Dipolar Recoupling in Magic-Angle-Spinning Nuclear Magnetic Resonance

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Abstract

This thesis concerns the development of radio-frequency pulse sequences in magic-angle-spinning solid-state nuclear magnetic resonance.

First, two classes of pulse sequences are presented which are synchronized with the sample rotation. Symmetry theorems are described which link the symmetry of the pulse sequences to selection rules for the recoupling and/or decoupling of certain spin interactions. Pulse sequences are demonstrated which recouple direct homonuclear dipolar interactions at high sample spinning frequencies. Several applications are shown, including the efficient excitation of double-quantum coherences, two-dimensional double-quantum spectroscopy, transfer of longitudinal magnetization and two-dimensional correlation spectroscopy. In addition, generalized Hartmann-Hahn sequences are demonstrated in which radio-frequency irradiation is applied simultaneously to two isotopic spin species. These sequences selectively recouple direct heteronuclear dipolar interactions and suppress all homonuclear interactions for both spin species. Experimental demonstrations are given of heteronuclear two-dimensional correlation spectroscopy, heteronuclear multiple-quantum spectroscopy and the estimation of heteronuclear dipolar couplings.

Second, a magic-angle-spinning nuclear magnetic resonance method is developed which directly estimates the backbone torsional angle $\psi$ in peptides and proteins. The method exploits multiple-quantum $^{13}$C coherence evolving under heteronuclear $^{13}$C-$^{15}$N dipolar interactions. Single torsional angles $\psi$ are determined with an accuracy of $5^\circ - 10^\circ$ in the tripeptides gly-gly-gly and ala-gly-gly by exploiting double-quantum and triple-quantum coherences respectively.
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III. Homonuclear zero-quantum recoupling in fast magic-angle-spinning nuclear magnetic resonance, A. Brinkmann, J. Schmedt auf der Günne, and M. H. Levitt, in manuscript.


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Part I
Basic Principles of NMR Spectroscopy
1 Introduction

The first successful nuclear magnetic resonance (NMR) experiments in condensed matter were carried out independently at the end of 1945 by Purcell, Torrey and Pound at Harvard University [1] and by Bloch, Hansen and Packard at Stanford University [2]. Purcell and his colleagues obtained their first NMR signal from the protons in solid paraffin wax, whilst Bloch and his colleagues observed the NMR signal from the protons in liquid water.

Since its discovery, NMR has become established as an important spectroscopic tool for investigating matter. Liquid-state NMR has had great success in the study of molecular structure and dynamics in chemistry, biology and medicine. The technique of magnetic resonance imaging (MRI) has become an essential part of modern medicine to obtain three-dimensional structures of biological tissues and to investigate organic functions. NMR in the solid-state faces considerable difficulties, as will be discussed in the following sections. Nevertheless, in recent years, many solid-state NMR methods have been developed for obtaining molecular structural information. These methods may be applied to systems which are unsuitable for diffraction methods or liquid-state NMR, such as membrane proteins.

In the following, a brief overview of the basic principles of NMR spectroscopy and the topics addressed in this thesis will be given. The discussion is kept very graphical at this point, an overview of the quantum-mechanical theory of NMR is given in part II of this thesis. A detailed introduction to NMR spectroscopy can be found in Refs. 3 and 4.
2 Nuclear Magnetism

2.1 Nuclear Spin and Magnetic Moment

Elementary particles, such as electrons, have certain intrinsic properties, such as mass, electric charge and spin. The spin of a particle is an intrinsic angular momentum, which is not produced by a rotation of the particle. The consequences of spin have to be described in the framework of quantum mechanics. In general, the total spin angular momentum may only take certain values, which are, according to quantum mechanics, given by $\hbar \sqrt{S(S+1)}$, where $\hbar$ is Planck’s constant divided by $2\pi$ and $S$ is called the spin quantum number. Each elementary particle has a certain value for $S$, which might be an integer (0, 1, 2, …) or a half-integer ($\frac{1}{2}$, $\frac{3}{2}$, …). One therefore speaks about spin-S particles, e.g., electrons are spin-$\frac{1}{2}$ particles, whereas photons are spin-1 particles.

Atoms are built of electrons and nuclei. While electrons are elementary particles, nuclei consist of nucleons (protons and neutrons), which are themselves build of elementary particles, the quarks. The properties of a nucleus are determined by the properties of its constituents. The nuclear spin emerges from the spin and orbital angular momenta of the nucleons, while the spin of the nucleons arises from the spin and orbital angular momenta of the quarks and the strong interaction particles, the gluons.

As a result, the nuclear spin quantum number may have an integer or half-integer value. NMR spectroscopy can only be performed on nuclei with spins not equal to zero. Table 2.1 contains a list of ground-state nuclear spins of a few isotopes common in biological molecules, such as peptides and proteins. This thesis will exclusively discuss the NMR spectroscopy of spin-$\frac{1}{2}$ nuclei, where the practical applications have been done solely on $^1$H, $^{13}$C and $^{15}$N.

<table>
<thead>
<tr>
<th>Isotope Notation</th>
<th>Spin$^a$</th>
<th>Natural Abundance [%]</th>
<th>Gyromagnetic Ratio $\gamma$ [10$^6$ rad s$^{-1}$ T$^{-1}$]</th>
<th>Larmor Frequency at $B_0 = 9.4$ T $\omega_0/2\pi$ [MHz]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>$I$ 1/2</td>
<td>$\sim$ 100</td>
<td>267.522</td>
<td>$-400.000$</td>
</tr>
<tr>
<td>$^{12}$C</td>
<td>0</td>
<td>98.9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>$S$ 1/2</td>
<td>1.1</td>
<td>67.283</td>
<td>$-100.602$</td>
</tr>
<tr>
<td>$^{14}$N</td>
<td>1</td>
<td>99.63</td>
<td>19.338</td>
<td>$-28.914$</td>
</tr>
<tr>
<td>$^{15}$N</td>
<td>$K$ 1/2</td>
<td>0.37</td>
<td>$-27.126$</td>
<td>$+40.559$</td>
</tr>
</tbody>
</table>

*a*nuclear spin in ground state

Table 2.1: A selection of nuclear isotopes and their properties [5].
2.2 Spin Precession and Larmor Frequency

The spin angular momentum is a vector $\mathbf{S}$, whose direction is called the spin polarization axis. Atomic nuclei with spins not equal to zero possess a microscopic magnetic moment, represented by the vector $\mathbf{\mu}$, which is parallel to the nuclear spin operator:

$$\mathbf{\mu} = \gamma \mathbf{S}$$  \hspace{1cm} (2.1)

where the proportionality constant $\gamma$ is called gyromagnetic ratio or magneto-gyric ratio. Table 2.1 contains a list of gyromagnetic ratios.

The magnetic moment of a nucleus is parallel or anti-parallel to the spin polarization axis, depending on the sign of the gyromagnetic ratio. It may be visualized as a magnetic dipole (e.g., a tiny bar magnet), which creates a magnetic dipole field. For example, the $^1\text{H}$ nuclear magnetic moment creates a magnetic dipole field of the order of 2.5 mT at a typical inter-atomic carbon-proton distance of 0.113 nm. The exact field size depends upon the relative orientation of the magnetic moment and the internuclear vector. This is considerably larger than the magnetic field of the Earth, which is of the order of 50 $\mu$T. The NMR experiments discussed in this thesis use samples which contain a large number ($> 10^{15}$) of nuclear spins. Their magnetic moments point in all possible directions in space and therefore do not give rise to any net macroscopic magnetic moment of the sample.

Nuclear spins interact with their surrounding and with each other. Usually, the interactions between nuclear spins in different molecules in the sample is considered to be small. The nuclear spins of a molecule therefore form a spin-system which is isolated from the spin-systems in other molecules. The collection of all spin-systems in the sample is called a spin ensemble.

2.2 Spin Precession and Larmor Frequency

If an external field is applied to the sample, the magnetic moments of the nuclei start precessing around the axis of the external magnetic field. The nuclear magnetic moments do not align with the external field, because the nuclei possess spin angular momentum. The spin polarization axis precesses around the external magnetic field with the Larmor frequency

$$\omega_0 = -\gamma B_0,$$ \hspace{1cm} (2.2)

where $B_0$ is the magnitude of the magnetic field at the nuclear site considered. The total sign of $-\gamma$ determines whether the spin polarization axis performs a right- or left-handed rotation around the magnetic field. Figure 2.1 shows the precession of the spin polarization axis.
2 Nuclear Magnetism

for a single spin with $\gamma > 0$, e.g., a $^{13}\text{C}$ nuclear spin. The frequency $\omega_0$ is an angular frequency and therefore given in units of rad s$^{-1}$. The unit rad for planar angles is defined as $1 \text{ rad} = 1 \text{ m m}^{-1} = 1$ and may therefore be left out. The frequency $\omega_0/2\pi$ is specified in Hz.

In modern NMR spectroscopy, external magnetic fields up to a size of 20 T are used. Table 2.1 contains the Larmor frequencies of selected nuclear isotopes in an external magnetic field with $B_0 = 9.4$ T.

2.3 Longitudinal Magnetization

The energy of a microscopic nuclear magnetic moment in an external magnetic field $B_0$ is given by

$$E = -\mathbf{\mu} \cdot \mathbf{B}_0.$$  \hspace{1cm} (2.3)

The resulting energy is lower for orientations of the magnetic moments parallel to the external field than for orientations anti-parallel to the external field. The interaction between an electron or nuclear magnetic moment with an external magnetic field is called the Zeeman interaction.

The nuclear magnetic moments are not completely isolated from their molecular surroundings, which leads to relaxation of the ensemble of magnetic moments into thermal equilibrium, where the probability that a single nuclear magnetic moment has a component parallel to the external field is slightly higher than the probability for a component anti-parallel to the field. As a result, the sample possess a macroscopic net magnetic moment parallel to the external field, called longitudinal magnetization, shown in Fig. 2.2(a). The difference in probabilities for the two orientations at room temperature is tiny, of the order of $10^{-5}$. The relaxation process is called longitudinal relaxation.

2.4 Transverse Magnetization and the NMR Signal

Because it is difficult to directly detect the longitudinal magnetization, in an NMR experiment the longitudinal magnetization is rotated into the $xy$-plane by rotating the spin polarization axis of the individual nuclear spins. This can be achieved by a radio-frequency (rf) pulse, equivalent to an oscillating magnetic field, perpendicular to the main field. The amplitude of the oscillating field can take values up to about 50 mT and is therefore much smaller than the static field. A strong effect on the nuclear spins is achieved by choosing the frequency resonant with the absolute value of the Larmor frequency of the spins. The net macroscopic magnetic moment in the transverse plane is called transverse magnetization.

The individual nuclear magnetic moments precess around the external static magnetic field, which leads to precession of the net macroscopic magnetic moment around the external field, shown in Fig. 2.2(b). For a single-spin ensemble
2.5 Nuclear Spin Interactions

Figure 2.2: (a) Longitudinal magnetization of a spin-ensemble in thermal equilibrium in an external magnetic field. (b) Transverse magnetization, precessing with the Larmor frequency $\omega_0$ around the external magnetic field. A laboratory reference frame $L$ is defined, so that the $z_L$-axis points in the direction of the external magnetic field.

The precession frequency is identical to the Larmor frequency of the nuclear spins. The precessing macroscopic transverse magnetization may be detected in a wire coil. The induced oscillating electric current is called the NMR signal or free-induction decay (FID).

The net transverse magnetization relies upon the coherent precession of the individual nuclear magnetic moments. This coherent state of the spins in the ensemble is called coherence. In an ensemble of spin-systems, which each contain several spins, different types of coherent spin-states might be obtained, as will be seen in detail later.

The transverse magnetization decays because the nuclear spins lose their coherence, due to small fluctuating fields produced by the molecular surroundings. This process is called transverse relaxation.

2.5 Nuclear Spin Interactions

Nuclear spins interact with their surroundings and with each other. The interactions may be divided into external and internal spin interactions. The interaction of the nuclear spins with the external static magnetic field and the oscillating transverse rf field are external spin interactions. Interactions with the molecular surroundings and other nuclear spins are internal spin interactions.

The external magnetic field induces currents in the electron clouds of a molecule in the sample. These currents themselves generate a magnetic field, so that the nuclear spin senses the sum of the external and induced magnetic fields. The resulting local magnetic field depends therefore upon the electronic
(chemical) structure of the molecule. This effect is called chemical shift and results in small differences in the Larmor frequencies of the nuclear spins in different molecular sites.

The magnetic moments of two nuclei interact directly with each other. The interaction is therefore called direct dipole-dipole coupling or direct dipolar coupling. The direct dipolar coupling is useful for molecular structure determination, since it depends upon the internuclear distance. In addition, nuclear spins interact indirectly with each other via bonding electrons. This interaction is called indirect dipole-dipole coupling or $J$-coupling.

Depending on whether or not a nuclear spin interaction depends upon the relative orientation of the molecule with respect to the external static field, it is referred to as either anisotropic (orientation-dependent) or isotropic (orientation-independent).

## 2.6 Fourier Transform NMR

Figure 2.3(a) shows the basic principle of a Fourier Transform NMR experiment. The pulse sequence diagram in Fig. 2.3(b) represents a simple NMR experiment, in which a single rf pulse is applied to the sample and the NMR signal is detected in the subsequent period.

The rf synthesizer generates the rf signal for the rf pulse, which has a certain reference frequency $\omega_{\text{ref}}$, phase, duration and amplitude. The rf signal is amplified and generates an oscillating transverse magnetic field $B_{\text{rf}}(t)$ in the rf coil, which is placed around the sample.

The detected NMR signal contains frequency components $\omega_j^0$, which correspond to the Larmor frequencies of different spin sites $j$ in the molecules of the sample. The quadrature receiver compares the NMR signal with the reference frequency $\omega_{\text{ref}}$ and outputs the complex NMR signal, which contains frequency components that correspond to the relative Larmor frequencies of the different spin sites:

$$\Omega_j^0 = \omega_j^0 - \omega_{\text{ref}}. \quad (2.4)$$

The time domain complex NMR signal is converted into the frequency domain NMR spectrum by a Fourier transformation. The positions of the peaks in the NMR spectrum are determined by the relative frequencies $\Omega_j^0$.

## 2.7 Liquid-State NMR

NMR spectroscopy is commonly performed on liquid and solid samples. The molecules in a liquid undergo translational and rotational motion, so that the orientation-dependent spin interactions become time dependent. If the time scale of the motion is fast compared to the time scale of the spin interaction
Figure 2.3: (a) Basic scheme of an NMR spectrometer including the processing of the NMR signal. (b) Pulse sequence diagram for a simple NMR experiment. Transverse magnetization is created by an rf pulse followed by signal detection.
(inverse of the size of the spin interaction), only the time-averaged spin interactions have a direct influence on the dynamics of the nuclear spins. The average over time is equivalent to an average over all possible molecular orientations.

In isotropic liquids, all molecular orientations have the same probability. In this case the orientational average over all anisotropic spin interactions disappears, leaving the isotropic spin interactions. This leads to NMR spectra with well resolved, narrow lines with high signal intensity and is the basis for the success of liquid-state NMR in chemistry and biology. On the other hand, the anisotropic spin interactions contain information on the molecular structure, which is not directly accessible in isotropic liquids. It should be noted that in this case structure determination is still possible, by studying the relaxation of the spin system, which is influenced by the anisotropic spin interactions.

In anisotropic liquids, the probabilities for different molecular orientations are not equal. Anisotropic spin interactions are not averaged out and allow the direct determination of molecular structural parameters, such as intermolecular distances.
3 Solid-State NMR

Since molecular motion is usually very restricted in solids, the dynamics of the nuclear spins is determined by the full anisotropic nature of the spin interactions. The consequences for the NMR spectrum depend on whether the structure of the solid is ordered or disordered.

Crystals are ordered solids with a periodic structure. NMR spectra of diluted, small nuclear spin systems in single crystals contain well-resolved resonance lines which allow the direct determination of the anisotropic spin interactions. However, it is often difficult to produce suitable crystals of sufficient size. Therefore, solid-state NMR is commonly performed on disordered samples, e.g., a powdered sample containing many tiny crystallites.

3.1 Powdered Samples

Figure 3.2(c) shows a $^{13}$C spectrum of a powdered sample of glycine. The structure of the amino acid glycine is shown in Fig. 3.1(a). The spectrum shows two broad peaks, which stem from $^{13}$C nuclei in the CO and C$_\alpha$ carbon positions.

The NMR signal of a powdered sample is a superposition of contributions from each crystallite, which are randomly oriented with respect to the external magnetic field. Since anisotropic spin interactions are orientation dependent, they are different in each crystallite, leading to peaks with different frequencies, resulting in a broad NMR spectrum. The broad powder lineshapes in the spectrum in Fig. 3.1(c) are mainly due to the anisotropic part of the chemical shift interaction. The spectral sensitivity and resolution of NMR spectra of powdered samples are generally low, limiting the application of this technique.

3.2 Cross Polarization and Heteronuclear Decoupling

In organic solids, the abundant $^1$H nuclear spins form a common network of direct dipolar couplings. This leads to a complicated behavior for the $^1$H nuclear spins. A common approach is therefore to obtain NMR spectra of nuclear spins

Figure 3.1: (a) Molecular structure of glycine. (b) Pulse sequence diagram for cross polarization to enhance the $S$-spin transverse magnetization, followed by strong proton decoupling during the detection of the $S$-spin NMR signal.
Figure 3.2: $^{13}$C spectra of a powdered sample of 99%-$^{13}$C$_2$-labelled glycine obtained with the pulse sequence shown in Fig. 3.1(b) at a magnetic field of $B_0 = 4.7$ T. (a) MAS spectrum at spinning frequency of $\omega_r/2\pi = 12000$ Hz. (b) MAS spectrum at spinning frequency of $\omega_r/2\pi = 870$ Hz. (c) Static spectrum.
3.3 Dilute Spins and Selective Labelling

with a low gyromagnetic ratio and a low natural abundance, while decoupling the abundant protons from the rare spins. Common examples for those rare spins are $^{13}\text{C}$ and $^{15}\text{N}$.

The sensitivity of NMR spectra obtained on spins with a low gyromagnetic ratio can be improved by transferring magnetization from the protons to the insensitive spins. This procedure is called cross polarization (CP) [6]. In addition, the rate by which experiments can be performed can be increased because the longitudinal relaxation of the $^1\text{H}$ spins is usually much faster than that of the insensitive spins.

Fig. 3.1(b) shows the pulse sequence diagram for the cross polarization scheme followed by strong proton decoupling. The row marked with $I$ denotes rf fields at the Larmor frequency of the abundant protons, while $S$ denotes the rf fields applied at the Larmor frequency of the rare spins (e.g., $^{13}\text{C}$). The rf fields at Larmor frequencies of different spin species can be adjusted independently from each other. The spectrometer is referred to as having several different rf channels.

First, transverse $I$-spin magnetization is produced by an appropriate rf pulse. Second, rf irradiation is applied simultaneously at the Larmor frequencies of both spin species. Magnetization is transferred from the $I$-spins to the $S$-spins if the magnitudes of the transverse magnetic fields $B_{rf}^I$ and $B_{rf}^S$ match the Hartmann-Hahn condition [7]:

$$|\gamma_I B_{rf}^I| = |\gamma_S B_{rf}^S|,$$

where $\gamma_I$ and $\gamma_S$ are the gyromagnetic ratios of the $I$- and $S$-spins respectively. The result is enhanced transverse magnetization on the $S$-spins. The enhancement is proportional to the ratio of the two gyromagnetic ratios, $|\gamma_I/\gamma_S|$.

In the subsequent period, the $S$-spin NMR signal is detected, while applying strong rf irradiation on the $I$-spins to decouple them from the $S$-spins. More sophisticated pulse sequences for heteronuclear decoupling such as TPPM [8] and C12 [9] may be used instead. The spectra in Fig. 3.2 were obtained using the pulse sequence scheme shown in Fig. 3.1(b).

3.3 Dilute Spins and Selective Labelling

Since the natural abundance of isotopes such as $^{13}\text{C}$ and $^{15}\text{N}$ is very low (see Table 2.1), their nuclear spins can be regarded as being isolated in the presence of strong proton decoupling. The resulting poor sensitivity of the NMR signals may be improved by selectively labelling the molecules with the isotopes of interest. The placement of several labels in a molecular fragment allows the study of spin dynamics in small dipolar-coupled spin systems. The spin systems may be isolated from each other by diluting the labelled molecules with unlabelled molecules.
3.4 Magic-Angle Spinning

The sensitivity and resolution of NMR spectra of disordered solids may be improved significantly by rapidly rotating the sample about an axis at the “magic angle”, \( \tan^{-1} \sqrt{2} \approx 54.74^\circ \), with respect to the static magnetic field (Fig. 3.3). This technique is called magic-angle spinning (MAS) [10,11]. The spatial rotation of the sample causes the orientation-dependent anisotropic spin interactions, such as the chemical shift anisotropies and the direct dipolar couplings, to become time-dependent and to be averaged out in the case of rapid sample spinning. The combination of CP and MAS is referred to as CP-MAS [12,13].

Figures 3.1(a) and (b) show \(^{13}\)C NMR spectra of glycine at spinning frequencies of (a) \( \omega_r/2\pi = 12000 \) Hz and (b) \( \omega_r/2\pi = 870 \) Hz. At high spinning frequencies, case (a), the anisotropic part of the chemical shift interaction is averaged out and two narrow spectral peaks are obtained, positioned at the isotropic chemical shifts, similar to a liquid-state NMR spectrum. At slow and intermediate spinning frequencies, case (b), the spectra contain sets of spinning sidebands, separated by the sample spinning frequency and centered at the respective isotropic chemical shifts.

3.5 Dipolar Recoupling

Most realistic applications of solid-state NMR in disordered solids require MAS to eliminate the effects of the anisotropic spin interactions (to a first order approximation). However, anisotropic spin interactions contain information about the molecular structure, e.g., the direct dipolar coupling depends upon the internuclear distance. Therefore, it is often desirable to temporarily recouple certain anisotropic spin interactions by applying pulse schemes of resonant rf fields to the nuclear spins, in order to suspend the averaging effect of the magic-angle rotation over a limited time interval (Fig. 3.4). These rf schemes are called...
The recoupling of dipolar couplings by rf pulse sequences is called *dipolar recoupling* and will be discussed in detail in part III of this thesis. Figure 3.5 shows one possible application of an rf pulse sequence, denoted RFDR [14,15], which recouples the dipolar couplings between spins of the same species. NMR spectra of multiply-labelled compounds contain many peaks which cannot usually be assigned easily to the corresponding spin sites in the molecule. Figure 3.5(a) shows a two-dimensional (2D) $^{13}$C spectrum of the tetrapeptide achatin-II (Gly-Phe-Ala-Asp) [15], shown in Fig. 3.5(b). The spectrum displays the isotropic chemical shifts of the molecular $^{13}$C sites in both dimensions. Cross peaks in the 2D spectrum indicate pairs of spins which are close in space to each other. Usually these are directly bonded $^{13}$C–$^{13}$C pairs. This type of 2D spectrum is called a 2D chemical shift correlation spectrum and greatly assists the assignment of MAS NMR spectra.

### 3.6 Torsional Angle Determination

Solid-state NMR has become an established tool to determine local molecular structural parameters in isotopically labelled molecules. Unlike diffraction methods, such as X-ray crystallography, solid-state NMR is not restricted to systems which form suitable crystals of sufficient size. Even in cases where the overall structure of large molecules has been solved using diffractions methods, the accuracy is often not sufficient to study fine details of the molecular structure. However, those details, like individual bond conformations, are often decisive for the function of those molecules in, for example, chemistry or biology. Unlike solution NMR, solid-state NMR has no restriction in the size of the molecules studied, nor does it require sufficient solubility. Solution NMR is particularly useful for systems of molecular mass below around 50000 molecular mass units, even though recent developments have extended this boundary to about 500000 molecular mass units in certain cases. Therefore, solid-state NMR has become increasingly successful in the study of large, disordered molecular systems, such as non-crystalline membrane proteins.

Solid-state NMR methods have been developed which allow the accurate determination of internuclear distances through the magnitude of the direct
Figure 3.5: (a) Chemical structure of [U-$^{13}$C, $^{15}$N]-achatin-II (b) Experimental 2D $^{13}$C-$^{13}$C chemical shift correlation spectrum obtained with the RFDR pulse sequence [14,15], at a magnetic field of $B_0 = 9.35$ T and a spinning frequency of $\omega_r/2\pi = 10.309$ kHz. Reprinted with permission from Ref.15. Copyright 1998, American Institute of Physics.
3.6 Torsional Angle Determination

Figure 3.6: The torsional angle $\phi$ in a molecular fragment A–B–C–D around the B–C bond axis is defined as the angle by which the bond C–D is rotated away from the bond B–A in the positive sense, when viewing along the B–C bond.

dipolar couplings. In certain cases, such as the determination of the torsional angle about a single chemical bond, this accuracy is often not sufficient. Therefore, several solid-state NMR techniques have been developed in recent years which allow the direct measurement of molecular torsional angles by determining the relative orientation of pairs of anisotropic spin interactions.

The definition of the torsional angle $\phi$ in a molecular fragment A–B–C–D around the B–C bond axis is shown in Fig. 3.6. The torsional angle $\phi$ is defined as the angle by which the bond C–D is rotated away from the bond B–A in the positive sense, when viewing along the B–C bond. Torsional angles are often very useful in describing molecular structure. Two examples are shown in Fig. 3.7. Figure 3.7(a) shows a polypeptide chain, which is built of several

Figure 3.7: Examples for the description of molecular structure by torsional angles: (a) and (b): Part of a polypeptide chain, illustrating the torsional angles $\phi$ and $\psi$. The peptide planes are indicated. (c) and (d): Two anomers of glucose, which can be distinguished by the indicated torsional angle: (c) $\alpha$-glucose, (d) $\beta$-glucose. (a) is reprinted with permission from Ref. 16. (c) and (d) by courtesy of C. E. Hughes.
individual amino acids connected by C'-N peptide bonds. A polypeptide chain consists, therefore, of a backbone of regularly repeating subunits and a variable part, the individual side chains (marked with “R”). The molecular fragment O–C’–N–H, including the peptide bond, forms a rigid planar structure, which is indicated in Fig. 3.7(a). A three dimensional view is shown in Fig. 3.7(b). Since the O–C’–N–H torsional angle is fixed to 180°, the only degrees of freedom which describe the conformation of the backbone at an individual amino acid are the torsional angles φ and ψ, which are indicated in Figs. 3.7(a) and (b). The complete backbone structure can therefore be defined by a set of (φ, ψ) torsional angles.

Figures 3.7(c) and (d) show another example of the usefulness of torsional angles to describe molecular structure. Two anomers of glucose are shown, α-glucose (c) and β-glucose (d). The measurement of the indicated H–C–C–H torsional angle allows the experimental distinction between these two anomers. In part IV of this thesis different solid-state NMR methods will be discussed which allow the direct determination of molecular torsional angles.
Part II
Theoretical Framework of Magic-Angle-Spinning NMR
4 Spin States

In quantum mechanics, the state of a physical system at a time point \( t \) is defined by specifying its state function \( \psi(t) \). Every measurable physical quantity is described by a hermitian operator. The only possible result of the measurement of a physical quantity is one of the eigenvalues of the corresponding hermitian operator. The time evolution of the quantum state \( \psi(t) \) is governed by the Schrödinger equation

\[
\frac{d}{dt} | \psi(t) \rangle = -i H(t) | \psi(t) \rangle,
\]

where \( H(t) \) is the hermitian operator, called the Hamiltonian, associated with the total energy of the system, expressed as an angular frequency, i.e., the eigenvalues of \( H(t) \) multiplied by Planck’s constant \( \hbar \) give the energy levels in Joules (J). In the following, the eigenvalues of all spin angular momentum operators will be dimensionless.

A macroscopic sample is, in principle, described by a state function, which includes the information about all the electrons and nuclei in the sample. In practice, the time scale of the electron dynamics is usually much shorter than that of the nuclear spin dynamics, which might therefore be described by a state function for the nuclear spin system and a Hamiltonian which only includes terms dependent upon the nuclear spins. This is called the spin Hamiltonian hypothesis [3].

4.1 Zeeman Eigenbasis

The state function \( | \psi_j(t) \rangle \) of a single spin \( S_j \) may be expressed in the basis of eigenfunctions \( | S, m_z \rangle \) of the operator \( S_{jz} \), i.e., the \( z \)-component of the spin angular momentum operator:

\[
S_{jz} | S, m_z \rangle = m_z | S, m_z \rangle.
\]

For \( S = \frac{1}{2} \), one obtains two eigenvalues, \( m_z = \pm \frac{1}{2} \). The corresponding eigenstates are called Zeeman states and are denoted |\( \alpha \rangle = | \frac{1}{2}, \frac{1}{2} \rangle \) and |\( \beta \rangle = | \frac{1}{2}, -\frac{1}{2} \rangle \):

\[
S_{jz} | \alpha \rangle = \frac{1}{2} | \alpha \rangle
\]
\[
S_{jz} | \beta \rangle = -\frac{1}{2} | \beta \rangle.
\]

In this basis, the spin state \( | \psi_j(t) \rangle \) may be expressed as

\[
| \psi_j(t) \rangle = c_\alpha(t) | \alpha \rangle + c_\beta(t) | \beta \rangle,
\]
4 Spin States

where \( c_\alpha(t) \) and \( c_\beta(t) \) are time-dependent complex coefficients which are normalized such that \( |c_\alpha(t)|^2 + |c_\beta(t)|^2 = 1 \) for all times \( t \). It can be shown that the spin state \( |\psi_j(t)\rangle \) is an eigenfunction, with the eigenvalue \(+1/2\), of the operator for the spin angular momentum component in a particular direction \( \mathbf{e}_p \) in space:

\[
S_j \cdot \mathbf{e}_p |\psi_j(t)\rangle = \frac{1}{2} |\psi_j(t)\rangle.
\]

(4.6)

The direction \( \mathbf{e}_p \) is the spin polarization axis, introduced in section 2.1.

In the case of a system of \( N_S \) spins-\(1/2\), a possible choice of basis set is the direct products of the Zeeman states of the individual spins. There are \( \mathcal{N} = 2^{N_S} \) basis states, denoted \( |r\rangle = |m_1 m_2 \ldots m_{N_S}\rangle \).

(4.7)

These basis states are eigenstates of the individual operators \( S_{jz} \) and the operator of the \( z \)-component of the total spin angular momentum, \( S_z \):

\[
S_z |r\rangle = M_r |r\rangle,
\]

(4.8)

with

\[
S_z = \sum_{j=1}^{N_S} S_{jz},
\]

(4.9)

and

\[
M_r = \sum_{j=1}^{N_S} m_j.
\]

(4.10)

The spin state \( |\psi(t)\rangle \) of a system of \( N_S \) spins-\(1/2\) may be expressed in the Zeeman product basis as:

\[
|\psi(t)\rangle = \sum_{r=1}^{\mathcal{N}} c_r(t) |r\rangle,
\]

(4.11)

where the coefficients \( c_r(t) \) are normalized such that \( \sum_r |c_r(t)|^2 = 1 \) for all times \( t \). The complex coefficients \( c_r(t) \) may be written as

\[
c_r(t) = |c_r(t)| \exp\{i \phi_r(t)\}.
\]

(4.12)
4.2 Time Evolution and Propagators

If the initial state \( |\psi(t_a)\rangle \) of a spin-system at time point \( t_a \) is known, the state \( |\psi(t)\rangle \) at a later time point \( t \geq t_a \) is determined by solving the Schrödinger equation (Eq. (4.1)). The propagator \( U(t, t_a) \) is defined as the operator which transforms the spin state \( |\psi(t_a)\rangle \) into the spin state \( |\psi(t)\rangle \):

\[
|\psi(t)\rangle = U(t, t_a) |\psi(t_a)\rangle,
\]

(4.13)

where the propagator \( U(t, t_a) \) solves the equation

\[
\frac{d}{dt} U(t, t_a) = -iH(t) U(t, t_a)
\]

(4.14)

\[
U(t, t_a) = \mathbb{1},
\]

(4.15)

which may be obtained by substituting Eq. (4.13) into the Schrödinger equation. The following cases may be considered:

(i) \( H \) is time-independent:

Equation (4.14) can easily be integrated:

\[
U(t, t_a) = \exp\{-iH(t - t_a)\}.
\]

(4.16)

(ii) \( [H(t'), H(t'')] = 0 \) for all time points \( t_a \leq t' \leq t'\prime \) \( t_a \leq t'' \leq t \):

This case is called *inhomogeneous* in the sense of Maricq and Waugh [17]. The propagator can also be derived for this case:

\[
U(t, t_a) = \exp\left\{-i \int_{t_a}^{t} dt'H(t')\right\}.
\]

(4.17)

(iii) \( [H(t'), H(t'')] \neq 0 \) for at least one pair of time points \( t_a \leq t' \leq t'\prime \):

This case is called *homogeneous* in the sense of Maricq and Waugh [17]. The propagator cannot, in general, be derived analytically in this case. However, consider a division of the time interval \( [t_a, t] \) into \( n \) small intervals of length \( \tau_k \) with \( k = 1, 2, \ldots, n \). Assume that \( H(t) \) is piecewise time-independent over the small intervals. The propagator in this case is given by

\[
U(t, t_a) = \exp\{-iH_n \tau_n\} \ldots \exp\{-iH_k \tau_k\} \ldots \exp\{-iH_1 \tau_1\},
\]

(4.18)

where \( H_k \) denotes the time-independent Hamiltonian operative in the \( k \)th time interval. In the case that \( H(t) \) is not piecewise time-independent, Eq. (4.18) can be considered to be a good approximation if \( n \) is sufficiently large.
4 Spin States

4.3 Rotation Operators

The operator for a rotation of spins $S$ through the angle $\xi$ around the axis $\chi = (x, y, z)$ is defined as

$$R_{\chi}(\xi) = \exp\{-i\xi S_{\chi}\}.$$  \hspace{1cm} (4.19)
5 The Nuclear Spin Hamiltonian

The nuclear spin Hamiltonian may be written as a sum over different spin interactions \( \Lambda \)

\[
H = \sum_{\Lambda} H^\Lambda. \tag{5.1}
\]

In the following, only interactions \( \Lambda \) in systems of coupled spins-\( \frac{1}{2} \) are considered.

5.1 Cartesian Representation

The Hamiltonian for the spin interaction \( \Lambda \) may be expressed with the help of a second rank cartesian tensor \( A^\Lambda \) in the following way:

\[
H^\Lambda = C^\Lambda (X^\Lambda)^T \cdot A^\Lambda \cdot Y^\Lambda
\]

\[
= C^\Lambda \left( X_x^\Lambda \quad X_y^\Lambda \quad X_z^\Lambda \right) \cdot \begin{pmatrix}
A_{xx}^\Lambda & A_{xy}^\Lambda & A_{xz}^\Lambda \\
A_{yx}^\Lambda & A_{yy}^\Lambda & A_{yz}^\Lambda \\
A_{zx}^\Lambda & A_{zy}^\Lambda & A_{zz}^\Lambda
\end{pmatrix} \cdot \begin{pmatrix}
Y_x^\Lambda \\
Y_y^\Lambda \\
Y_z^\Lambda
\end{pmatrix}, \tag{5.2}
\]

where \( X \) and \( Y \) are vector operators of the interacting physical quantities. These can be two nuclear spins or one nuclear spin and an external magnetic field. The constant \( C^\Lambda \) is characteristic for the interaction \( \Lambda \).

5.2 Spherical Representation

The components of the second rank cartesian tensor \( A^\Lambda \) can be decomposed into a scalar, \( A_0^\Lambda \), an antisymmetric first rank tensor, \( A_1^\Lambda \), and a traceless, symmetric second rank tensor, \( A_2^\Lambda \). The three resulting tensors \( A_i^\Lambda \) are the called irreducible spherical tensors of ranks \( l = 0, 1, 2 \) with corresponding cartesian tensors \( A_i^\Lambda \), which relate as

\[
A^\Lambda = A_0^\Lambda + A_1^\Lambda + A_2^\Lambda. \tag{5.3}
\]

The exact form of a tensor depends upon the coordinate system or reference frame it is expressed in. Consider two reference frames \( F \) and \( F' \), which are related to each other by rotations. The rotations from frame, \( F \) to \( F' \) can be described by three Euler angles \( \Omega_{FF'} = \{ \alpha_{FF'}, \beta_{FF'}, \gamma_{FF'} \} \). An irreducible spherical tensor \( A_i^\Lambda \) of rank \( l \) is composed of \( (2l + 1) \) components \( A_{iq}^\Lambda \), where \( q = -l, -l+1, \ldots, l \). Irreducible spherical tensors are defined as transforming in the following way under rotations of the coordinate system:

\[
[A_{iq}^\Lambda]^{F'} = \sum_{q'=-l}^{l} [A_{iq}^\Lambda]^{F} D_{q'q}^{l}(\Omega_{FF'}), \tag{5.4}
\]
where $D_{q'q}^l$ is a Wigner matrix element [18].

The Hamiltonian for the spin interaction $\Lambda$ may be expressed by a sum over scalar products of two irreducible spherical tensors, in the following way [19]:

$$H^\Lambda = C^\Lambda \sum_{l=0}^{2} \sum_{q=-l}^{l} (-1)^{q} \left[ \mathcal{A}_{lq}^\Lambda \right]^F \left[ \mathcal{T}_{l-q}^\Lambda \right]^F. \quad (5.5)$$

$\mathcal{A}_{lq}^\Lambda$ is called a spatial tensor. $\mathcal{T}_{lq}^\Lambda$ is the component $q$ of the irreducible spherical tensor of rank $l$, built from the two vector operators $X$ and $Y$ in Eq. (5.2) [19]. If $X$ and $Y$ are both spin operators, $\mathcal{T}_{lq}^\Lambda$ is called a spin tensor. If one of $X$ or $Y$ is a spin operator and the other is an external magnetic field, $\mathcal{T}_{lq}^\Lambda$ is called a spin-field tensor.

The exact form of the tensor elements depend upon the chosen reference frame $F$, whereas the scalar product of both tensors is independent of the choice of $F$. The tensors $\mathcal{A}_{lq}^\Lambda$ and $\mathcal{T}_{lq}^\Lambda$ for the different spin interactions can be found in Ref. 19.

### 5.3 Reference Frames in Solid State NMR

An overview over the relevant reference frames in solid state NMR is given in Fig. 5.1.

The principal axis system (PAS) of an interaction $\Lambda$, denoted $P^\Lambda$, is defined as the reference frame where the cartesian tensor $\mathcal{A}_{2}^\Lambda$ belonging to the second rank spatial tensor $\mathcal{A}_{2}^\Lambda$ is diagonal. The molecular frame, denoted $M$, is a reference frame fixed on the molecule and may be chosen arbitrarily. The rotor frame, denoted $R$, is a reference frame in which the $z_R$-axis coincides with the rotor axis. The laboratory frame, denoted $L$, is a reference frame in which the $z_L$-axis points in the direction of the external magnetic field.

Since the tensors $\mathcal{T}_{lq}^\Lambda$ are usually expressed in the laboratory frame $L$ of the experiment, it is necessary to transform the spatial tensors from the PAS to the laboratory frame:

$$\left[ \mathcal{A}_{lq}^\Lambda \right]^L = \sum_{q'=-l}^{l} \left[ \mathcal{A}_{lq'}^\Lambda \right]^P \left( D_{q'q}^l \right) (\Omega_{PL}^\Lambda), \quad (5.6)$$

where $\Omega_{PL}^\Lambda = \{ \alpha_{PL}^\Lambda, \beta_{PL}^\Lambda, \gamma_{PL}^\Lambda \}$ are the Euler angles relating the PAS of the interaction $\Lambda$ to the laboratory frame. Equation (5.5) becomes:

$$H^\Lambda = C^\Lambda \sum_{l=0}^{2} \sum_{q,q'=-l}^{l} (-1)^{q} \left[ \mathcal{A}_{lq'}^\Lambda \right]^P \left( D_{q'q}^l \right) (\Omega_{PL}^\Lambda) \left[ \mathcal{T}_{l-q}^\Lambda \right]^L. \quad (5.7)$$
5.4 High Field Approximation

To establish the notation in Papers I–III, the nuclear spin Hamiltonian is divided into the part describing the interaction with the rf field and a part describing the internal spin interactions and the interaction with the static field. The total spin Hamiltonian at time point $t$ may be written as

$$H(t) = H_{\text{rf}}(t) + H_{\text{int}}(t), \quad (5.8)$$

where the internal spin Hamiltonian $H_{\text{int}}(t)$ is time dependent if the molecules in the sample move or the whole sample is moved, while the rf spin Hamiltonian $H_{\text{rf}}(t)$ is time dependent because of the modulations of the rf fields. In a spin system containing two spin species $S$ and $K$, the internal Hamiltonian may be divided further:

$$H_{\text{int}}(t) = H_{\text{int}}^S(t) + H_{\text{int}}^K(t) + H_{\text{int}}^{SK}(t), \quad (5.9)$$

where $H_{\text{int}}^S(t)$ and $H_{\text{int}}^K(t)$ describe all homonuclear spin interactions of spins $S$ and $K$ respectively. The term $H_{\text{int}}^{SK}(t)$ describes the heteronuclear interactions between spins of species $S$ and $K$.

In high-field NMR of spin-$\frac{1}{2}$ nuclei, the internal spin Hamiltonian in Eq. (5.9) may be simplified by neglecting terms which do not commute with
$S_z$ and $K_z$. The remaining terms are referred to as secular terms, whereas the neglected terms are called non-secular terms. The neglecting of non-secular terms is called the secular or high-field approximation.

Since it is possible to rotate the spin angular momenta by external rf fields, the internal Hamiltonians will no longer be written in terms of components $T_{lq}^\Lambda$ of the spin or spin-field tensors, but in terms of components $T_{l,q}^{\Sigma_0}$ of pure irreducible spherical spin tensors which are defined in appendix A. This is allowed because the external magnetic field is constant and not subject to rotations. The ranks ranks $l$ and $\lambda$ are not necessarily the same. In addition, spatial tensors $A^{\Lambda}_l$ are defined, which are related to the spatial tensors $\mathcal{S}_l^\Lambda$ by numerical factors. Table 5.1 contains the relevant tensor components of $A^{\Lambda}_l$ for different spin interactions in the high-field approximation and Table 5.2 shows the relationship of the spatial tensors $A^{\Lambda}_l$ and $\mathcal{S}_l^\Lambda$ and the relationships of the spin tensor components $T_{l,q}^{\Sigma_0}$ and the components $T_{l,q}^{\Sigma}$ of the spin or spin-field tensors.

The internal Hamiltonians for homonuclear spin interactions for spins $S$ and $K$ may, in the high field approximation, be written as

$$ H_{\text{int}}^S(t) = \sum_{\Lambda_S,l,\Lambda_S} H_{l,\Lambda_S 0}^S(t) $$

$$ H_{\text{int}}^K(t) = \sum_{\Lambda_K,l,\Lambda_K} H_{l,\Lambda_K 0}^K(t), $$

where

$$ H_{l,\Lambda 0}^\Lambda(t) = \sum_{q=-l}^l [A^{\Lambda}_l]_q^P D_{q0}(\Omega^{\Lambda}_P) T_{l,\Lambda 0}^\Lambda. $$

The term $H_{l,\Lambda 0}^\Lambda(t)$ transforms as an irreducible spherical tensor of rank $l$ for spatial rotations and rank $\Lambda$ for spin rotations.

The internal Hamiltonians for heteronuclear spin interactions for spins $S$ and $K$ may, in the high field approximation, be written as

$$ H_{\text{int}}^{SK}(t) = \sum_{\Lambda_{SK},l,\Lambda_S,\Lambda_K} H_{l,\Lambda_S 0,\Lambda_K 0}^{SK}(t), $$

where the terms $H_{l,\Lambda_S 0,\Lambda_K 0}^{SK}(t)$ may be written in terms of products of the components of two irreducible spherical spin tensors, $T_{\Lambda_S 0}^{\Lambda_S} T_{\Lambda_K 0}^{\Lambda_K}$, for the spin species $S$ and $K$ respectively:

$$ H_{l,\Lambda_S 0,\Lambda_K 0}^{SK}(t) = \sum_{q=-l}^l [A^{SK}_l]_q^P D_{q0}(\Omega^{SK}_P) T_{l,\Lambda_S 0}^{\Lambda_S} T_{l,\Lambda_K 0}^{\Lambda_K}. $$
<table>
<thead>
<tr>
<th>Interaction</th>
<th>$\Lambda$</th>
<th>$F$</th>
<th>Space Part</th>
<th>Spin Part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z,CSA Zeeman and isotropic shift in rotating frame</td>
<td>Z,CS</td>
<td>$L$</td>
<td>$\omega_0$</td>
<td>$\Omega_j$</td>
</tr>
<tr>
<td>CSA isotropic shift</td>
<td>CS</td>
<td>$P$</td>
<td>$\omega_0\delta^j_{\text{iso}}$</td>
<td>0</td>
</tr>
<tr>
<td>homonuclear dipolar coupling</td>
<td>DD$_{jk}$</td>
<td>$P$</td>
<td>$\sqrt{6}b_{jk}$</td>
<td>0</td>
</tr>
<tr>
<td>homonuclear isotropic J-coupling</td>
<td>J$_{jk}$</td>
<td>$L$</td>
<td>$-\sqrt{3}2\pi J$</td>
<td>-</td>
</tr>
<tr>
<td>heteronuclear dipolar coupling</td>
<td>DD$_{SK}$</td>
<td>$P$</td>
<td>$2b_{jk}$</td>
<td>0</td>
</tr>
<tr>
<td>heteronuclear isotropic J-coupling</td>
<td>J$_{SK}$</td>
<td>$L$</td>
<td>$2\pi J$</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5.1: The space and spin parts for a list of spin interactions.
<table>
<thead>
<tr>
<th>Interaction</th>
<th>$\Lambda$</th>
<th>$C^\Lambda$</th>
<th>Space Part</th>
<th>Spin or Spin-Field Part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeeman</td>
<td>$Z$</td>
<td>$-\gamma_S$</td>
<td>$[\mathcal{A}^\Lambda_{00}]^L = -\frac{\sqrt{3}}{\omega_0} [A^\Lambda_{00}]^L$</td>
<td>$[\mathcal{T}^\Lambda_{00}]^L = -\frac{1}{\sqrt{3}} B_0 T^j_{10}$</td>
</tr>
<tr>
<td>isotropic shift</td>
<td>CS</td>
<td>$-\gamma_S$</td>
<td>$[\mathcal{A}^\Lambda_{00}]^L = -\frac{\sqrt{3}}{\omega_0} [A^\Lambda_{00}]^L$</td>
<td>$[\mathcal{T}^\Lambda_{00}]^L = -\frac{1}{\sqrt{3}} B_0 T^j_{10}$</td>
</tr>
<tr>
<td>CSA</td>
<td>CS</td>
<td>$-\gamma_S$</td>
<td>$[\mathcal{A}^\Lambda_{2q}]^P = \frac{\sqrt{3}}{\sqrt{2} 2\omega_0} [A^\Lambda_{2q}]^P$</td>
<td>$[\mathcal{T}^\Lambda_{20}]^L = \sqrt{\frac{2}{3}} B_0 T^j_{10}$</td>
</tr>
<tr>
<td>homonuclear dipolar coupling</td>
<td>DD$_{jk}$</td>
<td>1</td>
<td>$[\mathcal{A}^\Lambda_{2q}]^P = [A^\Lambda_{2q}]^P$</td>
<td>$[\mathcal{T}^\Lambda_{20}]^L = T^j_{20}$</td>
</tr>
<tr>
<td>homonuclear isotropic $J$-coupling</td>
<td>J$_{jk}$</td>
<td>$2\pi$</td>
<td>$[\mathcal{A}^\Lambda_{00}]^L = \frac{1}{2\pi} [A^\Lambda_{00}]^L$</td>
<td>$[\mathcal{T}^\Lambda_{00}]^L = T^j_{00}$</td>
</tr>
<tr>
<td>heteronuclear dipolar coupling</td>
<td>DD$_{SK}$</td>
<td>1</td>
<td>$[\mathcal{A}^\Lambda_{2q}]^P = \sqrt{\frac{3}{2}} [A^\Lambda_{2q}]^P$</td>
<td>$[\mathcal{T}^\Lambda_{20}]^L = \frac{1}{\sqrt{6}} (2 T^S_{10} T^K_{10} + T^S_{1-1} T^K_{11} + T^S_{11} T^K_{1-1})$</td>
</tr>
<tr>
<td>heteronuclear isotropic $J$-coupling</td>
<td>J$_{SK}$</td>
<td>$2\pi$</td>
<td>$[\mathcal{A}^\Lambda_{00}]^L = -\frac{\sqrt{3}}{2\pi} [A^\Lambda_{00}]^L$</td>
<td>$[\mathcal{T}^\Lambda_{00}]^L = -\frac{1}{\sqrt{3}} (T^S_{10} T^K_{10} - T^S_{1-1} T^K_{11} + T^S_{11} T^K_{1-1})$</td>
</tr>
</tbody>
</table>

Table 5.2: Relationships between the spatial tensor components $A^\Lambda_{lq}$ and $\mathcal{A}^\Lambda_{lq}$, and the spin tensor components $T^\Lambda_{\lambda 0}$ and the spin or spin-field tensor components $\mathcal{T}^\Lambda_{\lambda 0}$. 

5 The Nuclear Spin Hamiltonian
The term $H_{\lambda S0 \lambda K0}^{\lambda SK}(t)$ transforms as an irreducible spherical tensor of rank $l$ for spatial rotations, rank $\lambda_S$ for $S$-spin rotations and rank $\lambda_K$ for $K$-spin rotations.

### 5.5 Zeeman Interaction

The Hamiltonian of the Zeeman interaction of the $S$-spin nuclear magnetic moments with an external magnetic field $B_0 = B_0 \mathbf{e}_zL$ is given by

$$H^Z_S = -\mu \cdot B_0$$

$$= -\gamma_S S^T \cdot \mathbf{1} \cdot B_0$$

$$= -\gamma_S B_0 S_z$$

$$= \omega_0 S_z$$

$$= \sum_j [A_{00}^Z]^L T^j_0,$$

where $\omega_0 = -\gamma_S B_0$ is the Larmor frequency of the spin species $S$. Table 5.1 contains $[A_{00}^Z]^L$.

### 5.6 Interaction with Rf fields

During an rf pulse, the oscillating transverse magnetic field at time point $t$ is given by

$$B_{rf}(t) = B_{rt} \cos(\omega_{ref} t + \phi_p) \mathbf{e}_xL,$$

where $\omega_{ref}$ is the spectrometer reference frequency and $\phi_p$ is the rf phase, taking into account the sign of the gyromagnetic ratio [20, 21]. The reference frequency is defined as having the same sign as the Larmor frequency, $\omega_0$, of the irradiated spin species [20, 21]. The oscillating rf field may be decomposed into two counter-rotating parts. If $\omega_{ref} \approx \omega_0$, only the resonant part, rotating in the same sense as the Larmor precession, has a significant influence on the nuclear spins.

The Hamiltonian of the interaction of the $S$-spin nuclear magnetic moments with the resonant part of the oscillating transverse magnetic field is

$$H_{rt}(t) = -\frac{1}{2} \gamma_S B_{rt} \left( \cos(\omega_{ref} t + \phi_p) S_x + \sin(\omega_{ref} t + \phi_p) S_y \right).$$

The oscillatory time-dependence of the nuclear spin Hamiltonian may be removed by transforming it into the rotating frame, the reference frame rotating with the frequency $\omega_{ref}$ around the $z$-axis in the static laboratory frame. The resulting nuclear spin Hamiltonian is usually denoted $\hat{H}$ but this notation will
be used later for the Hamiltonian in the interaction frame of the rf field. From now on, all Hamiltonians are written in the rotating laboratory frame without a change in notation. The rf Hamiltonian in the rotating frame becomes:

\[ H_{\text{rf}}(t) = \omega_{\text{nut}} (\cos \phi_p S_x + \sin \phi_p S_y), \]  

(5.22)

where \( \omega_{\text{nut}} \) is the nutation frequency of the rf field, defined as

\[ \omega_{\text{nut}} = \frac{1}{2} \gamma_S B_{\text{rf}}. \]  

(5.23)

The transformation of the nuclear spin Hamiltonian into the rotating frame results in an additional term \( -\omega_{\text{ref}} S_z \), which might be included into the Zeeman interaction:

\[ H_Z^{\text{CS}} = \Omega_0 S_z, \]  

(5.24)

where the frequency offset \( \Omega_0 \) is defined as

\[ \Omega_0 = \omega_0 - \omega_{\text{ref}}. \]  

(5.25)

### 5.7 Chemical Shift Interaction

The interaction of a nuclear spin \( S_j \) with the magnetic field induced by the external fields in the electron clouds of the molecule can be described by the chemical shift tensor \( \delta_j \):

\[ H_j^{\text{CS}} = -\gamma_S S^T \cdot \delta_j \cdot B_0. \]  

(5.26)

The *deshielding* convention [3] is used for the chemical shift tensor. The cartesian tensor \( \delta^j \) may be decomposed into irreducible spherical tensors of rank \( l = 0, 1, 2 \), with the corresponding cartesian tensors \( \delta^j_l \).

The rank 0 part is the *isotropic chemical shift*. The rank 1 part is the anti-symmetric chemical shift, which can be ignored in the high-field approximation. The rank 2 part is the *chemical shift anisotropy*. The sum of the cartesian tensors for the isotropic chemical shift and the CSA, \( \delta_{\text{iso}}^j = \delta^j_0 + \delta^j_2 \), is diagonal in its own PAS. The diagonal elements \( \delta_{xx}^P, \delta_{yy}^P \) and \( \delta_{zz}^P \) are called the *principal values* of the chemical shift tensor. By convention, the principal values are labelled such that

\[ |\delta_{zz}^P - \delta_{\text{iso}}| \geq |\delta_{xx}^P - \delta_{\text{iso}}| \geq |\delta_{yy}^P - \delta_{\text{iso}}|, \]  

(5.27)

where the isotropic chemical shift is

\[ \delta_{\text{iso}}^j = \frac{1}{3} (\delta_{xx}^P + \delta_{yy}^P + \delta_{zz}^P). \]  

(5.28)
The CSA is associated with an anisotropic chemical shift frequency \( \omega_{\text{aniso}}^j \) and an asymmetry parameter \( \eta \), defined as

\[
\omega_{\text{aniso}}^j = \omega_0 (\delta_{zz}^j - \delta_{\text{iso}}^j),
\]

and

\[
\eta = \frac{\delta_{yy}^j - \delta_{xx}^j}{\delta_{zz}^j - \delta_{\text{iso}}^j}.
\]

The chemical shift Hamiltonian in the high-field approximation is given by

\[
H_{j}^{\text{CS}} = ([A_{00}^{\text{CS}}]_L + [A_{20}^{\text{CS}}]_L) S_{jz} + ([A_{00}^{\text{CS}}]_L + [A_{20}^{\text{CS}}]_L) T_{10}^j,
\]

where \([A_{00}^{\text{CS}}]_L \) (in the laboratory frame) and \([A_{20}^{\text{CS}}]_P \) (in the PAS) are listed in Table 5.1.

The Zeeman Hamiltonian for spin \( S_j \) in the rotating frame may be combined with the chemical shift Hamiltonian, defining a chemical shift frequency by

\[
\Omega_0^j = \omega_0 (1 + \delta_{\text{iso}}^j) - \omega_{\text{ref}}.
\]

The combined Hamiltonian has the same form as Eq. (5.31),

\[
H_{j}^{Z,\text{CS}} = ([A_{00}^{\text{Z,CS}}]_L + [A_{20}^{\text{CS}}]_L) T_{10}^j,
\]

where \([A_{00}^{\text{Z,CS}}]_L \) can be found in Table 5.1.

### 5.8 Direct Dipole-Dipole Interaction

#### 5.8.1 Homonuclear Direct Dipolar Couplings

The direct homonuclear dipolar coupling between two spins \( S_j \) and \( S_k \) can be described by the traceless symmetric cartesian tensor \( \mathbb{D}_{jk} \):

\[
H_{jk}^{\text{DD}} = S_j^T \cdot \mathbb{D}_{jk} \cdot S_k.
\]

In the high-field approximation, the Hamiltonian of the direct dipolar coupling may be written as

\[
H_{jk}^{\text{DD}} = [A_{20}^{\text{DD,}\lambda}]_L T_{jk}^{\lambda} = \omega_{jk} \frac{1}{\sqrt{6}} \left( 2S_{jz} S_{kz} - \frac{1}{2} (S_j^+ S_k^- + S_j^- S_k^+) \right),
\]

where \([A_{20}^{\text{DD,}\lambda}]_L \) is listed in Table 5.1.
where $[A_{20}^{DD,jk}]^P$ (in the PAS) is given in Table 5.1. The homonuclear dipolar coupling constant is defined as

$$b_{jk} = -\frac{\mu_0}{4\pi} \frac{\gamma_S^2 \gamma_k^2}{r_{jk}^3},$$  \hspace{1cm} (5.38)

where $r_{jk}$ is the spin-spin internuclear distance. For example, a $^{13}\text{C}^{-13}\text{C}$ distance of 0.152 nm corresponds to a dipolar coupling constant of $b_{jk}/2\pi = -2155$ Hz.

### 5.8.2 Heteronuclear Direct Dipolar Couplings

The direct heteronuclear dipolar coupling between two spins $S$ and $K$ is correspondingly given by

$$H_{SK}^{DD} = S^T \cdot D_{SK} \cdot K.$$  \hspace{1cm} (5.39)

In the high-field approximation, this reduces to

$$H_{SK}^{DD} = [A_{20}^{DD,SK}]^P T_{10}^S T_{10}^K$$  

$$= \omega_{SK} S_z K_z,$$  \hspace{1cm} (5.40)

$$= \omega_{SK} S_z K_z,$$  \hspace{1cm} (5.41)

where the definition of $[A_{20}^{DD,SK}]^P$ (in the PAS), given in Table 5.1, is unusual because it includes a factor of 2 which is usually kept together with the spin operator $S_z K_z$. For the purposes of this thesis, on the other hand, the definition in Eq. (5.40) and Table 5.1 is more practical. The heteronuclear dipolar coupling constant is defined as

$$b_{SK} = -\frac{\mu_0}{4\pi} \frac{\gamma_S \gamma_K \hbar}{r_{SK}^3}.$$  \hspace{1cm} (5.42)

For example, a $^{13}\text{C}^{-1}\text{H}$ distance of 0.113 nm corresponds to a dipolar coupling constant of $b_{SK}/2\pi = -20.94$ kHz. A $^{13}\text{C}^{-15}\text{N}$ distance of 0.133 nm corresponds to a dipolar coupling constant of $b_{SK}/2\pi = 1307$ Hz.

### 5.9 Indirect Dipole-Dipole Interaction

#### 5.9.1 Homonuclear $J$-Couplings

The electron mediated homonuclear $J$-coupling between two spins $S_j$ and $S_k$ can be described by the cartesian tensor $J_{jk}$:

$$H_{jk}^J = 2\pi \ S_j^T \cdot J_{jk} \cdot S_k.$$  \hspace{1cm} (5.43)

The cartesian tensor $J_{jk}$ may be decomposed into irreducible spherical tensors of rank $l = 0, 1, 2$. 

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The rank 0 part is the isotropic J-coupling. The Hamiltonian for the isotropic J-coupling is

\[ H^J_{jk} = 2\pi J_{jk} \mathbf{S}_j \cdot \mathbf{S}_k \]  

(5.44)

where \( J_{jk} \) is the homonuclear isotropic J-coupling constant which is given in Hz. \([A^J_{00}]^L\) is given in Table 5.1.

The rank 1 part is the antisymmetric J-coupling, which is usually ignored. The rank 2 part is the anisotropic J-coupling, which may be described by an anisotropic J-coupling constant and an asymmetry parameter. The coupling constant is usually of the same order of magnitude as the isotropic J-coupling constant. The corresponding irreducible spin tensor component for the anisotropic J-coupling is the same as for the direct homonuclear dipolar coupling, \( T^j_{20} \).

### 5.9.2 Heteronuclear J-Couplings

The heteronuclear J-coupling between two spins \( S \) and \( K \) is correspondingly given by

\[ H^J_{SK} = \mathbf{S}^T \cdot \mathbf{J}_{SK} \cdot \mathbf{K}. \]

(5.46)

The Hamiltonian for the isotropic J-coupling in the high-field approximation is

\[ H^J_{SK} = 2\pi J_{SK} S_z K_z \]  

(5.47)

\[ = [A^J_{00}]^L T^S_{10} T^K_{10}, \]  

(5.48)

where \( J_{SK} \) is the heteronuclear isotropic J-coupling and \([A^J_{00}]^L\) is given in Table 5.1.

### 5.10 Magic-Angle Spinning

For the description of MAS experiments, the transformation of the irreducible spherical spatial tensors \( A^\Lambda_l \) from the PAS to the laboratory frames is done in several steps, as shown in Fig. 5.1.

In the first step, the tensors for different spin interactions \( \Lambda \) are transformed into the molecular frame. The Euler angles \( \Omega^\Lambda_{PM} = \{\alpha^\Lambda_{PM}, \beta^\Lambda_{PM}, \gamma^\Lambda_{PM}\} \) describe the relative orientation of the PAS of the interaction \( \Lambda \) and the molecule-fixed frame.

\[ [A^\Lambda_{l0}]^L = \sum_{m', m'' = -l} [A^\Lambda_{lm''}]^P D^l_{m''m'}(\Omega^\Lambda_{PM}) D^l_{m'0}(\Omega_{ML}), \]  

(5.49)
The Nuclear Spin Hamiltonian

where the Euler angles \( \Omega_{ML} = \{\alpha_{ML}, \beta_{ML}, \gamma_{ML}\} \) describe the relative orientation of the molecular frame to the laboratory frame. Since, in a powdered sample, each crystallite is randomly oriented with respect to the laboratory frame, the Euler angles \( \Omega_{ML} \) are random variables.

In MAS NMR the transformation from the molecular frame to the laboratory frame is further divided. The interaction tensors are first transformed into the rotor frame. This transformation is described by the Euler angles \( \Omega_{MR} = \{\alpha_{MR}, \beta_{MR}, \gamma_{MR}\} \), which are random variables in a powdered sample. In the last step, the interactions are transformed from the rotor frame to the laboratory frame:

\[
[A_{lm}^\Lambda]^L = \sum_{m,m',m''=-l}^l [A_{lm''}^\Lambda]^P D_{m''m'}^l(\Omega_{PM}^\Lambda) D_{m'm}^l(\Omega_{MR}) D_{m0}^l(\Omega_{RL}),
\]

Assume that the sample is rotated at the magic angle, \( \beta_{RL} = \tan^{-1} \sqrt{2} \), with the angular frequency \( \omega_r \) and that \( \alpha_{RL}^0 \) defines the position of the rotor at time point \( t = 0 \). The Euler angles relating the rotor and the laboratory frame are at time point \( t \) conventionally given by

\[
\Omega_{RL}(t) = \{\alpha_{RL}, \beta_{RL}, \gamma_{RL}\} = \{\alpha_{RL}^0 - \omega_r t, \tan^{-1} \sqrt{2}, 0\},
\]

Using the definition of the Wigner matrices [18], the interaction tensor in the laboratory frame may be written as

\[
\omega^\Lambda(t) = [A_{00}^\Lambda]^L
= \sum_{m=-l}^l [A_{lm}^\Lambda]^R d_{m0}^l(\beta_{RL}) \exp\{i\omega_r t - \alpha_{RL}^0\},
\]

where \( [A_{lm}^\Lambda]^R \) is the interaction tensor in the rotor frame:

\[
[A_{lm}^\Lambda]^R = \sum_{m',m''=-l}^l [A_{lm''}^\Lambda]^P D_{m''m'}^l(\Omega_{PM}) D_{m'm}^l(\Omega_{MR}).
\]

Note that for exact magic-angle spinning, \( d_{00}^l(\beta_{RL}) = 0 \), i.e., the zeroth component of a rank 2 spatial interaction tensor is zero.

The homonuclear part of the internal Hamiltonian under MAS at time point \( t \) may be written:

\[
H_{\text{int}}(t) = \sum_{\Lambda,l,m,\lambda} H_{lm\lambda0}^\Lambda(t),
\]

where

\[
H_{lm\lambda\mu}^\Lambda(t) = \omega_{lm}^\Lambda \exp\{i\omega_r t\} T_{\lambda\mu}^\Lambda,
\]
\[ \omega_{lm}^A = [A_{lm}^A] R d_{m0}^l (\beta_{RL}) \exp\{-ima^0_{RL}\}. \] (5.56)

The heteronuclear part of the internal Hamiltonian under MAS at time point \( t \) may be written:

\[ H_{\text{int}}^{SK}(t) = \sum_{\lambda, l, m, \lambda_S, \lambda_K} H_{lm}^{\lambda} \lambda_S^0 \lambda_K^0(t), \] (5.57)

where

\[ H_{lm}^{\lambda_{SK}} \lambda_{S\mu} \lambda_{K\mu}(t) = \sum_{\lambda_{SK}, l, m, \lambda_S, \mu_S, \lambda_K, \mu_K} \omega_{lm}^{\lambda_{SK}} \exp\{im\omega_r t\} T_{\lambda_S \mu_S}^{\lambda} T_{\lambda_K \mu_K}^{\lambda}, \] (5.58)

and

\[ \omega_{lm}^{\lambda_{SK}} = [A_{lm}^{\lambda_{SK}}]^R d_{m0}^l (\beta_{RL}) \exp\{-ima^0_{RL} t\}. \] (5.59)
6 Density Operator

Consider an NMR sample containing an ensemble of spin-systems, isolated from each other. It is not practical to describe the complete spin-ensemble by a single state function. Instead, the spin ensemble is completely described by the density operator

\[ \rho(t) = |\psi_k(t)\rangle \langle \psi_k(t)| = \sum_k p_k |\psi_k(t)\rangle \langle \psi_k(t)|, \]  

(6.1)

where \( p_k \) is the probability that an individual spin-system is in the spin state \( |\psi_k(t)\rangle \), which, according to Eq.(4.11), may be expressed in the basis of the Zeeman product states:

\[ |\psi_k(t)\rangle = \sum_r^N c^k_r(t) |r\rangle. \]  

(6.2)

The statistical expectation value of an operator \( Q \) over the spin-ensemble is given by the average of the quantum-mechanical expectation values:

\[ \langle Q \rangle(t) = \sum_k p_k \langle \psi_k(t)| Q |\psi_k(t)\rangle = \text{Tr}\{\rho(t)Q\}. \]  

(6.3)

The density operator expressed in the Zeeman product basis has the form

\[ \rho(t) = \sum_{r,s} \rho_{rs}(t) |r\rangle \langle s|, \]  

(6.4)

where the matrix elements \( \rho_{rs}(t) = \langle r|\rho(t)|s \rangle \) are given by

\[ \rho_{rr}(t) = \frac{c^k_r(t)c^k_r(t)^*}{|c^k_r(t)|^2} \]  

(6.5)

\[ \rho_{rs}(t) = \frac{c^k_r(t)c^k_s(t)^*}{|c^k_r(t)||c^k_s(t)| \exp\{i(\phi_r(t) - \phi_s(t))\}}, \]  

(6.6)

The diagonal elements of the density operator, \( \rho_{rr}(t) \), are called the populations of the states \( |r\rangle \). The off-diagonal elements \( \rho_{rs}(t) \) \( (r \neq s) \) are called coherences between states \( |r\rangle \) and \( |s\rangle \). Equation(6.6) shows which conditions must be fulfilled for the existence of coherence between states \( |r\rangle \) and \( |s\rangle \): (i) The spin states of the individual spin-systems must be superposition states, involving \( |r\rangle \) and \( |s\rangle \). (ii) The phases \( \phi_r(t) \) and \( \phi_s(t) \) must be correlated over the ensemble. The existence of coherence in a spin-ensemble is equivalent to the existence of correlated spin states.

The operator \( |r\rangle \langle s| \) associated with the coherence \( \rho_{rs}(t) \) is an eigenoperator of \( S_z \):

\[ [S_z, |r\rangle \langle s|] = p_{rs} |r\rangle \langle s|, \]  

(6.7)
\[ p_{rs} = M_r - M_s. \quad (6.8) \]

where \( p_{rs} \) is the coherence order between states \(|r\rangle\) and \(|s\rangle\):

Table 6.1 gives some examples of spin operators and the coherences they are associated with.

Consider an ensemble of spin-systems containing spins of species \( S \). The density operator \( \rho_{eq} \) in an external magnetic field in thermal equilibrium at temperature \( T \) is, in the high-field approximation, given by

\[ \rho_{eq} = \frac{\exp\{-\frac{\hbar \hat{H}_S^Z}{k_B T}\}}{\text{Tr}\{\exp\{-\frac{\hbar \hat{H}_S^Z}{k_B T}\}\}}, \quad (6.9) \]

where \( k_B \) is the Boltzmann constant. In the limit of high temperatures (\( k_B T \gg |\hbar \omega_0| \), which is easily fulfilled at room temperature), this simplifies to

\[ \rho_{eq} \approx \frac{1}{2N_S} \mathbb{1} + \frac{1}{2N_S} \mathbb{B} S_z, \quad (6.10) \]

where the Boltzmann factor is defined as

\[ \mathbb{B} = \frac{\hbar \gamma_S B_0}{k_B T}. \quad (6.11) \]

Usually, the operator \( \mathbb{1} \), which does not contribute to any NMR signal, and the factors in Eq. (6.10), which determine the signal intensity, are neglected. Hence, the density operator in thermal equilibrium simplifies to

\[ \rho_{eq} \approx S_z. \quad (6.12) \]

Table 6.1: A selection of spin operators and their corresponding coherence orders.

| \( \rho \) | \( p_{rs} \) | Name
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( S_j^+ )</td>
<td>+1</td>
<td>((+1))-quantum coherence ( (1QC) )</td>
</tr>
<tr>
<td>( S_j^- )</td>
<td>-1</td>
<td>((-1))-quantum coherence ( (ZQC) )</td>
</tr>
<tr>
<td>( S_j^+ S_k^- )</td>
<td>0</td>
<td>zero-quantum coherence ( (ZQC) )</td>
</tr>
<tr>
<td>( S_j^- S_k^+ )</td>
<td>0</td>
<td>double-quantum coherence ( (2QC) )</td>
</tr>
</tbody>
</table>

where \( p_{rs} \) is the coherence order between states \(|r\rangle\) and \(|s\rangle\):
6 Density Operator

6.1 Time Evolution

The time evolution of the density operator $\rho(t)$ is governed by the Liouville-von Neumann equation

$$\frac{d}{dt}\rho(t) = -i[H(t), \rho(t)]. \quad (6.13)$$

If the initial density operator $\rho(t_a)$ of the spin-ensemble at time point $t_a$ is known, the density operator $\rho(t)$ at a later time point $t \geq t_a$ is obtained by

$$\rho(t) = U(t, t_a) \rho(t) U(t, t_a)^\dagger, \quad (6.14)$$

where the propagator $U(t, t_a)$ solves Eq. (4.14).

6.2 Rf Pulses

Consider an rf pulse with starting time point $t_a$, constant nutation frequency $\omega_{\text{nut}}$, constant phase $\phi$ and duration $\tau$. The flip angle $\xi$ of the pulse is given by

$$\xi = \omega_{\text{nut}} \tau, \quad (6.15)$$

and the standard notation for such an rf pulse is $\xi_{\phi}$, where $\xi$ and $\phi$ may be specified in degrees or radians. The propagator during the rf pulse at a time point $t_a \leq t \leq t_a + \tau$ is given by:

$$U_{\text{rf}}(t, t_a) = \exp\left\{ -i\omega_{\text{nut}}(\cos \phi S_x + \sin \phi S_y)(t - t_a) \right\} \quad (6.16)$$

$$= R_z(\phi) R_x(\omega_{\text{nut}}(t - t_a)) R_z(-\phi). \quad (6.17)$$

The propagator for the complete rf pulse is given by:

$$U_{\text{rf}}(t_a + \tau, t_a) = R_z(\phi) R_x(\xi) R_z(-\phi). \quad (6.18)$$

6.3 NMR Signal

Consider the case of signal detection on the $S$-spins with $\gamma_S > 0$. The output of the quadrature receiver (see Fig. 2.3) is the complex NMR signal which is digitized and stored in a computer. The digitized NMR signal $s(t)$ can be linked to the density operator $\rho(t)$ in the following way [20]:

$$s(t) \sim 2i \text{Tr}\left\{ \rho(t) \sum_{j=1}^{N_S} S_j^+ \right\} \exp\left\{ -i(\Phi_{\text{rec}} + \Phi_{\text{dig}}) \right\}, \quad (6.19)$$

where $\Phi_{\text{rec}}$ is the rf receiver phase and $\Phi_{\text{dig}}$ the post-digitization phase shift [20]. The consequence of Eq. (6.19) is that only $(-1)$-quantum coherences are detectable as NMR signals.


## 7 Average Hamiltonian Theory

Consider a spin-system evolving under the time-dependent Hamiltonian $H(t)$. The evolution from a time point $t_0^0$ to a later time point $t_0^0 + T$ is described by the propagator $U(t_0^0 + T, t_0^0)$, which, in general, cannot be determined analytically, as discussed in section 4.2. In the following, average Hamiltonian theory [22,23] is discussed, which allows the analytical approximation of the propagator $U(t_0^0 + T, t_0^0)$. The time-independent effective or average Hamiltonian, $\tilde{H}$, over the time interval $[t_0^0, t_0^0 + T]$ is defined by

$$U(t_0^0 + T, t_0^0) = \exp\{-i\tilde{H}T\}. \quad (7.1)$$

The effective Hamiltonian may be expanded in a series expansion, called the Magnus expansion [24]:

$$\tilde{H} = \tilde{H}^{(1)} + \tilde{H}^{(2)} + \tilde{H}^{(3)} + \ldots \quad (7.2)$$

where the first two orders are given by

$$\tilde{H}^{(1)} = T^{-1} \int_{t_0^0}^{t_0^0 + T} dt \, H(t) \quad (7.3)$$

$$\tilde{H}^{(2)} = (2iT)^{-1} \int_{t_0^0}^{t_0^0 + T} dt' \int_{t_0^0}^{t_0^0 + T} dt \, [H(t'), H(t)] \quad (7.4)$$

The Magnus expansion converges rapidly if, for any time $t_0^0 \leq t \leq t_0^0 + T$, the condition

$$||H(t)||T \ll 1 \quad (7.5)$$

is fulfilled, where the norm, $||H(t)||$, might, for example, be chosen to be

$$||H(t)|| = (\text{Tr}\{H(t)^2\})^{1/2}. \quad (7.6)$$

The condition in Eq. (7.5) is very conservative and is not necessary fulfilled in many practical applications of the Magnus expansion.

### 7.1 Interaction Frame

The convergence of the Magnus expansion may be speeded up by transforming the Hamiltonian $H(t)$ into a suitable interaction frame. Consider the case where the Hamiltonian may be expressed as a sum of two terms

$$H(t) = H_A(t) + H_B(t), \quad (7.7)$$
where both parts may be time-dependent and might not commute in general. The term $H_A(t)$ should be chosen in such a way that the propagator $U_A(t, t_a)$, solving the equations
\[
\frac{d}{dt} U_A(t, t_a) = -i H_A(t) U_A(t, t_a) \quad (7.8)
\]
\[
U_A(t_a, t_a) = 1, \quad (7.9)
\]
can easily be determined analytically. The interaction frame Hamiltonian is denoted $\tilde{H}(t)$ and is obtained by the transformation
\[
\tilde{H}(t) = U_A(t, t_a) H_B(t) U_A(t, t_a). \quad (7.10)
\]
Note that the propagator $U_A(t, t_a)$, which depends upon two time points, is used as the transformation operator, which strictly speaking should only depend upon the time point $t$ [25].

The propagator under the interaction frame Hamiltonian is denoted $\tilde{U}(t, t_a)$ and solves the equations
\[
\frac{d}{dt} \tilde{U}(t, t_a) = -i \tilde{H}(t) \tilde{U}(t, t_a) \quad (7.11)
\]
\[
\tilde{U}(t_a, t_a) = 1, \quad (7.12)
\]

The propagation under the total Hamiltonian $H(t)$ in Eq. (7.7) is consequently given by
\[
U(t, t_a) = U_A(t, t_a) \tilde{U}(t, t_a) \quad (7.13)
\]
The evolution of the spin-system from a time point $t_0$ to a later time point $t_0 + T$ is therefore described by
\[
U(t_0 + T, t_0) = U_A(t_0 + T, t_0) \tilde{U}(t_0 + T, t_0) \quad (7.14)
\]
The interaction frame propagator $\tilde{U}(t_0 + T, t_0)$ may be expressed in terms of an effective Hamiltonian analogous to Eq. (7.1) and analyzed by the Magnus expansion Eq. (7.2). The Hamiltonian in Eqs. (7.3) and (7.4) has to be replaced by the interaction frame Hamiltonian $\tilde{H}(t)$.

### 7.2 Rf pulse sequences under MAS

Consider a system of $S$-spins under MAS conditions to which an rf pulse sequence is applied. Suppose that the rf pulse sequence starts at time point $t_0$ and has a duration of $T$. The internal spin Hamiltonian in Eqs. (5.8) and (5.54) may be transformed into the interaction frame of the rf field, i.e., we identify $H_A(t) = H_{\text{rf}}(t)$ and $H_B(t) = H_{\text{int}}(t)$:
\[
\tilde{H}(t) = U_{\text{rf}}(t, t_0) H_{\text{int}}(t) U_{\text{rf}}(t, t_0). \quad (7.15)
\]
7.2 Rf pulse sequences under MAS

The interaction frame Hamiltonian at time point $t$ may, in general, be written as a series expansion in terms of the irreducible spin operators $T^A_{\lambda\mu}$:

$$\tilde{H}(t) = \sum_{\Lambda, l, m, \lambda, \mu} \tilde{H}^A_{lm\lambda\mu}(t), \quad (7.16)$$

where

$$\tilde{H}^A_{lm\lambda\mu}(t) = \frac{\text{Tr}\left\{T^A_{\lambda\mu} U_{\text{rf}}(t, 0) H^A_{lm\lambda0}(t) U_{\text{rf}}(t, t_0^0) T^A_{\lambda\mu}\right\}}{\text{Tr}\left\{T^A_{\lambda\mu} T^A_{\lambda\mu}\right\}} T^A_{\lambda\mu} \quad (7.17)$$

The first order average Hamiltonian may be written as

$$\bar{H}^{(1)} = \sum_{\Lambda, l, m, \lambda, \mu} \tilde{H}^A_{lm\lambda\mu}, \quad (7.18)$$

where

$$\bar{H}^A_{lm\lambda\mu} = T^{-1} \int_{t_0^0}^{t_0^0 + T} dt \tilde{H}^A_{lm\lambda\mu}(t). \quad (7.19)$$

In part III, rotor-synchronized rf pulse sequences will be discussed, which allow the derivation of simple selection rules which predict when a term $\bar{H}^A_{lm\lambda\mu}$ in the first order average Hamiltonian disappears, depending upon the chosen symmetry of the pulse sequence.
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Dipolar Recoupling in
Magic-Angle-Spinning NMR
8 Introduction

In section 3.5, the concept of recoupling pulse sequences was introduced, which suspend the averaging effect of MAS over a limited time interval and therefore recouple certain anisotropic spin interactions.

The averaging effect of the sample rotation can also be enhanced by appropriate radio frequency schemes, which are called decoupling pulse sequences. Isotropic spin interactions are not orientationally dependent and are therefore not averaged out by the sample rotation. They may only be removed by appropriate decoupling sequences. On the other hand, decoupling sequences might also be designed to decouple all spin interactions, but preserve certain isotropic spin interactions, such as the isotropic chemical shift and isotropic $J$-couplings.

Figure 8.1 gives an overview of different spin interactions, which might be recoupled or preserved using radio frequency pulse sequences. The spin interactions are divided into one-spin and two-spin interactions. The isotropic chemical shift might be preserved by decoupling pulse sequences, which remove all other spin interactions. The chemical shift anisotropy might be reintroduced by an appropriate CSA recoupling sequence. In the case of two-spin interactions, homonuclear and heteronuclear spin interactions can be distinguished. In each case there are the direct dipolar couplings and the isotropic $J$-couplings. In a homonuclear spin-system, homonuclear dipolar recoupling reintroduces the direct homonuclear dipolar interactions by a homonuclear dipolar recoupling sequence. The homonuclear isotropic $J$-coupling might be preserved by decoupling pulse sequences, which remove all other spin interactions.

In a heteronuclear spin-system rf irradiation might be applied to only one or to both spin species. The rf pulse schemes are referred to as single-channel and dual-channel sequences, respectively. Heteronuclear dipolar recoupling reintroduces the direct heteronuclear dipolar interactions by a heteronuclear dipolar recoupling sequence, whereas the heteronuclear isotropic chemical shift might be preserved by decoupling pulse sequences, which remove all other spin interactions.

This thesis will concentrate on dipolar recoupling: (i) homonuclear dipolar recoupling and (ii) heteronuclear dipolar recoupling using dual-channel rf pulse sequences. In general, these sequences have the following applications:

(i) Excitation of Multiple-Quantum Coherence:
Dipolar recoupling sequences may be used to excite multiple-quantum coherence (MQC) in multiple-spin systems. MQC can not be detected directly in an NMR experiment, therefore an experimental scheme has to be applied to excite MQC and at a later stage convert it into observable magnetization (single-quantum coherence). If the signals passing through MQC are selected by an appropriate phase cycle, the whole procedure is called multiple-quantum filtration (MQF). Not all dipolar recoupling se-
Figure 8.1: Overview of recoupling and preservation of spin interactions under MAS.
8.1 Homonuclear Dipolar Average Hamiltonians

quences are suitable for the excitation of MQC, will be seen later. MQF is especially useful if NMR experiments are performed on large molecules which are selectively labelled with an isotope of spin-$\frac{1}{2}$ in certain positions in a molecular fragment. The natural abundance background of signals from single spins may be suppressed by MQF, leaving only signals from the isotopically labelled multiple-spin systems.

(ii) Determination of Internuclear Distances:
An important application of dipolar recoupling sequences is to determine internuclear distances. In the simplest case of a two-spin system, the evolution of the NMR spectral intensities is measured as a function of the time interval during which a dipolar recoupling sequence is applied. The resulting curve is sensitive to the internuclear distance. The dipolar recoupling sequences used for this purpose may be suitable to excite MQC, which allows the distance determination between two isotopically labelled spin-sites in a large molecule.

(iii) Determination of Bond-Angles and Torsional Angles:
Dipolar recoupling sequences might be used in experiments which determine the relative orientation of pairs of anisotropic spin interactions. These experiments allow the direct determination of molecular bond angles and torsional angles. These type of experiments will be discussed in part IV of this thesis.

(iv) Magnetization Exchange:
In general, dipolar recoupling sequences may be used to transfer magnetization between spins. Suitable experiments using these techniques allow the identification of spins which are directly bonded or close in space to each other.

In the following, homonuclear and heteronuclear recoupling sequences are classified according to the possible first order dipolar average Hamiltonians they generate. Figure 8.1 contains a list of those Hamiltonians.

8.1 Homonuclear Dipolar Average Hamiltonians

For the case of homonuclear dipolar recoupling, consider a two-spin system $S_j$ and $S_k$. Equations (8.1)–(8.3) show three different time independent dipolar average Hamiltonians, which may be generated using a homonuclear dipolar recoupling rf pulse sequence.

The Hamiltonian in Eq. (8.1) is called a homonuclear zero-quantum average Hamiltonian and the rf pulse sequence generating this type of Hamiltonian is referred to as a homonuclear zero-quantum recoupling sequence. The Hamiltonian contains only two-spin terms with a total spin component of $\mu = 0$,
i.e., the spin term is given by the irreducible tensor operator $T_{20}^{jk}$. Strictly speaking, only the terms $S_j^-S_k^+$ and $S_j^-K_k^+$ are referred to as zero-quantum terms, whereas the term $S_jzS_kz$ is called longitudinal two-spin order. The average Hamiltonian in Eq. (8.1) has the same form as the Hamiltonian for the direct homonuclear dipolar coupling in Eq. (5.37). This type of average Hamiltonian is not suitable to excite higher order homonuclear MQC directly. On the other hand, it is ideal for the purpose of exchange of longitudinal magnetization. The zero-quantum average Hamiltonian conserves the total longitudinal magnetization in multiple-spin systems, leading to a uniform distribution of magnetization at long mixing intervals, during which the recoupling sequence is applied.

The Hamiltonian in Eq. (8.2) is called a homonuclear single-quantum average Hamiltonian and the rf pulse sequence generating this type of Hamiltonian is referred to as a homonuclear single-quantum recoupling sequence. The Hamiltonian contains only two-spin terms with a total spin component of $\mu = \pm 1$, i.e., the spin terms are given by the irreducible tensor operators $T_{2\pm1}^{jk}$. This type of Hamiltonian may be used to excite homonuclear MQC.

The Hamiltonian in Eq. (8.3) is called a homonuclear double-quantum average Hamiltonian and the rf pulse sequence generating this type of Hamiltonian is referred to as a homonuclear double-quantum recoupling sequence. The Hamiltonian contains only two-spin terms with a total spin component of $\mu = \pm 2$, i.e., the spin terms are given by the irreducible tensor operators $T_{2\pm2}^{jk}$. This type of Hamiltonian may be used to excite homonuclear MQC.

### 8.2 Heteronuclear Dipolar Average Hamiltonians

For the case of heteronuclear dipolar recoupling, consider a heteronuclear two-spin system $S_s$ and $K_k$. Four different time-independent heteronuclear dipolar average Hamiltonians are shown in Eqs. (8.4)–(8.7). These average Hamiltonians may be generated using a dual-channel heteronuclear dipolar recoupling rf pulse sequence.

The Hamiltonian in Eq. (8.4) will be referred to as a longitudinal two-spin average Hamiltonian. It contains only terms with the spin components $(\mu_s, \mu_K) = (0, 0)$, i.e., the spin term is given by the product $T_{10}^sT_{10}^k$ of irreducible tensor operators. These terms are also called heteronuclear longitudinal two-spin order. The resulting average Hamiltonian has the same form as the Hamiltonian for the direct heteronuclear dipolar coupling in Eq. (5.41) and therefore corresponds to the case of the homonuclear zero-quantum average Hamiltonian (Eq. (8.1)). This type of Hamiltonian has a favourable form, because the terms $S_{sz}K_{kz}$ commute for different spin pairs. This means that the evolution of a heteronuclear multi-spin system can be described as the superposition of the evolution of isolated spin pairs [26].
8.3 Dipolar Recoupling Rf Pulse Sequences

The Hamiltonian in Eq. (8.5) is called a heteronuclear zero-quantum average Hamiltonian and the rf pulse sequence generating this type of Hamiltonian is referred to as a heteronuclear zero-quantum recoupling sequence. The Hamiltonian contains only two-spin terms with spin components of $(\mu_S, \mu_K) = (\pm 1, \mp 1)$, i.e., the spin terms are given by the product $T^s_{1\pm 1} T^k_{1\mp 1}$ of irreducible tensor operators. As in the homonuclear case, this Hamiltonian is not suitable for exciting higher-order heteronuclear MQC, but is suitable for achieving magnetization exchange.

The Hamiltonian in Eq. (8.6) is called a heteronuclear single-quantum average Hamiltonian and the rf pulse sequence generating this type of Hamiltonian is referred to as a heteronuclear single-quantum recoupling sequence. The Hamiltonian contains only two-spin terms with a spin components of $(\mu_S, \mu_K) = (0, \pm 1)$, i.e., the spin terms are given by the product $T^s_{10} T^k_{11}$ of irreducible tensor operators. The case $(\mu_S, \mu_K) = (\pm 1, 0)$ is also possible, but is not shown here. This type of Hamiltonian may be used to excite heteronuclear MQC.

The Hamiltonian in Eq. (8.7) is called a heteronuclear double-quantum average Hamiltonian and the rf pulse sequence generating this type of Hamiltonian is referred to as a heteronuclear double-quantum recoupling sequence. The Hamiltonian contains only two-spin terms with a spin components of $(\mu_S, \mu_K) = (\pm 1, \pm 1)$, i.e., the spin terms are given by the product $T^s_{1\pm 1} T^k_{1\pm 1}$ of irreducible tensor operators. This type of Hamiltonian may be used to excite heteronuclear MQC.

8.3 Dipolar Recoupling Rf Pulse Sequences

The design of dipolar recoupling sequences under MAS conditions is a complex problem with many possible solutions. In general, dipolar recoupling sequences should have the following properties: (i) The size of the time-independent recoupled dipolar interaction (corresponding to $\omega_{jk}$ and $\omega_{sk}$ in Eqs. (8.1)–(8.7)), should be maximized. (ii) The sequence should be robust with respect to chemical shift anisotropies, isotropic chemical shifts and inhomogeneity of the rf fields. This means that the total first order average Hamiltonian generated by the dipolar recoupling sequence is ideally given by one or a mixture of the dipolar Hamiltonians Eqs. (8.1)–(8.7), with all higher order average Hamiltonians set to zero. (iii) In general, in powders, it is preferable if the recoupled dipolar interaction shows little dependence upon the molecular orientation. This can, for example, be achieved if the sequence is insensitive to one of the three Euler angles in a powdered sample. This Euler angle is usually the $\gamma_{MR}$ angle (see section 5.10), so that these type of sequences are referred to as being “$\gamma$-encoded” or “$\gamma$-independent”. (iv) The sequence should require a low rf field at the Larmor frequency of the recoupled spin species. This is especially important in the presence of an abundant spin species, which should be heteronuclear...
decoupled at the same time as the recoupling sequence is applied.

This thesis will discuss two classes of rf pulse sequences, denoted $CN^\nu_n$ and $RN^\nu_n$, which are synchronized with the sample rotation. The sequences are described by the three symmetry numbers, $N$, $n$, and $\nu$, which can be chosen according to simple theorems, in order to symmetry-allow certain terms in the average Hamiltonian. These sequences have been used to design rotor-synchronized homonuclear and heteronuclear recoupling sequences, which will be discussed in the following sections. First, a brief overview will be given of existing homonuclear and heteronuclear dipolar recoupling sequences.

8.3.1 Homonuclear Recoupling Sequences

In the past ten years, many techniques for homonuclear dipolar recoupling have been developed. A detailed review of these recoupling sequences is given in Refs. 27 and 28. For example, the rotational resonance [29–31], RFDR [14,15] and RIL [32–34] schemes are homonuclear zero-quantum recoupling methods. They generate a first order average Hamiltonian of the type in Eq. (8.1), where the average Hamiltonian at the rotational resonance condition only contains terms proportional to the spin operators $S_j^- S_k^+$ and $S_j^+ S_k^-$. In Paper III, homonuclear zero-quantum recoupling sequences, denoted $R4_1^4$ and $R6_6^2$, were designed using the general framework of the $RN^\nu_n$ sequences. Furthermore, RFDR can be identified as a special case of the $R4_1^1$ sequence.

Sequences like DRAMA [35,36], MELODRAMA (under certain conditions) [37] and DRAWS [38] produce mixtures of homonuclear zero-quantum and double quantum average Hamiltonians, Eqs. (8.1) and (8.3).

In the context of homonuclear dipolar recoupling techniques which generate a first order average Hamiltonian of the type in Eq. (8.3), sequences have been developed which are insensitive to one of the three Euler angles in a powdered sample. The first sequences of this type were the HORROR [39] and the C7 schemes [40]. Several improved variants of the C7 sequence were developed: the POST-C7 [41] and CMR7 [42] sequences are improvements in the compensation of chemical shift anisotropies, isotropic chemical shifts and rf inhomogeneity. As shown in Paper II, the performance of the POST-C7 sequence is better under certain circumstances than that of the CMR7 sequence. The SPC-5 [43] and SPC-5$_3$ [44] sequences have a lower rf field requirement and therefore allow operation at higher spinning frequencies.

In Ref. 9, the symmetry arguments leading to the original C7 sequence were generalized to a class of pulse sequences, denoted $CN^\nu_n$. This concept was used to address the problem of heteronuclear decoupling in rotating solids [9]. In Paper I, the concept of $CN^\nu_n$ sequences was explored to design a new sequence, denoted $C14_{14}^4$, which has a low rf field requirement and allows the operation at high spinning frequencies. In addition, a supercycled version of this sequence, denoted SC14, was developed, which is well compensated for chemical shifts. It
was shown that SC14 is roughly as broadband as previous recoupling schemes, while permitting application at high spinning frequencies.

In Paper II, a new class of rotor-synchronized pulse sequences, denoted $\text{RN}^\nu_n$, was introduced. This concept led to the design of a homonuclear double-quantum recoupling sequence, denoted $\text{R14}_2^6$, which has an improved performance compared to all previous techniques with respect to chemical shifts but is limited to use at moderate spinning frequencies.

### 8.3.2 Heteronuclear Recoupling Sequences

The recoupling of heteronuclear dipolar couplings by rf fields in solid state NMR has a long history. A review of heteronuclear dipolar recoupling techniques in rotating solids is given in Refs. 27 and 28. However, techniques originally developed for static solids are also widely used in rotating solids. In Paper IV, a detailed overview over different heteronuclear recoupling methods is given and their qualitative properties are shown. These techniques will only be listed here.

The following heteronuclear recoupling methods in MAS NMR involve application of rf fields to only one of the two spin species. The first method achieving this type of recoupling was rotary resonance recoupling (R$^3$) [45,46]. Different extensions of this method have appeared [47–49]. The most successful method so far is the REDOR scheme [50,51], in which discrete $\pi$ pulses are applied every half rotational period. An overview of the numerous applications of REDOR can be found in Ref. 28. The $\text{C}^\nu_n$ and $\text{RN}^\nu_n$ concept can also be used to design single-channel heteronuclear dipolar recoupling sequences. Examples of this type are the TC5 sequence [52], the T-MREV method [53,54], the R18$^7_1$ sequence [Paper II] and the recently presented C-REDOR method [55]. In Paper IV, solutions of the type $\text{RN}^\nu_n$ are presented, which may be regarded as variants of REDOR with four $\pi$ pulses per rotational period, including, for example, R12$^4_3$. In Table I in Paper II the qualitative properties of these sequences are listed. The general aim for all these sequences is to recouple the heteronuclear dipolar couplings, while decoupling homonuclear dipolar interactions and chemical shift interactions. There are two main drawbacks to these type of sequences; either the sequence is sensitive to the chemical shift anisotropy or cannot be designed to be $\gamma$-encoded. Examples of sequences which are $\gamma$-encoded but are sensitive to the CSA are TC5, T-MREV and R18$^7_1$. Sequences which are insensitive to the CSA, but are $\gamma$-dependent, are REDOR, C-REDOR and R12$^4_3$, where the latter two decouple the homonuclear dipolar interactions of the spin species they are applied to, whereas the original REDOR sequence does not.

The archetypal dual-channel heteronuclear solid state NMR method is the Hartmann-Hahn cross polarization (HH-CP) scheme [6,7], which is widely employed in solid-state NMR for the enhancement of signals from nuclei with low
gyromagnetic ratios and is an essential component of high-resolution NMR in solids. Several methods have been developed to reduce the sensitivity of the HH-CP method with respect to experimental imperfections and to improve performance in rapidly-spinning samples [56–59]. In its original version, HH-CP does not decouple the homonuclear interactions. Combining the idea of HH-CP with the idea of Lee-Goldburg homonuclear decoupling [60] has lead to the Lee-Goldburg Hartmann-Hahn cross-polarization (LG-HH-CP) method [61,62].

In Paper IV, the concept of $\mathrm{CN}_n^\nu$ and $\mathrm{RN}_n^\nu$ sequences were generalized to the case of heteronuclear spin systems exposed to simultaneous resonant irradiation on two rf channels. These type of sequences may be used for the purpose of heteronuclear dipolar recoupling, in which case they are referred to as generalized Hartmann-Hahn methods. Sequences were designed which generate $\gamma$-encoded heteronuclear double-quantum average Hamiltonians of the type in Eq. (8.7) and also remove all homonuclear dipolar interactions and chemical shift anisotropy terms for both spin species. This type of sequence might be used to excite heteronuclear multiple-quantum coherence and might be incorporated into experiments which correlate the isotropic shifts of neighbouring heteronuclear spins.
9 Rotor-Synchronized Pulse Sequences

The following will briefly discuss rotor-synchronized rf pulse sequences, denoted $CN_n^\nu$ and $RN_n^\nu$, which are applied at the Larmor frequency of one spin species, i.e., to one rf channel. A more detailed and general discussion is given in Paper IV and Ref. 9.

9.1 $CN_n^\nu$ Sequences

The general definition of a $CN_n^\nu$ sequence is given in Paper IV, but in the following one possible construction scheme for a $CN_n^\nu$ sequence is described. The pulse sequence is derived from a basic pulse sequence element denoted $C$, which is an rf cycle, i.e., the basic element $C$ provides no net evolution of the nuclear spin states when only the rf field is taken into account. The complete $CN_n^\nu$ sequence is built of $N$ basic elements $C$, which altogether span exactly $n$ rotational periods. This implies that the basic element $C$ has a duration of $\tau_E = n\tau_r/N$. The rf phases of consecutive cycles differ by $2\pi\nu/N$. The phase of the $q$th cycle is therefore given by $\phi_q = 2\pi\nu q/N$, with $q = 0, 1, \ldots, N - 1$. This leads to the sequence:

$$CN_n^\nu = (C)^{\phi_0} (C)^{\phi_1} \cdots (C)^{\phi_{N-1}}, \quad (9.1)$$

which has a total duration of $T = n\tau_r$. Note that in this case the first element in the $CN_n^\nu$ sequence is the same as the basic element $C$. The choice of $C$ is free as long it is an rf cycle, e.g., $C$ could be a simple $360^\circ$ pulse around the $x$-axis ($C = 360^0$), but other choices are also possible, as will be seen later.

An intuitive representation of $CN_n^\nu$ sequences is shown for two cases in Fig. 9.1. The sequence is represented by two helices, one for the spatial sample rotation and one for the rotation of the rf phases. The symmetry numbers $n$ and $\nu$ can be thought of as the “winding numbers” of these two helices. The spatial helix completes $n$ full turns during the complete $CN_n^\nu$ sequence of duration $T$, while the helix for the rotations of the rf phases completes $\nu$ turns during $T$.

9.2 $RN_n^\nu$ Sequences

The $RN_n^\nu$ sequence are defined generally in Paper IV, but in the following one possible construction scheme for a $RN_n^\nu$ sequence is described. The pulse sequence is derived from a basic element $R$ of duration $\tau_E = n\tau_r/N$, where $R$ rotates the spins by an odd multiple of $\pi$ around the $x$-axis in the rotating frame, e.g., $R$ could be a simple $180^\circ$ pulse around the $x$-axis ($R = 180^0$). Other choices, for example compensated pulses, are also possible and are often preferable, as will be seen later. The construction principle described here requires a second basic element, denoted $R'$, which is derived from $R$ by changing the sign.
of all rf phases. For example, if $R = 90_0180_090_0$, then $R' = 90_{-90}180_090_{-90}$. An $RN_n$ sequence may be constructed by concatenating $N/2$ phase-shifted $RR$ pairs, applying an additional phase shift of $\phi = \pi\nu/N$ in the following way:

$$RN_n = (R)_{\phi} (R')_{-\phi} (R)_{\phi} (R')_{-\phi} \ldots (R')_{-\phi}, \quad (9.2)$$

where the total duration of the pulse sequence is $T = n\tau_r$. Note that the first element of a $RN_n$ sequence is not equal to the basic element $R$.

In PaperIV it is shown that any $RN_n$ sequence is also a $C(N/2)_n$ sequence, even though its construction principles are quite different. The $RN_n$ symmetry is a stronger version of $C(N/2)_n$ symmetry. This is reflected in the more restrictive selection rules for $RN_n$ sequences compared to $CN_n$ sequences, which will be seen below.

### 9.3 Average Hamiltonian and Selection Rules

In section 7, it was shown that the internal spin Hamiltonian may be transformed into the interaction frame of the rf field and analyzed using the Magnus expansion [24]. In Eq. (7.18) the first order result for the the effective Hamiltonian was written as

$$\tilde{H}^{(1)} = \sum_{\Lambda,l,m,\lambda,\mu} H^{A}_{l,m,\lambda,\mu}, \quad (9.3)$$
9.3 Average Hamiltonian and Selection Rules

where the term $H_{lm}^{\Lambda}$ was generally defined in Eq. (7.19).

The exact appearance of the terms $H_{lm}^{\Lambda}$ depends upon the rotor-synchronized rf pulse sequence. The application of $CN_\nu^n$ or $RN_\nu^n$ sequences allows the derivation of simple selection rules which predict when a term $H_{lm}^{\Lambda}$ in the first order average Hamiltonian disappears, depending upon the chosen symmetry parameters $N$, $n$ and $\nu$. For this analysis, the knowledge of the exact basic elements $C$ and $R$ is not necessary. The derivation of the selection rules is presented in Ref. 9 for the case of the $CN_\nu^n$ sequences and in Paper IV for the case of the $RN_\nu^n$ sequences. In the following, only the results are presented.

(i) $CN_\nu^n$ sequences: The selection rules are:

$$H_{lm}^{\Lambda} = 0 \text{ if } mn - \mu \nu \neq NZ,$$

where $Z$ is any integer.

(ii) $RN_\nu^n$ sequences: The selection rules are:

$$H_{lm}^{\Lambda} = 0 \text{ if } mn - \mu \nu \neq \frac{N}{2}Z_\lambda,$$

where $Z_\lambda$ is an integer with the same parity as $\lambda$ (i.e., if $\lambda$ = even, then $Z_\lambda = 0, \pm 2, \pm 4, \ldots$; if $\lambda$ = odd, then $Z_\lambda = \pm 1, \pm 3, \pm 5, \ldots$).

The selection rules for the second order average Hamiltonian for the $CN_\nu^n$ and $RN_\nu^n$ sequences are discussed in Refs. 9, 63 and Paper IV.

In Paper I, space-spin selection diagrams (SSS diagrams) are introduced, which allows exploration of the consequences of Eqs. (9.4) and (9.5). Figure 9.2 shows SSS diagrams for a $CN_\nu^n$ type of sequence, namely the $C7_1^2$ sequence, while Fig. 9.3 shows SSS diagrams for a $RN_\nu^n$ type of sequence, namely the $R14_6^2$ sequence.

The levels in Figs. 9.2 and 9.3 indicate the total value of $mn - \mu \nu$, i.e., the left-hand sides of the inequalities in Eqs. (9.4) and (9.5). The superposition of $mn$ and $-\mu \nu$ is broken into two stages, to separate the effects of the spatial rotations and spin rotations. The “barriers” at the right-hand side of the diagrams have holes separated by $N$ units. The position of the holes depends on whether a $CN_\nu^n$ or an $RN_\nu^n$ sequence is chosen. In the case of the $CN_\nu^n$ sequence, the position of the holes is independent of the parity of the spin rank $\lambda$ and is given by multiples of $N$, which corresponds to the right-hand side of the inequality in Eq. (9.4). In the case of the $RN_\nu^n$ sequences, the position of the holes is dependent upon the parity of the spin rank $\lambda$. The positions are determined by the right-hand side of the inequality in Eq. (9.5). For homonuclear dipolar couplings, $\lambda$ is even and therefore the position of the holes are given by even multiples of $N/2$, i.e., $0, \pm 18, \pm 36, \ldots$. For the chemical shift anisotropy,
Figure 9.2: Space-spin selection diagram (SSS diagram) for C71. (a) Suppression of all CSA modulation components. (b) Selection of a single 2Q dipole-dipole component, with quantum numbers $(m, \mu) = (1, 2)$. The mirror image pathways stemming from $m = -1, m = -2$ have been omitted for simplicity.

$\lambda$ is odd so that the position of the holes correspond to odd multiples of $N/2$, i.e., $\pm 9, \pm 27, \ldots$. Pathways which are blocked by the barriers correspond to space-spin components which are removed (decoupled) in first order average Hamiltonian theory, i.e., the inequalities in Eqs. (9.4) and (9.5) are fulfilled. Pathways which pass through a hole in the barriers indicate space-spin components which are symmetry allowed in first order average Hamiltonian, i.e., the inequalities in Eqs. (9.4) and (9.5) are not fulfilled.

Figures 9.2(a) and 9.3(a) and show that all CSA components $(m = \{\pm 1, \pm 2\}$ and $\mu_K = \{0, \pm 1\})$ are suppressed by the symmetries C71 and R142 in the first order average Hamiltonian. Figure 9.2(b) shows that the C71 symmetry only allows homonuclear dipolar components with $(m, \mu) = (1, 2)$ (and, by implication, also $(m, \mu) = (-1, -2)$). Figure 9.3(b) shows that the R142 symmetry only allows homonuclear dipolar components with $(m, \mu) = (1, -2)$ (and, by implication, also $(m, \mu) = (-1, 2)$). The selection of terms with $\mu = \pm 2$ in both cases indicates double-quantum recoupling. The fact that in both cases the $\mu = +2$ term is associated with only one spatial component $(m = 1$ for C71; $m = -1$ for R142), leads to a $\gamma$-encoded average Hamiltonian, whose phase but not amplitude depends upon the Euler angle $\gamma_{MR}$.

The symmetry principles in Eqs. (9.4) and Eqs. (9.5) have, in addition to the dipolar recoupling sequences discussed here, led to a large selection of promising pulse sequences for heteronuclear decoupling [9, 63], and selection of homonuclear $J$-couplings [63–65]. In addition, the symmetry principles provide insight into a variety of existing methods, such as REDOR [50, 51], RFDR [14] and
9.4 Scaling Factors

The general symmetry properties of $C_N^\nu$ and $R_N^\nu$ sequences, which can be visualized using SSS diagrams, do not depend upon the detailed structure of the basic elements $\mathcal{C}$ and $\mathcal{R}$. However, the magnitudes of the symmetry allowed terms do depend upon the basic elements. In general, a symmetry-allowed term in the first order effective Hamiltonian has the form

$$\hat{H}_{lm\lambda\mu}^{\Lambda} = \kappa_{lm\lambda\mu} \left[ A_{lm}^{\Lambda} \right]^R \exp \left\{ -im(\alpha_{RL}^0 - \omega_{RL}^0) \right\} \cdot T_{\lambda\mu}^\Lambda, \quad (9.6)$$

where $\kappa_{lm\lambda\mu}$ is the scaling factor of the symmetry-allowed term with quantum numbers $(l, m, \lambda, \mu)$. The scaling factor is, in general, a complex number, whose absolute value is less than 1 and which depends upon the chosen basic element $\mathcal{C}$ or $\mathcal{R}$ but not on the chosen symmetry number $\nu$. In the case of an $R_N^\nu$ sequence the phase of the scaling factor depends upon the symmetry number $\nu$. The scaling factor $\kappa_{lm\lambda\mu}$ is defined and discussed in detail in Paper IV.
9.5 Phase-Time Relationships

Some important conclusions can be drawn from Eq. (9.6). Consider two identical rotor synchronized pulse sequences of type CN$_n^\nu$ or RN$_n^\nu$. The first pulse sequence has an overall phase shift of $\phi_0$ and starts at time point $t_0^0$. The second pulse sequence has an overall phase shift of 0 and starts at time point 0. According to Eq. (9.6), the average Hamiltonian components are related by

$$H_{lm\lambda\mu}^\Lambda(t_0^0, \phi_0) = H_{lm\lambda\mu}^\Lambda(0, 0) \exp\{-i(\mu \phi_0 - m \omega_r t_0^0)\}. \quad (9.7)$$

If $\mu \neq 0$ this can be written as:

$$H_{lm\lambda\mu}^\Lambda(t_0^0, \phi_0) = R_z(\phi_0 - \frac{m}{\mu} \omega_r t_0^0) H_{lm\lambda\mu}^\Lambda(0, 0) R_z(\phi_0 - \frac{m}{\mu} \omega_r t_0^0)^\dagger. \quad (9.8)$$

Assume that the average Hamiltonian, generated by a CN$_n^\nu$ or RN$_n^\nu$ sequence, only contains terms for which $\mu \neq 0$ and $m/\mu$ is constant, where the constant is identical for all terms. In this case, the dependence of the average Hamiltonian upon the starting time point of the pulse sequence can be removed by an appropriate overall phase shift of the pulse sequence:

$$\phi_0 = \frac{m}{\mu} \omega_r t_0^0. \quad (9.9)$$

The condition $\mu \neq 0$ and $m/\mu$ is constant is, for example, fulfilled by all $\gamma$-encoded pulse sequences. The SSS diagrams of two examples of CN$_n^\nu$ and RN$_n^\nu$ sequences have already been shown in Figs. 9.2 and 9.3.

9.6 Changing the Sign of $\nu$

Consider a rotor synchronized pulse sequence of class CN$_n^\nu$ or RN$_n^\nu$. For brevity, this sequence will be denoted C$^+$. The corresponding pulse sequence, where all rf phases within the pulse sequence are reversed in sign, will be denoted with C$^-$. In the case of C$^+ = \text{CN}_n^\nu$ with the basic element $\mathcal{C}$ this means that C$^- = \text{CN}^{-\nu}_n$ with the basic element $\mathcal{C}'$, where $\mathcal{C}'$ is obtained from $\mathcal{C}$ by changing the sign of all rf phases. In the case of C$^+ = \text{RN}_n^\nu$ with the basic element $\mathcal{R}$ this means that C$^- = \text{RN}^{-\nu}_n$, where the roles of $\mathcal{R}$ and $\mathcal{R}'$ are interchanged in Eq. (9.2).

In appendix A of Paper I the relationship between the corresponding average Hamiltonians (in first and second order) of the sequences C$^+$ and C$^-$ is derived. One important result from that derivation is that if the sequence C$^+$ was designed to generate a $\gamma$-encoded average Hamiltonian, the two sequences C$^+$ and C$^-$ cannot be concatenated without losing this property.

If, on the other hand, the sequence C$^-$ is bracketed by two strong $\pi$-pulses of phase 0, denoting the resulting sequence II C$^- \Pi$, the following relationship
of the average Hamiltonian components is valid:

\[ \hat{H}_{lm}^{\Lambda} (C^+) = (-1)^{\lambda} \hat{H}_{lm}^{\Lambda} (\Pi C^- \Pi). \]  

(9.10)

This has two consequences: (i) A supercycle can be constructed out of the original sequence \( C^+ \) and the bracketed sequence \( \Pi C^- \Pi \). If the original sequence \( C^+ \) generates a \( \gamma \)-encoded average Hamiltonian, so does the supercycled sequence. The first and second order average Hamiltonians for this supercycle are discussed in Paper I. It can be shown that in the first order average Hamiltonian terms with odd spin rank \( \lambda \) disappear, while in the second order average Hamiltonian cross terms involving interactions with spin ranks \( \lambda_1 \) and \( \lambda_2 \) disappear if the sum \( \lambda_1 + \lambda_2 \) is odd. This type of supercycle was used to construct the SC14 pulse sequence, discussed in Paper I. (ii) It is possible to insert an evolution interval, including a \( \pi \)-pulse, between a \( C^+ \) and a \( C^- \) sequence in order to refocus the evolution of the density operator under isotropic chemical shifts during the evolution interval. In Ref. 66, this principle was used in experiments to determine the decay rate constant of double-quantum coherence for \( ^{13} \)C spin pairs. In part IV of this thesis, this principle will be used in the context of experiments for the direct determination of molecular torsional angles.

9.7 Dual Rotor-Synchronized Pulse Sequences

The classification and theory of \( CN_n^{\nu} \) and \( RN_n^{\nu} \) sequences, which are applied simultaneously at the Larmor frequencies of two different spin species \( S \) and \( K \), is presented in Paper IV. Figure 9.4 shows the example of two simultaneous \( RN_n^{\nu} \) sequences on the \( S \)- and \( K \)-spins. The number of basic elements and the total number of rotor periods \( n \) is the same in both sequences. The increment parameter \( \nu \) is different for the two channels and denoted as \( \nu_S \) and \( \nu_K \) for the \( S \)- and \( K \)-spins respectively. The resulting synchronized dual R-sequence is denoted \( RN_n^{\nu_S,\nu_K} \).

The selection rules for the terms in first order average Hamiltonian theory describing homonuclear interactions are given by Eq. (9.5) for both spin species \( S \) and \( K \), if an \( RN_n^{\nu_S,\nu_K} \) sequence is applied. The selection rules for the terms
9 Rotor-Synchronized Pulse Sequences

in first order average Hamiltonian describing heteronuclear interactions are derived in Paper IV and for the case of an \( R_n^{\nu_S,\nu_K} \) sequence are given by

\[
\hat{H}_{lm\lambda_S\mu_S\lambda_K\mu_K} = 0 \quad \text{if} \quad m n - \mu_S \nu_S - \mu_K \nu_K \neq \frac{N}{2} Z_{\lambda_S + \lambda_K}. \tag{9.11}
\]

These selection rules permit a classification of the pulse sequences on the basis of their recoupling and decoupling properties. In section 10.3 an example for a generalized HH sequence is presented.
10 Applications

In the following, several applications are discussed of the general selection rules in Eqs. (9.4) and (9.5) to design $C N_n^\nu$ and $R N_n^\nu$ sequences, which lead to the desired time-independent first order average Hamiltonian. In all cases discussed below, certain components of the homonuclear or heteronuclear dipolar couplings are recoupled.

10.1 Homonuclear Double-Quantum Recoupling

Consider a homonuclear multiple-spin system, to which a homonuclear double-quantum recoupling sequence is applied, generating a time-independent homonuclear double-quantum average Hamiltonian, of the form:

$$\tilde{H}^{(1)} = \sum_{j<k} \left( \omega_{jk} \frac{1}{2} S_j^- S_k^- + \omega_{jk}^* \frac{1}{2} S_j^+ S_k^+ \right), \quad (10.1)$$

where $\omega_{jk}$ is the recoupled through-space homonuclear dipolar interaction.

The average Hamiltonian contains terms with the spin quantum numbers $(\lambda, \mu) = \{(2, 2), (2, -2)\}$. Nothing is assumed about the space quantum numbers, so, in principle, all combinations $(l, m) = \{(2, 1), (2, -1), (2, 2), (2, -2)\}$ are possible. Note that in the case of exact magic-angle spinning the terms with $(l, m) = (2, 0)$ disappear. As discussed in section 8.3, one of the desirable properties of a dipolar recoupling sequence is to generate a $\gamma$-encoded average Hamiltonian, which is achieved if a term for a certain spin quantum number $\mu$ is only associated with one spatial component (compare section 9.3).

To obtain a $\gamma$-encoded average Hamiltonian of the type in Eq. (10.1) using $C N_n^\nu$ or $R N_n^\nu$ sequences the selection rules in Eqs. (9.4) or (9.5) must be examined to achieve the following: (i) The terms $(l, m, \lambda, \mu) = \{(2, -m', 2, 2), (2, m', 2, -2)\}$ for either $m' = \pm 1$ or $m' = \pm 2$ should be symmetry allowed. (ii) All other homonuclear dipolar coupling components should be symmetry disallowed. (iii) All CSA components $(m = \{\pm 1, \pm 2\}$ and $\mu = \{0, \pm 1\}$) should be suppressed. Many solutions have been found which achieve these goals. Paper I contains a list of possible $C N_n^\nu$ symmetries, while Paper II contains a list of possible $R N_n^\nu$ symmetries. A few of these sequences, which are commonly used, are listed in Table 10.1. All these solutions recouple terms with the space component $m' = \pm 1$, which is, in general, preferable if a higher scaling factor for the recoupled dipolar interaction Eq. (9.6) is desired.

The recoupled through-space homonuclear dipolar interaction depends upon the molecular orientation $\Omega_{MR}$, the starting time point $t_0^0$ and the overall rf
| Sequence   | Symmetry | $(m, \mu)$ | $m'$ | $\mathcal{C}$                | $|\omega_{\text{mut}}/\omega_r|$ | $|\kappa|$ | Ref. | Remark      |
|------------|----------|------------|------|-----------------------------|--------------------------------|----------------|-----|-------------|
| C7         | $C7_2^1$ | (-1, -2)   | 1    | 360_180 360_180            | 7                             | 0.155         | 40  |             |
| POST-C7    | $C7_2^1$ | (-1, -2)   | 1    | 90_270 360_180 360_180    | 7                             | 0.155         | 41  |             |
| CMR7       | $C7_2^1$ | (-1, -2)   | 1    | 360_180 180_180           | 7                             | 0.155         | 42  | supercycled |
| SPC-5      | $C5_2^1$ | (-1, -2)   | 1    | 90_270 360_180 360_180    | 5                             | 0.135         | 43  | supercycled |
| SPC-5$_3$  | $C5_3^1$ | (-1, -2)   | 1    | 90_270 360_180 360_180    | 3.3                           | 0.091         | 44  | supercycled |
| C7$_5^1$   | $C7_5^1$ | (1, -2)    | -1   | 360_180                  | 2.8                           | 0.064         | 42  |             |
| C7$_5^1$   | $C7_5^1$ | (1, -2)    | -1   | 360_120 360_240           | 4.2                           | 0.144         | Paper I |             |
| C14$_4^5$  | $C14_4^5$| (1, -2)    | 1    | 360_240                  | 3.5                           | 0.157         | Paper I |             |
| SC14       | $C14_4^5$| (1, -2)    | 1    | 360_240                  | 3.5                           | 0.157         | Paper I | supercycled |
| R14$_2^6$  | $R14_2^6$| (-1, -2)   | 1    | 90_270 180_270           | 7                             | 0.172         | Paper II|             |

Table 10.1: Magnitudes of the scaling factor $\kappa$ and rf requirements for a selection of homonuclear double-quantum recoupling sequences based on $CN_n^\mu$ and $RN_n^\mu$ symmetries. The symmetry allowed terms are shown. $m'$ is the space component of the symmetry allowed term with $\mu = -2$. 
10.1 Homonuclear Double-Quantum Recoupling

phase $\phi_0$ of the recoupling sequence:

$$
\omega_{jk}(\Omega_{MR}, t^0_0, \phi_0) = \sqrt{6} b_{jk} \kappa e^{im'(\omega_r t^0_0 - \alpha_{RL} - \gamma_{MR}) + 2i\phi_0} 
$$

$$
\times \sum_{m=-2}^{2} d^{(2)}_{0m}(\beta_{PM}^{sk}) d^{(2)}_{mm'}(\beta_{MR}) e^{-im(\gamma^{sk}_{PM} + \alpha_{MR})},
$$

where $\kappa$ corresponds to scaling factor $\kappa_{2m',2} - 2$ in Eq. (9.6). The Euler angles $\Omega^{sk}_{PM} = \{\alpha^{sk}_{PM}, \beta^{sk}_{PM}, \gamma^{sk}_{PM}\}$ describe the transformation of each homonuclear dipole-dipole coupling from its principal axis system to a molecule fixed frame.

The scaling factor $\kappa$ is, in general, a complex number and might be written as:

$$
\kappa = |\kappa|e^{i\phi_\kappa}. \quad (10.3)
$$

The recoupled dipolar interaction $\omega_{jk}$ in Eq. (10.2) might also be expressed in a similar way as:

$$
\omega_{jk} = |\omega_{jk}|e^{i\phi_{jk}}, \quad (10.4)
$$

where the absolute value and argument are given by

$$
|\omega_{jk}|(\alpha_{MR}, \beta_{MR}) = \sqrt{6} |b_{jk}| |\kappa| \sum_{m=-2}^{2} d^{(2)}_{0m}(\beta^{sk}_{PM}) d^{(2)}_{mm'}(\beta_{MR}) e^{-im(\gamma^{sk}_{PM} + \alpha_{MR})}
$$

$$
\phi_{jk}(\gamma_{MR}, t^0_0, \phi_0) = \phi_\kappa + m'(\omega_r t^0_0 - \alpha_{RL} - \gamma_{MR}) + 2\phi_0 + \pi, \quad (10.5)
$$

where it should be noted that $b_{jk} = -|b_{jk}|$ for homonuclear dipolar couplings. That results in the additional $\pi$ in the phase. Since only the phase $\phi_{jk}$, and not the amplitude $|\omega_{jk}|$, depends upon the Euler angle $\gamma_{MR}$, the resulting double-quantum average Hamiltonian is $\gamma$-encoded. All sequences in Table 10.1 lead to $\gamma$-encoded average Hamiltonians. The recoupled terms are indicated for each sequence.

The scaling factor $\kappa$ depends upon the choice of the basic element $C$ or $R$. As discussed in section 8.3, it is desirable to choose the basic element so as to maximize the scaling factor $\kappa$. Even though, in first order average Hamiltonian theory, all CSA terms are suppressed, the CSA might have an important impact on higher order average Hamiltonian terms. It is desirable to choose a sequence which is robust with respect to chemical shift anisotropies as well as to isotropic chemical shifts and errors in the rf amplitude settings. In addition, the applied rf fields during the recoupling sequence should be minimized. This is particularly important in systems where abundant I-spins should be decoupled during the recoupling sequence. Table 10.1 lists, for each
pulse sequence, the basic element $C$ or $R$, the absolute value of the scaling factor $\kappa$, and the ratio of the nutation frequency of the required rf field and the spinning frequency, $|\omega_{\text{out}}/\omega_s|$. If a pulse sequence should be suitable for use at high spinning frequencies, this ratio must be as small as possible, to limit the required rf field during the recoupling sequence.

The $Cn^k$ sequences C7 [40], POST-C7 [41] and CMR7 [42] all have the same symmetry, namely $C7_1^2$, but use different basic elements $C$. The CMR7 sequence applies an additional supercycle, which will not be discussed here. All three sequences have the same scaling factor and the same rf requirement; that the rf field, expressed as a nutation frequency, must be given by seven times the sample spinning frequency. The sequence R14_2 [PaperII], with the indicated basic element, has the same rf requirement but provides a higher scaling factor. The large rf requirement of all these sequences usually limits their use to spinning frequencies below 10,000 kHz.

The sequences SPC-5 [43] and SPC-5_3 [44] use the reduced symmetries $C5_1^1$ and $C5_1^3$ respectively, which do not suppress all CSA terms in first order average Hamiltonian. Therefore, an additional supercycle must be applied, which doubles the length of the sequence but which removes the unwanted terms in the average Hamiltonian [43,44]. These two sequences have a lower rf requirement but also have lower scaling factors compared to the previously discussed sequences.

The symmetry $C7_1^5$ together with the basic element $C = 360_5 360_{180}$ [44] has a very low rf requirement, but on the other hand has a very small scaling factor $\kappa$, which makes this pulse sequence unsuitable in practice. A better choice is the basic element $C = 360_0 360_{120} 360_{240}$ [PaperI], which leads to a scaling factor which is nearly the same as for the C7 sequence but which has a considerably lower rf requirement.

In PaperI, a pulse sequence $C14_5^0$ was designed, which uses a single 360° pulse as basic element ($C = 360_0$). This leads to a high scaling factor for the recoupled homonuclear dipolar interactions and a low ratio of required rf field to spinning frequency of 3.5. PaperI discusses in detail the fact that the resulting pulse sequence is well compensated with respect to rf amplitude errors. This sequence is not, on the other hand, well compensated with respect to isotropic chemical shift and chemical shift anisotropies. Supercycles of $C14_5^0$ have been constructed based on the principles discussed in section 9.6. This supercycle is called SC14 and is presented and discussed in detail in PaperI. For clarity, the supercycle is briefly shown here:

$$ SC14 = C14_5^0 \cdot [\Pi_0^{-1} \cdot C14_4^{-5} \cdot \Pi_0]_{\pi/7} \cdot [C14_4^5]_{\pi} \cdot [\Pi_0^{-1} \cdot C14_4^{-5} \cdot \Pi_0]_{8\pi/7}, $$

where the notation $\Pi_0$ indicates the insertion of a $\pi$ pulse element with phase $\phi = 0$ and $\Pi_0^{-1}$ indicates the deletion of a $\pi$-pulse element. The notation $[\ldots]_{\phi}$ indicates an overall phase shift of the bracketed sequence by $\phi$. The sequence
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$[\Pi_0^{-1} \cdot C14^{-5} \cdot \Pi_0]_{\pi/7}$ is therefore a phase-shifted cyclic permutation of the C14 sequence. The SC14 sequence is roughly as broadband as other recoupling schemes, such as POST-C7, SPC5 and R142, while permitting application at higher spinning frequency.

All the sequences in Table 10.1 recouple homonuclear double-quantum terms $(m, \mu) = \{(-m', 2), (m', -2)\}$, where $m' = \pm 1$. It should be noted that the ratio of the spatial and the spin component is in each case a constant, which is independent from the symmetry allowed term. For example, in the case of the symmetry C72 the ratio is $m/\mu = 1/2$ for both symmetry allowed terms $(m, \mu) = \{(-1, -2), (1, 2)\}$. In this case, the phase-time relationship in Eq. (9.8) is valid for the complete recoupled homonuclear double-quantum average Hamiltonian:

$$
\tilde{H}^{(1)}(t_0, \phi_0) = R_z(\phi_0 + \frac{m'}{2} \omega_r t_0^0) \tilde{H}^{(1)}(0, 0) R_z(\phi_0 + \frac{m'}{2} \omega_r t_0^0)^\dagger.
$$

(10.7)

In this case, the dependence of the average Hamiltonian on the starting time point of the recoupling sequence can be removed by choosing the phase $\phi_0$ according to Eq. (9.9):

$$
\phi_0 = -\frac{m'}{2} \omega_r t_0^0.
$$

(10.8)

10.1.1 Excitation of Double-Quantum Coherence

The following will discuss how homonuclear double-quantum recoupling sequences can be used to excite homonuclear double-quantum coherences. A basic rf pulse sequence for this purpose is shown in Fig. 10.1(a). The row marked I denotes rf fields at the Larmor frequency of the abundant protons, while S denotes the rf fields applied at the $^{13}$C Larmor frequency. Specific time points within the pulse sequence are denoted 1–5. The sequence starts with ramped cross polarization to enhance the S-spin magnetization [56]. The following $\pi/2$-pulse converts the transverse magnetization into longitudinal S-spin magnetization, time point 1. The homonuclear double-quantum recoupling sequence of duration $\tau_{ex}$ converts the sum longitudinal magnetization into homonuclear ($\pm 2$)-quantum coherence, time point 2. An optional time interval $t_1$ might be inserted between the excitation and reconversion sequence. The excited double-quantum coherence is converted into longitudinal magnetization by the reconversion sequence of duration $\tau_{re}$, time point 4. The longitudinal magnetization is converted into observable magnetization by a $\pi/2$ read pulse. The S-spin NMR signal is detected in the subsequent period.

Figure 10.1(b) shows the coherence transfer pathway diagram (CTP diagram) [4] of the pulse sequence. In order to select signals passing through ($\pm 2$)-quantum coherences at time point 3 and longitudinal magnetization at
Figure 10.1: (a) Rf pulse sequence for passing S-spin signals through homonuclear double-quantum coherence. (b) The coherence transfer pathway diagram. (c) Excitation and reconversion of 2QC for a single molecular orientation in the $\{1, 4\}$-subspace. (d) Excitation and reconversion of 2QC in the $\{1, 4\}$-subspace in a powdered sample.
time point \( t_4 \), it is necessary to add the NMR signals from different experiments
which were obtained by phase-cycling \([4]\) the overall phases of the reconversion
pulse sequence, the \( \pi/2 \) read pulse and the post-digitization phase shift \([20,21]\).
The exact phase-cycle is specified in Paper I.

The following gives an intuitive picture of how the homonuclear double-
quantum average Hamiltonian in Eq. (10.1) with the recoupled dipolar inter-
action in Eq. (10.2) might be used to excite homonuclear double-quantum co-
herence. Consider an isolated pair of spins-\( \frac{1}{2} \), \( S_1 \) and \( S_2 \). The four states
\( |1\rangle = |\alpha\alpha\rangle \), \( |2\rangle = |\alpha\beta\rangle \), \( |3\rangle = |\beta\alpha\rangle \) and \( |4\rangle = |\beta\beta\rangle \) can be used as the basis
states of the two-spin system. The spin dynamics of this pair under a double-
quantum average Hamiltonian of the form in Eq. (10.1) can be described using
single-transition operators \([67,68]\). The only single-transition operators neces-
sary in this case are those which have non-vanishing matrix elements between
the basis states \( |1\rangle \) and \( |4\rangle \). In the case of a spin-\( \frac{1}{2} \) pair, these operators are
given by

\[
S_{x}^{(1,4)} = \frac{1}{2} (S_1^- S_2^- + S_1^+ S_2^+) \quad (10.9)
\]

\[
S_{y}^{(1,4)} = \frac{i}{2} (S_1^- S_2^- - S_1^+ S_2^+) \quad (10.10)
\]

\[
S_{z}^{(1,4)} = \frac{1}{2} (S_{1z} + S_{2z}) \quad (10.11)
\]

\[
S_z^{+ (1,4)} = S_{x}^{(1,4)} + i S_{y}^{(1,4)} = S_1^+ S_2^+ \quad (10.12)
\]

\[
S_z^{- (1,4)} = S_{x}^{(1,4)} - i S_{y}^{(1,4)} = S_1^- S_2^- \quad (10.13)
\]

The single-transition operators fulfill the cyclic commutation relationships

\[
\left[ S_{x}^{(1,4)}, S_{y}^{(1,4)} \right] = i S_{z}^{(1,4)} \quad \bigcirc . \quad (10.14)
\]

The symbol \( \bigcirc \) was introduced in Ref. 3 and indicates that Eq. (10.14) is valid for
all cyclic permutations of the indices \( x, y \) and \( z \). The commutation relationships
in Eq. (10.14) are analogous to the relationships for the single spin operators
\( S_x, S_y \) and \( S_z \). If the dynamics of the two-spin system can be entirely described
using the single transition operators in Eqs. (10.9)–(10.11), the two-spin system
is said to behave like a fictitious spin-\( \frac{1}{2} \) in the \( |1\rangle, |4\rangle \)-subspace \([67,68]\).

The double-quantum average Hamiltonian in Eq. (10.1) for an isolated pair
of spins-\( \frac{1}{2} \), \( S_1 \) and \( S_2 \), may be expressed using the single-transition operators:

\[
\tilde{H}^{(1)} = |\omega_{12}| \left\{ S_x^{(1,4)} \cos \phi_{12} + S_y^{(1,4)} \sin \phi_{12} \right\} . \quad (10.15)
\]

This Hamiltonian looks like the Hamiltonian of the radio frequency field in
the rotating frame in Eq. (5.22). The evolution of the fictitious spin-\( \frac{1}{2} \) in the
\( |1\rangle, |4\rangle \)-subspace under a Hamiltonian of the type in Eq. (10.15) is therefore
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comparable to the evolution of a single spin-$1/2$ under an rf pulse. The evolution of the spin-$1/2$ pair in the $\{|1\rangle, |4\rangle\}$-subspace may therefore be visualized in a coordinate system using the single-transition operators $S_x^{(1,4)}$, $S_y^{(1,4)}$ and $S_z^{(1,4)}$ as axes. The average Hamiltonian in Eq. (10.15) for a single molecular orientation $\Omega_{MR}$ is a pseudo-field of strength $|\omega_{12}|$, represented by a vector in the $S_x^{(1,4)}S_y^{(1,4)}$-plane, as shown in Fig. 10.1(c). The angle between the the pseudo-field and the $S_x^{(1,4)}$-axis is given by the phase $\phi_{12}$. Note that this phase depends upon the Euler angle $\gamma_{MR}$, the starting time point of the recoupling sequence, $t_0^0$, and the overall phase of the recoupling sequence, $\phi_0$.

In the following, this picture is used to briefly describe the excitation and reconversion of $S$-spin double-quantum coherence during the pulse sequence shown in Fig. 10.1(a) for a single molecular orientation $\Omega_{MR}$. The density operator of the two spin system at time point $\odot$ is given by sum longitudinal $S$-spin magnetization:

$$\rho_{\odot} = S_z^{(1,4)}. \tag{10.16}$$

During the excitation sequence of duration $\tau_{ex}$ the density operator in Eq. (10.16) evolves under the average Hamiltonian in Eq. (10.15). The phase $\phi_{12}$ for the average Hamiltonian during the excitation sequence is, according to Eq. (10.6), given by

$$\phi_{12}(\gamma_{MR}, t_0, \phi_0^{ex}) = \phi_\kappa + m'(\omega, t_0) - \alpha_0^{RL} - \gamma_{MR} + 2\phi_0^{ex} + \pi, \tag{10.17}$$

where $\phi_0^{ex}$ is the overall rf phase of the excitation sequence. Figure 10.1(c) shows, how $\rho_{\odot}$ is rotated around the pseudo-field by the angle $|\omega_{12}|\tau_{ex}$. The density operator at time point $\odot$ is given by

$$\rho_{\odot} = S_z^{(1,4)} \cos |\omega_{12}|\tau_{ex}$$

$$+ \sin |\omega_{12}|\tau_{ex} \left\{ S_x^{(1,4)} \cos (\phi_{12}^{ex} - \pi/2) + S_y^{(1,4)} \sin (\phi_{12}^{ex} - \pi/2) \right\} \tag{10.18}$$

$$= S_z^{(1,4)} \cos |\omega_{12}|\tau_{ex}$$

$$+ \frac{1}{2} \sin |\omega_{12}|\tau_{ex} \left\{ S^{-(1,4)} e^{-i(\phi_{12}^{ex} - \pi/2)} + S^{+(1,4)} e^{-i(\phi_{12}^{ex} - \pi/2)} \right\}. \tag{10.19}$$

The terms containing $S^{-(1,4)}$ and $S^{+(1,4)}$ correspond to homonuclear $S$-spin $(-2)$-quantum and $(+2)$-quantum coherences respectively. The phase of the excited double-quantum coherence depends upon the phase $\phi_{12}^{ex}$ and therefore on the Euler angle $\gamma_{MR}$. The amplitude of the excited double-quantum coherence depends upon $|\omega_{12}|\tau_{ex}$ and therefore on the Euler angles $\alpha_{MR}$ and $\beta_{MR}$. For a single molecular orientation, the condition $|\omega_{12}|\tau_{ex} = \pi/2$ leads to the maximal amplitude for the double-quantum coherences. This corresponds to the
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In the case that \( \rho_2 \) is given by a vector in the \( S_x^{(1,4)} S_y^{(1,4)} \)-plane. In a powdered sample, this condition cannot be fulfilled for all molecular orientations. In this case, the selection of double-quantum coherences by the phase-cycling corresponds to taking the projection of \( \rho_2 \) onto the \( S_x^{(1,4)} S_y^{(1,4)} \)-plane.

If no evolution occurs during the interval \( t_1 \) and the phase cycling is taken into account, the density operators at time points \( \mathcal{Q}_2 \) and \( \mathcal{Q}_3 \) are given by

\[
\rho_\mathcal{Q}_3 = \rho_\mathcal{Q}_2 = \frac{1}{2} \sin |\omega_{12}| \tau_{\text{ex}} \left\{ S^{-(1,4)} e^{i \left( \phi_{12} - \frac{\pi}{2} \right)} + S^{+(1,4)} e^{-i \left( \phi_{12} - \frac{\pi}{2} \right)} \right\}.
\]

(10.20)

The phase \( \phi_{12} \) for the average Hamiltonian during the reconversion sequence is given by

\[
\phi_{12}^{\text{re}}(\gamma_{MR}, t_\mathcal{Q}, \phi_{12}^{\text{ex}}) = \phi_\kappa + m'(\omega_r t_\mathcal{Q} - \alpha_{RL}^0 - \gamma_{MR}) + 2\phi_0^{\text{re}} + \pi,
\]

(10.21)

where \( \phi_0^{\text{re}} \) is the overall rf phase of the reconversion sequence.

It is desired to reconvert the double-quantum coherence back into longitudinal magnetization on the \( S \)-spins. This can be achieved if the pseudo-field during the reconversion sequence points in the opposite direction to the pseudo-field during the excitation sequence (see Fig. 10.1(c)). This can be achieved if the following relationship for the phases of the average Hamiltonians during the excitation and reconversion sequence is valid:

\[
\phi_{12}^{\text{re}}(\gamma_{MR}, t_\mathcal{Q}, \phi_{12}^{\text{ex}}) = \phi_{12}^{\text{ex}}(\gamma_{MR}, t_\mathcal{Q}, \phi_{12}^{\text{ex}}) + \pi.
\]

(10.22)

This condition is fulfilled if

\[
\phi_0^{\text{re}} - \phi_0^{\text{ex}} = -\frac{m'}{2} \omega_r (t_\mathcal{Q} - t_\mathcal{Q}) + \frac{\pi}{2}.
\]

(10.23)

This is basically the phase-time relationship in Eq. (10.8). The additional phase of \( \pi/2 \) ensures that positive longitudinal magnetization is obtained at time point \( \mathcal{Q}_4 \). A phase of 0 would lead to negative longitudinal magnetization at time point \( \mathcal{Q}_4 \). If the phase cycle selects longitudinal magnetization at time point \( \mathcal{Q}_4 \), the density operator at this time point is given by

\[
\rho_\mathcal{Q}_4 = f_{2Q}(\tau_{\text{ex}}, \tau_{\text{re}}, \Omega_{MR}) S_z^{(1,4)},
\]

(10.24)

where \( f_{2Q}(\tau_{\text{ex}}, \tau_{\text{re}}, \Omega_{MR}) \) is called the filtering efficiency of the double-quantum coherence, which depends upon the molecular orientation and is given by

\[
f_{2Q}(\tau_{\text{ex}}, \tau_{\text{re}}, \Omega_{MR}) = \sin(|\omega_{12}| \tau_{\text{ex}}) \sin(|\omega_{12}| \tau_{\text{re}}).
\]

(10.25)

Note that the final filtering efficiency does not depend upon the Euler angle \( \gamma_{MR} \). A reduced dependence on the molecular orientation leads often to a higher double-quantum efficiency in non-oriented samples.
Figure 10.2: Simulated double-quantum filtering efficiencies in a two-spin $^{13}$C system as a function of $\tau_{\text{ex}} + \tau_{\text{re}}$ using the POST-C7 [41] sequence at a spinning frequency of $\omega_r/2\pi = 5.500$ kHz. Only homonuclear dipolar couplings are included in the simulations. (a) For an internuclear $^{13}$C–$^{13}$C distance of $r_{jk} = 0.152$ nm, which corresponds to a dipolar coupling of $b_{jk}/2\pi = -2156$ Hz. (b) For an internuclear $^{13}$C–$^{13}$C distance of $r_{jk} = 0.252$ nm, which corresponds to a dipolar coupling of $b_{jk}/2\pi = -477$ Hz.

In a powdered sample, all possible molecular orientations are present. Figure 10.1(d) visualizes the excitation and reconversion of double-quantum coherences in a powdered sample. The direction of the rotation axis depends upon the Euler angle $\gamma_{MR}$, while the flip angles depend upon the Euler angles $\alpha_{MR}$ and $\beta_{MR}$. The double-quantum filtering efficiency Eq. (10.25) has to be integrated over all possible orientations $\Omega_{MR}$:

$$\bar{f}_{2Q}(\tau_{\text{ex}}, \tau_{\text{re}}) = \langle f_{2Q}(\tau_{\text{ex}}, \tau_{\text{re}}, \Omega_{MR}) \rangle_{\Omega_{MR}}.$$  

(10.26)

Two typical curves for the double-quantum filtering (2QF) efficiency in a powdered sample as a function of the pulse sequence interval $\tau_{\text{ex}} + \tau_{\text{re}}$ are shown in Fig. 10.2. Such curves can be obtained experimentally by the pulse sequence shown in Fig. 10.1(a), using $t_1 = 0$. The excitation and reconversion intervals $\tau_{\text{ex}}$ and $\tau_{\text{re}}$ are kept equal to each other, both being incremented at the same time. The maximal theoretical 2QF efficiency in a powder is 73%. The curves were calculated for two different internuclear $^{13}$C–$^{13}$C distances using Eq. (10.26). Figure 10.2(a) shows the result if a internuclear distance of 0.152 nm is assumed. This corresponds to a homonuclear $^{13}$C–$^{13}$C dipolar coupling of $-2156$ Hz. Figure 10.2(b) shows the result if a internuclear $^{13}$C–$^{13}$C distance of 0.252 nm is assumed. This corresponds to a homonuclear $^{13}$C–$^{13}$C dipolar coupling of $-477$ Hz.

The curves have no strict periodic behavior, but one can still speak loosely about “oscillations” and the “frequency” of these oscillations. The oscillation frequency of the curves depends upon the recoupled dipolar interaction in
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10.2 Homonuclear Zero-Quantum Recoupling

Eq. (10.2) and therefore on the internuclear distance. It is therefore possible to measure internuclear distances by obtaining experimental double-quantum build-up curves and comparing them with simulations [44, 69]. This technique is especially useful in large molecules which are specifically labelled at two molecular sites, because the double-quantum filtering suppresses the natural abundance signal from other molecular sites.

10.2 Homonuclear Zero-Quantum Recoupling

In Paper III, the symmetry principle in Eq. (9.5) was used to design homonuclear zero-quantum recoupling sequences which provide an time-independent dipolar average Hamiltonian of the form

\[ \tilde{H}^{(1)} = \sum_{j \lt k} \left( \omega_{jk} \frac{1}{\sqrt{6}} \left( 2S_j z S_k z - \frac{1}{2} (S_j^+ S_k^+ + S_j^- S_k^-) \right) + 2\pi J_{jk} \mathbf{S}_j \cdot \mathbf{S}_k \right), \]

(10.27)

where \( J_{jk} \) is the \( J \)-coupling between spins \( S_j \) and \( S_k \), and \( \omega_{jk} \) is the recoupled through-space dipolar interaction, which is a real number.

Table I in Paper III presents solutions for \( \text{RN}_n \) sequences suitable for homonuclear zero-quantum recoupling. Two examples are the symmetries \( \text{R4}_4 \) and \( \text{R6}_6 \). These sequences recouple the homonuclear dipolar coupling terms with quantum numbers \( (l, m, \lambda, \mu) = \{(2, 1, 2, 0), (2, -1, 2, 0), (2, 2, 2, 0), (2, -2, 2, 0)\} \) in the first order average Hamiltonian. At the same time, all other homonuclear dipolar interactions, all chemical shift anisotropies, all isotropic chemical shifts and all heteronuclear couplings are removed. The RFDR sequence is identified as a special case of the \( \text{R4}_4 \) sequence. Supercycles are employed to improve the robustness of the \( \text{R4}_4 \) and \( \text{R6}_6 \) sequences with respect to isotropic chemical shifts and chemical shift anisotropies. The resulting supercycled sequences are denoted \( \text{SR4}_4 \) and \( \text{SR6}_6 \) and are discussed in detail in Paper III.

One of the major applications of zero-quantum recoupling sequences is the homonuclear correlation spectroscopy of uniformly \(^{13}\text{C}\)-labelled peptides and proteins. The \( \text{SR6}_6 \) sequence is compared with RFDR and RIL by simulating the longitudinal magnetization transfer for three typical cases, corresponding to spin-\( \frac{1}{2} \) pairs in a labelled peptide or protein. The \( \text{SR6}_6 \) sequence proves to be a good candidate for operation at high spinning frequencies and magnetic fields. The \( \text{SR4}_4 \) and \( \text{SR6}_6 \) sequences are demonstrated experimentally by obtaining longitudinal magnetization transfer curves for \([^{13}\text{C}_2, ^{15}\text{N}]\)-glycine and by acquiring 2D homonuclear correlation spectra of \([\text{U}-^{13}\text{C}]\)-L-tyrosine.

10.3 Heteronuclear Double-Quantum Recoupling

Section 8.2 discussed possible average Hamiltonians which are suitable for heteronuclear dipolar recoupling in spin-systems consisting of spins of type \( S \) and
K. Paper IV concentrated on the design of \( \gamma \)-encoded rf pulse sequences which recouple the heteronuclear dipolar interactions but which also decouple all the homonuclear dipolar interactions and all chemical shift anisotropies. As discussed there, this can only be achieved by applying \( \text{CN}_n \) and \( \text{RN}_n \) sequences to both spin species at the same time. Dual-channel heteronuclear double-quantum recoupling sequences were designed, which generate a time-independent heteronuclear double-quantum average Hamiltonian of the form:

\[
H^{(1)} = \sum_{s < k} (\omega_{sk} S^-_s K^-_k + \omega^*_{sk} S^+_s K^+_k),
\]

(10.28)

where \( \omega_{sk} \) is the recoupled through-space heteronuclear dipolar interaction and the sum is taken over all heteronuclear spin pairs.

Tables IV and V in Paper IV present solutions for \( \text{CRN}^\nu_{nS,\nu K} \) and \( \text{RN}^\nu_{nS,\nu K} \) sequences which generate \( \gamma \)-encoded heteronuclear double-quantum average Hamiltonians, of the form given in Eq. (10.28), and also remove all homonuclear dipolar interactions and chemical shift anisotropy terms for both spin species, \( S \) and \( K \). One example is the dual sequence \( R24^8;7 \), i.e., the symmetry \( R24^8 \) on the \( S \)-spins and the symmetry \( R24^7 \) on the \( K \)-spins. This dual sequence recouples the heteronuclear double-quantum terms \( (l, m, \lambda_S, \mu_S, \lambda_K, \mu_K) = \{(2, 1, 1, -1, 1, -1), (2, -1, 1, 1, 1, 1)\} \).

Heteronuclear double-quantum recoupling sequences may be used to excite heteronuclear double-quantum coherence. This is analogous to the case of exciting homonuclear double-quantum coherence by homonuclear double-quantum recoupling sequences. Figure 10.3(a) shows a sketch of an rf pulse sequence for passing \( S \)- and \( K \)-spin signals through heteronuclear double-quantum coherence. The rows marked with \( I \), \( S \) and \( K \) denote rf fields at the Larmor frequencies of the abundant protons, the \( S \)- and the \( K \)-spins; in this case \( S = ^{13}\text{C}, K = ^{15}\text{N} \). Enhanced longitudinal magnetization is prepared on the \( S \)- and \( K \)-spins by successive ramped cross polarization and a following \( \pi/2 \) pulse on the \( S \)- and \( K \)-spins. The heteronuclear double-quantum recoupling sequence of duration \( \tau_{ex} \) converts the sum longitudinal magnetization into heteronuclear \( (\pm 2) \)-quantum coherence. For the experiments described in this section, \( t_1 = 0 \). The excited heteronuclear double-quantum coherence is converted into longitudinal magnetization by the reconversion sequence of duration \( \tau_{re} \). The longitudinal magnetization is converted into observable magnetization by a \( \pi/2 \) read pulse. The \( S \)-spin NMR signal is detected in the subsequent period.

Experimental results for 10% \( [2,^{13}\text{C},^{15}\text{N}] \)-glycine are shown in Figs. 10.3(b) and (c). The experimental heteronuclear 2QF efficiency is plotted as a function of the pulse sequence interval \( \tau_{ex} + \tau_{re} \). The results were obtained using the dual sequence \( R24^8;7 \) with the basic elements \( R^S = R^K = 90_{180}360_{0}180_{180}90_{0} \) on the \( S \)- and \( K \)-spins. In Fig. 10.3(b) the excitation and reconversion intervals \( \tau_{ex} \) and \( \tau_{re} \) were kept equal to each other, both being incremented at the same
10.3 Heteronuclear Double-Quantum Recoupling

(a) Rf pulse sequence for passing S-spin signals through heteronuclear double-quantum coherence. (b) and (c) Symbols: Experimental double-quantum filtered efficiencies obtained on 10% labeled [2-13C,15N]-glycine as a function of $\tau_{ex} + \tau_{re}$ using the sequence $R_{24}^{8,7}$ with $B^S = B^K = 90_{180}360_0180_{180}90_0$. (b) The excitation and reconversion intervals are incremented simultaneously ($\tau_{ex} = \tau_{re}$). (c) The excitation interval was fixed to $\tau_{ex} = 2.250$ ms, while $\tau_{re}$ was varied. Solid lines: accurate numerical simulations of the amplitudes for $b_{SK}/2\pi = 888$ Hz. Dashed lines: average Hamiltonian simulations ignoring the heteronuclear $J$-coupling, using the same value for $b_{SK}/2\pi$ as for the solid line curves.
time. In Fig. 10.3(c), the excitation interval giving the maximum efficiency for the case $\tau_{\text{ex}} = \tau_{\text{re}}$ was first determined. The excitation interval was fixed to this value while the reconversion interval was incremented, starting at $\tau_{\text{re}} = 0$. This method usually provides larger amplitude modulations [69].

The solid lines in Figs. 10.3(b) and (c) are the result of accurate two-spin simulations, using the spin interaction parameters of $[2^{-13}\text{C},^{15}\text{N}]$-glycine. The heteronuclear dipolar coupling constant was varied to obtain the best fit between numerical simulations and experimental results. The determined dipolar coupling constant is in good agreement with the result obtained by REDOR studies [70]. The dashed lines in Figs. 10.3(b) and (c) are the results of simulations using the average Hamiltonian in Eq. (10.28). The average Hamiltonian simulations agree very well with the numerically exact simulations.

The details for the experiments and simulations can be found in Paper IV. In addition, experimental results and numerical simulations are shown for further heteronuclear double-quantum recoupling sequences of type CR$N_n^\nu S,\nu K$ and RN$N_n^\nu S,\nu K$.

## 10.4 Double-Quantum Spectroscopy

Homonuclear and heteronuclear double-quantum recoupling sequences may be used to study the dynamics of double-quantum excitation in a multiple-spin system. Here, the principles are discussed for the case of homonuclear double-quantum recoupling.

Consider a homonuclear two-spin system, $S_1$ and $S_2$, with the isotropic chemical shifts $\Omega_0^1$ and $\Omega_0^2$ respectively. Section 10.1.1 discussed how the pulse sequence shown in Fig. 10.1(a) creates homonuclear $(\pm 2)$-quantum coherence between $S_1$ and $S_2$ at time point 2. If $t_1 > 0$, CSA modulations are ignored and the experiment is performed far from rotational resonance [29–31], the double-quantum coherence evolves under the Hamiltonian of the isotropic chemical shifts of spins $S_1$ and $S_2$. The $(\pm 2)$-quantum coherence oscillates with the frequency $+(\Omega_0^1 + \Omega_0^2)$, while the $(\pm 2)$-quantum coherence oscillates with the frequency $+(\Omega_0^1 + \Omega_0^2)$. During the reconversion sequence and the following $\pi/2$-pulse, the double-quantum coherence is reconverted into observable $(\pm 1)$-quantum coherence on spins $S_1$ and $S_2$, oscillating at frequencies $+\Omega_0^1$ and $+\Omega_0^2$ respectively. The pulse sequence shown in Fig. 10.1(a) may be used to acquire a two-dimensional data matrix $s(t_1,t_2)$ by acquiring experiments with incrementation of the interval $t_1$. A two-dimensional spectrum $S(\omega_1,\omega_2)$ may be obtained by subjecting $s(t_1,t_2)$ to a complex Fourier transform in the $t_2$ dimension and a cosine Fourier transform in the $t_1$ dimension.

Paper I discusses that, under suitable conditions, the final 2D spectrum contains pure absorption double-quantum peaks. In case of the two spin system, two double-quantum peaks appear in the spectrum. One peak has frequency coordinates $(\omega_1,\omega_2) = (\Omega_0^1 + \Omega_0^2,\Omega_0^1)$. This corresponds to the transfer of 2QC
between $S_1$ and $S_2$ at time point $\mathbb{3}$ to longitudinal magnetization on $S_1$ at time point $\mathbb{4}$ by the homonuclear double-quantum recoupling sequence. The other peak has frequency coordinates $(\omega_1, \omega_2) = (\Omega_{11} + \Omega_{22}, \Omega_{22}^\prime)$, which corresponds to the transfer of double-quantum coherence between $S_1$ and $S_2$ to longitudinal magnetization on $S_2$.

Paper I studied the development of the 2D double-quantum peak amplitudes as a function of the pulse sequence intervals $\tau_{ex}$ and $\tau_{re}$. The experiments were performed on the three spin-system of $^{13}$C$_3$-L-alanine, shown in Fig. 10.4. Figure 10.5 shows experimentally obtained 2D double-quantum spectra of $^{13}$C$_3$-L-alanine. The experimental details can be found in Paper I. In both cases the excitation interval was given by $\tau_{ex} = 441.5 \, \mu s$.

Figure 10.5(a) shows the 2D double-quantum spectrum for a reconversion interval $\tau_{re} = 207.8 \, \mu s$. In the $\omega_1$ dimension, the frequencies of the three possible double-quantum coherences are labelled with the spin-sites involved in the double-quantum coherence. In the $\omega_2$ dimension, the frequencies of the three $(−1)$-quantum coherences are labelled with the corresponding spin-sites. For one 2D double-quantum peak, the underlying transfer process is sketched. The double-quantum coherence between the $\alpha$ and $\beta$ carbon sites is transferred during the reconversion part of the pulse sequence into single-quantum coherence of the $\alpha$ carbon site. In general, peaks representing transfer of double-quantum coherence between two spins into single-quantum coherence of a spin within the same pair are called direct double-quantum peaks. The 2D spectrum in Fig. 10.4 contains all six possible direct peaks for a three-spin system.

Figure 10.5(a) shows the 2D double-quantum spectrum for a reconversion interval $\tau_{re} = 1168.8 \, \mu s$. In this case, additional peaks are observed. For one of those the underlying transfer process is sketched. The double-quantum coherence between the $\alpha$ and $\beta$ carbon sites is transferred during the reconversion part of the pulse sequence into single-quantum coherence of the CO carbon site. In general, peaks representing transfer of double-quantum coherence between two spins into single-quantum coherence of a third spin are called indirect double-quantum peaks. The 2D spectrum in Fig. 10.4 contains, in addition to the six direct peaks, all three possible indirect peaks for a three-spin system.

It should be noted that the peak amplitudes of the direct peaks for short reconversion time $\tau_{re}$ are proportional to the reconversion time $\tau_{re}$, while the peak amplitudes of the indirect peaks are proportional to the cube of $\tau_{re}$. This allows effective suppression of the indirect peaks in double-quantum spectra, which makes assignment of the double-quantum peaks to spin-sites easier.

The amplitudes of the 2D double-quantum peaks may be measured as a
Figure 10.5: Experimental 2D double-quantum $^{13}\text{C}$ spectra of 98% $^{13}\text{C}_3$-L-alanine, obtained using the pulse sequence in Fig. 10.1(a) at $B_0 = 4.7$ T and a spinning frequency of $\omega_r/2\pi = 11.000$ kHz with $\tau_{\text{ex}} = 441.5$ $\mu$s and (a) $\tau_R = 207.8$ $\mu$s, (b) $\tau_R = 1168.8$ $\mu$s.
function of the reconversion interval $\tau_{re}$. In Fig. 11 of Paper I, the amplitudes of the nine 2D double-quantum peaks of $^{13}$C$_3$-L-alanine are plotted as a function of the reconversion interval $\tau_{re}$. The agreement between the experimental results and numerical simulations is good.

In Paper IV, dual rotor-synchronized heteronuclear double-quantum recoupling sequences of the type discussed in section 10.3 were used to obtain 2D heteronuclear double-quantum spectra.
11 Outlook

In part III of this thesis, the general symmetry principles for rotor-synchronized pulse sequences, denoted $CN_n^\nu$ and $RN_n^\nu$, were presented. These principles were successfully applied to different problems of recoupling in homonuclear and heteronuclear spin systems. The selection rules for the first order average Hamiltonian terms give a general idea about the recoupling properties of rotor-synchronized pulse sequences of type $CN_n^\nu$ and $RN_n^\nu$. However, to judge the performance of a pulse sequence under realistic conditions, in addition, the analysis of higher order average Hamiltonian terms or numerically exact simulations is required. So far, higher order average Hamiltonian terms have only been counted using higher order selection rules, in order to explain the different performance of different pulse sequences with the same properties in first order average Hamiltonian [9, Paper II]. Clearly, this type of analysis is improved considerably by also determining the sizes of the higher order terms.

A successful approach to identify well-compensated pulse sequences has been to systematically numerically simulate a selection of candidate sequences, obtained by evaluating the first order selection rules, together with different basic elements [Papers III, IV]. This approach may be largely automatized and is expected to help to effectively identify recoupling sequences with certain desired properties. In addition, it has been shown [Papers I, III] that supercycles can improve the performance of recoupling sequences considerably. Clearly, the inclusion of supercycling schemes in the average Hamiltonian selection rules (e.g., for SC14 in Paper I), further simplifies the design of rotor-synchronized pulse sequences.

The dual rotor-synchronized pulse sequences need not only be applied to the problem of selective heteronuclear recoupling. An important application is expected to be to achieve homonuclear recoupling for a rare spin species while heteronuclear couplings to an abundant spin species are decoupled. So far, this is achieved by applying a strong unmodulated rf field to the abundant spin species during the homonuclear recoupling sequence on the rare spins. In particular, the application of homonuclear recoupling sequences at high spinning frequencies requires a clear improvement in the heteronuclear decoupling. The principles of dual rotor-synchronized pulse sequences may assist the solving of this problem.
Part IV

Determination of Molecular Torsional Angles by Solid-State NMR
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In section 3.6 the measurement of molecular torsional angles by solid-state NMR was introduced. In the following, a brief overview is given of the existing techniques which have been developed in static and MAS solid-state NMR to directly determine molecular torsional angles. All these techniques are based on the determination of the relative orientation of pairs of anisotropic spin interaction tensors. The chemical shift anisotropy and the direct dipolar coupling are suitable anisotropic spin interactions. For that reason, methods have been developed to determine relative orientations between two CSA tensors, two dipolar coupling tensors or one CSA and one dipolar coupling tensor. However, to determine a molecular torsional angle from the relative orientations of two anisotropic tensors both their orientations with respect to suitable molecular segments must be known. Therefore, the choice of dipolar couplings is preferable, because the dipolar interaction tensor is always symmetric and is oriented along the internuclear vector, whilst the orientation of a CSA tensor with respect to a suitable molecular segment is, in general, not known in advance and must be measured separately.

In some cases, such as for the CSA tensor of the C' (i.e., CO) site in a polypeptide backbone (see Fig. 3.7), assumptions of the tensor orientations might be made with extreme care, but this reduces the accuracy of the torsional angle determination. There have been two general approaches taken to determine the relative orientation of anisotropic spin interaction tensors. (i) Techniques based on two-dimensional exchange NMR spectroscopy [71] and (ii) techniques using the evolution of multiple-quantum coherence under anisotropic spin interactions.

12.1 Techniques Based upon 2D Exchange NMR Spectroscopy

The basic idea of the techniques based upon two-dimensional exchange NMR is to let magnetization for one isotopically labelled spin-site evolve under the first anisotropic spin interaction (CSA, dipolar coupling) for a certain time interval, transfer this magnetization to another isotopically labelled spin-site and then let it evolve under the second anisotropic spin interaction for a another time interval.

Experiments of this type were performed in static solids to determine the relative orientation of a $^{13}$C CSA tensor and a $^{13}$C-$^{13}$C dipolar coupling tensor [72, 73]. The experiment is referred to as 2D DD/CSA (two-dimensional dipole-dipole/chemical-shift anisotropy) NMR exchange spectroscopy. Single-quantum coherence evolves under the $^{13}$C-$^{13}$C dipolar couplings for an interval $t_1$. ($^{13}$C chemical shifts are removed by a Carr-Purcell sequence.) Magnetization transfer is achieved by spin-diffusion of longitudinal $^{13}$C-spin magneti-
ization. Finally, single quantum coherence is allowed to evolve under the $^{13}\text{C}$ chemical shifts and the $^{13}\text{C}$$-^{13}\text{C}$ dipolar couplings for an interval $t_2$, while being detected. This experimental implementation results in two-dimensional powder patterns, which can be simulated to extract the relative orientation of the $^{13}\text{C}$ CSA tensor and the $^{13}\text{C}$$-^{13}\text{C}$ dipolar coupling. The same basic approach was used for the 2D CSA/CSA NMR exchange spectroscopy [73]. Both types of experiments have the disadvantage that the sensitivity and resolution is low, because they are performed in a static non-crystalline solid. In systems with diluted labelled molecules, the resulting two-dimensional spectra contain contributions from natural abundance $^{13}\text{C}$ which make the analysis more difficult.

The sensitivity and resolution of such experiments can be increased considerably by operating under MAS spinning conditions. At moderate spinning frequencies, 2D CSA/CSA MAS exchange spectroscopy might be used to obtain the relative orientation of two $^{13}\text{C}$ CSA tensors [74,75]. During the two evolution periods, $t_1$ and $t_2$, single-quantum coherence evolves under the CSA and direct dipolar couplings. During $t_2$, the signal is detected. The polarization transfer is again achieved by spin diffusion. The peak amplitudes of the cross peaks between sidebands in the 2D spectrum depend upon the relative orientation of the two CSA tensors. If two successive carbonyl sites in the peptide backbone are chosen (see Fig. 3.7), this experiment allows the determination of the relative torsional angles $\phi$ and $\psi$ [74,75]. Again, the two-dimensional spectrum will contain contributions from natural abundance $^{13}\text{C}$. In addition, this technique cannot be used at higher spinning frequencies, because spinning sidebands are no longer obtained. In this case, CSA recoupling under MAS during the evolution intervals is needed [PaperII].

The name relayed anisotropy correlation (RACO) NMR was chosen of Ishii et al. for the same type of experiments based on 2D exchange NMR spectroscopy [76]. They determined the relative orientation of a $^{13}\text{C}$ CSA tensor and a $^{13}\text{C}$$-^{1}\text{H}$ dipolar coupling tensor to determine an O–C–C–H torsional angle. In peptides, the torsional angle $\psi$ is defined by such an O–C–C–H torsional angle (see Fig. 3.7). Different implementations of this experiment have been reported [76,77]. The main difference lies in the choice of the sequences to recouple the $^{13}\text{C}$ CSA during one evolution interval and perform homonuclear $^{1}\text{H}$$-^{1}\text{H}$ dipolar decoupling, leaving the heteronuclear $^{13}\text{C}$$-^{1}\text{H}$ dipolar couplings, during a second evolution interval. In addition, the sequences chosen for polarization transfer between those two evolution intervals are different. However, the results are, in both cases, 2D spectra, containing powder-patterns, which are sensitive to the relative orientation of the $^{13}\text{C}$ CSA tensor and the $^{13}\text{C}$$-^{1}\text{H}$ dipolar coupling tensor. The main disadvantage, as discussed before, is the lack of suppression of signals from natural abundance $^{13}\text{C}$.

The same basic idea might be used to determine the relative orientation of two dipolar coupling tensors. The choice of heteronuclear over homonuclear
12.2 Techniques Using Multiple-Quantum Coherence

dipolar couplings has the advantage that the Hamiltonians for heteronuclear dipolar couplings for different spin pairs commute which each other [26]. Experiments of this type were performed to determine the torsional angles in H–C–C–H [78] and H–N–C–H [79, 80] molecular fragments. Again, the main differences lie in the choice of the homonuclear $^1$H–$^1$H decoupling sequence to allow evolution under only the heteronuclear $^{13}$C–$^1$H or $^{13}$C–$^{15}$N dipolar couplings and the choice of polarization transfer sequence, which connects the two evolution intervals. In all these experiments, the two evolution intervals were incremented independently from each other, leading to two-dimensional powder-like spectra which are sensitive to the relative orientation of the two dipolar coupling tensors.

Another possibility is to synchronously increment the two intervals, during which evolution under the chosen heteronuclear dipolar couplings occur. This approach was chosen to determine the torsional angles in H–C–C–H [44], H–N–C–H [44] and H–N–N–H [54] molecular fragments. This approach reduces the experimental time drastically. It becomes possible to acquire 2D spectra which correlate the isotropic chemical shifts of the different spin-sites as a function of the identical lengths of the two intervals during which evolution under the heteronuclear dipolar couplings is allowed. These experiments are performed at high spinning frequencies, so that the influence of $^{13}$C–$^{13}$C or $^{13}$C–$^{15}$N couplings during these two evolution intervals become negligible. Altogether, this allows the application in multiply-labelled systems to obtain several torsional angles at the same time [44, 54].

12.2 Techniques Using Multiple-Quantum Coherence

The basic idea behind such techniques is to excite a correlated spin-state (multiple-quantum coherence, MQC) and allow it to evolve under the influence of anisotropic spin interactions, such as CSA or heteronuclear dipolar couplings. The evolution of the MQC is not influenced by the dipolar couplings between the different spins-sites which are involved in the MQC [81]. The modulation of the MQC by the anisotropic spin-interactions is sensitive to their relative orientation. A typical case is the excitation of $^{13}$C MQC. In this case, signals stemming from natural abundance $^{13}$C are automatically suppressed.

The first experiment of this type was performed on a static non-crystalline sample to determine the relative orientation between a $^{13}$C$_\alpha$ CSA tensor and a $^{13}$CO CSA tensor [82]. Double-quantum coherence is created between the $\alpha$ and CO $^{13}$C sites, allowed to evolve freely under the $^{13}$C chemical shifts for a time interval $t_1$, then reconverted into observable magnetization and detected during the time interval $t_2$. This method allows the measurement of the torsional angle $\psi$ in a peptide backbone. The main advantage of this approach is that it only requires the isotopic labelling of one amino acid in the peptide or protein. Another technique in static solids determines the relative orientation between
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a $^{13}\text{CO}$ CSA tensor and a $^{13}\text{C}_\alpha-^{1}\text{H}$ heteronuclear dipolar coupling tensor [83].

As with experiments discussed previously, operation under MAS condition increases the sensitivity and resolution of such experiments. The free evolution of $^{13}\text{C}$ double-quantum coherences at moderate spinning-frequencies allows the determination of the relative orientation of two $^{13}\text{C}$ CSA tensors [84,85]. When applied to peptides, in order to determine the relative orientation of two $^{13}\text{CO}$ CSA tensors in the peptide backbone, the torsional angles $\phi$ and $\psi$ can be extracted [85].

Another class of experiments which uses the evolution of MQC under heteronuclear dipolar couplings is called multiple-quantum heteronuclear local field (MQ-HLF) spectroscopy. These experiments operate under MAS conditions and the basic idea is to excite MQC and allow them to evolve under the heteronuclear local fields of neighboring spins. The evolution of the MQC is sensitive to the correlation of the heteronuclear local fields, which makes it possible to determine torsional angles. In a basic implementation of such an experiment in a heteronuclear four-spin system, $K_1-S_1-S_2-K_2$, double-quantum coherence is excited between the $S$-spins and allowed to evolve under the heteronuclear dipolar $S-K$ couplings. In this case, the relative orientation of the two heteronuclear dipolar coupling tensors $S_1-K_1$ and $S_2-K_2$ can be determined, from which the torsional angle $K_1-S_1-S_2-K_2$ can be obtained.

The MQ-HLF experiments have the following properties: (i) Operation under MAS conditions guarantees good sensitivity and resolution. (ii) No knowledge is required of the orientations of CSA tensors. (iii) In isotopically labelled molecules, signals from natural abundance spins are suppressed. (iv) They prove to be very sensitive to the torsional angle in a certain range.

However, this technique also has some disadvantages: (i) Isotopic labelling is required. This is not, in general, a disadvantage because all previously discussed methods require isotopical labelling. However, if the determination of one torsional angle requires 4 specific labels (e.g. $S=^{13}\text{C}$ and $K=^{15}\text{N}$ in the example above), this is more costly than to use a method which determines the relative orientation of two CSA tensors. (ii) The excitation of MQC might be very inefficient, especially for higher order MQC.

The first MQ-HLF experiment was designed to measure the torsional angle in an $^{1}\text{H}-^{13}\text{C}-^{13}\text{C}-^{1}\text{H}$ molecular fragment [86]. This experiment is referred to as the HCCH-2Q-HLF experiment and will be discussed in detail in the next section. $^{13}\text{C}$ double-quantum coherence is exited and allowed to evolve under the heteronuclear $^{13}\text{C}-^{1}\text{H}$ dipolar couplings. The accuracy of the torsional angle determination is around $\pm 10^\circ$ near a torsional angle of $180^\circ$ (trans conformation) and around $\pm 20^\circ$ near a torsional angle of $0^\circ$ (cis conformation). The HCCH-2Q-HLF experiment has found several applications in biological molecules. In the light receptor protein rhodopsin the H–C10–C11–H molecular torsional angle in the isomerization region of the chromophore (retinal) was
determined in the ground state of rhodopsin [87] and in the metarhodopsin-I photointermediate [88]. The measurement of the H–C1–C2–H torsional angle in glucose allows the distinction between the α- and the β-anomer [89]. The HCCH-2Q-HLF experiment was one of the methods used to determine the structure of different forms of the polymorphic drug compound cimetidine [90]. In this case, the relative orientation between a $^{13}$CH and a $^{12}$CH$_3$ group was determined, where the two $^{13}$C labels where separated by a distance of 0.55 nm, which allowed the excitation of $^{13}$C double-quantum coherence with an efficiency of only about 1%.

The concept of MQC-HLF spectroscopy was applied to measure the torsional angle $\phi$ in a peptide by determining the torsional angle in the $^1$H–$^{15}$N–$^{13}$C–$^1$H molecular fragment in the peptide backbone (see Fig. 3.7) [91,92]. This method exploits the evolution of heteronuclear $^{15}$N–$^{13}$C double- and zero-quantum coherence under the heteronuclear $^{15}$N–$^1$H and $^{13}$C–$^1$H dipolar couplings. The peptide torsional angle $\phi$ is, in the ideal case, related to the H–N–C–H torsional angle in an L-amino acid by $\phi = \phi_{H-N-C-H} + 60^\circ$. However, in practice, this relationship might deviate from the ideal case by up to about 10°, which detracts from the accuracy of the determination of $\phi$ [44]. The torsional angle $\phi_{H-N-C-H}$ takes very different values in different secondary structures of proteins, about 165°–180° in β-sheets and about 120° in α-helices [93]. This allows the design of an experiment to obtain two-dimensional $^{15}$N–$^{13}$C chemical shift correlation spectra in multiple $^{15}$N- and $^{13}$C-labeled proteins, where signals from α-helical conformations are removed while signals from β-sheet conformations are retained [93].

The torsional angle $\psi$ in a peptide backbone might be determined with the help of NCCN-2Q-HLF experiments [49, Paper V]. These experiments determine the torsional angle in an $^{15}$N–$^{13}$C–$^{13}$C–$^{15}$N molecular fragment by exploiting the evolution of $^{13}$C double-quantum coherence under the recoupled $^{13}$C–$^{15}$N heteronuclear dipolar couplings. The experiment has also been generalized to the case where $^{13}$C triple-quantum coherence is allowed to evolve under the heteronuclear fields of neighboring $^{15}$N spins [Paper VI]. These experiments are in general referred to as NCCN-MQ-HLF experiments and will be discussed in detail below.
13 The HCCH-2Q-HLF Experiment

13.1 Homonuclear Decoupling

For the HCCH-2Q-HLF experiment, the excited correlated spin state between the two $^{13}$C spins (double-quantum coherence) is allowed to evolve under the heteronuclear local fields of the neighboring $^1$H spins for an interval $t_1$. During $t_1$ the $^{13}$C–$^1$H heteronuclear dipolar couplings need to be present. In the experiments described below, this is achieved by applying a homonuclear decoupling sequence during the interval $t_1$. Homonuclear $^1$H–$^1$H dipolar couplings are suppressed, leaving the heteronuclear $^{13}$C–$^1$H interactions, usually scaled down by a factor $\kappa < 1$. In the original implementation of the HCCH-2Q-HLF experiment [86], the MREV8 sequence [94] was used for homonuclear decoupling. In the implementation discussed below, the Frequency Switched Lee-Goldburg scheme [95] was used, which proves to be more robust in practical applications.

Both these homonuclear recoupling sequences generate a time dependent average Hamiltonian of the form

$$H_{IS}(t) = \kappa \sum_{j,k} \omega_{jk}^{IS}(t) I_{jz} S_{kz}, \quad (13.1)$$

where the $\omega_{jk}^{IS}(t)$ are given by Eq. (5.52). The advantage of this type of average Hamiltonian is that the terms $I_{jz} S_{kz}$ commute for different spin pairs. This makes it easy in the analysis discussed below to include additional heteronuclear $^{13}$C–$^1$H couplings [26]. Due to the time-dependence of $\omega_{jk}^{IS}(t)$, the evolution of the double-quantum coherence of the $^{13}$C is refocused after one rotational period $\tau_r = 2\pi/\omega_r$, where $\omega_r$ is the sample rotation frequency. Therefore this type of time dependent average Hamiltonian is only useful for application to the HCCH-2Q-HLF experiment if the scaled $^{13}$C–$^1$H heteronuclear dipolar couplings are larger or about the same as the sample spinning frequency. The scaling factor $\kappa$ typically has a value of $0.3 < \kappa < 0.6$ and therefore the sample spinning frequency should not exceed 10.000 kHz. Below is discussed a way of preventing the refocusing of the double-quantum evolution for another rotational period, so that the sample spinning frequency should not exceed 20.000 kHz in this case. In addition, the average Hamiltonian in Eq. (13.1) is obtained under the assumption that the nutation frequency of the rf field on the $^1$H is large compared to the sample rotation frequency ($\omega_{nut}^H \gg \omega_r$). This is called the “quasi-static” approximation. So, with increasing sample spinning frequency, one also has to increase the nutation frequency of the rf field on the $^1$H channel to be able to use Eq. (13.1).

At higher spinning frequencies it is necessary to apply heteronuclear recoupling sequences for use in rotating solids. A few of them were mentioned in section 8.3.2. Sequences like TC5 [52], T-MREV [53,54] and R187 [PaperII] re-
couple the $^{13}\text{C}^{-1}\text{H}$ heteronuclear dipolar couplings, while decoupling the $^1\text{H}^{-1}\text{H}$ homonuclear dipolar couplings, if they are applied on the $^1\text{H}$.

### 13.2 Experimental Implementation

Two possible rf pulse sequences to perform the HCCH-2Q-HLF experiment are shown in Fig. 13.1. The row marked $I$ denotes the rf fields at the Larmor frequency of the abundant protons, while $S$ denotes the rf fields applied at the $^{13}\text{C}$ Larmor frequency.

The sequence in Fig. 13.1(a) is based on the original implementation, presented in Ref. 86. The excitation and reconversion of homonuclear double-quantum coherences is discussed in section 10.1. The POST-C7 sequence [41] was used as the homonuclear double-quantum recoupling sequence (see Table 10.1). The excited double-quantum coherences are allowed to evolve for one rotation period, $\tau_r$. The constant interval is divided into two parts. The first part is given by an interval $t_1$, occupied by a homonuclear decoupling sequence, applied on the $I$-spins, FSLG in this case. The second part is an interval $\tau_r - t_1$, during which unmodulated high-power proton decoupling is applied to decouple the heteronuclear $^{13}\text{C}^{-1}\text{H}$ interactions. A series of experiments is acquired in which $t_1$ is increased. This implementation requires that the rf reference frequency is exactly set to the mean value of the isotropic chemical shifts of the two $S$-spins, in order to avoid evolution of the double-quantum coherence under the chemical shifts.

The sequence in Fig. 13.1(b) is a modification of that in Fig. 13.1(a). The excited double-quantum coherences on the $S$-spins are allowed to evolve for two rotation periods. In the middle of the evolution period a $\pi$-pulse is applied on the $S$-spins in order to refocus the evolution of the double-quantum coherences under the isotropic chemical shifts of the $S$-spins. Due to the change in the phases of the double-quantum coherences induced by the $\pi$-pulse, a reconversion
sequence with opposite winding number $\nu$, denoted $CN_n^{-\nu}$, has to be applied to reconvert the double-quantum coherences into longitudinal $S$-spin magnetization (see section 9.6). The interval $t_1$, occupied by the homonuclear decoupling sequence, is divided into two parts which are incremented in the directions shown in Fig. 13.1(b). Without the $\pi$-pulse on the $S$-spins the evolution of the double-quantum coherences under the heteronuclear $^{13}$C-$^1$H couplings would be refocused at $t_1 = \tau_r$. The $S$-spin $\pi$-pulse prevents that and the evolution is first refocused at $t_1 = 2\tau_r$.

### 13.3 Theory

In the following, only a brief overview of the theory for the experiments in Figs. 13.1(a) and (b) is given. The theory of the original sequence in Fig. 13.1(a) is presented in Refs. 86 and 16. The theory of the modified sequence in Fig. 13.1(b) has not been presented before, but is a straightforward extension of that in Ref. 16.

Consider the heteronuclear four spin system $I_1-[S_1,S_2]-I_2$. For the pulse sequences in Fig. 13.1(a) and (b) the double-quantum filtered signal amplitude for a single molecular orientation $\Omega_{MR}$ as a function of $t_1$ is given by

$$ a(t_1, \Omega_{MR}) = \frac{1}{2} f_{2Q}(\tau_{ex}, \tau_{re}, \Omega_{MR}) \left\{ \cos \Phi_{\text{evol}}^{\alpha \alpha}(t_1) + \cos \Phi_{\text{evol}}^{\alpha \beta}(t_1) \right\}, \quad (13.2) $$

where the Euler angles $\Omega_{MR} = \{\alpha_{MR}, \beta_{MR}, \gamma_{MR}\}$ relate the molecular fixed frame to a frame fixed on the rotor. The symbols $\alpha$ and $\beta$ are used as shorthand for the quantum numbers $m_z = \frac{1}{2}$ and $m_z = -\frac{1}{2}$. The factor $f_{2Q}(\tau_{ex}, \tau_{re}, \Omega_{MR})$ is the filtering efficiency of the homonuclear double-quantum recoupling sequence chosen. It depends upon the excitation and reconversion intervals and on the molecular orientation $\Omega_{MR}$ and is defined by Eq. (10.25) in section 10.1.1.

The phase $\Phi_{\text{evol}}^{m_1 m_2}(t_1)$ depends on whether the pulse sequence in Fig. 13.1(a) or (b) is chosen:

(i) Pulse sequence in Fig. 13.1(a):

$$ \Phi_{\text{evol}}^{m_1 m_2}(t_1) = \Phi_{IS}^{m_1 m_2}(t_1, 0). \quad (13.3) $$

(ii) Pulse sequence in Fig. 13.1(b):

$$ \Phi_{\text{evol}}^{m_1 m_2}(t_1) = \Phi_{IS}^{m_1 m_2}(0, -\frac{t_1}{2}) - \Phi_{IS}^{m_1 m_2}(\frac{t_1}{2}, 0). \quad (13.4) $$

The negative sign for the second phase is created by the $\pi$-pulse on the $S$-spins. A positive sign at this point would lead to the expression $\Phi_{IS}^{m_1 m_2}(t_1/2, -t_1/2)$ on the right hand side of the equation, which would then correspond to Eq. (13.3).
In both cases the accumulate phase $\Phi_{I_S}^{m_1 m_2}(t_b, t_a)$ is given by

$$\Phi_{I_S}^{m_1 m_2}(t_b, t_a) = \kappa \int_{t_a}^{t_b} dt \sum_{j=1, s=1}^{2} m_j \omega_{j s}^{I S}(t),$$

(13.5)

where the sums over $j$ and $s$ are taken over all spins $I_j$ and $S_s$ respectively. Note the definition of $\omega_{j s}^{I S}(t)$ by Eqs. (5.52) and (5.41), which contain an additional factor of 2. For a powdered sample Eq. (13.2) must be averaged over all possible molecular orientations $\Omega_{MR}$:

$$a(t_1) = \langle a(t_1, \Omega_{MR}) \rangle_{\Omega_{MR}},$$

(13.6)

13.4 Results and Discussion

Figures 13.3(a) and (b) show experimental results for diammonium 2,2'-$^{13}$C$_2$-fumarate (DAF), obtained using the pulse sequences in Figs. 13.1(a) and (b) respectively. The H–C–C–H fragment in DAF is in the trans conformation (Fig. 13.2). In both cases, the experimental 2QF signal amplitudes (symbols) are plotted as a function of the pulse sequence interval $t_1$. The signal amplitudes were estimated by integrating the experimental double-quantum filtered spectra. The integrals were normalized to the spectral integral for $t_1 = 0$. In addition, Figs. 13.3(a) and (b) show simulations of modulation curves for the H–C–C–H fragment in DAF in which the bond lengths and angles are kept constant (the same parameters were used as in Ref. 86). The only free parameters in the simulations were the H–C–C–H torsional angle $\phi$ and the scaling factor $\kappa$. The simulations are only sensitive to the absolute value of the torsional angle, $|\phi|$. The solid lines in both figures represent the best fit between the experimental data and the simulated curves, which were obtained by varying the torsional angle and $\kappa$. In addition, simulated curves are shown for a few different values of the torsional angle, but keeping the same scaling factor $\kappa$.

In Fig. 13.3(a), the torsional angle for the best fit is given by $|\phi| = 180^\circ$, which is the one expected from the trans conformation of DAF. The simulated curves for the same value of $\kappa$ but torsional angles of 160$^\circ$ and 170$^\circ$ differ from the curve for 180$^\circ$.

In Fig. 13.3(b), on the other hand, the torsional angle for the best fit is given by $|\phi| = 170^\circ$, which is 10$^\circ$ smaller than expected. The differences between simulated curves for different torsional angles and the same value of $\kappa$ are more pronounced in this experiment. This suggests that the angular resolution of the
Figure 13.3: (a) and (b): Symbols: Experimental 2QF signal amplitudes as a function of $t_1$, obtained on diammonium $2,2'$-$^{13}$C$_2$-fumerate at a field of $B_0 = 4.7$ T and a spinning frequency of $\omega_r/2\pi = 5.600$ kHz. The experimental 2QF efficiency was around 44%. The proton nutation frequency during FSLG was 82.3 kHz in both cases. Lines: Numerical simulations of the signal amplitudes, assuming different H–C–C–H torsional angles. (a) Results obtained by using the pulse sequence in Fig. 13.1(a). All simulations use $\kappa = 0.54$, which was obtained for the best fit at the torsional angle of 180°. (b) Results obtained by using the pulse sequence in Fig. 13.1(b). All simulations use $\kappa = 0.57$, which was obtained for the best fit at the torsional angle of 170°. (c) and (d): Root mean square deviation between pairs of simulated signal amplitude curves as a function of the two torsional angles. The contour lines correspond to the heights 0.020, 0.075, 0.133, 0.195, 0.260, 0.328 and 0.400 (lowest values correspond to the dark areas). (c) For the pulse sequence in Fig. 13.1(a) using $\kappa = 0.54$, (d) for the pulse sequence in Fig. 13.1(b) using $\kappa = 0.57$. 

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experiment in Fig. 13.1(b) is significantly higher than that for the experiment in Fig. 13.1(a). However, this makes it more difficult to explain the deviation. Possible causes might be: (i) additional protons on neighboring molecules, which are not taken into account but might influence the signal modulation, (ii) rf inhomogeneity, (iii) non-uniform cross-polarization.

Figures 13.3(c) and (d) show the sensitivity of the experiments in Figs. 13.1(a) and (b) to the H–C–C–H torsional angle. In both cases, the signal amplitudes were simulated for torsional angles in the range $[0^\circ, 180^\circ]$, again taking all other conformational parameters from DAF. The scaling factor $\kappa$ was the same for all these simulations. The root mean square deviation was calculated between each pair of curves and plotted as a function of the two torsional angles. These plots give an estimation on how much the two curves for two different torsional angles differ from each other. The more the two curves differ, the larger is the value for the root mean square deviation. The ideal case is, therefore, a deep valley along the diagonal of those plots. Figures 13.3(c) and (d) show that both the experiments in Figs. 13.1(a) and (b) are very sensitive to the torsional angle in the range $[130^\circ, 180^\circ]$. Figure 13.3(c) shows clearly ambiguities in the range $[20^\circ, 90^\circ]$. Also, Fig. 13.3(d) shows ambiguities, but they are less pronounced. In addition, the valley in the sensitive region $[130^\circ, 180^\circ]$ is considerably steeper in Fig. 13.3(d), which corresponds to a higher resolution in the torsional angle in the sensitive region of these experiments.

It should be noted that a similar approach as presented in Fig. 13.1(b) was used to improve the sensitivity of an HNCH experiment, based on 2D exchange NMR, to determine the peptide torsional angle $\phi$ [92].
14 The NCCN-MQ-HLF Experiment

14.1 Heteronuclear Recoupling

For the NCCN-MQ-HLF experiment, the excited $M$-quantum coherence between $^{13}$C spins is allowed to evolve under the heteronuclear local fields of the neighboring $^{15}$N spins. The $^{15}$N spins are as rare as the $^{13}$C spins and the $^{15}$N–$^{15}$N dipolar couplings are small and therefore no homonuclear $^{15}$N–$^{15}$N decoupling is needed during the evolution interval of the MQC. In principle, experiments of the type shown in Figs. 13.1(a) and (b), but with strong proton decoupling during the whole evolution interval, could be used for this type of experiments. The typical size of dipolar couplings between directly bonded $^{13}$C and $^{15}$N is about 1.000 kHz. Following the arguments of section 13.1, the spinning frequency should not exceed 1.000 kHz for experiments of type Fig. 13.1(a) and not exceed 2.000 kHz for experiments of type Fig. 13.1(b). This slow spinning has, on the other hand, several disadvantages: (i) The signal to noise ratio in the $^{13}$C spectra is reduced, because the signal intensity is distributed over several spinning sidebands. (ii) The spectral resolution of the $^{13}$C spectra is reduced for the same reason. As discussed in section 13.1, at higher spinning frequency it is necessary to apply heteronuclear recoupling sequences for use in rotating solids, in this case to regain the heteronuclear $^{13}$C–$^{15}$N dipolar couplings.

A few possible sequences were discussed in section 8.3.2. In the implementations of the NCCN-MQ-HLF experiments which will be briefly discussed below, different versions of the REDOR method [50, 51] are used. The other implementation of the NCCN-2Q-HLF experiment uses a modification of the rotary resonance recoupling ($R^3$) technique [45, 46], which is denoted SPI-$R^3$ [49]. These techniques all create time-independent average Hamiltonians of the type

$$\tilde{H}_{SK} = \sum_{s,k} \omega_{sk}^{SK} S_{sz} K_{kz}. \quad (14.1)$$

The advantage of this type of average Hamiltonian is that the terms $S_{sz} K_{kz}$ commute for different spin pairs [26], similar to the case discussed in section 13.1.

14.2 Different NCCN-MQ-HLF Experiments

The first implementations of the of the NCCN-MQ-HLF experiment used $^{13}$C–$^{13}$C double-quantum coherences evolving under the heteronuclear dipolar $^{13}$C–$^{15}$N couplings [49, Paper V] and is referred to as NCCN-2Q-HLF experiments. They enable the measurement of the torsional angle $\psi$ in a $^{15}$N–$^{13}$C–$^{13}$C–$^{15}$N fragment in the backbone of a polypeptide or protein.
14.2 Different NCCN-MQ-HLF Experiments

The NCCN-2Q-HLF experiment requires the selective labelling of the molecular fragment of interest, which is more costly than to use a uniformly $^{13}\text{C, }^{15}\text{N}$-labeled peptide instead. However, in a uniformly labelled peptide the simple strategy, presented in Paper V, is no longer sufficient for two reasons: (i) The $^{13}\text{C}\alpha-{ }^{13}\text{CO}$ double-quantum coherences do not only evolve under heteronuclear dipolar $^{13}\text{C}-{ }^{15}\text{N}$ couplings but also under the additional homonuclear $^{13}\text{C}-{ }^{13}\text{C}$ couplings. (ii) In a simple experimental setup, it is not possible to distinguish signals stemming from different $^{13}\text{C}-{ }^{13}\text{C}$ double-quantum coherences which were excited during the homonuclear double-quantum recoupling sequence. There are different possibilities for how to overcome problems (i) and (ii).

The first possibility includes the following two steps. First, it is necessary to remove the $^{13}\text{C}-{ }^{13}\text{C}$ dipolar couplings during the evolution interval of the $^{13}\text{C}-{ }^{13}\text{C}$ double-quantum coherences, while recoupling the heteronuclear $^{13}\text{C}-{ }^{15}\text{N}$ dipolar couplings. Pulse sequences which achieve this exist and include the generalized HH sequences [Paper IV], discussed in section 10.3, multiple-pulse decoupled REDOR (MPDR) [96], C-REDOR [55] and variants of REDOR, based on $R_{N_{0}}$ symmetries, briefly discussed in Paper IV.

Second, the pulse sequence for the NCCN-2Q-HLF experiment must incorporate an additional double-quantum evolution interval during which the double-quantum coherences are allowed to evolve under the $S$-spin chemical shifts. The result will be a series of two-dimensional double-quantum spectra, where the double-quantum peak intensities are modulated by the heteronuclear $^{13}\text{C}-{ }^{15}\text{N}$ couplings. This type of two-dimensional spectra are discussed in section 10.4.

Another approach to overcome problems (i) and (ii) is to excite the extreme coherences in the system of coupled $^{13}\text{C}$ spins in the peptide of interest. The extreme coherences in a system of $M$ coupled $S$-spins are the ones of order $\pm M$. These coherences are called $M$-quantum coherences (MQC). They do not evolve under the homonuclear $S$-spin interactions [81]. A special case of this concept is the evolution of $^{13}\text{C}$ double-quantum coherences in a $^{15}\text{N}-{ }^{13}\text{C}-{ }^{15}\text{N}$ molecular fragment. However, uniformly $^{13}\text{C, }^{15}\text{N}$-labelled glycine is the only example of a uniformly labelled amino acid which provides isolated $^{13}\text{C}-{ }^{13}\text{C}$ pairs. In other amino acids higher order multiple-quantum coherences would have to be excited to avoid evolution under additional $^{13}\text{C}-{ }^{13}\text{C}$ couplings. Examples are triple-quantum coherences (3QC) in alanine, cysteine and serine, or four-quantum coherences (4QC) in threonine, asparagine and aspartic acid. It should be noted that some of these peptides contain additional $^{15}\text{N}$ spins if they are uniformly $^{15}\text{N, }^{13}\text{C}$-labelled. However, additional $^{13}\text{C}-{ }^{15}\text{N}$ couplings can easily be taken into account because the Hamiltonians of different heteronuclear couplings commute when using the REDOR sequence as the recoupling sequence [26]. The excitation of $^{13}\text{C}$-MQC and evolution under heteronuclear
The NCCN-MQ-HLF Experiment

14.3 Experimental Implementation

Figures 14.1 and 14.2 show possible experimental implementations of the NCCN-MQ-HLF experiment. These experiments are triple-resonance experiments, i.e., they involve rf irradiation at the Larmor frequencies of three different spin species. The spin species are identified as: $I = ^1\text{H}$, $S = ^{13}\text{C}$ and $K = ^{15}\text{N}$.

The pulse sequences shown in Figs. 14.1(a) and (b) exploit homonuclear $S$-spin 2QC. The excitation and reconversion of 2QC using homonuclear double-quantum recoupling sequences of type $CN_n^\nu$ or $RN_n^\nu$ was discussed in general in section 10.1 and in the context of the HCCH-2Q-HLF experiment in section 13.2.

The pulse sequence shown in Fig. 14.2 exploits $S$-spin 3QC, which is excited by applying a homonuclear recoupling sequence of type $CN_n^\nu$ or $RN_n^\nu$ directly after enhanced transverse $S$-spin magnetization is prepared by ramped cross-polarization [97]. In general, this allows the excitation of multiple-quantum coherences on the $S$-spins of odd order [97, 98]. We select 3QC on the $S$-spins by appropriate phase-cycling [4].
14.3 Experimental Implementation

The total evolution interval $t_1$ of the MQC in the implementations in Figs. 14.1(a) and (b) is variable. Such experiments are referred to as *variable-time* experiments. The evolution interval $t_1$ is, in both cases, given by an integer number of rotor periods: $t_1 = n\tau_r$. In both cases, during the evolution period, strong rf pulses of flip angle $\pi$ and duration $\tau_p$ are applied at the $K$-spin Larmor frequency at intervals of $\tau_r/2$. This corresponds to the REDOR technique [50,51], which inhibits the coherent averaging of the $^{13}\text{C}^{15}\text{N}$ dipolar couplings by the magic-angle rotation. A series of experiments is acquired in which $n$ is increased. Since the length of the total evolution interval is varied, the variable-time implementation requires the determination of the relaxation time constant of the MQC during the evolution interval.

The pulse sequence in Fig. 14.2 employs a *constant-time* implementation of the REDOR sequence [51]. This means that the total evolution period of the MQC is constant. A constant number of $\pi$-pulses is applied on the $K$-spins during that interval. A series of experiments is performed by moving half of the $\pi$-pulses on the $K$-spins in the fashion shown in Fig. 4 of Paper VI. Note that, in the following, $t_1$ will be used as the time interval by which the $\pi$-pulses are moved. The constant-time implementation allows the determination of the relaxation time constant of the MQC during the evolution interval to be dispensed with.

The implementation in Fig. 14.1(a) requires that the $S$-spin rf reference frequency during $t_1$ is set exactly to the mean value of the isotropic chemical shifts of the two $S$-spins, in order to avoid evolution of the double-quantum coherence under the isotropic chemical shifts. The implementations in Figs. 14.1(b) and 14.2 lift that restriction, because the evolution of the MQC under the isotropic chemical shift is refocused by the $\pi$-pulse on the $S$-spins in the middle of the

![Figure 14.2: (a) Pulse sequence diagram for the constant-time implementation of the NCCN-3Q-HLF experiment. (b) The coherence transfer pathway diagram. Reprinted with permission from Ref. 99.](image-url)
total evolution period, which replaces the π-pulse on the $K$-spins at this position. Section 14.7 will show that this π-pulse on the $S$-spins also refocuses the evolution of the MQC under the Bloch-Siegert shift.

14.4 Theory

The theory of the general NCCN-MQ-HLF experiment is discussed in Paper VI. Here, only a brief overview of the results will be given. Consider the heteronuclear spin system $K_1-[S_1, S_2, \ldots, S_M]-K_2$, where nothing is assumed about the geometrical conformation of the $M$ $S$-spins. The multiple-quantum filtered (MQF) signal amplitude for the coherences of order $M$ for a single molecular orientation $\Omega_{MR}$ as a function of $t_1$ is given by:

$$ a(t_1, \Omega_{MR}) = \frac{1}{2} f_{MQ}(\tau_{ex}, \tau_{re}, \Omega_{MR}) \left\{ \cos \Phi_{evol}^{\alpha_1}(t_1) + \cos \Phi_{evol}^{\alpha_2}(t_1) \right\}, \quad (14.2) $$

where the factor $f_{MQ}(\tau_{ex}, \tau_{re}, \Omega_{MR})$ is the filtering efficiency for the excitation and reconversion of the $S$-spin MQC, which depends upon the excitation and reconversion intervals and on the molecular orientation $\Omega_{MR}$. In section 10.1.1, Eq. (10.25), the expression for $f_{2Q}(\tau_{ex}, \tau_{re}, \Omega_{MR})$ is given. In Ref. 97 and Paper VI the expression for $f_{3Q}(\tau_{ex}, \tau_{re}, \Omega_{MR})$ can be found.

For the variable-time implementation shown in Figs. 14.1(a) and (b), the following expressions for the phase $\Phi_{evol}^{m_1 m_2}(t_1)$ are obtained, assuming infinitely strong π-pulses ($\tau_p = 0$) on the $K$-spins:

$$ \Phi_{evol}^{m_1 m_2}(t_1) = n \tau_r = n \left\{ \Phi_{SK}^{m_1 m_2} \left( \frac{\tau_r}{2}, 0 \right) - \Phi_{SK}^{m_1 m_2}(\tau_r, \frac{\tau_r}{2}) \right\}. \quad (14.3) $$

For the constant-time implementation shown in Fig. 14.2, the following expressions for the phase $\Phi_{evol}^{m_1 m_2}(t_1)$ are obtained, assuming infinitely strong π-pulse on the $K$-spins:

$$ \Phi_{evol}^{m_1 m_2}(t_1) = \Phi_{SK}^{m_1 m_2}(t_1, 0) - \Phi_{SK}^{m_1 m_2}(0, -t_1). \quad (14.4) $$

Paper VI contains additional expressions for the case of pulses of finite duration. Note the similarity of Eqs. (14.4) and (13.4). This shows that the modulation of the MQC by the $^{13}C-{^{15}}N$ heteronuclear dipolar couplings in the constant-time implementation of the NCCN-MQ-HLF experiment is analogous to the modulation of the 2QC by the $^{13}C-{^1}H$ heteronuclear dipolar couplings in the implementation of the HCCH-2Q-HLF experiments shown in Fig. 13.1(b).

In both cases, Eqs. (14.3) and (14.4), the accumulate phase $\Phi_{SK}^{m_1 m_2}(t_b, t_a)$ is given by

$$ \Phi_{SK}^{m_1 m_2}(t_b, t_a) = \int_{t_a}^{t_b} dt \sum_{s=1}^{M} \sum_{k=1}^{2} m_k \omega_{sk}^{SK}(t), \quad (14.5) $$
where the sums over $s$ and $k$ are taken over all spins $S_s$ and $K_k$ respectively. Note also in this case the definition of $\omega_{sk}^{SK}(t)$ by Eqs. (5.52) and (5.41), which contain an additional factor of 2. As in the case of the HCCH-2Q-HLF experiment, Eq. (14.2) must be averaged over all possible molecular orientations $\Omega_{MR}$ for a powdered sample:

$$a(t_1) = \langle a(t_1, \Omega_{MR}) \rangle_{\Omega_{MR}}.$$  \hspace{1cm} (14.6)

### 14.5  Torsional Angle Estimation in Gly-Gly-Gly

The pulse sequences shown in Fig. 14.1 were used to determine the torsional angle $\psi$ of a $^{15}\text{N}^{13}\text{C}^{13}\text{C}^{15}\text{N}$ fragment in the isotopically labelled tripeptide $[^{15}\text{N}, ^{13}\text{C}_2\text{-gly-}[^{15}\text{N}\text{-gly-}^{15}\text{N}]\text{gly}$ (GGG). The structure and labelling is shown in Fig. 14.3(a).

Paper V used the pulse sequence shown in Fig. 14.1(a) to determine the torsional angle $\psi$. The experimental results are shown in Fig. 14.4(a) and were obtained at a static field of $B_0 = 9.4$ T and a spinning frequency of $\omega_r / 2\pi = 5.059$ kHz. The homonuclear double-quantum recoupling sequence to excite $S$-spin double-quantum coherence was given by a C7 sequence [40] ($C_{1/2}$, $\mathcal{C} = 360_0360_{120}360_{240}$). The $S$-spin nutation frequency during the recoupling sequence was 35.413 kHz. For further details of the experiment and the sample GGG, see Paper V.

The experimental results shown in Fig. 14.4(b) were obtained on the same sample at a static field of $B_0 = 4.7$ T and a spinning frequency of $\omega_r / 2\pi = 10.349$ kHz using the pulse sequence shown in Fig. 14.1(b). This required a different double-quantum recoupling sequence, with a lower rf field requirement (see discussion in section 10.1). The excitation sequence was a $C_{15}^7$ sequence, with the basic pulse sequence element $\mathcal{C} = 360_0360_{120}360_{240}$, while the reconversion sequence was a $C_{15}^{-1}$ sequence, with the basic element $\mathcal{C} = 360_0360_{240}360_{120}$. This means that all rf phases were reversed in sign between the excitation and reconversion sequences (see section 9.6). The $S$-spin nutation frequency during the recoupling sequence was 36.222 kHz and was therefore...
14 The NCCN-MQ-HLF Experiment

Figure 14.4: Signal amplitudes for the GGG sample as a function of the evolution interval *t*₁. (a) Symbols: Experimental results obtained with the pulse sequence shown in Fig. 14.1(a) at a static field of *B₀* = 9.4 T and a spinning frequency of *ωᵣ/2π* = 5.059 kHz. The experimental 2QF efficiency was around 35%. Three different versions of the *¹⁵N* π pulses were used: well calibrated π pulses (Exp1), misset π pulses (Exp2) and composite π pulses (Exp3). The lines correspond to simulations for the indicated torsional angles. (b) Symbols: Experimental results obtained with the pulse sequence shown in Fig. 14.1(b) at a static field of *B₀* = 4.7 T and a spinning frequency of *ωᵣ/2π* = 10.349 kHz. The experimental 2QF efficiency was around 23%. The lines correspond to simulations for the indicated torsional angles.

In both Figs. 14.4(a) and (b), the experimental double-quantum filtered signal amplitudes, marked with symbols, are plotted as a function of the evolution interval *t*₁ = *nπᵣ*. These experimental dephasing curves can be compared with numerical simulations. The numerical simulations were performed using: (i) the bond lengths and bond angles of the N–C–C–N fragment in GGG (see Paper V for details), (ii) the experimentally determined decay constant of the double-quantum coherences during the interval *t*₁ and (iii) the N–C–C–N torsional angle, which was the only free parameter in these simulations. The simulations are only sensitive to the absolute value of the torsional angle, |ψ|.

The solid lines in both Figs. 14.4(a) and (b) correspond to the best fits between the experimental data points and the simulated curves. In addition, both figures include simulations for a few other values of the torsional angle |ψ|, using the same double-quantum decay constant. Figure 14.4(a) includes experimental results for a variety of π-pulses. The best fits between simulations and experiments for Figs. 14.4(a) and (b) were obtained for torsional angles |ψ| = 162° and |ψ| = 166° respectively, which are both within 3° of the X-ray value, which is *ψ* = 164.8°. The torsional angle estimation is at least comparable to that used in the experiments to obtain the results in Fig. 14.4(a).
as good as ±5°. Note the reproducible discrepancies between the simulations and the experiments. Paper VI suggests different possible reasons for these discrepancies, such as asymmetric molecular vibrations, pulse imperfections, non-uniform cross-polarization or minor spin-interactions, all of which are not taken into account in the simulations.

Figure 14.5 shows a contour plot of the simulated signal amplitudes for the experiments in Fig. 14.1, as a function of the evolution interval and the torsional angle $|\psi|$. The experiment is particularly sensitive to the torsional angle in the region $140^\circ \leq |\psi| \leq 180^\circ$. In addition, the plot shows ambiguities in the range $0^\circ \leq |\psi| \leq 140^\circ$.

14.6 Torsional Angle Estimation in Ala-Gly-Gly

The NCCN-3Q-HLF experiment was used to obtain the torsional angle $\psi$ in a molecular fragment of the isotopically labelled tripeptide $[^{15}\text{N}, ^{13}\text{C}_3\text{-ala}]^{[^{15}\text{N}-\text{gly}]}\text{-gly}$ (AGG). The structure and labelling is shown in Fig. 14.3(b).

For the excitation of the 3QC the POST-C7 sequence [41] was used, i.e., the symmetry $C7_1$ together with the basic pulse element $\mathcal{C} = 90_0360_{180}270_0$. For the reconversion sequence the symmetry $C7_2^{-1}$ was used together with the basic element $\mathcal{C} = 270_0360_{180}90_0$, which was experimentally found to result in a slightly higher triple-quantum filtered (3QF) efficiency. For further details about the sample and the experiment, see Paper VI.

The experimentally obtained triple-quantum filtered signal amplitudes, marked with symbols, plotted as a function of $t_1$, are shown in Fig. 14.6. In
Figure 14.6: Signal amplitudes for the GGG sample as a function of the interval $t_1$. Symbols: Experimental results obtained with the pulse sequence shown in Fig. 14.2 at a static field of $B_0 = 4.7$ T and a spinning frequency of $\omega_r/2\pi = 5.000$ kHz. The experimental 3QF efficiency was around 2.5%. The error bars represent the standard deviation of the spectral noise. The lines correspond to simulations for the indicated torsional angles.

In addition, numerical simulations for different torsional angles $\psi$ are shown. The numerical simulations used the bond lengths and bond angles obtained from the AGG X-ray structure (see Paper VI), while the torsional angle was the only free parameter in the simulations. The solid line corresponds to the best fit between the experimental data points and the simulated curves. The results shown here correspond to a molecular torsional angle of $\psi = 161^\circ$. A best fit of the same quality is obtained for a torsional angle of $\psi = -152^\circ$. The positive value is in good agreement with the result obtained by X-ray diffraction $\psi = 160.5^\circ$.

These results show that the NCCN-3Q-HLF experiment is sensitive to the sign of the torsional angle. However, in general, one obtains two distinct values with opposite sign. This ambiguity can be overcome by performing both the NCCN-2Q-HLF and the NCCN-3Q-HLF experiment. The torsional angle estimation for the NCCN-3Q-HLF experiment is at least as good as $\pm 10^\circ$. Also, in this case, the discrepancies between the best fit and the experimental data points should be noted, as they were discussed in the previous section for the case of the NCCN-2Q-HLF experiment.
14.7 Bloch-Siegert Shift

Rf irradiation, which is applied at the Larmor frequency of the $K$-spins, usually does not affect the $S$-spins. If, however, the difference between the absolute values of the Larmor frequencies of the two spin species is small, the rf irradiation on the $K$-spins also directly affects the evolution of $S$-spins by imposing an additional rotation of the $S$-spins around the $z$-axis. This can also be interpreted as a shift of the isotropic chemical shift of the $S$-spins. This effect is called the \textit{Bloch-Siegert shift} and is usually very small.

Assume that rf irradiation with the reference frequency $\omega_{\text{ref}}^K$ and nutation frequency $\omega_{\text{nut}}^K = \frac{1}{2} \gamma_K B_{\text{rf}}^K$ is applied to the $K$-spins, where $B_{\text{rf}}^K$ is the peak value of the oscillating magnetic rf field. The nutation frequency for the $S$-spins is given by $\omega_{\text{nut}}^S = \frac{1}{2} \gamma_S B_{\text{rf}}^K = |\gamma_S/\gamma_K| \omega_{\text{nut}}^K$. The rf Hamiltonian of the $S$-spins is viewed from a frame rotating with the reference frequency $\omega_{\text{ref}}^S$. Both $\omega_{\text{ref}}^K$ and $\omega_{\text{ref}}^S$ have the same sign as the respective Larmor frequencies for the $K$- and $S$-spins. In that case, the rf Hamiltonian of the $S$-spins in the rotating frame of the $S$-spins contains an additional term in second order average Hamiltonian theory [23]:

$$H_{BS} = \omega_{BS} S_z \quad (14.7)$$

$$\omega_{BS} = \frac{1}{2} \left( \frac{\gamma_S}{\gamma_K} \omega_{\text{nut}}^K \right)^2 \left\{ \frac{1}{\omega_{\text{ref}}^K + \omega_{\text{ref}}^S} + \frac{1}{\omega_{\text{ref}}^K - \omega_{\text{ref}}^S} \right\} S_z, \quad (14.8)$$

where $S_z$ is the operator for the $z$-component of the total angular momentum of the $S$-spins and $\omega_{BS}$ is called the Bloch-Siegert shift.

Table 14.1 contains four examples to give an idea of the importance of the Bloch-Siegert shift in practical cases. Typical values for the nutation frequency $\omega_{\text{nut}}^S$ were chosen and the Bloch-Siegert shift was calculated for two different values for the external static field $B_0$. As is already clear from Eq. (14.8),

<table>
<thead>
<tr>
<th>$B_0$</th>
<th>$\omega_{\text{ref}}^S/2\pi$</th>
<th>$\omega_{\text{ref}}^K/2\pi$</th>
<th>$\omega_{\text{nut}}^K/2\pi$</th>
<th>$\omega_{BS}/2\pi$</th>
<th>$\omega_{BS}'/2\pi^c$</th>
<th>$\omega_{BS}'/2\pi^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[T]</td>
<td>[MHz]</td>
<td>[MHz]</td>
<td>[kHz]</td>
<td>[Hz]</td>
<td>[Hz]</td>
<td>[Hz]</td>
</tr>
<tr>
<td>4.7</td>
<td>-50.301</td>
<td>20.280</td>
<td>62.5$^a$</td>
<td>-570.5</td>
<td>-46.2</td>
<td>-94.5</td>
</tr>
<tr>
<td>4.7</td>
<td>-50.301</td>
<td>20.280</td>
<td>79.4$^b$</td>
<td>-919.9</td>
<td>-58.6</td>
<td>-120.0</td>
</tr>
<tr>
<td>9.4</td>
<td>-100.602</td>
<td>40.559</td>
<td>62.5$^a$</td>
<td>-285.3</td>
<td>-23.1</td>
<td>-47.2</td>
</tr>
<tr>
<td>9.4</td>
<td>-100.602</td>
<td>40.559</td>
<td>79.4$^b$</td>
<td>-460.0</td>
<td>-29.3</td>
<td>-60.0</td>
</tr>
</tbody>
</table>

$^a \tau_p = 8.0 \mu s. \quad ^b \tau_p = 6.3 \mu s. \quad ^c \omega_r/2\pi = 5.059 \text{ kHz}. \quad ^d \omega_r/2\pi = 10.349 \text{ kHz}.$

Table 14.1: Examples for Bloch-Siegert shifts for $\gamma_S^{(13C)} = 67.283 \times 10^6 \text{ rad s}^{-1} \text{T}^{-1}$ and $\gamma_K^{(15N)} = -27.126 \times 10^6 \text{ rad s}^{-1} \text{T}^{-1}$. 

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Figure 14.7: (a) Signal amplitudes for a sample of $^{13}$C$_2$-glycine as a function of the evolution interval $t_1$. Symbols: Experimental results for different values of $\tau_p$ obtained with the pulse sequence shown in Fig. 14.1(a) at a static field of $B_0 = 4.7$ T and a spinning frequency of $\omega_r/2\pi = 10.349$ kHz. The lines correspond in each case to best fits for the absolute values of the effective Bloch-Siegert shift. The results of the fits are indicated in the inset table. (b) Signal amplitudes for the GGG sample as a function of the evolution interval $t_1$. Symbols: Experimental results obtained with the pulse sequence shown in Fig. 14.1(a) at a static field of $B_0 = 4.7$ T and a spinning frequency of $\omega_r/2\pi = 10.349$ kHz. Solid Line: Simulation using the indicated torsional angle and the effective Bloch-Siegert shift.

The necessity to apply the pulse sequence in Fig. 14.1(b) to obtain the experimental data in Fig. 14.4(b) using the experimental conditions as discussed above is shown in Fig. 14.7. Figure 14.7(b) shows the experimental results obtained on GGG with the pulse sequence shown in Fig. 14.1(a) using C7\textsubscript{15} with $\mathcal{C} = 360_360_{120}360_{240}$ as the excitation and reconversion sequence at a static field of $B_0 = 4.7$ T and a spinning frequency of $\omega_r/2\pi = 10.349$ kHz. The difference between these experimental results and that shown in Fig. 14.4(b) is significant. The double-quantum filtered signal amplitudes in Fig. 14.7(b) reflect an additional oscillation of the double-quantum coherences during the evolution interval $t_1$. This oscillation may be attributed to the evolution of the $S$-spin double-quantum coherences under the Bloch-Siegert shift due to the rf irradiation applied to the $K$-spins during $t_1$.

In order to demonstrate this, the same experiment was performed on a sample of $^{13}$C$_2$-glycine using the pulse sequence shown in Fig. 14.1(a). Because there are no $^{15}$N spins present in the sample, no $^{13}$C-$^{15}$N recoupling is established by applying pulses at the $^{15}$N Larmor frequency. A series of
14.7 Bloch-Siegert Shift

Experiments were done in which the nutation frequency of the pulses at the $^{15}$N Larmor frequency was kept constant and the length of the pulses $\tau_p$ is varied. The experimental results are shown in Fig. 14.7(a). In each case a $\cos |\omega'_0| t_1 \exp\{-\lambda t_1\}$ modulation was fitted to the experimental curves. The inset table in Fig. 14.7(a) contains the absolute values for the fitted effective Bloch-Siegert shift $|\omega'_0| = |\omega'_0|/2\pi$ for the different pulse lengths $\tau_p$. It should be noted that the Bloch-Siegert shift, $\omega_{BS}$, given by Eq. (14.8), is constant during the pulses in all experiments because the nutation frequency is constant. However, the length of the pulses $\tau_p$ is changing and therefore the effective Bloch-Siegert shift $\omega'_0$ over the total evolution interval is proportional to the pulse length:

$$\omega'_0 = \frac{2\tau_p}{\tau_r} \omega_{BS}. \quad (14.9)$$

The solid line in Fig. 14.7(b) was obtained by simulating the double-quantum signal amplitudes on GGG as a function of the torsional angle $|\psi|$ and in addition applying the function $\cos |\omega'_0| t_1$ to the simulated results. The solid line is the result of fitting the torsional angle $|\psi|$ and the effective Bloch-Siegert shift $|\omega'_0|$ to the experimental data. The result shows that the Bloch-Siegert shift during the rf pulses on the $K$-spins is responsible for the additional modulation of the $S$-spin double-quantum coherences during the interval $t_1$. In the pulse sequence shown in Fig. 14.1(b), the $\pi$-pulse on the $S$-spins in the middle of the evolution time $t_1$ refocuses not only the evolution of the $S$-spin double-quantum coherences under the $S$-spin isotropic chemical shifts but also the evolution under the Bloch-Siegert shift, because the same number of rf pulses are applied on the $K$-spins before and after the $\pi$-pulse on the $S$-spins.

There are three reasons why the Bloch-Siegert shift is so pronounced in the experiment in Fig. 14.7(b) and could not be noticed in experiment in Fig. 14.4(a): (i) The Bloch-Siegert shift for a certain rf nutation frequency is, according to Eq. (14.8), mainly proportional to the inverse of the difference of the absolute values of the $S$- and $K$-spin Larmor frequencies. Table 14.1 shows that therefore the Bloch-Siegert shift is larger by a factor 2 at a static field of $B_0 = 4.7$ T than at a static field of $B_0 = 9.4$ T for the same rf nutation frequency. (ii) The Bloch-Siegert shift depends quadratically upon the rf nutation frequency. (iii) The effective Bloch-Siegert shift (Eq. (14.9)) depends upon the ratio of pulse lengths on the $K$-spins and the rotational period $\tau_r$. This means the effective Bloch-Siegert shift is directly proportional to the sample spinning frequency if the rf nutation frequency is kept constant. The shaded values in Table 14.1 show that the Bloch-Siegert shift for the experimental parameters used to obtain the results in Figs. 14.4(b) and 14.7(b) is more than a factor 5 larger than for the experimental parameters used to obtain the results in Fig. 14.4(a).
15 Conclusions

In part IV of this thesis, the general idea of MQ-HLF spectroscopy is presented. These experiments allow the determination of molecular torsional angles with a good sensitivity in the range \([140^\circ, 180^\circ]\). The experiments are theoretically easy to describe and do not need any information about the orientations of CSA tensors. If the extreme coherences in the \(S\)-spin cluster are exploited, neither the homonuclear direct dipolar couplings nor the homonuclear \(J\)-couplings have to be taken into account.

The HCCH-2Q-HLF and NCCN-MQ-HLF experiments were discussed. The latter was implemented as a 2QC and a 3QC version. The 2Q-HLF methods are, in general, only sensitive to the absolute value of the torsional angle, whereas the 3Q-HLF methods are also sensitive to the sign of the torsional angle, although usually one positive and one negative torsional angle describe the experimental results equally well. If 2Q-HLF and 3Q-HLF methods are combined, on the other hand, the determination of the definite torsional angle is possible.

The spectral sensitivity of the MQ-HLF methods is very low for orders of MQC higher than two, because the excitation of such MQC under MAS condition is relatively low at present. From this perspective, the use of three-dimensional double-quantum spectroscopy appears to be a good alternative for use in multiply-labelled samples. It allows the determination of several molecular torsional angles in a single experiment. A similar approach was used in the case of the methods based on 2D exchange NMR spectroscopy [44]. However, two-dimensional double-quantum spectra, correlating double-quantum with single-quantum spectral frequencies, have the advantages discussed in section 10.4, mainly the suppression of indirect peaks with respect to direct peaks. In this case, high MAS spinning frequencies are necessary, so that the influence of homonuclear dipolar couplings during the evolution of the 2QC under the heteronuclear local fields might be neglected. It should be noted, however, that the homonuclear isotropic \(J\)-couplings have to be considered in this case if the heteronuclear dipolar couplings have the same order of magnitude as the homonuclear \(J\)-couplings [Paper IV].

The possible improvement of the torsional angle determination by orientational weighting by rf pulse sequences or partially oriented samples is discussed in detail in Ref. 75 in the context of 2D exchange spectroscopy and briefly in Ref. 99 in the context of MQ-HLF spectroscopy. The basic idea, in the context of the MQ-HLF experiment, is to weight the excitation of MQC depending upon the crystallite orientations. This can be done by suitable rf pulse sequences or by partially orienting the sample. This should increase the torsional angle resolution of the MQ-HLF experiment in different regions of the possible torsional angles and solve ambiguities.
Part V
Appendices
A Irreducible Spherical Spin Tensor Operators

A.1 One Spin
The single Spin-$\frac{1}{2}$ tensor operators $T^{ij}_{\lambda\mu}$ are:

\begin{align*}
T_{10}^j &= S_{jz} \\
T_{i\pm 1}^j &= \mp \frac{1}{\sqrt{2}} S_{j}^{\pm}
\end{align*}
(A.1)\hspace{1cm}(A.2)

A.2 Two Spins
The two Spin-$\frac{1}{2}$ tensor operators $T^{ij}_{\lambda\mu}$ are:

\begin{align*}
T_{00}^{jk} &= -\frac{1}{\sqrt{3}} S_{j} \cdot S_{k} \\
T_{10}^{jk} &= \frac{1}{2\sqrt{2}}\left(S_{j}^{+}S_{k}^{-} - S_{j}^{-}S_{k}^{+}\right) \\
T_{1\pm 1}^{jk} &= \frac{1}{2}\left(S_{j}^{+}S_{kz} - S_{jz}S_{k}^{\pm}\right) \\
T_{20}^{jk} &= \frac{1}{\sqrt{6}}\left(3S_{jz}S_{kz} - S_{j} \cdot S_{k}\right) \\
T_{2\pm 1}^{jk} &= \mp \frac{1}{2}\left(S_{j}^{\pm}S_{kz} + S_{jz}S_{k}^{\pm}\right) \\
T_{2\pm 2}^{jk} &= \frac{1}{2}S_{j}^{\pm}S_{k}^{\pm}
\end{align*}
(A.3)\hspace{1cm}(A.4)\hspace{1cm}(A.5)\hspace{1cm}(A.6)\hspace{1cm}(A.7)\hspace{1cm}(A.8)
References


References


References


References


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References


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Part VI

Included Papers
Paper I
Paper II
Paper III
Paper IV
Paper V
Paper VI