1 - \omega_2, \omega_2 - \omega_3, t) - 6G'_{123}(\omega_1 - \omega_2, \omega_2 + \omega_3, t) \ight.$$

$$- 6G'_{123}(\omega_1 + \omega_2, \omega_2 - \omega_3, t) + 36G'_{123}(\omega_1 + \omega_2, \omega_2 + \omega_3, t)$$

$$- (G'_{12}(\omega_1 - \omega_2, t)G_{23}(\omega_2 - \omega_3, t) + G_{12}(\omega_1 - \omega_2, t)G'_{23}(\omega_2 - \omega_3, t))$$

$$+ 6 (G'_{12}(\omega_1 + \omega_2, t)G_{23}(\omega_2 - \omega_3, t) + G_{12}(\omega_1 + \omega_2, t)G'_{23}(\omega_2 - \omega_3, t))$$

$$+ 6 (G'_{12}(\omega_1 - \omega_2, t)G_{23}(\omega_2 + \omega_3, t) + G_{12}(\omega_1 - \omega_2, t)G'_{23}(\omega_2 + \omega_3, t))$$

$$- 36 (G'_{12}(\omega_1 + \omega_2, t)G_{23}(\omega_2 + \omega_3, t) + G_{12}(\omega_1 + \omega_2, t)G'_{23}(\omega_2 + \omega_3, t)) \right\}$$

$$= \frac{d}{dt} \left( \Delta_{123} - \frac{1}{4} \Delta_{12} \Delta_{23} \right) = \left( \frac{d}{dt} \Delta_{123} - \frac{1}{4} (\Gamma_{12} \Delta_{23} + \Delta_{12} \Gamma_{23}) \right)$$

where $\frac{d}{dt} \Delta_{123}$ lables the 4th order two step process term such as spin diffusion. As demonstrated, the fourth order term is under certain conditions not equal to zero.

Note, that in the Redfield limit $\frac{d}{dt} G_m(\omega = 0, t \gg \tau_c) \approx J_i(0)$ and hence, $\Delta_{ij} \propto J_i(\omega) \cdot t$ while $\Gamma_{jk} \propto J_j(\omega)$ and finally $\Gamma_{ij} \Delta_{jk} \propto J_i(\omega) \cdot J_j(\omega) \cdot t$ as expected.

In the case of uncorrelated motion the fourth order term is zero (e.g. $\Gamma_{123} = 0$) and the second order process is described by the product of the individual transfer rate constants.

### 8.4.5 Simulations of a two step process in the presence of three spins for small rate constants

*The simulations presented in this and the next section were performed by M. Ernst*

To study the influence of correlated and non-correlated motion on two-step polarization-transfer processes, numerical simulations using the spin-simulation environment GAMMA [58] were used in addition to the theoretical calculations presented so far. The simulated spin system consisted of two or three dipolar-coupled spins $1/2$ at a Larmor fre-
quency of 600 MHz corresponding to proton spins at a static magnetic field of 14 T. The spins were put on a linear chain with a distance of 3 Å between two neighbouring spins. The simulations were carried out in the laboratory-frame of reference using the full time-dependent Hamiltonian consisting of the Zeeman term, the isotropic chemical-shift term, and the full untruncated dipolar Hamiltonian. The spin system was undergoing isotropic rotational tumbling with a correlation time of 7.8 ns extracted from two-spin simulations without internal mobility. The rotational tumbling was introduced by stochastic rotations of the molecule every 10 ps where the rotation angle had a Gaussian distribution with a mean value of 1.8 degree. To obtain an ensemble average over the stochastic processes, 300 simulations with different starting orientations of the molecule were averaged. On top of the overall rotational tumbling correlated or uncorrelated jumps of the spins around the equilibrium position of 3 Å were implemented by stochastic jumps of ±1 Å with a correlation time of 80 ps. The simulation of the two-spin system without internal mobility was used to compare the results of the numerical simulations to calculations based on the Redfield theory to calibrate the rotational correlation time. For the three-spin system, three sets of simulations were performed: (i) simulations without internal mobility, i.e., the distance between neighbouring spins was fixed at 3 Å. (ii) Simulations with correlated jumps with a correlation time of 80 ps, i.e., the distance between neighbouring spins was 2 or 4 Å and changed simultaneously. (iii) Simulations with uncorrelated jumps with a correlation time of 80 ps, i.e., the distance between neighbouring spins was 2 or 4 Å and changed independently.

The simulation started with selective magnetization on spin \( I_1 \) only. The time evolution of the ensemble averaged operators \( I_{1z} \), \( I_{2z} \) and \( I_{3z} \) are presented in Figure 8.9. In details, Figure 8.9 shows the relaxation of \( I_{1z} \), the simulated NOE build up of \( I_{2z} \) as well as the direct NOE from \( I_{1z} \) to \( I_{3z} \) as well as the spin-diffusion contribution from \( I_{1z} \) via \( I_{2z} \) to \( I_{3z} \) versus the time \( t \). The comparison between the simulation and the Redfield theory is not straight forward, as the timescale of internal motion is not separated suffi-
ciently from the overall tumbling time. As one can see in Figure 8.10B, the theoretical (straight) lines, which follow distance averaging in accordance to fast internal motion (see Table 5.30), have a clear discrepancy to the simulated ones. To predict the simulated relaxation curves, a continued solution of the Redfield theory (eqs. (5.28) and (5.30)) for all timescales is used. As one can clearly see in Figure 8.9A, the theoretical curves and the simulations for the correlated case fits nearly perfect (straight and curly curves; blue curve for the auto - relaxation of Spin 1, green curves for cross-relaxation between Spin 1 and 2, and green for cross - relaxation and spin diffusion contribution for Spin 1 and Spin 3). In Figure 8.9B, a slight discrepancy can be seen even for the solution valid for all timescales, which is attributed to the relaxation pathway between the $I_{1z}$ and the $4I_{1z}I_{2z}I_{3z}$ operator. The fourth order has no effect to the relaxation, as the process under study is too slow and the correlation averages out (see above). The simulations clearly reiterate the finding from the theoretical considerations, that the fourth order term albeit dependent on correlated motion is not present in NMR experiments and in particular in NOESY experiments and that a second order approximation is sufficient to describe NMR observables and in particular spin diffusion in NOE experiments.
Figure 8.9: Three spin relaxation simulations for a linear three jump model optimized for a correlation time of $\tau_c = 7.8$ ns. Two independent simulations were performed assuming a jump model with a correlation time of the jumps of 80 ps. The blue lines are the values derived for the auto-relaxation, the green ones represent the cross-relaxation process between spin $I_1$ and $I_2$ and the red curves represent the cross-relaxation and spin-diffusion process between spin $I_1$ and $I_3$. The theoretical curves are calculated using the equations (5.29) and (5.31) derived in chapter 5.1.2. The black lines follows a inverse power of sixth dependency of the distance assuming only fast internal motion.

Figure A: A correlated motion between the atoms is simulated. The distance between spin $I_1$ and $I_2$ as well as between $I_2$ and $I_3$ can jump between 2 Å and 4 Å while the distance between spin $I_1$ and $I_3$ can only jump between 4 Å and 8 Å as a correlation between the two individual jumps is present.

Figure B: A un-correlated motion between the atoms is simulated. Here, the distance between the first and third spin can jump between 4 Å, 6 Å and 8 Å while the probability of the intermediate distance is twice the probability of the other two.
Figure 8.10: Three spin relaxation simulations for a linear three jump model optimized for a correlation time of $\tau_c = 7.8$ ns. Two independent simulations were performed. Figure A: The static simulations are an indication for the quality of the values derived. As for longer calculation time the influence of errors within the processors get more important this is a test for the high performance and the confidence interval of the simulations.

Figure B: The simulations are the same as in Figure 8.9A. The theoretical curves are calculated for the limit of fast internal motion. However the factor of 78.1 between the time scale for internal motion and the correlation time is not enough to separate the two motional regimes. The deviations between the straight (theoretical) lines and the simulated curves show, that the motion is not in the limit of fast internal motion. A better agreement is achieved by using the exact three spin solution for correlated motion as described by eq. (5.29) (see figure 8.9).
8.4.6 Simulations of a two step process in the presence of three spins for fast processes

In the case of electron paramagnetic resonance (EPR) the rotation correlation time can be quite long while the anisotropy of the dipole-dipole Hamiltonian gets higher due to the higher gyromagnetic ratio of the electrons. In this case, the Redfield limit is no longer valid. Hence, one has to go back to the assumptions made in the derivation of the theory and check which ones are still valid and which are truncated.

The first assumption was, that the second order is sufficient only if the timescale of $t$ modulating the process is much larger in the sense, that $\tilde{\sigma}(t)$ differs only little from its starting value at $t = 0$. The second assumption is the high temperature limit where the density matrix can be assumed to be diagonal and will trend towards the thermal equilibrium density matrix. The third assumption is that the Hamilton can be decomposed into a spin part and a random function of time where one assumes, that each spin operator oscillates at one frequency. As the spin part is time independent, one can define a correlation function as the time averaged product of the two random time functions at different time points. The fourth and last assumption is, that the time scale on which the correlation function decays is much smaller than the observation time of the process. This is called the Redfield limit.

The second and third assumptions are independent of time scales and hence not violated by longer correlation times. The first assumption might be still valid if one takes higher order contributions into account. Hence, a fourth order expansion provides a better description of the relaxation process. Finally one has to rethink the Redfield limit. If the correlation time is much longer than the observation time of the process, the integration of the correlation function is not a Fourier Transformation any more. Assuming, that the correlation function can still be described by an exponential decay where the characteristic time is given by $\tau_c$, the integral can be solved analytically. Doing so one
can calculate the second and fourth order auto- and cross-relaxation rate constants. To validate the theoretical results derived in subsection 8.4.4 GAMMA simulations were performed for EPR Parameters. The simulated spin system consisted again of two or three dipolar-coupled spins $\frac{1}{2}$ at a Larmor frequency of 95 GHz corresponding to electron spins at a static magnetic field of 3.4 T. The spins were again put on a linear chain with a distance of 15 Å between to neighbouring spins. The simulations were carried out under the same conditions as in section 8.4.5 with different correlation times. In Figure 8.11, such a two spin simulation for a rotational tumbling time of 100 ns is shown. The rotational tumbling was introduced by stochastic rotations of the molecule every 10 ps where the rotation angle had a Gaussian distribution with a mean value of 1.8 degree. To obtain an ensemble average over the stochastic processes, 300 simulations with different starting orientations of the molecule were averaged. On top of the overall rotational tumbling correlated or uncorrelated jumps of the spins around the equilibrium position of 15 Å were implemented by stochastic jumps of $\pm 3$ Å every 1 ns. As one can see in Figure 8.11, the blue curves representing the simulations are better fitted by the green fourth order solution then by the second order ones (red). For all theoretical curves, the correlation function is only integrated to the maximal mixing time $t_{\text{max}}$. Hence, beyond the Redfield-limit, the higher order simulations suggest that correlated motions have an influence on the buildup and might be detectable therein.
Figure 8.11: Two spin relaxation simulations for a linear two jump model optimized for a correlation time of $\tau_c = 100$ ns. An EPR system at a proton frequency of 95 GHZ was performed assuming a jump model with a statistical jump every 1 ns and jump distances of $r_1 = 12 \text{ Å}$ and $r_2 = 18 \text{ Å}$. The blue lines are the simulations. For longer mixing time the evolution of the density matrix can not longer be neglected resulting in a modulation of the build up. The black lines are the 2 spin Redfield solutions whereas the red lines are the second order solutions obtained by the exact integration of the correlation function. The green lines are the 4th expansion.
8.4.7 Discussion

The semi-classical relaxation theory by Redfield usually used to study NMR relaxation phenomena is a solution of the Liouville - von Neumann equation up to second order. Here, we extended the solution to a fourth order approximation using the cumulant expansion. The theoretical considerations show that the fourth order term is in principle sensitive to concerted motion and equal to zero in absence of concerted motion as already mentioned by Freed [59]. Furthermore, even in the presence of concerted motion the 4th order term is only unequal to zero if the rate constant of the process is faster than the rotational correlation time of the molecule studied.

Since NMR-related rate processes are in general slower than the rotational correlation time of the molecule, NMR experiments appear to be well described by the Redfield theory and an expansion to the fourth order approximation is not necessary. Assuming as an example a NOE transfer in NMR, which is caused by a spin flip due to the random fluctuating magnetic field, the time scale of the process is slow. As the spin flip rate is given mainly by the relaxation time $T_1$, this process is in the order of seconds. Hence, the energy transfer rate is slow compared to the correlation time, which is in the order of ns. For a NOE experiment in the limit $\tau_{flip} \gg \tau_c$ the well known Redfield limit is correct, while the fourth order has to be taken into account for time scales $\tau_{flip} \leq \tau_c$. It is noteworthy to mention that this conclusion is possible only by a fourth order expansion of the Liouville - von Neuman equation presented here by a detailed interpretation of the 4th cumulant (8.9) as discussed above. Other argumentations usually put forward are wrong. This includes the notion that the integration of the correlation function (eq. 8.15 and8.16) resulting in the functional $G$ (eq. 8.23 and8.24) can be integrated up to infinity because the correlation falls apart fast. This approach is not correct because (i) it results in an infinite value for the spectral density function unless tricks established by Abragam and Goldmann are used (see Apendix A for more details). Both tricks are
only effective for a double integral over time and hence are limited to the second order approximation. In addition, (ii) the request of integration to infinity for the individual integrals within the fourth order term decouples evidently potential correlations and or interactions between functions to be integrated and concomittantly decouples any correlations of motions. Obviously, this assumption by itself results in a vanishing fourth order term. While the expansion of the relaxation theory to fourth order appears to be unnecessary for established NMR processes, scenarios of having a fast rate process in respect to the overal rotational correlation time of the system studied can be envisioned as shown both by simulations (Figure 8.11) and theoretical considerations (chapter 8.4.4 and Appendix G). Experimental evidence of such phenomena by EPR (electron paramagnetic resonance-spectroscopy) for example remain to be demonstrated.
Part IV

Application of exact NOEs (eNOE) to the model protein ubiquitin
9 Ubiquitin as a model system for the extraction of exact distances

As ubiquitin is used in all studies presented in the following, a short section is given about its function and structure. Ubiquitin (from latin ubique "everywhere") is found in almost all eukaryotic cells. It consists of 76 amino acids and has a molecular mass of 8.57 kDa [61]. The sequence and the secondary structure is shown in figure 9.1. The characterization of the function of ubiquitin was performed in the early 1980s and the nobel price for chemistry was awarded in 2004 "for the discovery of ubiquitin-mediated protein degradation" [62]. We used ubiquitin as a model protein, since a high resolution X-ray structure [56] as well as an NMR bundle [54] is available for comparison and many NMR measurements to describe dynamics have been applied to ubiquitin [63, 50, 64]. As we want to establish a new method, a known and well studied protein is useful to show the high accuracy of the method presented above, as we shall see in the following.
Figure 9.1: The upper part of the figure shows the secondary structure of ubiquitin. Below, the one letter amino acid code of the ubiquitin sequence is shown. The numbers in the upper part correspond to the amino acid listed in the sequence.

### 9.1 Sample preparation

Ubiquitin with the human sequence was expressed recombinantly in E. coli in the triple labelled medium from Silantes E. coli - OD2 CDN (²H > 95 %, ¹³C, ¹⁵N) and purified in H₂O, giving a MALDI-TOF mass of 9500.2 Da. The sample was measured in H₂O with 3 % of D₂O at a concentration of 4.3 mM and at a pH of 5.8 in a 20 mM K₂PO₄ buffer. The sample measured is nearly perdeuterated with the exception of the exchangeable backbone and side-chain amide protons.
10 Exact distances and internal dynamics of perdeuterated ubiquitin from NOE buildups

10.1 Introduction

Following the theoretical considerations in chapter 5, the experimental set ups and the discussion of potential problems for the translation of NOEs to accurate averaged distances presented in part III, it is the aim of this chapter to get experimental support for the conclusions drawn and apply the the approach of eNOEs to a well known system - the protein ubiquitin. The measurements of NOE buildups in perdeuterated human ubiquitin results in distances between two amide-protons up to 5 Å that shown a random error of $\Delta r = 0.07$ Å when compared to the X-ray structure [56]. This error is smaller than the corresponding pairwise rmsd of 0.20 Å using X-ray [56] methods to determine the distances. Comparison of exact eNOE-derived distances with the X-ray structure furthermore reveals dynamical informations, showing distinct motions in $\beta-$sheets, $\alpha-$helices or loops.
10.2 Methods

The experiments for this chapter were performed by T. Segawa. Data analysis was first done by B. Vögeli and revised for this thesis by D. Leitz.

All experiments were performed on a Bruker 700 MHz spectrometer equipped with a triple resonance cryoprobe at 284 K. The resonances were assigned using a TROSY-HNCA and a 3D $^{15}$N-resolved HMQC-NOESY experiment [25]. The 3D $^{15}$N-resolved HMQC-NOESY experiment (see Figure 7.1) was also used to measure NOE buildup rate constants. Spectra were acquired with the mixing times $\tau_m = 0.03$ s, 0.06 s, 0.09 s and 0.20 s. The global correlation time $\tau_c$ was calculated from the ratio $T_2/T_1$, where the $T_2$ time was back-calculated using $T_1$ and $T_{1\rho}$ measurements. The relaxation delays for the $T_1$ measurements were $\tau_m = 0.032$ s, 0.128 s, 0.192 s and 0.256 s while for the $T_{1\rho}$ measurements relaxation delays of $\tau_m = 0.03$ s, 0.06 s and 0.09 s were used. As a rotational model, isotropic overall tumbling was assumed. This simplification is justified because the diffusion anisotropy is 1.17 [49] and the expected experimental error is maximally 5 % for an individual rate constant. All spectra were processed with the program PROSA [65] and analyzed with the program XEASY [66].

10.3 Comparison between exact distances extracted from NOE and distances obtained from the X-ray structure

From the NOE buildups, we extracted exact distances from the normalized cross-peak intensities following eq. (5.7). Figure 10.1 is a correlation plot between experimentally derived effective distances from NOE buildups versus distances obtained from the X-ray

95
structure [56]. As one can clearly see, for distances up to 5 Å a very good correlation is achieved. For distances larger than 5 Å, only obtained for distances within loops or across β− strands, an underestimation of the distance is observed. But the overall correlation is still good, resulting in a slope between the two data sets compared of 0.95 (see table (10.1)). In detail, the effective distances between spins located in consecutive β−strands and non-consecutive α− helix yield the best correlation with a slope of 0.98 and 0.99, respectively. But the deviation of these distances is quit high, resulting in a low correlation coefficient. The best correlation with the highest correlation coefficient is therefore given for distances between β−strands. The effective distances between spins located in loops are underestimated, indicated by a slope of 0.93. This may be attributed in part due to conformational artifacts but also to internal motion, reducing the cross-relaxation rate constant as indicated by eq. (5.3). A better correlation is obtained by taking only the distances below the 5 Å limit into account, as for them a higher accuracy due to a better signal to noise ratio is achieved (table (10.1)). All slopes get closer to one beside the slope of the effective distances of residues located in consecutive β−strands and consecutive α−helix which stays the same. This is attributed to the fact, that consecutive residues in the sequence are below the 5 Å limit and the cut-off has no effect on them. A pairwise rmsd of 0.20 Å between the effective distances and those obtained from the x-ray structure at 1.8 Å resolution results into an averaged error of 5%. As a conclusion, the extraction of effective distances from NOE builds up is possible with high accuracy which is attributed to the dependancy of the NOE on the inverse sixth power of the distance.
Table 10.1: Slopes $s$ and Pearson’s correlation coefficient $r$ between effective experimental $H^N - H^N$ distances and those extracted from the X-ray structure for ubiquitin. Spin diffusion corrections are already taken into account.

<table>
<thead>
<tr>
<th>atom pair</th>
<th>consecutive $\beta$-strand$^2$</th>
<th>between $\beta$-strands</th>
<th>consecutive $\alpha$-helix</th>
<th>non-cons. $\alpha$-helix</th>
<th>loops$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s$</td>
<td>0.95</td>
<td>0.98</td>
<td>0.94</td>
<td>1.04</td>
<td>0.99</td>
</tr>
<tr>
<td>$r$</td>
<td>0.89</td>
<td>0.47</td>
<td>0.96</td>
<td>0.51</td>
<td>0.17</td>
</tr>
<tr>
<td>$s$</td>
<td>1.00</td>
<td>0.98</td>
<td>1.00</td>
<td>1.04</td>
<td>1.01</td>
</tr>
<tr>
<td>$r$</td>
<td>0.97</td>
<td>0.47</td>
<td>0.99</td>
<td>0.51</td>
<td>0.75</td>
</tr>
</tbody>
</table>

1 distances are obtained from the X-ray structure 1UBQ [56]; first two rows: all distances are taken into account; last two rows: only distances < 5 Å are counted.

2 all spin pairs where at least one spin is located in a loop.

Figure 10.1: Correlation plot between $H^N - H^N$ distances derived from the X-ray structure, where the protons are placed at ideal positions and the distances derived from the experimental cross-relaxation rate constants. The distances are sorted with respect to secondary structure elements. Red triangles and squares represent distances between and within $\beta$-strands, respectively, while purple triangles label distances across $\alpha$-helices and purple squares distances between consecutive $\alpha$-helices. Blue circles finally label distances between protons in loops. The black line indicates a perfect correlation of one.
10.4 Order parameters for ubiquitin and the Internal Motion encoded in them

The exact effective distances measured can now be used to extract informations on internal motions. A measure of the amount of motion in the system is given by the order parameter defined in eq. (5.4). Comparing the cross-relaxation rate constant of a rigid structure with the one derived from the NOE measurements gives a measure for the amount of motion present in the particular system studied. Assuming the X-ray structure 1UBQ [56] to be rigid, one can calculate pairwise order parameters for each distance available. One has to keep in mind, that the X-ray structure is not a true ensemble averaged conformation and hence the motional network map shown in figure 10.2 is only meant as a sketch of the motions present. To avoid therefore an over-intepretation of these data, not individual order parameters will be discussed in the following, but averaged ones over individual secondary structure elements. As mention above in chapter 5, the order parameter can be approximately decomposed into an angular component \((S_{\text{fast}}^{\text{HH}})_{\text{ang}}^2 \leq 1\) and a radial \((S_{\text{fast}}^{\text{HH}})_{\text{rad}}^2 \geq 1\) one. Order parameters smaller than one can therefore be assigned to (mainly) angular motion, while order parameters larger then one can be assigned to distance fluctuations. We present in Table 10.2 averaged order parameters derived from a linear regression between different secondary structure elements of the cross-relaxation rate constants back calculated from the X-ray structure and those extracted from the NOE buildups. These averaged order parameters are compared to those obtained for a different sample (e.g. GB3) but using the same method as described above. Before these two sets of order parameters are compared, we focus on the ones derived for ubiquitin. The overall order parameter for \(^1\text{H}N - \(^1\text{H}N\) distances in ubiquitin is 0.83 for a temperature of \(T = 284\ \text{K}\) which is close to the value of 0.79 for \(^{15}\text{N} - \(^1\text{H}\) vectors measured at \(T = 288\ \text{K}\) [50]. This two values are not exactly com-
Table 10.2: Slopes $s$ and Pearson’s correlation coefficient $r$ between experimental cross-relaxation rate constants and rigid ones back-calculated from the X-ray structure. The averaged values were derived by linear regression of the predicted cross-relaxation rate constants from the X-ray structure and those measured experimentally.

<table>
<thead>
<tr>
<th>Sample</th>
<th>All</th>
<th>Cons. $\beta$-strand</th>
<th>between $\beta$-strand</th>
<th>Cons. $\alpha$-helix</th>
<th>Non-cons. $\alpha$-helix</th>
<th>loops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubq</td>
<td>s</td>
<td>0.830</td>
<td>1.130</td>
<td>0.793</td>
<td>0.845</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>0.983</td>
<td>0.627</td>
<td>0.981</td>
<td>0.591</td>
<td>0.747</td>
</tr>
<tr>
<td>GB3*</td>
<td>s</td>
<td>0.870</td>
<td>1.103</td>
<td>0.978</td>
<td>0.906</td>
<td>0.918</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>0.973</td>
<td>—</td>
<td>0.945</td>
<td>0.639</td>
<td>—</td>
</tr>
</tbody>
</table>

* for GB3 a HMQC-NOESY was measured; for the calculation of the order parameter an anisotropic tumbling was used (see [55]).

parable but as explained in chapter 8.2, the motion of the $^{15}\text{N} - ^1\text{H}$ bond has a direct influence on the $^1\text{H}^\text{N} - ^1\text{H}^\text{N}$ distance. Averaged order parameters for spins located in consecutive $\beta$-strands have a slope of 1.13 which indicates a rigid angular behavior but some distance fluctuations. All other averaged order parameters are smaller than one indicating mostly angular flexibility. Compared with the order parameters derived for GB3, using the same pulse sequence as in this paper [55], a similar trend is observed (see Table 10.2). Only the values for distances between $\beta$-strands seems to be more conserved in GB3, indicated by a slope closer to one, as compared to ubiquitin. Overall, the slopes of the order parameters in different secondary structure elements suggest, that (i) there is motion present and (ii) the motion between the different secondary structures and loops appear to be distinct from each other and (iii) the motion within a structural type of element appears to be conserved as both GB3 and ubiquitin show similar trends of the slope. However, the motion present in the system is of very different nature and an exact interpretation of its origin is complicated, as a "model-free" approach was used for the extraction.
Figure 10.2: Order parameters, extracted from effective distances, depicted on the ribbon representation of ubiquitin. \((S_{HH})^2\) order parameters between consecutive spins in the sequence are colored within the secondary structure element, while non-consecutive residues are marked by a colored stick. The color code is: yellow < 0.7; red 0.7 to 0.9; purple 0.9 to 1.1; blue > 1.1 and white if no value is available. The \(^1\)H-\(^{15}\)N bonds are represented by black sticks. The two graphs were prepared using the program MolMol [67].

### 10.5 Conclusion

The aim of this chapter was to show, that the conversion of Nuclear Overhauser Effects (NOEs) into relatively precise distances for detailed structural studies of proteins is possible with high accuracy. To this purpose it is demonstrated that the measurement
of NOE buildups between amide protons in perdeuterated human ubiquitin enables the
determination of $^1\text{H}_N^1\text{H}_N$ distances up to 5 Å with high accuracy and precision. These
NOE-derived distances have an experimental random error of 0.07 Å, which is smaller
than the pairwise rmsd (root mean square deviation) of 0.24 Å obtained with corres-
ponding distances extracted from an X-ray structure (pdb codes: 1UBQ). Finally, order
parameters were calculated using the ratio between the experimentally derived exact
distances and those obtained from the X-ray structure. Matching these order parameters
to secondary structure elements allows predictions on the kind of motion present. Com-
parison with order parameters obtained for GB3 gives a good correlation which leads to
the supposition, that motion seems to be conserved in secondary structure elements.
11 Temperature-dependence of $^{1}\text{H}_{N} - ^{1}\text{H}_{N}$ distances in ubiquitin as studied by exact measurements of NOEs

11.1 Introduction

Although NMR relaxation phenomena provide a great deal of insight into local molecular dynamics, the dynamic picture of biomacromolecules is still largely incomplete, since no method is available to detect motions between atoms that are far apart in the sequence. The investigations shown in chapter 9 indicate that extraction of exact effective distances from NOE rate constants may allow the determination of such motions. Using this approach, we measured exact effective distances between amide protons in $^{15}\text{N,}^{13}\text{C,}^{2}\text{H}$-labelled ubiquitin at three temperatures (284 K, 307 K, 326 K), while those at 284 K have already been discussed in chapter 9. The comparison between the three data sets reveals that, while the correlation time corrected cross-relaxation rate constants increase by 18 % from 284 K to 307 K, those at 326 K increase by 32 % as compared to those at 284 K. Since theoretical considerations indicate that the NOE is largely insensitive to fast motion (see chapter 8.2), as long as the local order parameter (e.g. $S_{NH}^2$) is larger than 0.5 the effective distance can be calculated from the NOE using its $\langle r^{-6}\rangle$ dependency. Doing so, the average NOE increase translate into effective distance changes of 2.4 % and 4.0 % in the temperature regimes measured. The data presented demon-
strate that the determination of quantitative NOEs is a powerful tool to extract small structural and dynamical changes in a biomolecule.

11.2 Methods

11.2.1 NMR and data analysis

All experiments were performed on a Bruker 700 MHz spectrometer equipped with a triple resonance cryoprobe at 284 K, 307 K or 326 K. The resonances were assigned using a 3D ct-TROSY-HNCA and a 3D $^{15}$N-resolved HMQC-NOESY experiment [25]. The 3D $^{15}$N-resolved HMQC-NOESY experiment [36] was also used to measure NOE buildup rate constants. Spectra were acquired with the mixing times $\tau_m = 0.03$ s, 0.06 s, 0.09 s and 0.20 s at 284 K, $\tau_m = 0.03$ s, 0.06 s, 0.09 s and 0.12 s at 307 K, and $\tau_m = 0.06$ s, 0.09 s, 0.12 s and 0.15 s at 326 K taking into account the decreasing rotational correlation time of ubiquitin with increasing temperature. The measurements at 284 K have been published previously [36]. Furthermore, we recorded a $^{13}$C, $^1$H]-HSQC with a constant time of 54 ms with a $^{13}$C, $^{15}$N labelled ubiquitin sample [25]. $T_1$, $T_{1\rho}$ - $^{15}$N relaxation measurements were performed with relaxation delays of $\tau_m = 0.032$ s, 0.128 s, 192 s and 256 s for the $T_1$ measurements and $\tau_m = 0.03$ s, 0.06 s, 0.09 s for the $T_{1\rho}$ measurements. To elucidate cross-correlated relaxation delays TROSY/Anti-TROSY spectra were measured with a relaxation delay of $\tau_m = 0.06$ s. All data were processed with the programs Prosa [65] or nmrPipe [47] and analyzed with the programs XEASY [66] or nmrDraw [47], respectively. For the extraction of the cross-relaxation rate constants, the same procedure as described in reference [36] was followed including the time-dependent spin diffusion analysis. The translation of NOEs into distances requires knowledge of the overall rotational correlation time of the
molecule $\tau_c$ (see chapter 5 or eq. (5.17)).

11.2.2 CD spectroscopy

CD spectra were measured on a JASCO J-815 at 20 $\mu$M ubiquitin in a 0.1 cm path-length cuvette in NMR sample buffer from 275 K to 345 K in 5 K steps with an equilibration time of 1 h for each temperature measurement. Each spectra is the average of three measurements for which the scan speed was 10 nm/min, slit width at 1.5 nm and scan range from 200 nm to 260 nm.

11.2.3 MD simulations

This chapter was taken from [36] and kindly provided by W. van Gunsteren The set of structures is a Boltzmann ensemble obtained from a 20 ns MD simulation of ubiquitin in water at a constant temperature of 284K and a pressure of 1 atm performed with the GROMOS software [68]. All Arg, Cys and His residues and the amino terminus were protonated (ph=5.6). The GROMOS 53A6 force field [69] was used in conjunction with SPC water [70]. The initial structure was 1 UBQ. Truncated octahexal periodic boundary conditions were applied. The box contained 7331 water molecules and one Cl$^-$ Ion to balance the protein change. All protein bond lengths and the geometry of the water molecules were constrained with a precession of $10^{-4}$ [71]. The MD integration time step was 2 fs. A triple-range scheme was used to evaluate the non-bonded interactions. The interactions between charge groups at a distance between 0.8 nm and 1.4 nm were only updated every 5 steps, while the electrostatic interactions beyond 1.4 nm were approximated using a reaction field with $\epsilon = 66$. The "center of mass" motion was removed every 100 ps. The temperature and pressure relaxation times were $\tau_\sigma = 0.1$ ps.
and $\tau_p = 0.5$ ps respectively [72]. The compressibility used for the pressure coupling was $45.75 \cdot 10^{-5} \text{ mol} \cdot \text{nm}^2 / \text{kJ}$. After an equilibration period, 100 trajectory structures at 200 ps intervals were saved for analysis. The RMSD of the trajectory structures with respect to the initial (X-ray) calculated for the C$^\alpha$ atoms of residue 2-73 was about 0.15 nm. Only Eq. 16 was used for this MD ensemble, as the 20 ns simulation does not cover slow protein motion.

11.2.4 Temperature normalized cross-relaxation rate constants

For a better analysis of the increase in motion, a reduced NOE rate constant termed NOE*, using as a reference the NOEs derived at 284 K, is used:

$$\sigma^* = \sigma \cdot \frac{\tau_c (284 \text{K})}{\tau_c (307 \text{K or 326 K})}$$

(11.1)

11.3 Temperature-dependent $^1\text{H}_N - ^1\text{H}_N$ NOE rate constants of ubiquitin

The NOE rate constants between pairs of $^1\text{H}_N$ spins in human ubiquitin have been extracted from NOE buildups at three temperatures (284 K, 307 K, and 326 K) using the $^{15}\text{N}$-resolved HMQC-NOESY experiment (see Figure 7.1). Due to the magnetization pathway symmetry of a spin pair two buildups can be evaluated. These two buildups correspond to the magnetization transfers from $^1\text{H}_N$ of residue x to $^1\text{H}_N$ of residues y and vice versa. The differences between the two buildups arise from imbalances during the pulse sequences stemming from the WATERGATE element [40] as well as small contributions from experimental uncertainties such as intensity read-out (see chapter 7.1). The data fits very well to theoretical curves and the NOE rate constants can be extrac-
ted with confidence. This finding is supported by a repetition of the experiment at 307 K, which yielded very similar NOE rate constants with an average deviation between the two measurements of 3%. At 284 K a total of 140 NOE rate constants could be evaluated with 78 from both pathways and 62 from only one pathway. Similarly, at 307 K a total of 94 NOE rate constants were determined with 55 from both pathways, and 39 from one pathway only. At 326 K a smaller number of NOEs could be determined due to the smaller rotational correlation time that results in a decrease of cross peak intensities (which was partially countered by longer mixing times). Furthermore several resonances were lost due to fast exchange with water and the presence of motion. Thus, only 79 NOEs in total, 47 for both pathways and 32 with one pathway, could be determined.

Inspection of Figure 7.3 indicates a decrease of the NOE buildups with temperature. This finding is only in part due to the decrease of the rotational correlation time $\tau_c$, which changes by more than a factor of 2 between 284 K ($\tau_c = 7.72$ ns) and 326 K ($\tau_c = 3.02$ ns) (Figure 8.2). Comparison of representative, $\tau_c$-corrected NOE buildups between residues 4 and 67 (Figure 11.1) with the uncorrected one (Figure 7.3) shows that the buildup is smaller at higher temperatures than expected for a protein with temperature-independent conformation and dynamics. Similar findings are observed throughout the entire protein structure, including the $\beta$-sheets as well as the $\alpha$-helix (Figure 11.2). These differences are attributed to a temperature-dependent change of motion and/or the average distance between the two spins involved.
Figure 11.1: $\tau_c$ - corrected normalized intensities of the representative cross peaks $^1H_N^4$ - $^1H_N^{67}$ versus the mixing time for the three temperatures 284 K (blue), 307 K (green) and 326 K (red). Intensities are taken from the measurements and scaled by the ratio of the two correlation times (in accordance to eq. (11.1)).
11.4 Comparison between measured NOEs and those calculated from various structural models

Table 11.1: Slopes $s$ and Pearson’s correlation coefficient $r$ between experimental and predicted cross-relaxation rate constants $\sigma$ for ubiquitin. For each temperature all individually extracted distances were taken into account.

<table>
<thead>
<tr>
<th>Model</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>284 K</td>
</tr>
<tr>
<td>X-ray structure (pdb code 1Ubq) [56]</td>
<td>$s$</td>
</tr>
<tr>
<td></td>
<td>0.820</td>
</tr>
<tr>
<td>NMR pdb structure 1D3Z: HN distance set to $d_{HN} = 0.98$ Å [57]</td>
<td>0.803</td>
</tr>
<tr>
<td>NMR pdb structure 1D3Z: HN distance set to $d_{HN} = 1.01$ Å [57]</td>
<td>0.814</td>
</tr>
<tr>
<td>NMR bundle 2k39 (average distance *) [54]</td>
<td>0.978</td>
</tr>
<tr>
<td>GROMOS simulation (average distance *)</td>
<td>1.769</td>
</tr>
</tbody>
</table>

* The NMR bundle as well as GROMOS distances were linearly averaged.

Before the observed temperature-induced changes of the $\tau_c$ - corrected NOEs are discussed in detail, the data are first compared with corresponding values calculated from representative structures similar to chapter 9. However, here we compare the eNOE derived distances not only with the X-ray structure (pdb code 1Ubq), but also with the NMR structure (1D3Z), where the $^{15}$N - $^1$H bonds are either fixed at 0.98 Å or 1.01 Å [57], with the bundle 2k39 derived from RDCs and MD simulations [54], and with MD simulation using the software package GROMOS [36]. Table 11.1 shows the slope $s$ providing an "averaged" experimental order parameter (eq. (5.4)) as well as
the correlation coefficient $r$ for a linear regression (experimental versus predicted $\sigma$). Concentrating on the comparison with the experimental data set at 284 K, it should be noted that compared to [36] this data set is an improvement as the $\tau_c$ was determined more accurately. The X-ray structure fits well with this data set as previously documented [36] (although a less accurate $\tau_c$ was used therein). The NMR bundle 2k39 fits even better to the NOE data set for local interactions (i.e. intra and sequential NOEs), while across $\beta-$strands it fits rather poor when compared to the X-ray structure (Table S1). Overall, the GROMOS simulation compares poorly with the experimental NOEs, having apparently too much internal motion (Table 11.1). However, while for the X-ray structure as well as the NMR structure and the Griesinger bundle the correlation gets worse with increasing temperature (although the latter is based on experimental values measured at 298 K), the GROMOS MD simulation gets better. Most importantly for the context of interest is that at 326 K the cross-relaxation rate constants fits poorly with all the structures and structural models listed as examplified by the slope $s$ (Table 11.1). Note that the apparent small improvement of the Pearson’s correlation coefficient $r$ is not interpreted here because it may be due to the different extent of the data sets used). The slopes $s$, which provide an “averaged” order parameter are ranging between 0.69 and 1.46 indicating an increase of internal motion of ubiquitin for high temperatures.

### 11.5 Temperature-dependent $^1H_N - ^1H_N$ distance in ubiquitin

The comparison between NOE rate constants at the three temperatures of interest and corresponding calculated values from various structural models (Table 11.1) documented above (Figures 11.2) strongly indicate a structural and/or dynamical change of ubiquitin with increasing temperature. To evaluate these temperature-dependent changes
more quantitatively, the NOE rate constants have been translated into effective distances \( (r_{\text{eff}}) \) using eq. (5.10), since in accordance to the findings above, the \(^1\text{H}_N - ^1\text{H}_N\) NOEs appear to be insensitive to fast motions. This approach enables a detailed explanation of the temperature-dependent structural and dynamical changes of ubiquitin. As illustrated in Figure 11.3 the distances measured at 284 K are in excellent agreement with the X-ray structure 1Ubq irrespective of whether the distances extracted are of sequential or long-range nature, or whether they are located in the \(\alpha\)-helix, \(\beta\)-strands or loop regions. This observation is in line with our previous comparison although at that time the rotational correlation time \(\tau_c\) was not accurately determined [36, 55]. Overall, for the temperature change from 284 K to 307 K an average decrease of the \(\tau_c\)-corrected NOE rate constant in the order of 18 \% and a concomitant average increase of the distance by 2.4 \% is measurable throughout the protein structure, including the \(\alpha\)-helical as well as \(\beta\)-sheet secondary structures (Figure 11.2).

Another increase of 19 K to 326 K yielded changes in the same order indicating structural and/or dynamical changes. An average decrease of the \(\tau_c\)-corrected NOE rate constant in the order of 14 \% and concomitantly an average increase of the distance by 1.6 \% is measured. Again, these distance increases are observed throughout the protein structure (Figure 11.3). Comparing the dataset at 284 K and 326 K the average decrease of the NOE rate constants is 32 \%, translating into a distance change of 4.0 \%.
Figure 11.2: Relative temperature-dependent changes of NOE rate constants $\sigma^*$. The blue bars show the $\tau_c$-corrected changes in NOE buildup rate constants in % between 284 K and 307 K, while the red bars show the changes between 284 K and 326 K, respectively. The $^1$H$_N$ involved are labelled with their corresponding residue number.
Figure 11.3: NOE - derived effective distances $r_{eff}$ between two $^1H_N$ atoms in ubiquitin measured for three temperatures (blue 284 K, green 307 K, red 326 K). The black circles show extracted distances from the X-ray structure (1Ubq). Error bars shown are based on the $\sigma$ determined individually from the two pathways. If only one pathway could be determined, no error bar is shown. The $^1H_N$ involved are labelled with their corresponding residue number.
A detailed inspection of the temperature-dependence of the distance within the $\alpha$-helix further reveals that the average distance increase in the temperature regime 307 K - 326 K is more prominent towards the C-terminus of the $\alpha$-helix indicating a breathing of the helix from its C-terminus (Figure 11.3 and Table 11.2).

To convert the distance increase into a dynamic picture, eq. (5.4) may be used because it defines an order parameter which, dependent on its type of motion, can be smaller or larger than 1 and 1 if rigid (see text in chapter 5). Using eq. (5.4) with the reduced cross-relaxation rate constant $\sigma^*$ under the assumption that the structure of ubiquitin at 284 K represents a compact conformation on top of which the protein breathes or partially unfolds. Following this assumption and using the reduced cross-relaxation rate constant $\sigma^*$, one gets from eq. (5.4) a $\Delta S^2$ for the two temperature regimes 284 K – 307 K and 284 K – 326 K. As highlighted for the $\beta$ - sheet ($\beta_1 - \beta_5$) and the $\alpha$ - helix in Figure 11.4, the dynamics is changing moderately between 284 K and 307 K and increases further in a quasi linear manner with temperature. Hence, when compared with the data at 284 K a pronounced increase in motion is observed at 326 K (Figure 11.4). While the helix seems to be mostly temperature insensitive at its N-terminal side, larger changes in the cross-relaxation rate constant as well as in the distance can be observed towards its C-terminal end. The changes in NOE and distance across beta sheets are also pronounced (Figure 11.3,11.4B). It appears that for the loops overall larger changes are observed indicating more increase in flexibility than in secondary structural elements. This changes include in particular the termini of the secondary structural elements. It is not surprising that the increase of the temperature results in a less well compact structure, since towards the melting temperature the structure of ubiquitin should go towards a random coil conformation because of the unfolding process. Overall, the increase of motion between 284 K and 326 K as determined by the $\Delta S^2$ is only little but significant.
Figure 11.4: Temperature-dependent changes of the NOE-derived order parameters in ubiquitin.

(A) On the left hand side the relative $\tau_c$-corrected change from 284 K to 307 K within the $\alpha$-helix is shown, whereas on the right hand side the change from 284 K to 326 K is shown.

(B) On top changes of the NOE-derived order parameter between 284 K and 307 K within and between the first and last $\beta$-strand are shown, while the bottom representation indicates the changes of the order parameter between 284 K and 326 K. The backbone of the secondary structures is drawn by black lines and the residues are labelled according to the amino acid sequence position.

The colour code is as follows: grey: $-40\% < \Delta S^2 < -20\%$, violet: $-20\% < \Delta S^2 < 0\%$, blue: $0\% < \Delta S^2 < 20\%$, yellow: $20\% < \Delta S^2 < 40\%$, red: $\Delta S^2 > 40\%$. 
Table 11.2: Temperature-dependent changes of $^1$H$_N$-$^1$H$_N$ distances within the $\alpha$-helix of ubiquitin derived from NOE measurements.

A: The distances were extracted from the X-ray structure (pdb code 1Ubq [56]).

### 11.6 Comparison of the $^1$H$_N$ - $^1$H$_N$ NOE rate constants to other temperature-sensitive probes

*The CD spectra were recorded by J. Greenwald*

The presented study on temperature-sensitive $^1$H$_N$ - $^1$H$_N$ NOE rate constants is compared with corresponding measurements of $^1$H$_N$, $^{13}$C$^\alpha$ and $^{13}$C$^\beta$ chemical shifts, scalar couplings across hydrogen bonds [63] and circular dichroism. Figure 11.5 shows representative correlation plots between the $\tau_c$-corrected NOE - rate constant changes and the corresponding $^1$H$_N$ chemical shift changes with temperature. Although there is a
correlation present it is of complex nature. This is expected since the chemical shift depends on the electronic environment of its spin while the NOE shows mostly a distance dependency. Although still of complex nature, the $^{13}\text{C}^{\alpha}$ and $^{13}\text{C}^{\beta}$ shifts depend mainly on backbone angles and hence their deviation from random coil shifts can be used to investigate secondary structures and changes thereof. In order to correlate the NOE changes with the carbon shift changes, constant-time HSQC [25] spectra at the three different temperatures (284 K, 307 K and 326 K) with $^{15}\text{N}$, $^{13}\text{C}$-labelled ubiquitin were recorded. To compensate for temperature induced offsets in the chemical shift reference, we calculated the difference of the $^{13}\text{C}^{\alpha}$ and $^{13}\text{C}^{\beta}$ shifts [73] depicted in Figure 11.6. This difference is plotted versus the corresponding NOE ($\delta^{^{13}\text{C}^{\alpha}}_{(i)} - \delta^{^{13}\text{C}^{\beta}}_{(i)}$ is plotted versus $\sigma_{(i,j)}$). Overall, the carbon chemical shifts change with increased temperature towards the value derived for “random coil” configurations of the corresponding amino acid residue. Following the discussions above (Figure 11.2-11.4), the chemical shift changes of the C-terminus of the $\alpha$–helix (residues 28 - 37) and the end of the $\beta$–strand 5 close to the C-terminus (residues 69 and 70) are of special interest. While in the $\alpha$–helix only little chemical shift changes are observed at the center, more pronounced changes at its C-terminus are observed. Similarly, temperature-induced chemical shift changes are observed for the $\beta$–strand 5 with increased changes towards the C-terminus. As highlighted by Figure 11.6, analogous trends were found by the NOE measurements. Particular noteworthy is that the chemical shift difference as well as the NOEs appear to change proportionally to each other with the temperature change for most examples shown (Figure 11.6). However, the temperature dependence of the NOEs appears to be much more sensitive than for the carbon shifts. This finding is attributed to the proportionality between the deviation of carbon shifts from random coil and secondary structure content [74], while the NOE shows a power to six dependency on the distance. An even more impressive correlation between NOEs and the scalar couplings across
hydrogen bonds [63] is observed attributed to the fact that both measurements are strongly dependent on distance changes (Figure 11.7). The only outlier is Residue 42/70 for which the scalar coupling behaves untypical. Albeit the good overall correlation between NOE and scalar-couplings, an estimation of the change of the hydrogen bond distance based on a statistical relationship between scalar coupling and hydrogen bond length (0.03 Å) [63] appears to be smaller than the effective distance increase between two corresponding amide protons as measured by NOEs (0.20 Å for $\Delta r_{(326 K - 284 K)}$).

The nature of this apparent discrepancy is not known but may be attributed to the not entirely understood relationship between scalar coupling and hydrogen bond length. Finally, for an independent measurement of the temperature-sensitivity of the secondary structure in ubiquitin we turned to circular dichroism (CD) spectroscopy. The high thermal denaturation temperature of ubiquitin makes complete thermodynamic analysis difficult to do, so the change in the CD signal with temperature was measured instead. We found that there was a reversible change in the intensity of the 223 nm band at the same temperatures used in the NMR measurements (Figure 11.8). The negative ellipticity in this region of the CD spectrum arises from $\alpha$-helix and to a lesser degree from $\beta$-sheet, and the shift towards less negative signal at higher temperature is indicative of a temperature-dependent decrease in the content of these secondary structural elements. Furthermore, the signal decrease of the negative ellipticity changes proportionally with the temperature as can be depicted from the inset of Figure 11.8. This observation is well in line with the NOE rate constants which also change linearly with temperature in the temperature regime measured.

In summary, all NMR probes as well as the CD measurements support the observation that with a temperature increase within the ambient temperature regime conformational and dynamical changes appear in ubiquitin.
Figure 11.5: Temperature-dependent $^1$H$_N$ chemical $\delta(^1H)$ shifts versus $\tau_c$—corrected NOE rate constants $\sigma^*$ measured at the temperatures 284 K, 307 K and 326 K. A complex correlation is observed as expected (see text). The values measured at 284 K are coloured blue, at 307 K green and at 326 K red, respectively. The $^1$H$_N$ involved are labelled with their corresponding residue number.
Figure 11.6: Correlation plots between differences in chemical shifts of $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ versus the corresponding $\tau_c$-corrected NOE $\sigma^*$ for 284 K (blue circles), 307 K (green circles) and 326 K (red circles). The black lines indicate the difference $\delta^{13}\text{C}^\alpha - \delta^{13}\text{C}^\beta$ in a “random coil” entity calculated from [75]. Glycines are not taken into account as no $^{13}\text{C}^\beta$ shift is present. No error bars are included in the figure because the error is two orders of magnitude smaller than a typical chemical shift difference. The error in determining the chemical shifts can be estimated by the ratio between the line width (full width at half maximum FWHM) and 2 times the Signal/Noise (S/N) ratio [76]. Typical values are FWHM = 20 Hz, $N = 80\,000$, $S_{\min} = 10^7$ resulting in an error of $5 \cdot 10^{-4}$ ppm. The $^1\text{H}_N$ involved are labelled with their corresponding residue number.
Figure 11.7: $^{3h}J_{NC'}$ scalar coupling constants across hydrogen bonds extracted from ref. [63] versus $\tau_c$ - corrected NOE rate constants $\sigma^*$ measured at the temperatures 284 K, 307 K and 326 K. The values measured at 284 K are coloured blue, at 307 K green and at 326 K red, respectively. The $^1$H$_N$ involved are labelled with their corresponding residue number.
Figure 11.8: CD spectra of ubiquitin at the three temperature used for this study (284K: blue, 307K: green, 326K: red). The inset depicts the temperature-dependence of the negative band at 223 nm that is characteristic of $\alpha$-helices.

11.7 Unfolding pathway of ubiquitin

While with low resolution techniques ubiquitin appears to be a classical example for a simple protein folding pathway (two state process [77, 78]) high resolution techniques show that the folding $\rightleftharpoons$ unfolding is more complex. For example, the temperature-dependent measurements of scalar couplings across hydrogen bonds indicate that around loop 1 (residues 8-11) a rearrangement of hydrogen bonds is observed in the temperat-
ure regime 298 K - 328 K [63]. Unfolding in urea indicates that a significant amount of the ubiquitin molecules are still hydrogen bonded in the segment including residues 2-18 ($\beta$-strands 1 and 2) at 8 M urea [79] which implies a large stability of this segment. Fourier-transform Infrared Spectroscopy indicates that the protein segment following the $\alpha$-helix unfolds first with temperature [80], which is in good agreement with the measured NOEs indicating an increase of motion towards the C-terminus of the $\alpha$-helix (Figure 11.4, Table 11.2). Similarly, pressure-induced unfolding of ubiquitin suggests that the $\alpha$-helix unfolds, starting with a displacement of the C-terminal end of the helix and the connected loop. The pressure induced unfolding also indicates that the flexibility of the C-terminus (i.e. residues 72-76) is extended which finds support by the NOE measurements at 326 K (Figure 11.3 and11.4) [81, 82].

The presented structural changes with increasing temperature derived from NOE measurements are not only in good agreement with other data but also give quantitative insights into the breathing of ubiquitin with increasing temperatures. This finding indicates that before ubiquitin unfolds it takes up the increasing thermal energy by partial opening of its structure by breathing.

11.8 Conclusion

The NOESY experiment comprises dynamical and structural information from the chemical shifts, scalar couplings, line width of cross peaks and most importantly NOEs. While most often NOEs were only used in a qualitative manner for structure determination [10] or elucidation of dynamics [83], we recently demonstrated that the determination of NOE rate constants in a protein results in precise effective distances [36]. One remaining problem was that the relationship between NOE and distance depends on the type of motion present. Here, we showed first that the NOE is insensitive to fast
motion as long as the local order parameter $S_{NH}^2 > 0.5$. Next, we demonstrated on the model-protein ubiquitin that the quantitative NOE rate constants can be used to highlight small conformational and dynamical changes of the protein backbone upon change of the temperature. The measurements indicate that at 326 K the protein ubiquitin appears to breathe more than at 284 K in agreement with molecular dynamics simulations on other systems [84]. Hence, quantification of NOEs appears to be an excellent probe for the characterization of both, the protein 3D structure as well as its motion.
12 The influence of Binding on the Distances Studied by Measurements of Ubiquitin/UBM2 complexes

12.1 Introduction

So far, the extraction of eNOEs yielded a detailed insight into dynamics and its increase with increasing temperature within the model protein ubiquitin. Here, it is the aim to apply the eNOE approach presented to reveal small structural and dynamical changes of ubiquitin upon binding of the protein partner UBM2, which binds in the micromolar range to ubiquitin.

12.2 Methods

A reference sample with no UBM2 was used for a unbound distance map of double labeled $^{13}$C - $^{15}$N ubiquitin at a concentration of 3 mM. The sample was measured in H$_2$O with 10 % of D$_2$O at a pH of 6 in a 25 mM NaPO$_4$ buffer at a temperature of 284 K. The correlation time was, due to the lower concentration and the different buffer with $\tau_c = 7.17$ ns somewhat lower than the triple-labeled sample used above (section 8.1). Two other samples were produced in the same buffer and measured under the same
conditions. One contained deuterated UBM2 at a concentration of 0.75 mM together with 2.5 mM of ubiquitin. Hence, ubiquitin is partially bound resulting in a higher correlation time of $\tau_c = 8.94$ ns. The third sample contains 4.3 mM UBM2 together with 1 mM ubiquitin. All ubiquitin molecules are bound causing a relaxation time of $\tau_c = 13.80$ ns. The correlation times were extracted with the procedure explained above (see chapter 7.2). One of the TROSY spectra recorded was used for assignment (see Figure 12.1).

Figure 12.1: TROSY spectra of ubiquitin for different concentration of UBM2. Blue is the reference sample of free double labeled ubiquitin. The green peaks represent the intermediate state with 2.3 mM ubiquitin and 1 mM UBM2. The red peaks are the extreme case with 0.75 mM ubiquitin and 2.5 mM UBM2. Binding of UBM2 to ubiquitin is evident from the chemical shift changes. Many cross-peaks disappear at the intermediate state, indicating slow conformational exchange of ubiquitin between the free and complex form.
12.3 Determination of NOE rate constants

In section 7.4 Figure 7.3 shows buildup curves for a triple-labeled sample were only $^1\text{H}_N$-$^1\text{H}_N$ interactions could be observed. The deviation of buildups for $A \rightarrow B$ and $B \rightarrow A$ was obvious but rarely significant. A complete other scenario occurs for the buildups measured with the double-labeled sample. The deviation between the two buildup curves for the same transfer in both directs differs significantly. In Figure 12.2, two representative buildups are shown for cross-relaxation involving side-cain atoms. As one can clearly see, the two buildup curves have a big discrepancy. Theoretically, the transfer should be symmetric for both pathways, as the cross-relaxation rate constant is symmetric under particle permutation. Hence, the deviation must be related to experimental errors which are not enlightened yet. As we used the NOESY-HXCQ element to avoid $^1\text{H}_\alpha$ suppression (see section 7.1), we will focus in the following on interactions, were both pathways are available, to get a comprehensive data analysis. Taking single pathways into account too would result in a large error as already mentioned and are not used for the determination of distances but might be used for a structure determination later on.
Figure 12.2: Experimental NOE buildup curves for pairs of $^1$H-$^1$H residues in pure ubiquitin (i.e. shown are buildups for A $\rightarrow$ B as well as B $\rightarrow$ A) for two representative examples. The x-axis shows the mixing time ([11.21 16.21 20.21 26.21] ms), whereas the normalized intensities are plotted on the y-axis. Experimentally measured values are indicated by red circles while the black lines illustrate the fit of the data using the exact two spin solution.

### 12.4 Binding pocket of UBM2

The spectrum above (Figure 12.1) shows that the extraction of the binding informations of ubiquitin and UBM2 might be difficult. Especially for the intermediate sample with low UBM2 concentration, many peaks disappear. Nevertheless, some conclusions can be extracted from the backbone TROSY. For high concentrations of UBM2, where all ubiquitin molecules are bound, the H$_N$ resonance of residue 9 appears while some H$_N$ resonances shift significantly (e.g. peaks of residue 8 and 10). In accordance to [85], one binding interface includes residue 8. Therefore, the H$_N$ of residue 9 is protected and fast exchange with D$_2$O is no longer possible. For the other binding interfaces (i.e. residues 44 and 70) the chemical shift differences are not so prominent. The NOE spectra might give further verifications of the binding process. For a first analysis of the adaptability of the method to binding studies, we will concentrate on the residues
involved in the binding process. UBM2 contains a hydrophobic pocket where residue 8 and 70 of ubiquitin are involved [85]. For this reason we focus on the environment of residue 8 to study the changes in $^1$H-$^1$H distances. In Figure 12.3A a correlation plot between the exact effective distances of spin pairs located near the binding pocket and the distances obtained from the X-ray structure [56] is shown. Distances obtained from the reference sample (only ubiquitin at 3 mM concentration) are indicated by blue circles, while distances obtained from the sample with the highest UBM2 concentration (1 mM ubiquitin and 4.3 mM UBM2) are shown in red. For the free ubiquitin a good correlation is present with a slope of a linear regression of 0.98. A slight underestimation of the distances can be observed for the bound state, indicated by a slope of 0.96. In Figure 12.3B a direct comparison between the exact effective distances for the two samples are shown. An overall trend towards smaller distances for the ubiquitin/UBM2 complex is present. Especially for interactions involving residue 10 a big change can be observed. A decrease in the $^1$H - $^1$H distances might be attributed to a decrease in motion. As the first loop of ubiquitin includes the residues 8 - 11 (see Figure 9.1) due to the binding of residue 8 to UBM2 the flexibility of this region might become limited and the effective distances modulated by this motion as a consequence become shorter. But as no data is available for residue 9 for the free ubiquitin sample due to the fast exchange explained above and a limited amount of useable cross-relaxation rate constants as both pathways are necessary, further investigations have to be performed for a comprehensive picture. However, as a starting point, the surrounding of the binding pocket in ubiquitin reveals that the binding process can be studied with the method presented in this thesis.
Figure 12.3: (A) Correlation plot between $\text{H}_N - \text{H}_N$ distances derived from the X-ray structure and those derived experimentally from the binding studies of pure ubiquitin (blue circles) and of ubiquitin with 4.3 mM UBM2 (red circles).

(B) Bar plot for the comparison of experimental distances for pure ubiquitin (blue bars) and the bounded form with 4.3 mM UBM2 (red bars). The labels beneath the x-axis show the involved spins $i$ and $j$, in accordance to their position in the sequence.
12.5 Conclusion

We analysed two NOESY spectra recorded at 284K for the binding study of ubiquitin with UBM2. As a reference, pure ubiquitin at a concentration of 3 mM was used in the same buffer as the sample containing 0.75 mM ubiquitin and 2.5 mM UBM2. The diagonal and cross-peaks were assigned and cross-relaxation rate constants were extracted for the binding pocket in the surroundings of residue 8, in accordance to the procedure explained in part III. Exact averaged distances could be obtained for $^1$H-$^1$H interactions, for which both pathways were available. The distances were compared to the X-ray structure of ubiquitin and to each other and an overall decrease in effective distance could be observed. This might be addressed to restrictions in fluctuations due to the binding. Again, the method presented in this thesis is applicable to binding studies too, albeit further analysis has to be performed to get a denser spin map.
Part V

Conclusion and Outlook
We presented a protocol for the determination of exact distances from NOE experiments. Although several problems have to be faced, the only issue one has to encounter for is the influence of spin diffusion. In particular, as fast internal motion has no significant influence, the conversion of cross-relaxation rate constants into distances has no angular but a simple inverse sixth root distance dependency. The theoretical considerations, including the protocol, were applied to the well studied model protein ubiquitin. Measuring the cross-relaxation rate constants at a temperature of 284 K demonstrates, that the method presented results in precise average distances. These distances were translated into a dynamical picture of secondary structural elements. To utilize the potential of motional informations stored in the cross-relaxation rate constants, further measurements of ubiquitin at two higher temperatures (e.g. 307 K and 326 K) were performed. An increase of the distances, and therewith the internal motion, could be measured. Finally, the method was applied to binding studies of ubiquitin with UBM2. Again, the method proved to be applicable to binding studies.

As the method is now established, it can be used for exact distance measurements in biological important proteins. Furthermore, if the data derived are good and cover the whole protein, an ensemble calculation, using the exact distances, can be performed. This method is currently developed in our group and will be tested on GB3. Once it is established it may be a powerful new tool in the field of structure determination because it may allow a comprehensive determination of both structure and motion in proteins.
Bibliography


Part VI

Appendix
A Detailed derivation of the master equation of relaxation

A.1 The Liouville - von Neumann equation and its formal Picard - Lindelöf solution

In the following we want to give a more detailed derivation of the the semi-classical relaxation theory following the description by [1], [31] and [59]. The state of the spin system can be described by a density matrix \( \sigma \in H \) (where \( H \) denotes a Hilbert space) whose evolution is described by the Liouville-von Neumann equation:

\[
\frac{d\sigma}{dt} = -\frac{i}{\hbar}[H, \sigma]
\]  

Towards calculating the evolution of the density matrix, the Hamiltonian \( H(t) \) is described by the sum of a static Hamiltonian \( H_0 \) and a perturbation Hamiltonian \( H^p(t) \) which is the Hamiltonian of the spin-lattice coupling. \( H^p(t) \) represents interactions between spin states and their modulation by stochastic rate processes. Next, the average over all identical molecules of the sample, which is also its time average for any given
molecule, is taken.

\[ \frac{d \langle \sigma \rangle}{dt} = -\frac{i}{\hbar} \langle [\mathcal{H}(t), \sigma] \rangle \]  \hspace{1cm} (A.2)

This may seem strange, since the concept of the density matrix includes already statistical average. That a further average is necessary stems from the fact that the operator \( \mathcal{H}^p(t) \) is a random function of time [1]. Hence, different representatives of a Gibbs ensemble have different history and ergo different density matrices \( \sigma \).

To isolate the effect of the spin-lattice coupling, one can use an interaction representation which incorporates the entire static Hamiltonian \( \mathcal{H}_0 \). It is conventional to define such a representation by

\[ \hat{Q} = e^{\frac{i}{\hbar} \mathcal{H}_0 t} \langle Q \rangle e^{-\frac{i}{\hbar} \mathcal{H}_0 t} \]  \hspace{1cm} (A.3)

with \( \hat{Q} : \mathcal{H} \rightarrow \mathcal{H} \) whereas the evolution equation for the density matrix in this representation is

\[ \frac{d \hat{\sigma}}{dt} = -\frac{i}{\hbar} \langle [\mathcal{H}^p(t), \hat{\sigma}] \rangle \]  \hspace{1cm} (A.4)

This last equation can be formally integrated

\[ \hat{\sigma}(t) = \hat{\sigma}(0) - \frac{i}{\hbar} \int_0^t \langle [\mathcal{H}^p(t'), \hat{\sigma}(t')] \rangle \, dt' \]  \hspace{1cm} (A.5)
which results after an iterative process of eq. (A.4) into eq. (A.5) in

\[
\dot{\sigma}(t) = \dot{\sigma}(0) - \frac{i}{\hbar} \int_0^t \langle [\mathcal{H}_P(t'), \dot{\sigma}(0)] \rangle \, dt' \\
- \frac{1}{\hbar^2} \int_0^t \int_0^{t'} \langle [\mathcal{H}_P(t'), [\mathcal{H}_P(t''), \dot{\sigma}(0)]] \rangle \, dt'' \, dt'
+ \frac{i}{\hbar^3} \int_0^t \int_0^{t'} \int_0^{t''} \langle [\mathcal{H}_P(t'), [\mathcal{H}_P(t''), [\mathcal{H}_P(t'''), \dot{\sigma}(0)]]] \rangle \, dt''' \, dt'' \, dt'
+ \frac{1}{\hbar^4} \int_0^t \int_0^{t'} \int_0^{t''} \int_0^{t'''} \langle [\mathcal{H}_P(t'), [\mathcal{H}_P(t''), [\mathcal{H}_P(t'''), [\mathcal{H}_P(t''''), \dot{\sigma}(0)]]]] \rangle \, dt'''' \, dt''' \, dt'' \, dt'
+ \ldots
\]

(A.6)

Since the spin-lattice coupling has a zero average, the first integral of eq. (A.6) vanishes by definition and the third integral does not contribute to longitudinal relaxation under a dipole-dipole Hamiltonian. As discussed in section 8.4, the second order term of eq. (A.6) describes the cross relaxation rate constants and no further terms of the expansion has to be treated for NRM studies. Thus, we take only the first non-vanishing term into consideration, the second order one. Hence, one finds

\[
\dot{\sigma}(t) \approx \dot{\sigma}(0) - \frac{1}{\hbar^2} \int_0^t \int_0^{t'} \langle [\mathcal{H}_P(t'), [\mathcal{H}_P(t''), \dot{\sigma}(0)]] \rangle \, dt'' \, dt'
\]

(A.7)

As a consequence of the finite lattice temperature, we must replace

\[
\dot{\sigma}(t) \rightarrow \bar{\dot{\sigma}}(t) - \dot{\sigma}_{eq} := \bar{\dot{\sigma}}
\]

(A.8)

where \( \dot{\sigma}_{eq} = 1 - \alpha \mathcal{H}_0 \) is the thermal-equilibrium form of the density matrix and \( \alpha \) is the inverse temperature \( \alpha = -\frac{\text{Tr}\{\dot{\sigma}_{eq} \mathcal{H}_0\}}{\text{Tr}\{\dot{\sigma}\}} \). We then obtain

\[
\bar{\dot{\sigma}}(t) = \bar{\sigma}(0) - \frac{1}{\hbar^2} \int_0^t \int_0^{t'} \langle [\mathcal{H}_P(t'), [\mathcal{H}_P(t''), \bar{\sigma}(0)]] \rangle \, dt'' \, dt'
\]

(A.9)
where $\langle \cdot \rangle$ describes the ensemble average. This is (almost) the master equation for the evolution of the density matrix under the effect of a random perturbation [1].

### A.2 Dipole - Dipole Hamiltonian

Towards a master equation of relaxation, a useful description of the Hamiltonian is needed and developed in the following. We will concentrate on pure dipole-dipole interactions between the spins. The classical interaction energy between two magnetic dipole moments $i$ and $j$ is given by

$$E = \frac{\mu_0}{4\pi} \frac{1}{r_{ij}^3} \left( \vec{\mu}_i \vec{\mu}_j - \frac{3}{r_{ij}^2} \left( \vec{\mu}_i \vec{r}_{ij} \right) \left( \vec{\mu}_j \vec{r}_{ij} \right) \right)$$  \hspace{1cm} (A.10)

where $\vec{r}_{ij}$ is the position vector between these two moments. Using the quantum-mechanical relation $\vec{\mu} = \gamma \hbar \vec{I}$ one obtains the dipole-dipole Hamiltonian between the two spins $I_i$ and $I_j$:

$$\mathcal{H}_{DD}^{(ij)} = \frac{\mu_0 \gamma_i \gamma_j \hbar^2}{4\pi} r_{ij}^3 \left( \vec{I}_i \cdot \vec{I}_j - \frac{3}{r_{ij}^2} \left( \vec{I}_i \vec{r}_{ij} \right) \left( \vec{I}_j \vec{r}_{ij} \right) \right) = \vec{I}_i A^{(ij)} \vec{I}_j$$  \hspace{1cm} (A.11)

where $A^{(ij)} = \begin{pmatrix} A_{xx} & A_{xy} & A_{xz} \\ A_{yx} & A_{yy} & A_{yz} \\ A_{zx} & A_{zy} & A_{zz} \end{pmatrix}$ is a Cartesian tensor described by a $3 \times 3$ matrix. In the principle axes system (PAS) of the Cartesian tensor, the tensor is diagonal and the distance vector is parallel to the $z$-axis ($\vec{r}_{ij} = \vec{e}_z$) one obtains $A^{(ij)} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -2 \end{pmatrix}$

Instead of using a Cartesian tensor representation, an irreducible representation is used taking the symmetry of the system into account. This is done
by using the spherical tensor notation as the system has a rotational symmetry with re-
spect to the $z$-axis. Following the description by Ernst [42], a general tensor of rank $k$

 cannot be expressed in terms of a symmetry-adapted basis that belongs to the representation

 $D_0, \ldots, D_k$ of the rotation group. For a second-rank spatial tensor in the PAS (where

 the symmetric part of the matrix representation is diagonal and the anti-symmetric one

 is fully described by $A_{xy}, A_{xz}$ and $A_{yz}$) one finds

$$
\rho_{00} = -\frac{1}{\sqrt{3}} (A_{xx} + A_{yy} + A_{zz})
$$

$$
\rho_{10} = -\frac{i}{\sqrt{2}} (A_{xy} - A_{yx})
$$

$$
\rho_{1,\pm 1} = -\frac{1}{2} (A_{xx} - A_{zz} \pm i (A_{xy} - A_{yz}))
$$

$$
\rho_{20} = \frac{1}{\sqrt{6}} (3A_{zz} - (A_{xx} + A_{yy} + A_{zz}))
$$

$$
\rho_{2,\pm 1} = \mp\frac{1}{2} (A_{xx} + A_{zz} \pm i (A_{xy} + A_{yz})) = 0
$$

$$
\rho_{2,\pm 2} = \frac{1}{2} (A_{xx} - A_{yy} \pm i (A_{xy} - A_{yx}))
$$

(A.12)

where the representations $\rho$ in our case of a pure dipole-dipole Hamiltonian are given

 by

$$
\rho_{00} = 0
$$

$$
\rho_{10} = 0
$$

$$
\rho_{1,\pm 1} = 0
$$

(A.13)

$$
\rho_{20} = -\sqrt{6} \frac{\mu_0}{4\pi} \frac{\gamma_i \gamma_j \hbar^2}{r_{ij}^3}
$$

(A.14)

$$
\rho_{2,\pm 1} = 0
$$

$$
\rho_{2,\pm 2} = 0
$$
Instead of using the PAS (where the tensor is symmetric), one sometimes uses three other values for characterizing the tensor, namely the isotropic average \( a = \text{Tr} \{ A \} = A_{xx} + A_{yy} + A_{zz} = 0 \), the anisotropy \( \delta : \delta = A_{zz} - \frac{a}{3} = -\frac{2 \mu_{0} \gamma_{i} \gamma_{j}}{4\pi} \frac{\hbar^{2}}{r_{ij}^{3}} \) and the asymmetry \( \eta = \frac{A_{yy} - A_{xx}}{\delta} = 0 \) as done in subsection 8.4.4.

To rewrite our Hamiltonian in the laboratory frame (where the observation takes place) as a sum of products of time-invariant spin operators \( T_{l,-m}^{(I,S)} \) and time-dependent space tensor components \( A_{l,m}^{(I,S)} \)

\[
\mathcal{H}_{DD}^{(I,S)} = \sum_{l=0}^{2} \sum_{m=-l}^{l} (-1)^{m} A_{l,m}^{(I,S)} T_{l,-m}^{(I,S)},
\]

one has to find the spherical tensor components \( (A_{l,m}^{(I,S)}) \) by back rotating the \( \rho \)'s of eq. (A.14) using rotation matrices with the three Euler angles \( \xi, \vartheta, \varphi \). The spin tensor operators \( T_{l,-m}^{(I,S)} \) are normally already given in spherical coordinates. So one ends up with

\[
A_{l,m} = \sum_{m'=-l}^{l} \rho_{l,m'} D_{m'm}^{l}(\xi, \vartheta, \varphi)
\]

Since \( \eta = 0 \iff A_{l,m} \) is axially symmetric, \( A_{l,m} \) is only dependent of \( \vartheta \) and \( \varphi \) and can be written as

\[
A_{l,m}(\Omega_{ij}) = A_{l,m}(\vartheta, \varphi) = \sqrt{\frac{6\pi}{2l+1}} \cdot \delta \cdot Y_{l,m}(\vartheta, \varphi)
\]

where \( Y_{l,m}(\vartheta, \varphi) \) are the spherical harmonics. For a pure dipole-dipole interaction, one has to take into account only interactions of tensors up to rank two, as higher order terms
occurs mere for nuclei with spin \( I \geq \frac{3}{2} \). So one gets

\[
A_{lm}(\Omega_{ij}) = -\sqrt{6} \cdot \frac{\mu_0}{4\pi} \gamma_i \gamma_j R^2 \sqrt{\frac{4\pi}{2l+1}} \frac{Y_{l,m}(\theta, \varphi)}{r_{ij}^3} := B_{2l}^{ij} F_{l}^{m}(\Omega(t)) \quad l \leq 2
\]  

(A.18)

where we defined \( F_{l}^{m}(\Omega(t)) = \sqrt{\frac{4\pi}{2l+1}} \frac{Y_{l,m}(\theta, \varphi)}{r_{ij}^3} \) and \( B_{2l}^{ij} = -\sqrt{6} \cdot \frac{\mu_0}{4\pi} \gamma_i \gamma_j R^2 \).

### A.3 Master equation of relaxation

To derive the master equation of relaxation, a pure dipole-dipole Hamiltonian is assumed. As derived above (eq. (A.15)), the Hamiltonian consists of the sum of scalar products between time-invariant spin operators \( T^m \) and time-dependent space tensor components \( F_{l}^{m}(\Omega(t)) \) that account for the relaxation-inducing stochastic process:

\[
\mathcal{H}^p(t) = \sum_{i<j} \sum_{l=0}^{2} \sum_{m=-l}^{l} (-1)^{m} B_{2l}^{ij} T_{l}^{i} T_{l}^{j}^{\dagger} F_{l}^{m}(\Omega_{ij}(t)) \quad (A.19)
\]

where the indices \( ij \) label the spin interaction between spin \( i \) and \( j \). (Note: the number of spins is in principle arbitrary. However, in the following a two spin system is considered). To change back into the laboratory frame (where the observation takes place), the transformation \( T_{m,\text{int}} = e^{i\mathcal{H}_0 t} T_{m} e^{-i\mathcal{H}_0 t} \) is used. For the calculation \( T_{m} \) is expanded into a sum of eigenoperators \( V_p \) of the Hamiltonian \( \mathcal{H}_0 \), which fulfills \( [\mathcal{H}_0, V_p] = \omega_p V_p \). Hence, \( T_{m} \) can be expanded in this basis of eigenoperators which results in

\[
T_{m} = \sum_p v_{m,p}^{\dagger} V_p e^{i\omega_p t} \quad \text{and} \quad T_{m}^{\dagger} = \sum_p v_{m,p}^{\ast} V_p^{\dagger} e^{-i\omega_p t} \quad (A.20)
\]
For further calculations the identity

\[ T_{l,ij}^m (\Omega_{ij}(t)) = (-1)^m (T_{m,ij}^l)^\dagger (-1)^m F_{l}^{-m*}(\Omega_{ij}(t)) = T_{m,ij}^{l\dagger} F_{l}^{-m}(\Omega_{ij}(t)) \]  

(A.21)
is used. Eq. (A.9) can now be rewritten using the relation

\[ \hat{\sigma}(t) - \hat{\sigma}(0) = - \sum_{i<j} \frac{1}{\hbar^2} (B_{ij}^2)^2 \sum_{l=m-l} \sum_{p,p'} v_p^m v_{p'}^{m*} [V_p, [V_{p'}^\dagger, \hat{\sigma}(0)]] \]

\[ \int_0^t \int_0^t C_{\tau_l}(t_1) e^{i(\omega_{p} - \omega_{p'}^{t_1})t_1} e^{i\omega_{p'}^{t_1} \tau} dt_1 d\tau \]  

(A.22)
The random time functions \( F_{l}^m(\Omega(t)) \) are statistically independent (so the ensemble average would give zero) unless \( l'=l \) (which includes - because \( l \leq m \leq l \) - the same equality for \( m \)). Next, a correlation function is defined by

\[ C_{mm'}(t_1 - t_2) = \left\langle F_{l}^m(\Omega_{ij}(t_1)) F_{l'}^{m'}(\Omega_{ij}(t_2)) \right\rangle \]

\[ = C_{mm'}(\tau) = \left\langle F_{l}^m(\Omega_{ij}(\tau)) F_{l'}^{m'}(\Omega_{ij}(0)) \right\rangle \]  

(A.23)

with \( \tau = t_1 - t_2 \). Note, that \( t \geq t_1 \geq t_2 \) and the correlation function is only dependent on the difference between the time points. By including terms \( 1 = e^{-i\omega_\alpha t_{\beta}} . e^{i\omega_\alpha t_{\beta}} \) (where \( \alpha = p', q' \) and \( \beta \) is the index for the corresponding time) one obtains (for an easier reading, we define \( Z_{ij}^2 = \frac{1}{\hbar} B_{ij}^2 = -\sqrt{6} \cdot \frac{m_0}{4\pi \gamma_i \gamma_j \hbar} \) :

\[ \hat{\sigma}(t) - \hat{\sigma}(0) = - \sum_{i<j} (Z_{ij}^2)^2 \sum_{l=m-l} \sum_{p,p'} v_p^m v_{p'}^{m*} [V_p, [V_{p'}^\dagger, \hat{\sigma}(0)]] \]

\[ \int_0^t \int_0^t C_{\tau_l}(t_1) e^{i(\omega_{p} - \omega_{p'}^{t_1})t_1} e^{i\omega_{p'}^{t_1} \tau} dt_1 d\tau \]  

(A.24)

Here, we already changed the integration boundaries with respect to the new variables \( t_1 \) and \( \tau \). The procedure is shortly explained in the following
Integration of the correlation function

First, we define $\tau(t_1, t_2) = t_1 - t_2$. We want to solve the integral within the new variables $t_1$ and $\tau$. The Jacobian is

$$
\det(J) = \begin{vmatrix}
\frac{\partial t_1}{\partial t_1} & \frac{\partial t_1}{\partial \tau} \\
\frac{\partial t_2}{\partial t_1} & \frac{\partial t_2}{\partial \tau}
\end{vmatrix} = \begin{vmatrix}
1 & 0 \\
0 & -1
\end{vmatrix} = -1 \quad (A.25)
$$

Therefore we can replace $dt_2 dt_1$ by $dt_1 d\tau$ (note, that in the transformation law only the absolute value of the Jacobian determinant is needed and so the order of derivation is unimportant). With this transformation we still keep in mind, that $t_2$ is related to $t_1$ (because the latter is an upper limit for $t_2$). This relation still holds when changing to $\tau$ since it is still a function of $t_1$. Let us consider the new integration boundaries: The integral can be seen as a limit of the Riemann sum. Therefore, we can formally regard the two integrals as two sums ending up (using the nomenclature $t_1 = t^\prime$ and $t_2 = t^\prime$) with

$$
\lim_{N \to \infty} \sum_{k=0}^{N} \sum_{i=0}^{k} f(t^\prime_{i,1}, t^\prime_{i,k}) \Delta t^\prime_{i} \Delta t^\prime_{k} = \lim_{N \to \infty} \left\{ \left( f(t^\prime_0, t^\prime_0) \Delta t^\prime_0 \Delta t^\prime_0 \right) + \left( \sum_{i=0}^{1} f(t^\prime_i, t^\prime_1) \Delta t^\prime_i \Delta t^\prime_1 \right) + \left( \sum_{i=0}^{2} f(t^\prime_i, t^\prime_2) \Delta t^\prime_i \Delta t^\prime_2 \right) + ... + \left( \sum_{i=0}^{n} f(t^\prime_i, t^\prime_n) \Delta t^\prime_i \Delta t^\prime_n \right) + ... \right\} \quad (A.26)
$$

As one can see, this is similar to an integration over the triangle shown in the right subfigure of Figure A1.1. This integration can be replaced by the integration paths shown in the left subfigure. The resulting integration set is the same, since the integration over a set gives the same result iff the two decompositions of the set are isomorphic. The last figure shows now the new integration set. We can easily identify it with the original one.
and therefore we end up with the identity

\[ F(t) = \int_0^t \int_0^{t_1} f(t_1 - t_2)dt_2dt_1 = \int_t^0 \int_0^\tau f(\tau)dt_1d\tau \quad (A.27) \]

which can be rewritten and solved as

\[ F(t) = \int_0^t \int_\tau^t f(\tau)dt_1d\tau = \int_0^t (t - \tau) \cdot f(\tau)d\tau \quad (A.28) \]
1. In the first graph, the upper and lower sum is shown. In this spacial case, the Riemann sum and the upper sum are equivalent. One can easily see, that with rising index $k$ the contribution of $\Delta t_i$ gets bigger.

2. In the second graph the original integration set is shown.

3. By changing the variables from $t, t'$ to $t, \tau = t' - t'$ the new integration path is shown in the lower left graph.

4. The new boundaries can now easily be derived from the lower right graph by comparison between chart 2 and 4.
For a further treatment of eq. (A.24), the variable transformation explained above can be used. However, one has to make a case differentiation for \( p = p' \) and \( p \neq p' \):

- \( p = p' \)
  In this case, the term \( e^{i(\omega_p - \omega_p')t_1} = 1 \) and eq. (A.24) simplifies to

\[
\tilde{\sigma}(t) - \tilde{\sigma}(0) = - \sum_{i < j} (Z^i_j)^2 \sum_{m=-l}^{l} \sum_{p} |v_p^m|^2 [V_p, [V_p^\dagger, \tilde{\sigma}(0)]]
\int_0^t \int_\tau^t C^m(\tau)e^{i\omega_p\tau} dt_1 d\tau \quad (A.29)
\]

Which results after evaluating the first integral in:

\[
\tilde{\sigma}(t) - \tilde{\sigma}(0) = - \sum_{i < j} (Z^i_j)^2 \sum_{m=-l}^{l} \sum_{p} |v_p^m|^2 [V_p, [V_p^\dagger, \tilde{\sigma}(0)]]
\int_0^t (t - \tau)C^m(\tau)e^{i\omega_p\tau} d\tau \quad (A.30)
\]

We now introduce the correlation time \( \tau_c \) as the characteristic time for the decay of the correlation function. Under the assumption that this correlation time \( \tau_c \) is much shorter than the evolution time of the density matrix \( \tilde{\sigma}(t) \), the correlation function \( C^m(\tau) \) is negligibly small for times \( t \gg \tau_c \) but still short enough for the evolution of \( \tilde{\sigma}(t) \) to be small. This assumption enables us to simplify eq. (A.30)
by using $t \to \infty$ and therefore $t \gg \tau$.

\[
\tilde{\sigma}(t) - \tilde{\sigma}(0) \approx -\sum_{i<j} \left( Z_{2}^{ij} \right)^{2} \sum_{m=-l}^{l} \sum_{p} |v_{p}^{m}|^{2} [V_{p}, [V_{p}^{\dagger}, \tilde{\sigma}(0)]]
\]

\[
t \int_{0}^{\infty} C_{m}(\tau)e^{i\omega p \tau} d\tau
\]

\[
= \sum_{i<j} \left( Z_{2}^{ij} \right)^{2} \sum_{m=-l}^{l} \sum_{p} |v_{p}^{m}|^{2} [V_{p}, [V_{p}^{\dagger}, \tilde{\sigma}(0)]] t \frac{1}{2} J_{m}(\omega_{p})
\]

(A.32)

Here, we introduced the spectral density function

\[
J_{m}(\omega) = \int_{-\infty}^{\infty} C_{m}(\tau)e^{i\omega \tau} d\tau
\]

(A.33)

which is the Fourier transform of the correlation function.

**The complex nature of the density function**

A Fourier transform is normally defined for boundaries from $-\infty$ to $\infty$. The integral in eq. (A.31) has a lower boundary of 0 and not $-\infty$ and hence one has to calculate

\[
\int_{0}^{\infty} C_{m}(\tau)e^{i\omega \tau} d\tau = \frac{1}{2} \int_{-\infty}^{\infty} C_{m}(\tau)e^{i\omega \tau} d\tau + \frac{1}{2} \int_{-\infty}^{\infty} C_{m}(\tau) \text{sign}(\tau) e^{i\omega \tau} d\tau
\]

\[
= \frac{1}{2} J_{m}(\omega) + \frac{1}{2} iK_{m}(\omega)
\]

(A.34)

This is correct due to the real, even-valued functions $C(\tau)$ of $\tau$. The imaginary part of the expression usually leads only to a small dynamic frequency shift and can be incorporated into $H_{0}$. Hence, the equality defined in eq. A.33 is formally correct.
• $p \neq p'$

For large times $t$, eq. (A.24) is described in a good approximation by

$$
\tilde{\sigma}(t) - \tilde{\sigma}(0) \approx - \sum_{i<j} \left( Z^{ij}_2 \right)^2 \sum_{m=-l}^{l} \sum_{p,p'} v^m_p v^m_{p'} [V_p, [V_{p'}, \tilde{\sigma}(0)]] \frac{1}{i(\omega_p - \omega_{p'})} \\
\frac{1}{2} \left( e^{i(\omega_p - \omega_{p'})t} J^m(\omega_p) - J^m(\omega_{p'}) \right)
$$

(A.35)

The expression in the brackets of eq. (A.35) contains oscillating terms $\sim e^{i(\omega_p - \omega_{p'})t}$ and constants $\langle J^m(\omega) \rangle$. So, if we choose a value for time $t$ so that $(\omega_p - \omega_{p'})t \gg 1$ (in which the assumption for large $t$ from above is included) the contributions for $p \neq p'$ will rapidly average to zero and can be neglected in the following (see e.g. [1]).

Although eq. (A.32) is a formal solution for the second order expansion of the density operator, the differential equation up to second order, which is called the "master equation" of relaxation, is often used as a starting point. It is derived by taking the time derivative of the density operator which yields

$$
\frac{d\tilde{\sigma}(t)}{dt} = - \sum_{i<j} \left( Z^{ij}_2 \right)^2 \sum_{m=-l}^{l} \sum_{p} [v^m_p]^2 [V_p, [V_{p'}, \tilde{\sigma}(0)]] \frac{1}{2} J^m(\omega_p)
$$

(A.36)

A.4 From the spectral density to the cross-relaxation rate constants

To describe the cross-relaxation rate constants from the formal solution up to second order of the Liouville-von-Neuman equation (A.32) or/and from the corresponding master equation of relaxation (eq. (A.36)) in presence of a dipole - dipole Hamiltonian, this term is further treated in the following.
Starting from eq. (A.36), one has to calculate all occurring commutators. Therefore, the basis operators $v_{m,p}^l$ of the unperturbed Hamiltonian for a dipole-dipole Hamiltonian of the form (here we used the fact, that $l \leq 2$ for $I < \frac{3}{2}$)

$$
\mathcal{H}_{ij}(t) = -\sqrt{6} \frac{\mu_0}{4\pi} \gamma_i \gamma_j \hbar^2 \sum_{m=-2}^{2} T_{2,m}^{(ij)} \sqrt{\frac{4\pi}{5}} Y_{2,m}(\varphi_{ij}(t)) \frac{r_{ij}^3}{r_{ij}^2(t)}
$$

(A.37)

can be achieved by finding the eigenoperators and eigenfrequencies of $\mathcal{H}_0$, which have to fulfill the eigenvalue equation $[\mathcal{H}_0, V_p] = \omega_p V_p$ (if we assume for example an unperturbed Hamiltonian of the form $\mathcal{H}_0 = \omega_1 I_{1z} + \omega_2 I_{2z}$ one eigenoperator is $I_{1z} I_{2z}$ since $[\mathcal{H}_0, I_{1z} I_{2z}^\dagger] = \omega_2 I_{1z} I_{2z}^\dagger$). A complete set of these eigenoperators is given in the following Table A.1 [42]:

<table>
<thead>
<tr>
<th>$l, m$</th>
<th>$v_{m}^l V_p$</th>
<th>$v_{m}^l V_p$</th>
<th>$\omega_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 0</td>
<td>$\frac{2}{\sqrt{6}} I_{1z} I_{2z}$</td>
<td>$\frac{2}{\sqrt{6}} I_{1z} I_{2z}$</td>
<td>0</td>
</tr>
<tr>
<td>2, 0</td>
<td>$-\frac{1}{\sqrt{6}} I_{1z}^+ I_{2z}$</td>
<td>$-\frac{1}{\sqrt{6}} I_{1z}^+ I_{2z}$</td>
<td>$\omega_1 - \omega_2$</td>
</tr>
<tr>
<td>2, 0</td>
<td>$-\frac{1}{\sqrt{6}} I_{1z}^- I_{2z}$</td>
<td>$-\frac{1}{\sqrt{6}} I_{1z}^- I_{2z}$</td>
<td>$\omega_1 - \omega_2$</td>
</tr>
<tr>
<td>2, ±1</td>
<td>$\mp \frac{1}{2} I_{1z} I_{2z}^\pm$</td>
<td>$\mp \frac{1}{2} I_{1z} I_{2z}^\pm$</td>
<td>$\omega_2$</td>
</tr>
<tr>
<td>2, ±1</td>
<td>$\mp \frac{1}{2} I_{1z} I_{2z}^\pm$</td>
<td>$\mp \frac{1}{2} I_{1z} I_{2z}^\pm$</td>
<td>$\omega_1$</td>
</tr>
<tr>
<td>2, ±2</td>
<td>$\frac{1}{2} I_{1z} I_{2z}^\pm$</td>
<td>$\frac{1}{2} I_{1z} I_{2z}^\pm$</td>
<td>$\omega_1 + \omega_2$</td>
</tr>
</tbody>
</table>

Table A.1: Listed are the eigenoperators of the unperturbed Zeeman-Hamiltonian. In the first column, the quantum numbers $l$ and $m$ are listed. In the next two columns the eigenoperators and the corresponding adjoint operators are given. In the last column the eigenfrequency of the corresponding eigenoperator is given.

Next, one has to calculate all the occurring commutators resulting from the decomposition of the spin operators. Starting with an initial magnetization $\hat{\sigma}(0) = I_{1z}$, the nine commutators are listed in the following table:
Table A.2: Listed are the double Commutators for dipole-dipole relaxation with an initial magnetization of $\tilde{\sigma}(0) = I_z$.

The cross-relaxation terms between the two spins involved (namely $I_1$ and $I_2$) can be obtained by calculating the expectation value between the commutators calculated before and the final spin. Theses values are listed in the Table A.3.

Table A.3: Expectation values for dipole-dipole auto- and cross-interaction for spin $I_{1z}$ and $I_{2z}$, respectively. In the first column, the expectations values for the double commutators listed in Table A.2 and the final spin are listed, while in the last column the frequencies are given. At these frequencies, the spectral density function is sampled.
To get an idea of the calculation, we want to derive step by step the expectation value for the first term

\[
\frac{1}{24} \langle I_{2z} | I_{1z} - I_{2z} \rangle = \frac{1}{24} \left\{ \langle \alpha \alpha | I_{2z} I_{1z} - I_{2z}^2 | \alpha \alpha \rangle + \langle \alpha \beta | I_{2z} I_{1z} - I_{2z}^2 | \alpha \beta \rangle \right. \\
\left. \langle \beta \alpha | I_{2z} I_{1z} - I_{2z}^2 | \beta \alpha \rangle + \langle \beta \beta | I_{2z} I_{1z} - I_{2z}^2 | \beta \beta \rangle \right\}
\]

\[
= \frac{1}{24} \left\{ \frac{1}{2} \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} 1 \\ 0 \\ 0 \\ 0 \end{pmatrix} + \ldots \right\}
\]

\[
= \frac{1}{24} \left\{ 0 - \frac{1}{2} \frac{1}{2} + 0 \right\} = -\frac{1}{24}
\]  

where \( \alpha \) and \( \beta \) label the pure states of the spin system.

**Matrix representation**

So far, we started from the Liouville - von Neumann equation (A.1) and derived a general expression for the time dependence of the density function (A.36). This last equation is of the form \( \frac{d \tilde{\sigma}(t)}{dt} = \frac{dK(t)}{dt} \cdot \tilde{\sigma}(t) \) and defined in the Liouville space. Hence, the density operator is represented by a vector, while \( \frac{dK(t)}{dt} \) is a matrix. The diagonal elements of this matrix are the auto-relaxation rate constants \( \rho \) while the off-diagonal elements are the cross-relaxation rate constants \( \sigma \). For a two spin system, the equation representing the system is given by

\[
\frac{d}{dt} \begin{pmatrix} I_{1z} \\ I_{2z} \end{pmatrix} = - \begin{pmatrix} \rho_1 & \sigma_{12} \\ \sigma_{12} & \rho_2 \end{pmatrix} \begin{pmatrix} I_{1z} \\ I_{2z} \end{pmatrix}
\]  

(A.39)
Using the numerical values for the commutators and expectation values derived above, we end up with an auto-relaxation rate constant for a two-spin system of

\[
\rho_1 = -6 \frac{1}{\hbar^2} \gamma_1^2 \gamma_2^2 h^4 \mu_0^2 \frac{1}{2} \left\{ \frac{2}{24} J(\omega_1 - \omega_2) + \frac{2}{8} J(\omega_1) + \frac{2}{4} J(\omega_1 + \omega_2) \right\}
\]

\[
= -\frac{\gamma_1^2 \gamma_2^2 h^2 \mu_0^2}{(4\pi)^2} \frac{1}{4} \left\{ J(\omega_1 - \omega_2) + 3J(\omega_1) + 6J(\omega_1 + \omega_2) \right\}
\]  

(A.40)

and a cross-relaxation coefficient:

\[
\sigma_{12} = -\frac{1}{\hbar^2} \frac{\gamma_1^2 \gamma_2^2 h^4 \mu_0^2}{(4\pi)^2} \frac{1}{2} \left\{ \frac{2}{24} J(\omega_1 - \omega_2) - \frac{2}{4} J(\omega_1 + \omega_2) \right\}
\]

\[
= -\frac{\gamma_1^2 \gamma_2^2 h^2 \mu_0^2}{(4\pi)^2} \frac{1}{4} \left\{ J(0) - 6J(\omega_1 + \omega_2) \right\}
\]  

(A.41)

For spins of the same kind (e.g., hydrogen atoms) we get \(\omega_1 = \omega_2 = \omega_0\) and \(\gamma_1 = \gamma_2 = \gamma\), and therefore

\[
\rho_1 = \rho_2 = -\frac{\gamma^2 \gamma_2 h^2 \mu_0^2}{(4\pi)^2} \frac{1}{4} \left\{ J(0) + 3J(\omega_0) + 6J(2\omega_0) \right\}
\]  

(A.42)

\[
\sigma_{12} = -\frac{\gamma^4 h^2 \mu_0^2}{(4\pi)^2} \frac{1}{4} \left\{ J(0) - 6J(2\omega_0) \right\}
\]  

(A.43)

which are the known auto- and cross-relaxation rate constant terms ([23],[25]).
B Programs

B.1 Simulating fast internal motion

```matlab
%This program calculates the fast HH order parameters for individual spin pairs. As an input it needs a pdb structure as well as a file with local HH order parameters.

clear all

%For NOE calculations from distances one needs these constants.
mu0 = 4*pi*10^(-7);
hbar = 1.05457148*10^(-34);
gamma = 2.6752*10^8;
tauc = 7.72*10^(-09);
omegaH = 700*2*pi*10^6;

%The delta is a constant pre-factor for the NOE calculation from the constants defined above. The Js are the spectral density functions at a given frequency omega.
delta = 2*mu0/(4*pi)*(hbar*gamma^2);
J0 = 2/5*tauc;
Jp = 2/5*tauc/(1+(2*omegaH*tauc)^2);

%faktor is the proportionality factor between the inverse sixth power of the distance and the NOE
Faktor = (delta/4)^2*(-J0 + 6*Jp);

%In the file HN the pdb structure is already matlab readable and sorted for each H-H distances together with the associated order parameters
load HN.txt %combined pdb & order parameter values

Hvector1 = HH(:,1:5); %H-vector of the 1st cone
Nvector1 = HH(:,6:8); %N-vector of the 1st cone
Hvector2 = HH(:,9:11); %H-vector of the 2nd cone
Nvector2 = HH(:,12:14); %N-vector of the 2nd cone
betamax1 = HH(:,15); %opening angle of the 1st cone (in degrees)
betamax2 = HH(:,16); %opening angle of the 2nd cone (in degrees)

%intersection vector between H and N of the 1st cone
HN1 = [Hvector1(:,1) - Nvector1(:,1), Hvector1(:,2) - Nvector1(:,2), Hvector1(:,3) - Nvector1(:,3)];

%intersection vector between H and N of the 2nd cone
HN2 = [Hvector2(:,1) - Nvector2(:,1), Hvector2(:,2) - Nvector2(:,2), Hvector2(:,3) - Nvector2(:,3)];

%the length between N and H should always be the same
LenghtHH = [norm(HN1(:,1));norm(HN2(:,1))];
```

165
%vector between each individual H-H distance
HHvector = [Hvector2(:,1) - Hvector1(:,1), Hvector2(:,2) - Hvector1(:,2), Hvector2(:,3) - Hvector1(:,3)];

%polar angles of the HN vectors
Drehwinkel1 = acos(HN1(:,3)./LenghtHN(1,1));
Drehwinkel2 = acos(HN2(:,3)./LenghtHN(2,1));

%individual spin pairs are written to the output file Simul
Simul(:,1:2) = HN(:,1:2);

%sampling steps for phi
SamplingPhi = 20;

%loop over the individual spin pairs
for i = 1:size(HN,1)
    %for not causing problems the variables defined within this loop
    %are deleted (the keep command keeps all the rest)
    keep i OveralldistII Simul HHvector SamplingPhi Drehwinkel1 ... Drehwinkel2 betamax1 betamax2 rHN1 rHN2 HN1 HN2 ... Hvector1 Hvector2 Hvector1 Hvector2 Faktor
    i %to see how far the simulation is
    %distance between the two HN spins according to the pdb file
    HHdistcalc = norm(HHvector(i,:));
    %-------------------------------------------------------------
    %first cone
    %generate normal distribution
    %normal distribution between 0 and 1
    Gauss1 = randn(600,1);
    Help1 = (Gauss1 < 0 ).*0 + (Gauss1 > 0).*1;
    Gauss1a = nonzeros(Help1.*Gauss1);
    %define standart deviation
    Salone1 = 1/2*cos(betamax1(i,1)/180*pi)*(1+cos(betamax1(i,1)/180*pi));
    Maxbeta1 = sqrt((1-(1+2*Salone1)/3)/2);
    %generate theta angles between zero and betamax as a normal
    %distribution around the cone axis
    thetacone1a = Maxbeta1./pi*180.*Gauss1a;

    %-------------------------------------------------------------
%set all angles bigger than betamax to zero (to skip them later)

\[ \text{Help1a} = \begin{cases} 0 & \theta_{\text{cone1a}} > \text{betamax1}(i,1) \\ 1 & \theta_{\text{cone1a}} < \text{betamax1}(i,1) \end{cases}; \]

%Defining a vector with all simulated theta angles for the first cone
\[ \theta_{\text{cone1}} = \text{nonzeros}(\text{Help1a} \times \theta_{\text{cone1a}}); \]
\[ \Theta_{\text{cone1}} = \theta_{\text{cone1}} / 180 \times \pi; \]

%Defining a vector with phi angles for the first cone %from 0 to 360 in SamplingPhi Stepps.
\[ \phi_{\text{cone1}} = 0: \text{SamplingPhi}: 360; \]
\[ \Phi_{\text{cone1}} = \phi_{\text{cone1}} \times \pi / 180; \]

%Defining a 3D matrix of theta and phi angles for combining %each phi angle with every theta angle
\[ \text{MatrixPhicone1} = \text{repmat}(\Phi_{\text{cone1}}, 1, \text{size}(\Theta_{\text{cone1}}, 1)); \]
\[ \text{MatrixThetacone1} = \text{repmat}(\Theta_{\text{cone1}}, 1, \text{size}(\Phi_{\text{cone1}}, 1)); \]

% x, y, and z component of every simulated position on the %cone surface. These points are normal distributed.
\[ x_{\text{vectorcone1}} = L_{\text{HN1}}(1,1) \times \cos(\text{MatrixPhicone1}) \times \sin(\text{MatrixThetacone1}); \]
\[ y_{\text{vectorcone1}} = L_{\text{HN1}}(1,1) \times \sin(\text{MatrixPhicone1}) \times \sin(\text{MatrixThetacone1}); \]
\[ z_{\text{vectorcone1}} = L_{\text{HN1}}(1,1) \times \cos(\text{MatrixThetacone1}); \]

% second cone
% the same procedure as above

\[ \text{Gauss2} = \text{randn}(600,1); \]
\[ \text{Help2} = \begin{cases} 0 & \text{Gauss2} < 0 \\ 1 & \text{Gauss2} \geq 0 \end{cases}; \]
\[ \text{Gauss2a} = \text{nonzeros}(\text{Help2} \times \text{Gauss2}); \]
\[ \text{Salone2} = 1/2 \times \cos(\text{betamax2}(i,1)/180 \times \pi) \times (1 + \cos(\text{betamax2}(i,1)/180 \times \pi)); \]
\[ \text{Maxbeta2} = \sqrt{(1 - (1 + 2 \times \text{Salone2})/3) / 2}; \]
\[ \theta_{\text{cone2a}} = \text{Maxbeta2} \times \pi / 180 \times \text{Gauss2a}; \]
\[ \text{Help2a} = \begin{cases} 0 & \theta_{\text{cone2a}} > \text{betamax1}(i,1) \\ 1 & \theta_{\text{cone2a}} < \text{betamax1}(i,1) \end{cases}; \]
\[ \text{thetacone2} = \text{nonzeros}(\text{Help2a} \times \theta_{\text{cone2a}}); \]
\[ \Theta_{\text{cone2}} = \theta_{\text{cone2}} / 180 \times \pi; \]
\[ \phi_{\text{cone2}} = 0: \text{SamplingPhi}: 360; \]
\[ \Phi_{\text{cone2}} = \phi_{\text{cone2}} \times \pi / 180; \]
MatrixPhicone2 = repmat(Phicone2,1,size(Thetacone2,1));
MatrixThetacone2 = repmat(Thetacone2,1,size(Phicone2,1))';
xvectorcone2 = LenghtHN(2,1).*cos(MatrixPhicone2).*sin(MatrixThetacone2);
yvectorcone2 = LenghtHN(2,1).*sin(MatrixPhicone2).*sin(MatrixThetacone2);
zvectorcone2 = LenghtHN(2,1).*cos(MatrixThetacone2);

%---------------------------------------------------
%For matching the pdb structure one has to rotate the two cones.
%Therefore the angles Drehwinkel1 and Drehwinkel2 were used. This
%rotation is performed by multiplying \(\{x,y,z\}\) with the corresponding
%rotation matrix.
%rotation of the first cone
meinconevec1a = [ 0;0;LenghtHN(1,1)];
norm1a = cross(meinconevec1a,HN1(i,1:3));
norm1r = norm1a./norm(norm1a);
tt = 1 - cos(Drehwinkel1(i,1));
cc = cos(Drehwinkel1(i,1));
ss = sin(Drehwinkel1(i,1));
rotation matrix
A11 = [tt*norm1r(1,1)^2 + cc ...
       tt*norm1r(1,1)*norm1r(1,2) - ss*norm1r(1,3) ...
       tt*norm1r(1,1)*norm1r(1,3) + ss*norm1r(1,2); ...
       tt*norm1r(1,2)^2 + cc ...
       tt*norm1r(1,2)*norm1r(1,3) - ss*norm1r(1,1); ...
       tt*norm1r(1,2)*norm1r(1,3) - ss*norm1r(1,2); ...
       tt*norm1r(1,3)^2 + cc];
%new coordinates of the first cone
xvectorcone1neu = Nvector1(i,1) + A11(1,1).*xvectorcone1 + . ..
                    A11(1,2).*yvectorcone1 + A11(1,3).*zvectorcone1 ;
yvectorcone1neu = Nvector1(i,2) + A11(2,1).*xvectorcone1 + ...  
                    A11(2,2).*yvectorcone1 + A11(2,3).*zvectorcone1 ;
zvectorcone1neu = Nvector1(i,3) + A11(3,1).*xvectorcone1 + ...  
                    A11(3,2).*yvectorcone1 + A11(3,3).*zvectorcone1 ;
%rotation of the second cone

meinconevec1a = [ 0;0;LengthHN(2,1)];
normla = cross(meinconevec1a,HN2(i,1:3));
norm1a = normla./norm(normla);

tt = 1 - cos(Drehwinkel2(i,1));
cc = cos(Drehwinkel2(i,1));
ss = sin(Drehwinkel2(i,1));

%rotation matrix

A11 = [tt*norm1r(1,1)^2 + cc ...
   tt*norm1r(1,1)*norm1r(1,2) - ss*norm1r(1,3) ...
   tt*norm1r(1,1)*norm1r(1,3) + ss*norm1r(1,2); ...
   tt*norm1r(1,2)^2 + cc ...
   tt*norm1r(1,2)*norm1r(1,3) - ss*norm1r(1,1); ...
   tt*norm1r(1,1)*norm1r(1,3) - ss*norm1r(1,2) ...
   tt*norm1r(1,3)^2 + cc];

%new coordinates of the second cone

xvectorcone2neu = Nvector2(i,1) + A11(1,1).*xvectorcone2 + ...
   A11(1,2).*yvectorcone2 + A11(1,3).*zvectorcone2;
yvectorcone2neu = Nvector2(i,2) + A11(2,1).*xvectorcone2 + ...
   A11(2,2).*yvectorcone2 + A11(2,3).*zvectorcone2;
zvectorcone2neu = Nvector2(i,3) + A11(3,1).*xvectorcone2 + ...
   A11(3,2).*yvectorcone2 + A11(3,3).*zvectorcone2;

%---------------------------------------------------
%This comment here is only for visualisation of the two cones in space.

kval = 0:0.01:1;
Hvvector1 = Hvvector1(i,1) + kval .* HHvector1(i,1);
Hvvector2 = Hvvector1(i,2) + kval .* HHvector1(i,2);
Hvvector3 = Hvvector1(i,3) + kval .* HHvector1(i,3);
cone1vecx = Hvvector1(i,1) + kval .* BH1(i,1);
cone1vecy = Hvvector1(i,2) + kval .* BH1(i,2);
cone1vecz = Hvvector1(i,3) + kval .* BH1(i,3);
cone2vecx = Hvvector2(i,1) + kval .* BH2(i,1);
cone2vecy = Hvvector2(i,2) + kval .* BH2(i,2);
cone2vecz = Hvvector2(i,3) + kval .* BH2(i,3);
```matlab
% This is the final step. The distance between each point on the surface of
% the first cone and each point of the second cone is calculated. Each
% distance is written in the matrix d. Furthermore the angle between each
% distance vector and the intersection vector between the two cone axes is
% calculated and written to Y20 (as all other contributions averages out.

count1 = 0;
for a1=1:size(xvectorcone1neu,1)
    for a2=1:size(xvectorcone1neu,2)
        count1 = count1+1;
        d(:,:,count1) = sqrt((xvectorcone1neu(a1,a2)-xvectorcone2neu).^2 +
            (yvectorcone1neu(a1,a2)-yvectorcone2neu).^2 +
            (zvectorcone1neu(a1,a2)-zvectorcone2neu).^2);
        VectorZaehler = (xvectorcone1neu(a1,a2) -
            xvectorcone2neu).*HHvector(i,1) +
            (yvectorcone1neu(a1,a2) -
            yvectorcone2neu).*HHvector(i,2) +
            (zvectorcone1neu(a1,a2) -
            zvectorcone2neu).*HHvector(i,3);
        VectorNenner1 = sqrt((xvectorcone1neu(a1,a2)-xvectorcone2neu).^2 +
            (yvectorcone1neu(a1,a2)-yvectorcone2neu).^2 +
            (zvectorcone1neu(a1,a2)-zvectorcone2neu).^2);
        VectorNenner2 = sqrt(HHvector(i,1).^2 + HHvector(i,2).^2 +
            HHvector(i,3).^2);
        Winkel = VectorZaehler./((VectorNenner1.*VectorNenner2));
        Y20(:,:,count1) = 1/4*sqrt(5/(pi)).*sqrt(4*pi/5).*(3*(Winkel).^2 -1);
    end
end
```
Now each inverse distance to the power of three is weighted with the angle

\[ \text{distancefast} = \frac{Y20}{d^3}; \]

and summed up in accordance to the formula for fast internal motion. The effective distance is calculated by taking the pdb distance as reference.

\[ \text{dist0fast} = \text{distancefast}(1); \]
\[ \text{Singlesum} = 1/(\text{sum(dist0fast)./size(dist0fast,1)})^{(1/6)}; \]
\[ \text{dist4fastav} = (1/(1./\text{HHdistcalc}^3))^{(1/6)}.\text{Singlesum}; \]

The same procedure as above. Here, the effective distance is calculated by taking both cones individually as a reference.

\[ d1 = (1/(1./d^3))^{(1/6)}; \]
\[ \text{fast4two} = d1(1).\text{Singlesum}; \]
\[ \text{dist4fast} = (\text{sum(fast4two)./size(fast4two,1))}; \]

Output file. From the distances one can calculate a NOEs on the basic of pure fast internal motion. With this NOEs one can take the ratio between the rigid (pdb) NOE and the simulated one to determine an order parameter S2.

\[ \text{Simul}(i,3) = (\text{Faktor}./(\text{dist4fastav}10^{-10})^{(1/6)}; \]
\[ \text{Simul}(i,4) = (\text{Faktor}./(\text{dist4fast}10^{-10})^{(1/6)}; \]
\[ \text{Simul}(i,5) = (\text{Faktor}./(\text{HHdistcalc}10^{-10})^{(1/6)}; \]
\[ \text{Simul}(i,6) = \text{Simul}(i,3)./\text{Simul}(i,5); \]
\[ \text{Simul}(i,7) = \text{Simul}(i,4)./\text{Simul}(i,5); \]

save('sigma.txt','Simul','-ascii')

end

Figure B1.1: Source code of the MATLAB program for the calculation of the influence of fast internal motion on the $^1$H$_N$-$^1$H$_N$ order parameter using the local $^1$H-$^{15}$N order parameter and the pdb file for a certain molecule. The principle idea of the program is explained in section 8.2.
B.2 Simulating spin diffusion in proteins

In the following, the master file of the program for the calculation of the influence of spin diffusion on the NOE buildup as described in section 8.3 is listed followed by a short description of each individual subprogram.

```
%Master file
%all input parameters are defined as well and all subfiles are called

clear all

%input of the pdb file
DistanceFile = input('What is the name of your pdb file (with ending!)? ','s');

%input of auto-relaxation values for the corresponding correlation time
%if no experimental data exist the program simulates them
rhoFile = input('What is the name of your rho file (with ending!)? If you do not have one type n ','s');

%correlation time in ns and seconds
taucin = input('Which correlation time does your molecule have (in ns)? ');
tauc = taucin*10^-9;

%time points measured
%in principle only the longest mixing time is necessary
Tp = input('Enter your measuring time points in vector form [t1 t2 t3 ...] (in ms): ');
Timepoints  = Tp*10^-3;

%deuteration level of the HA protons
deut_HA = input('Enter the deuteration level of the alpha-protons (in %): ');
DeutHA = deut_HA/100;

%deuteration level of all other carbon bound protons
deut_Prot = input('Enter the deuteration level of all other carbon bound protons (in %): ');
DeutPro   = deut_Prot/100;

%deuteration level of the methyl protons
deut_Met = input('Enter the deuteration level of the Methyl protons (in %): ');
DeutMet  = deut_Met/100;

%deuteration level of the amide protons
deut_Amid = input('Enter the deuteration level of the water (in %): ');
DeutAmid = deut_Amid/100;

%if wanted, the program uses pseudo atoms for the methyl groups; only if defined in the pdb file!
pseudo = input('Do you want to use Pseudo-Atoms for the Methyl-Groups? (y/n): ','s');
```

end of user input
% out of the pdb file the HN-HN distances are extracted; furthermore a
% numeral code for labeling the protons is assigned to each spin pair
Distances = pdbread(DistanceFile,pseudo);

% auto-relaxation rates rho for all protons are simulated here
% at the end experimental values for the rhos are used if available
rhofile = rhosim(tauc,DeutHA,DeutPro,DeutMet,DeutAmid,Distances,rhoFile);

% for each individual spin pair all possible spin partners nearby (within a
% radius of six Angstrom) are assigned
Pathway = allpath(Distances,rhofile);

% finally, for each spin pair the Solomon equation for each intermediate
% spin defined in Pathway is calculated and finally summed up
Final = simulations(tauc,DeutBA,DeutPro,DeutMet,DeutAmid,Timepoints,Pathway);

% if pseudo atoms for the methyl groups are used the final output (with
% different labeling is backassigned) has to be slightly modified
if strcmp(pseudo,'n') == 1
    Finalneu = Methyladd(Final);
    together(Finalneu);
else
    together(Final);
end

Figure B1.2: Source code of the MATLAB master program for the calculation of the influence of spin diffusion in NOE build up curves using a pdb file as input. The principle idea of the program is explained in the following.
The master program for the calculation of the influence of spin diffusion in NOE buildups is divided in two parts. In the first part the variables for the inputs made by the user are defined. As an input the user needs

1. a pdb file of the molecule

2. a text file where the auto-relaxation rate constants for each individual spin is stored. The first column should contain the residue number, the second one the atom assignment and the third one finally the auto-relaxation rate constant. If no such file exists the program simulates these rate constants (within an error of 20 %) itself.

3. the correlation time of the molecule is needed to calculate the cross-relaxation rate constants from the distances from the pdb file

4. a vector with the mixing times used in the NOE experiment. In principle only the longest mixing time is necessary unless one doesn’t want a buildup simulation.

5. the following four inputs for the deuteration level of

   (a) the $\alpha$ protons

   (b) all other carbon bound protons

   (c) the protons in methyl groups

   (d) the water; this effects all nitrogen bound protons too. Note, that the NOE itself get lowered by this factor too. However, this effect is not included in the final correction.

6. the last input is about the use of pseudo-atoms for methyl groups. This option can only be used if the pdb structure has pseudo-atoms for the methyl group.
After the user entered these values the subprograms are called. First the program called "pdbread" is used. The pdb file is loaded into the working memory. An internal number code is assigned to the different protons in the molecule and each proton-proton distance within a specified radius is calculated. As default this maximal distance is set to 10 Å. For a shorter calculation time one can lower this limit taking into account slight inaccuracies. The output called "Distances" consists of five columns. The first four contain the the two proton residues and atoms numbers and the last one the distance between them.

The next subprogram called is "rhosim". It simulates the auto-relaxation rate constant of each proton in the pdb-file using the distances calculated above and summing up all contributions of surrounding protons. Finally the program replaces the simulated auto-relaxation rate constants by experimentally derived ones stored in a text file if available.

The subprogram "allpath" takes as an input the distances and the cross-relaxation rate constants and assigns to each individual H N-H N pair all possible spin partners. The output contains column-wise the first and second spin with its residue and atom number, the corresponding cross-relaxation rate constant for the two spin system and the distance between them. Finally each intermediate spin label within the distance defined above (e.g. 10 Å ) with its cross-relaxation rate constant and the two distances are listed (e.g. the distance between spin one and the intermediate and between the intermediate and spin two).

The program "simulations" finally solves the Solomon equation for each individual three spin system. Each buildup is weighted by the deuteration level of the spins involved. The deviation between the buildup of the pure two spin solution and the sum of all three spin contributions is calculated and gives the correction factor for the cross-relaxation rate constant.
Afterwards the optional program "Methyladd" sorts out the contributions of each spin in a methyl group if the user decided not to take pseudo-atoms.

The subprogram "together" finally creates an output where row-wise the first and second spin with its residue number and atom name (in accordance to the pdb code) followed by the pure two spin cross-relaxation rate constant and the cross-relaxation rate constant with the spin diffusion contributions is listed. In the last two rows the correction factor in % and the number of spin partners derived from the pdf code is given.
Curriculum vitae

Personal Data

Name                  Dominik Leitz
Date of birth         29. September 1982
Place of birth        Singen, Germany
Citizenship           German
Education

1989 - 1993  elementary school in Singen (Germany)

1993 - 2002  Friedrich-Wöhler-Gymnasium Singen
(general grammar school, Germany)

2002  Abitur (diploma from German secondary
school qualifying for university admission)

2002 - 2004  basic studies of physics at the University of Konstanz, Germany

2004 - 2007  main study period at the ETH Zürich, Switzerland

summer 2006  Semester work in the group of Prof. Ott, ETH Zürich on
"Magnetic susceptibility of BaVS₃"

spring 2007  Master thesis in the group of Prof. Esslinger, ETH Zürich on
"Realization of a two-dimensional magneto-optical trap for potassium"

2007 - 2011  Ph.D. thesis in the group of Prof. Riek, ETH Zürich (to be awarded)
Publications


Poster Presentations
