We review a variety of recently developed $^1$H–X heteronuclear recoupling techniques, which rely only on the homonuclear decoupling efficiency of very-fast magic-angle spinning. All these techniques, which are based on the simple rotational-echo, double-resonance (REDOR) approach for heteronuclear recoupling, are presented in a common context. Advantages and possibilities with respect to the complementary application of conventionally X and $^1$H-inversely detected variants are discussed in relation to the separability and analysis of multiple couplings. We present an improved and more sensitive approach to the determination of $^1$H–X dipolar couplings by spinning-sideband analysis, termed REREDOR, which is applicable to XH$_n$ groups in rigid and mobile systems and bears some similarity to more elaborate separated local-field methods. The estimation of medium-range $^1$H–X distances by analyzing signal intensities in two-dimensional REDOR correlation spectra in a model-free way is also discussed. More specifically, we demonstrate the possibility of combined distance and angle determination in H–X–H or X–H–X three-spin systems by asymmetric recoupling schemes and spinning-sideband analysis. Finally, an $^1$H–X correlation experiment is introduced which accomplishes high sensitivity by inverse ($^1$H) detection and is therefore applicable to samples with $^{15}$N in natural abundance.

Key Words: REDOR; fast MAS; rotor encoding; spinning sidebands; Q8M8; rotational dynamics; distance and angle determination; hydrogen bonds; inverse detection; natural abundance.

1. INTRODUCTION

Heteronuclear correlation techniques, which involve protons and are able to provide a reasonable chemical-shift resolution, quantitative dipole–dipole couplings, or both, have been a long-standing challenge in solid-state NMR. Since the advent of the simple CP-based 2D WISE technique operating at moderate magic-angle spinning (MAS) frequencies [1, 2], which only provides qualitative information on $^1$H lineshapes, chemical-shift, and $^1$H–X dipolar contacts, the recent years have seen
tremendous advances with respect to the development of more quantitative approaches.

Using different homonuclear decoupling schemes, the most popular and commonly used of which seems to be the frequency-switched Lee-Goldburg (FSLG, [3]) scheme or its even more robust phase-modulated version [4], techniques have been devised which provide $^1\text{H}$ linewidths of less than 0.2 ppm [5] and quantitative $^1\text{H}$$-^1\text{H}$$-^{13}\text{C}$ distances of up to 2.5 Å [6]. Besides the technological progress concerning the RF capabilities of modern NMR spectrometers, which make such elaborate pulse sequences feasible, recent advances in MAS probe technology [7, 8] have a considerable share in opening up the application of conceptually simpler pulse sequences [9–14]. Probes with spinning frequencies of up to 50 kHz are now available [7], and experiments relying on the homonuclear decoupling performance of MAS alone can rival the more quantitative and elaborate approaches in many areas of application [9, 14–19].

The $^1\text{H}$ line-narrowing effect of very-fast MAS is now well understood [20]. In essence, the complicated response of a tightly coupled $^1\text{H}$ spin system can be described well by a sum of individual pair couplings, if the sample is spun only fast enough. The ensuing considerable simplification in the analysis of homonuclear $^1\text{H}$ spectra in terms of a spin-pair approach, in particular the analysis of $^1\text{H}$$-^1\text{H}$ double-quantum correlation spectra based on simple excitation schemes [10], has already opened up the way to a multitude of applications concerning structure and dynamics in supramolecular systems [9, 14–19] and polymers [21, 22]. In much the same way, very-fast MAS simplifies the analysis of heteronuclear correlation spectra.

In recent years, we have presented various $^1\text{H}$$-^{13}\text{C}$ (or $^1\text{H}$$-^{15}\text{N}$) heteronuclear correlation techniques [11–13, 23, 24] which are all based on the popular rotational-echo, double-resonance (REDOR) heteronuclear dipolar recoupling scheme introduced in the late 80s by Gullion and Schaefer [25]. These techniques differ only (i) in the nucleus from which the recoupling process is started (either $^1\text{H}$ or any X-nucleus whose polarization is enhanced by CP), (ii) in the detected nucleus (either conventional X- or inverse $^1\text{H}$-detection), and (iii) in the coherence state probed in the indirect dimension of the 2D experiment.

In this contribution, we will first review the variety of these techniques on a common basis and highlight their modes and areas of application. A particular focus will be the analysis of spinning-sideband patterns, which appear in the indirect dimension of the experiments. They are generated by the mechanism of rotor encoding [26, 27] and contain very precise information on dipole–dipole couplings. We will further introduce a new, simpler and more efficient technique for the observation of such sideband patterns, show how medium-range couplings can be estimated from an analysis of spectral intensities in 2D shift correlation spectra, and demonstrate how sideband patterns can even be used to measure distances and angles in three-spin systems. Finally, we show an $^1\text{H}$$-\text{X}$ correlation experiment whose signal sensitivity is enhanced by an inverse, i.e. $^1\text{H}$, detection scheme to such an extent that samples naturally abundant in $^{15}\text{N}$ can be investigated.

It should be mentioned that, even though being designed for $^1\text{H}$$-\text{X}$ spin systems, the concepts to be detailed in the following can be just as well applied to any heteronuclear X–Y spin system, with protons serving merely as a source of
polarization. All these techniques are still awaiting fruitful applications in areas where the conventional REDOR approach, as used for spin-$\frac{1}{2}$ systems such as $^{13}$C–$^{15}$N, reaches its limit of applicability, namely in the case of multiple spins of either species.

2. THEORETICAL DESCRIPTION

2.1. Dipolar Time Evolution

The treatment of a system of multiple heteronuclear-coupled spins is straightforward; all couplings commute and can be evaluated independently. Previous studies [11, 12, 20] have shown that this holds well for systems containing protons with strong perturbing homonuclear couplings, provided that the spinning frequency is sufficiently high [10]. The time-dependent heteronuclear dipole–dipole coupling under MAS is best described by phase factors, as derived from first-order average Hamiltonian theory. The general phase acquired under transverse evolution of a spin $i$, dipolar-coupled to a spin $j$, reads

$$\Phi^{(ij)}(t_a; t_b) = \int_{t_a}^{t_b} d_{IS}^{(ij)}(\omega_R t) \, dt.$$  \hspace{1cm} (1)

The time-dependent coupling term, $d_{IS}^{(ij)}(\omega_R t)$, contains all the information about the coupling strength and the tensor orientations. $\Phi^{(ij)}(t_a; t_b)$ vanishes for $t_b - t_a = N\tau_R$, which reflects the averaging process of MAS. The phase acquired under a single rotor period of REDOR recoupling [25], where $\pi$-pulses with a separation of $\frac{1}{2}\tau_R$ prevent the averaging to zero, is calculated from the integral over half a rotor period:

$$\tilde{\Phi}^{(ij)} = 2\Phi^{(ij)}(t; t + \tau_R/2).$$  \hspace{1cm} (2)

As a specific example, the phase factor for a simple two-spin system (where no relative orientations of different dipole–dipole tensors are to be considered) over one rotor period of REDOR recoupling reads

$$\tilde{\Phi}_{11} = -\frac{D_{IS}}{\omega_R} 2\sqrt{2} \sin 2\beta \sin(\omega_R t_1 + \gamma).$$  \hspace{1cm} (3)

$D_{IS}$ is the heteronuclear dipole–dipole coupling constant, $\beta$ and $\gamma$ are the Euler angles defining the orientation of the dipole–dipole vector with respect to the rotor axis. For the more general case of multiple IS couplings, more complicated relationships apply. The response of a multi-spin system to the application of various pulse sequences can now be calculated within the framework of simple product operator theory [28], where the dipolar phase factors simply replace the product of time-independent coupling constant and evolution time known from solution-state NMR. The evolution of a single transverse coherence straightforwardly results in a cosine modulation of the initial coherence and a sine-modulated
antiphase coherence:

$$\hat{S}_{x/y} \rightarrow \hat{S}_{x/y} \cos \Phi \pm \hat{S}_{y/x} \hat{I}_z \sin \Phi.$$  \(\text{(4)}\)

Analogously, an antiphase coherence may be converted back to a transverse coherence state in a second evolution interval upon acquisition of a second sine term. The coupling of an \(S\) spin to many \(I\) spins is evaluated in an equivalent fashion; then, multi-spin antiphase coherences of the type $$\hat{S}_{x/y} \hat{I}^{(1)}_z \hat{I}^{(2)}_z \ldots$$ are the result. A detailed description of the theory including explicit expressions for the phase factors for the general multi-spin case can be found in Ref. [12].

2.2. The Second Dimension

An important variable in Eqs. (2) and (3) is \(t_1\), which specifies the rotor position at the beginning of the calculated recoupling interval. Its main effect arises when \(t_1\) is identified with the time lag between two different recoupling periods flanking the indirect spectral dimension in the experiments to be discussed below. The shift in the relative rotor phase between two recoupling periods is the main source of the versatile spinning sidebands, from which distance and angle information can be derived. This way of extracting dipolar coupling information from 2D spectra is particularly valuable in that it represents an alternative and independent way to the more common analysis of spectral intensities. Both approaches will be demonstrated below in the context of multiple couplings.

The spinning sidebands are generated by a mere change in the rotor phase associated with a particular recoupling interval, and the mechanism has been termed reconversion rotor encoding (RRE) [27]. It is special in that the number of observed sidebands can be “pumped” to a convenient level by proper choice of the overall recoupling time (given in multiples of rotor periods, \(N_{\text{exc}}\) and \(N_{\text{rec}}\)). The \(N_{\text{exc}}/N_{\text{rec}}\) terminology is derived from homonuclear \(^1\text{H}\)–\(^1\text{H}\) double-quantum (DQ) spectroscopy, where the two recoupling periods are identified with the DQ excitation and reconversion periods [26, 29].

In fact, REDOR is the heteronuclear equivalent of the homonuclear back-to-back pulse sequence [30], which is used in most of the cited \(^1\text{H}\) DQ MAS investigations: the dipolar phase factor of the latter differs from Eq. (3) only by the usual factor of \(\frac{3}{2}\) appearing between homo- and heteronuclear dipole–dipole couplings. In comparison with homonuclear DQ spectroscopy, heteronuclear REDOR-based 2D spectroscopy is more flexible as far as the choice of the coherence state present in the indirect dimension is concerned. In Fig. 1, the basic building blocks of such 2D experiments, as well as their relation to conventional REDOR, are depicted schematically (with Figs. 1(a) and 1(b) to be discussed in the next section). During the recoupling periods, antiphase coherences are present, while, depending on the up to four 90°-pulses with appropriate phases, which can be placed before and directly after the \(t_1\) time interval on either channel, heteronuclear multiple-quantum, antiphase magnetization, heteronuclear dipolar order, or just longitudinal magnetization can be selected in \(t_1\).
In our recent publications [11–13, 24], heteronuclear single-quantum correlation (HSQC, which involves antiphase magnetization in $t_1$; see also Fig. 2) and heteronuclear multiple-quantum correlation (HMQC) were the methods of choice for shift correlation experiments, while heteronuclear dipolar-order rotor encoding (HDOR) and rotor-encoding of longitudinal magnetization (RELM) were used for efficient measurement of the RRE spinning-sideband patterns. The former two experiments exhibit $^1$H chemical-shift resolution in $t_1$ and are usually conducted in a rotor-synchronized fashion ($\Delta t_1 = N \tau_R$), so as to avoid the appearance of spinning sidebands. In contrast, the latter two experiments lack modulations due to chemical shifts in $t_1$, which speeds up the measurement because a small number of slices covering a single rotor period in $t_1$ is sufficient for the generation of the sideband spectrum [11].

**FIG. 1.** Pulse sequences for heteronuclear correlation spectra based on REDOR. The preparation periods for the generation of $z$-magnetization (none for $^1$H, CP for low-$\gamma$ heteronuclei) and the detection periods are omitted for clarity.
In Figs. 1(c) and 1(d), different variants of the HDOR experiment are shown, which differ in the pathway followed by the transverse contribution of the mixed-spin coherence during recoupling: in the DIP (dipolar) experiment the transverse component remains on one channel (SS experiment, or, for inverse detection, II experiment), while in the recoupled polarization transfer (REPT) experiment (IS or SI), two INEPT-type [31] 90°-pulses switch the transverse component of the antiphase coherence from one channel to the other (\(I_S^z, I_I^y\to I_I^y, I_S^z\)). Analogously, as depicted in Fig. 2, the recoupling periods of HSQC experiments can be chosen such that the transverse component of interest follows an SS (a), II (b), IS (c) or SI (d) pathway during recoupling. Accordingly, either I-spin (a,c) or S-spin (b,d) chemical-shift evolution takes place during \(t_1\), while the other spin species is detected during \(t_2\), respectively. Consequently, IS chemical-shift correlation spectra are obtained in all cases, but by choosing the pathway during recoupling, i.e., by directing the recoupling, multiple-spin geometries can be probed in different ways, as will be discussed in Section 2.4.
2.3. A New Experiment

The simplest way to generate the above-mentioned spinning-sideband patterns can be inferred by comparison of Figs. 1(a) and 1(b). The presentation is simplified; pulse sequence blocks for the generation of sufficient $z$-magnetization such as CP, or some saturation pulses to remove unwanted polarization, have to be appended as appropriate. Starting with the conventional REDOR pulse sequence (a), the central $\pi$-pulse is skipped and instead, a variable $t_1$ delay is inserted. The second $t_1$ interval at the end of the pulse sequence and the placement of the central $\pi$-pulse of the two independent recoupling periods (now identified as excitation and reconversion periods) on the other channel simply serve to refocus $S$ spin chemical-shift evolution. The time evolution is in this case most conveniently calculated using the sum of the phases acquired under the action of the whole sequence \{excitation–$t_1$–reconversion–$t_1$\}:

$$S \propto \left\langle \prod_i \cos(N_{exc}\Phi^{(i)}_0 - \Phi^{(i)}(0; t_1) - N_{rec}\Phi^{(i)}_{t_1} + \Phi^{(i)}(t_1; 2t_1)) \right\rangle. \quad (5)$$

The product represents the coupling of the spin $S$ to more than one $I$ spin. In analogy with our previous work [11, 12], the spinning-sideband patterns generated by this experiment are always dominated by the strongest couplings, which in the case of CH, CH$_2$, and CH$_3$ groups are the directly bonded protons only. While being not very informative in the rigid limit, the experiment can straightforwardly be used to study the dynamic averaging of the intra-moiety couplings by dynamic processes. An example will be presented in Section 4.1.

As far as its scope for applications is concerned, the experiment is very similar to the technically more involved traditional separated local-field (SLF) experiments [32–34] conducted under MAS [35–38]. A much simplified approach for an SLF experiment under MAS conditions was recently published by Frydman and coworkers [39], who could show that the complicated homodecoupling schemes such as MREV-8 can actually be left out in such an experiment, thus leaving the removal of perturbing homonuclear couplings to moderate MAS (up to 15 kHz) alone. However, the sideband patterns observed in Frydman’s experiment are essentially the normal dipolar-modulated sideband patterns commonly observed in MAS experiments ($\propto \langle \cos(\Phi(0; t_1)) \rangle$), in which higher-order sidebands vanish once the coupling constant becomes small (due to dynamics) or the MAS frequency increases (which is desirable to remove still present perturbing effects of homonuclear couplings).

Our approach lifts this restriction because the sidebands are mainly generated by the rotor-encoding mechanism inherent to the recoupling process [27], and can be pumped to arbitrary order by increasing $N_{exc}$ and $N_{rec}$ (which are usually equal). This is shown in Fig. 3(a) for the example of a single $IS$ coupling. Along these lines, we propose the acronym REREDOR (for rotor-encoded REDOR) for this experiment. Under conditions of very-fast MAS and/or weak coupling constants, the two $\Phi^{(i)}(t_a; t_b)$ terms in Eq. (5) (they describe the residual dipolar evolution during the two $t_1$ intervals) become small compared to the terms associated with the recoupling and
may safely be neglected. Restricting the argument to a single coupling, simple algebraic manipulations using addition theorems of trigonometric functions may be used to rearrange Eq. (5):

\[ S \propto \langle \cos(N_{\text{exc}} \Phi_0 - N_{\text{rec}} \Phi_1) \rangle \\
\propto \langle \sin N_{\text{exc}} \Phi_0 \sin N_{\text{rec}} \Phi_1 \rangle + \langle \cos N_{\text{exc}} \Phi_0 \cos N_{\text{rec}} \Phi_1 \rangle. \] 

At this point, the similarity to our recently published techniques becomes obvious. The sin–sin term corresponds to the modulation associated with the two-spin terms in the HSQCC, HMQC, and HDOR varieties [12], while the cos–cos term corresponds to the single-spin modulation detected in the RELM experiment [24]. The former term leads to a spinning-sideband pattern with odd-order sidebands only, while the latter term leads to even-order sidebands only. This is depicted in Fig. 3(b).

Naturally, the new experiment is more sensitive because the spectra exhibit more sidebands than spectra generated by each of the other experiments. In addition, the pulse sequence has only a minimum of pulses (all the 90° pulses necessary for coherence selection are missing), which removes timing problems and signal losses due to other experimental imperfections.

A few analogies should be noted at this point: firstly, the experiment bears similarity to the first published version of REDOR, which was, in fact, a 2D technique [40]. In the so-called XDM (for extended dipolar modulation) experiment, a \( t_1 \)-shift of \( \pi \)-pulses on one of the two channels leads to a similar time-modulation pattern from which dipolar couplings can be extracted. The experiment has, however, never received the popularity of the 1D version, and its potential as a
powerful SLF experiment for the study of reorientational dynamics of $^1$H–$^{13}$C dipolar tensors has not yet been acknowledged. Secondly, Eq. (5) is structurally analogous to the formula for the time signal generated by the CSA-filter sequence published by Hong [41], who exploited the fact that a pulse sequence featuring an analogous train of REDOR $\pi$-pulses may just as well be used for the recoupling, thus measuring, of $^{15}$N CSA tensors.

2.4. Detection Schemes, Directing, and Angular Dependence

As mentioned earlier, the insertion of 90°-pulses before or after the $t_1$ period serves to select a specific coherence state (rather than a single-spin coherence), by which signals from uncoupled nuclei are no longer detected. As an example, the comparison of Figs. 1(b) and 1(c) shows how four additional 90°-pulses select heteronuclear dipolar order in $t_1$. The mixed spin state contributes the sin–sin-modulated signal contribution, as shown above. The intensity of this contribution increases with increasing recoupling time when $t_1 = 0$ or $N\tau_R$. It represents the integral intensity for a signal detected in the direct dimension, which builds up and levels out at 0.5 [12], and is analogous to a conventional REDOR curve (the cos–cos contribution decreases from 1.0 to the same level of 0.5).

We will now consider a simple spin system consisting of abundant $I$ spins and rare $S$ spins, as for instance represented by a system with $^1$H and $^{13}$C in natural abundance, under the application of one of the pulse sequences probing a heteronuclear two-spin state (HDOR, HSQC, HMQC). Note that isotopic labeling schemes [42] and a specific topology of the spin system can lead to a reversal of the role of $I$ and $S$ spins. In the various types of heteronuclear correlation experiments, different pathways of transverse coherences can be chosen. The potential of this option lies in the fundamental difference of the coupling topologies when one of the two spin species considered is isotopically dilute (or spatially isolated). The two possible cases, SLF (separated local field [32]) and PDLF (proton-detected local field [43]), are illustrated in Fig. 4. In the case of PDLF, transverse $I$ coherences evolve as

$$\sum_i \sum_i \phi_i \tilde{i}_x \tilde{s}_z \cos \phi_i \pm \sum_i \sum_i \phi_i \tilde{i}_y \tilde{s}_z \sin \phi_i, \quad (7)$$

while in the case of SLF a transverse $S$ coherence evolves as

$$\tilde{s}_{x/y} \sum_i \phi_i \tilde{i}_y \tilde{s}_z \Pi_i \cos \phi_i \pm \sum_i \tilde{s}_{x/y} \tilde{i}_z \Pi_i \sin \phi_i, \quad (8)$$

Apart from the missing higher-order antiphase coherences in Eq. (8), which are negligible in most of the relevant cases [12], the structure of the modulation experienced by evolving $I$ spins (Eq. (7)) is much simpler: it consists of a sum of pair couplings. When $I$ spins are transverse for the whole course of the experiment, i.e., an $II$ experiment is performed (see Fig. 2b), the final signal is a
The superposition of pair signals:

\[ S_{II} \propto \sum_i \left\langle \sin N_{\text{exc}} \Phi_0^{(i)} \sin N_{\text{rec}} \Phi_{\tau_1}^{(i)} \right\rangle. \tag{9} \]

In the corresponding 2D spectrum, the detected \( I \) spins can be resolved from each other by their chemical shift, while in the indirect dimension, two options are possible: one can choose to observe the sideband pattern (HDOR), which may be a sum of two-spin patterns as a result of \( I \)-spin couplings to different \( S \) spins (at different sites in the sample, though, as a result of isotopic dilution). If a unique analysis is not possible, the HSQC experiment can provide the necessary chemical-shift separation also in the indirect dimension. The necessary spectral resolution will, however, necessitate a large number of \( t_1 \) slices, such that one might also consider the analysis of intensity build-up in terms of two-spin REDOR curves using a rotor-synchronized HSQC.

Direct \( S \) detection (SS experiment, see Fig. 2a), on the other hand, leads to modified signals of the type

\[ S_{SS} \propto \sum_i \left\langle \sin N_{\text{exc}} \Phi_0^{(i)} \sin N_{\text{rec}} \Phi_{\tau_1}^{(i)} \prod_{j \neq i} \left( \cos N_{\text{exc}} \Phi_0^{(j)} \cos N_{\text{rec}} \Phi_{\tau_1}^{(j)} \right) \right\rangle. \tag{10} \]

The additional cosine terms lead to the known problems in REDOR-based correlation experiments: they introduce an angular, thus model, dependence into the experimental data [44, 45]. Note that the complete sum signal corresponds to what is usually detected in a 1D \( S \)-detected REDOR experiment. Spectral separation by individual \( I \)-spin contributions can again be achieved using a 2D HSQC variant. As was shown by Michal and Jelinski [46], approximate distance determination is then possible in a uniformly labeled \( {}^{13}\text{C} - {}^{15}\text{N} \) system, despite the presence of the perturbing cosine terms. This aspect will be explored in more detail in Section 4.2 for the case of multiple \( {}^{13}\text{C} - {}^{1}\text{H} \) couplings.
Our previous investigations have shown that spinning-sideband analysis in an SS experiment is also possible when only the dominating couplings are to be determined \[12\]. Then, the cosine terms representing weak-coupling perturbations only lead to a slight increase of the resulting couplings. When the second coupling reaches a similar magnitude as the primary one, the analysis of the pattern in terms of a single spin-pair becomes critical. Including the second coupling, however, we have found that different excitation and reconversion times (\(N_{\text{exc}}\) and \(N_{\text{rec}}\), respectively) give rise to spinning-sideband patterns which are surprisingly sensitive to the angle between the two involved coupling vectors. This \(I_2S\) case is a situation in which the large potential of directed recoupling concept can be appreciated: the individual coupling constants can be determined precisely by performing an \(II\) experiment (which might not necessarily be an inverse-detected experiment, i.e., when the \(S\) spin is a proton), while the SS experiment can be used to determine the angle, using the known coupling constants as fixed parameters. This will be illustrated in Section 4.3.

We are now left with the discussion of the polarization-transfer schemes (REPT), examples of which are shown in Figs. 1(d), 2(c) and 2(d). The IS experiment (Fig. 2(c), with \(S\) being identified with \(^{13}\text{C}\) in natural abundance) is conceptually simple because the pulse sequence does not contain a cross polarization, and has already proven to be useful in a variety of molecular dynamics applications \[16, 18, 47, 48\], where the reductions of CH dipolar coupling constants due to fast anisotropic dynamics were measured quantitatively. Its signal modulation reads

\[
S_{IS} \propto \sum_i \left\langle \sin N_{\text{exc}} \Phi_0^{(i)} \sin N_{\text{rec}} \Phi_1^{(i)} \prod_{j \neq i} \cos N_{\text{rec}} \Phi_1^{(j)} \right\rangle.
\]

This leads to sideband patterns which, in comparison with the SS experiment, are even less influenced by the remote couplings represented by the cosine terms \[11\]. Only the first-order sidebands suffer from remote couplings to an appreciable degree, and can be excluded from the fit. It should be noted that the 1D version of REPT is also known as TEDOR (transferred-echo, double-resonance) \[49\]. The main effect of the cosine terms, once they attain an appreciable magnitude, is a damping of the spectral intensity at longer recoupling times. This interference effect does not arise in the SS experiment due to the additional existence of the cosine terms corresponding to the excitation period. The REPT experiment allows us to check the consistency of dipole–dipole couplings determined by one of the other variants. Even though the REPT approach is not very useful beyond the determination of dominant coupling constants, the multi-spin interference effect may be used to advantage in spectral editing applications \[11\].

The inverse-detected REPT (SI experiment, Fig. 2d) features essentially the same advantages and shortcomings as the IS experiment. The need for an initial CP step to transfer the initial magnetization to the X nucleus and the subsequent TEDOR transfer back to \(^1\text{H}\) may at a first glance represent a serious disadvantage as compared to the other three variants. However, it turns out that the SI experiment is the only inverse variant so far which is applicable to samples naturally abundant in \(^{13}\text{C}\) or \(^{15}\text{N}\). This will be dealt with in Section 4.4.
3. EXPERIMENTAL PART

All NMR experiments were carried out on Bruker DSX/DRX spectrometers, operating at $^1$H Larmor frequencies of 500 and 700 MHz. Double-resonance MAS probes supporting rotors of 2.5 mm outer diameter were used, and the samples were spun at 30 kHz under ambient temperature conditions. Due to friction, this leads to effective sample temperatures of approx. 50°C. In the dipolar recoupling pulse sequences, the RF power levels were usually set to 100 kHz on $^1$H and X channels, corresponding to 90° pulse lengths of 2.5 µs. For $^{13}$C detection, the TPPM $^1$H decoupling scheme [50] was applied at an RF field strength of 100 kHz.

Methyl group motions were investigated on Q8M8 [51], which is distributed by Bruker as a reference substance for $^{29}$Si NMR [52]. Q8M8 is an octamer siloxane of double four-ring structure (a cube with Si at the corners and –O– along the edges, sum formula $\text{Si}_8\text{O}_{12}$) with eight $\text{O}–\text{Si}((\text{CH}_3)_3$ groups attached to the Si corners.

U–$^{13}$C-l-alanine, U–$^{13}$C-l-tyrosine and l-histidine HCl·H₂O were purchased from CIL and Sigma-Aldrich, respectively, and used without further purification. The tyrosine was converted into its hydrochloride salt by recrystallizing the material from an HCl solution. The N–H···N angle determination was carried out on $N$-butylaminocarbonyl-6-tridecyl-isocytosine (in the “enol” form with a pyrimidin-4-ol unit, see also Fig. 12),

The $^{13}$C resonance of the methyl groups in Q8M8, even though expected to be a single peak, consists of two resolved lines at 1.9 and 2.3 ppm; indicating a symmetry break in the cubic molecular structure [55]. The spinning-sideband patterns arising from the C–H dipolar couplings within the individual methyl groups shown in Fig. 5 are the sum of the contributions from these two signal positions, since no difference was discernible between the two individual patterns. This implies that the same type

4. RESULTS AND DISCUSSION

We will now experimentally demonstrate some of the principles of rotor encoding and directed recoupling. Each application highlights a different aspect regarding the advantages provided by the flexibility of specifically choosing the pathways of the IS coherences.

4.1. Spinning-Sideband Analysis Using REREDOR

We present here the application of spinning-sideband analysis to the identification and quantification of a yet unreported motional process in Q8M8. We focus on the $^1$H–$^{13}$C dipolar couplings within the terminal methyl groups for which REREDOR, as opposed to REPT-HDOR, gives meaningful sideband patterns already at much shorter recoupling times.

The $^{13}$C resonance of the methyl groups in Q8M8, even though expected to be a single peak, consists of two resolved lines at 1.9 and 2.3 ppm, indicating a symmetry break in the cubic molecular structure [55]. The spinning-sideband patterns arising from the C–H dipolar couplings within the individual methyl groups shown in Fig. 5 are the sum of the contributions from these two signal positions, since no difference was discernible between the two individual patterns. This implies that the same type

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2 Kindly provided by S. H. M. Sontjens, M. H. P. van Genderen and R. P. Sijbesma, Technical University of Eindhoven, NL.
of motion is present for all terminal OSi(CH$_3$)$_3$-groups. Figure 5(b) shows the results from least-squares fitting of the experimental data in Fig. 5(a) to the methyl group formulae

\[ S \propto \langle \cos^3(N_{rc\,pl}\Phi_0^{(i)} - \Phi^{(i)}(0; t_1) - N_{rc\,pl}\Phi_{t_1}^{(i)} + \Phi^{(i)}(t_1; 2t_1)) \rangle \]  

(12)

for the REREDOR patterns and

\[ S \propto \langle \sin N_{rc\,pl}\Phi_0 \sin N_{rc\,pl}\Phi_{t_1} \cos^2 N_{rc\,pl}\Phi_{t_1} \rangle \]  

(13)

for the REPT-HDOR pattern. The two recoupling periods were here chosen to have an equal length of $N_{rc\,pl}\tau_R$.

The results nicely demonstrate that consistent results for the CH dipolar coupling constant can be obtained from the sideband patterns, which exhibit almost arbitrary sideband orders as generated by increasing the recoupling time. Notably, the structure of Eq. (13) for the REPT-HDOR pattern at the top of Fig. 5 is responsible for the appearance of meaningful fifth-order sidebands (indicated by arrows) only at very long recoupling times, at which the overall signal is already substantially decreased by apparent T$_2$ relaxation under the recoupling pulse sequence. Analyzable REREDOR patterns are already obtained for the shortest recoupling time of $8\tau_R$ shown here. An intuitive reason for this phenomenology might be provided by the argument that the third power appearing in Eq. (12) leads to a prefactor of 3 for the dipolar phase factors in the arguments of the trigonometric function after some rearrangement using addition theorems. In contrast, Eq. (13)
features the sin–sin-term typical for a single spin pair, which is doubly cos-modulated by the phase factor associated with the second recoupling period only. This is due to the polarization transfer character of the pulse sequence, where the coupling topology gives rise to a PDLF and an SLF situation during the first and the second recoupling period, respectively. The two scenarios are depicted in Fig. 4. In REREDOR, the coupling topology is SLF during the whole sequence, which leads to a constructive action of all three CH dipolar couplings in the CH$_3$ group.

Some deviations from the simple single-CH$_3$ theory used for data analysis deserve discussion. Firstly, the very large, thus unreliable, first-order sidebands in the REPT pattern are due to secondary couplings involving coherences of distant protons with the methyl carbon at which the pattern is detected. The pattern is in fact a heterogeneous superposition of the primary intra-CH$_3$ pattern and such secondary contributions, which are restricted to the first-order sidebands at reasonable recoupling times [11]. This is why long recoupling times need to be employed in order to generate appreciable fifth-order sideband intensity.

Secondly, deviations between experimental spectra and fits are also apparent for the REREDOR patterns. The influence of remote couplings on REREDOR patterns is more subtle than in the case of REPT. Generally, the SLF coupling geometry leads to the observation of systematic changes in the overall shape of the pattern, causing the extraction of higher coupling constants than would be expected for isolated methyl groups. We have shown that such deviations are, however, not large [12]; the patterns are still dominated by the largest coupling constant. The size of this effect may be estimated by comparison of the averaged REREDOR result ($D_{\text{CH}}^{\text{avg}}/2\pi = (2200 \pm 125)$ Hz) with the result of the REPT fit (1990 Hz), which is slightly lower. Notwithstanding the deviations of experiments and fits, stable fitting was possible in all cases. The notable deviations in the centerband intensities are attributed to either imperfections in the phase cycle or specific heteronuclear relaxation mechanisms. Such effects lead to systematic changes in the spectral intensity of the detected $^{13}$C resonance, which are not a function of the rotor encoding during $t_1$, and were recently shown to be present in spectra using a different type of heteronuclear recoupling sequence [42].

The mere knowledge of the averaged CH dipolar coupling constant in Q8M8 does not yet permit any detailed interpretation of the motional process. Assuming a rigid siloxane cube structure, we hypothesize that the whole OSi(CH$_3$)$_3$-unit performs a rotation or a three-site jump around the O–Si bond, in analogy with the motion of the three CH vectors around the Si–C bond in each of the three methyl groups. The coupling constant in a rigidly rotating CH$_3$-group, $D_{\text{CH}}^{\text{new}}/2\pi$, was previously determined to be $6.9$ kHz [11]. The reduction factor for the second motional averaging is related to the second Legendre polynomial: $|P_2(\cos \alpha)| = D_{\text{CH}}^{\text{new}}/D_{\text{CH}}^{\text{avg}}$. This yields an angle $\alpha = 110.3 \pm 1.2^\circ$ between the O–Si bond and the Si–C bonds (the second solution, $\alpha = 137.7^\circ$, calculated assuming a positive $P_2(\cos \alpha)$, seems unlikely in view of our hypothesis). The local geometry of O–Si(CH$_3$)$_2$–O in linear and cyclic dimethylsiloxane oligomers is usually close to tetrahedral [56] ($\alpha \approx 109.5^\circ$), so our result for $\alpha$ supports the hypothesis of a well-defined rotation or a three-site jump of the OSi(CH$_3$)$_3$-units in Q8M8.
4.2. Analysis of Spectral Intensities Using X-Detected DIP-HSQC

Sideband analysis has just been shown to be the method of choice for precision measurements of directly bonded H–X distances and the elucidation of dynamics which average the dominant couplings. Consequently, we now address the question whether REDOR-based recoupling techniques can also be used to measure secondary, i.e., medium-range, couplings. As is apparent from Eq. (10), spinning-sideband patterns, which require recoupling times well in the plateau region of the corresponding coherence build-up, cannot be expected to allow the determination of a weak coupling in the presence of a stronger one—the latter will dominate the cosine terms and contribute largely to the sideband pattern [12]. However, when just intensity measurements at short recoupling times with small $N_{\text{exc}} = N_{\text{rec}}$ are performed, the signal,

$$S_{\text{SS}} \propto \sum_{i} \left( \sin^2 N_{\text{exc}} \Phi_0^{(i)} \prod_{j \neq i} (\cos^2 N_{\text{exc}} \Phi_0^{(j)}) \right),$$

(14)

can be expected to mainly reflect the build-up due to the coupling associated with the sine terms. The cosine terms will be close to one. The spectral separation of different $S$ and $I$ spins in the direct and indirect dimensions, respectively, of a 2D heteronuclear correlation spectrum of the SS type (obtainable by the pulse sequence shown in Fig. 2a) offers a way to assess these intensities experimentally. In particular, the individual components of the sum correspond to the separation by $^1\text{H}$ chemical shift in the indirect dimension.

In order to evaluate the feasibility of such an approach, explicit calculations of the intensity build-up in small 3- and 4-spin systems using Eq. (14) were performed. We consider a CH-group with two further remote spins (Fig. 6(a)) and a spin system with distances typical for a quaternary carbon (Figs. 6(b) and 6(c)). We show calculations spanning the whole range of possible relative orientations in such spin systems. Our starting configuration is a single CH coupling tensor oriented along $z$. When a second proton is added to the CH spin system, the Euler angle $\beta_1$ between the principal axes of the two heteronuclear dipolar coupling tensors can vary between $0^\circ$ and $90^\circ$; larger values are redundant due to the symmetry of the tensors. We show calculations for seven values with an increment of $15^\circ$: Two Euler angles, $\beta_2$ (in-plane rotation around the $y$-axis) and $\gamma_2$ (out-of-plane rotation) are needed to locate the principal axis of a third heteronuclear coupling tensor. Again, due to the symmetry of the coupling tensors, the angular range for a coverage of all possible configurations is limited: $0^\circ \leq \beta_2 < 180^\circ$, $0^\circ \leq \gamma_2 < 90^\circ$. Our calculations comprise curves for 14 different pairs of $\beta_2, \gamma_2$ covering two octants of a sphere, which yields a total of 98 curves when combined with the seven values of $\beta_1$. The families of curves corresponding to the 3- and 4-spin systems are shown in dark and light gray, respectively, in Fig. 6.

At a first glance, the wide range of intensities covered by the multi-spin curves based on different relative orientations, especially in the regions behind the first maxima, renders a model-free analysis of the whole curve hopeless. This problem is general to REDOR, and has been addressed by several authors [44, 45]. It was
concluded that the prospects for the separability of multiple couplings in REDOR are not good, and that there is only hope to determine the dominant coupling. However, the full REDOR signal is represented by the sum of the signals associated with all \( I \) spins (index \( i \)) in Eq. (14), while the spectral separation of the components in a 2D spectrum makes it possible to analyze the initial rise for each coupling individually. This was already experimentally shown by Michal and Jelinski on a \(^{13}\text{C}/^{15}\text{N}\) system with \(^{13}\text{C}\) detection [46]. Even though the authors mentioned that their data analysis was also based on the spin-pair approach, it is not clear from the cited communication how these authors dealt with the important issues of (i) the plateau value which is assumed for the analysis, and (ii) how this value was experimentally determined.

Figure 6(a) illustrates that the build-up curve for a secondary coupling (C–H2) of a carbon directly bonded to H1 is actually very well described by its corresponding pair solution, with very weak angular dependence, but with one important difference: the plateau intensity is a factor of two lower. This is expected, since in a 3-spin system, the two protons finally share the signal intensity; and the contribution due to the strong coupling reaches the plateau region already at the shortest accessible recoupling times. Thus, the build-up of the secondary coupling should be fitted with a spin-pair solution scaled to a plateau value of \( \frac{1}{3} \). When the
perturbing influence of a third proton is included in the calculation, the spin-pair solution is still seen to be a good approximation for recoupling times up to \(100 \, \mu s\) (\(1 \ldots 3t_R\) at 30 kHz MAS). Further calculations of the build-up associated with C–H3 in the same spin system (not shown) indicate that this argument holds similarly well for the third, even weaker coupling.

Note that the plateau value for the weak-coupling build-up curve is scaled to 1/6 when the carbon in question is a CH\(_2\) group. More simulations are necessary to explore the situation for CH\(_3\) groups, where the three dominant couplings, \(D_{CH}/2\pi\), are motionally averaged (three-site jump) to about 6 kHz as opposed to 20 kHz for a rigid bond. The separation of dominant-to-secondary couplings might not be sufficient in this case for the application of a scaled-plateau analysis.

Angular dependences are much more serious when a quaternary carbon is considered (Figs. 6(b) and 6(c)). More protons in its vicinity may be located at similar distances, which increases the spread of data introduced by the orientational dependence. The range of recoupling times for which the spin-pair approach represents an acceptable approximation is seen to be limited to \(1\) or \(2t_R\) at 30 kHz MAS. Note that the spin-pair curve represents an upper limit in all cases (leading to an underestimation of coupling constants, thus an overestimation of distances). Scaling the plateau of the fit curve down to lower values is not expected to lead to an improvement of the fit of the initial rise. We found that a scaling of the plateau is only feasible when one (or more) coupling constants clearly dominate by about a factor of \(4\).

Having established these simple rules of thumb for the analysis of build-up data from 2D REDOR correlation experiments, we now turn to an experimental test. We have performed rotor-synchronized 2D \(^1\)H–\(^{13}\)C DIP-HSQC experiments \cite{12} with four different recoupling times (\(1 \ldots 4t_R\) at 30 kHz MAS) on U–\(^{13}\)C-l-tyrosine hydrochloride. Figure 7 shows a graphical representation of the part of the molecule and the distances which we attempted to determine. In order to separate individual \(^1\)H–\(^{13}\)C antiphase coherences associated with a specific \(^{13}\)C nucleus, slices along the indirect \(^1\)H dimension were deconvoluted into the different signals. In order to obtain stable fits, we first determined the line position and the width for each individual proton using data taken at short recoupling times, at which individual slices are dominated by the closest proton only. Figures 8(a) and 8(b) shows two examples. The results of the deconvolution procedure are shown in Table 1.

The spectra become increasingly crowded and overlapped at longer recoupling times (Fig. 8(c)). However, fixing the chemical shifts and linewidths allowed us to obtain reliable deconvolutions. It is then straightforward to calculate the fraction of the intensity of a specific carbon signal associated with the coupling to a specific proton. In order to determine its intensity on an absolute scale, we are only left with measuring the absolute intensity of the carbon signal itself, which is in principle given by its REDOR fraction. Analogously, we used the first slice of the respective 2D spectrum after Fourier transformation over the \(^{13}\)C dimension (which corresponds to the sum of coupled-\(^{13}\)C intensity) and normalized it to a 1D reference experiment taken with the same number of scans. We used the same pulse sequence as for the 2D spectrum (\(t_1 = 0\)) and just changed the phase cycle so as to acquire a full echo instead of selecting heteronuclear coherence states.
The so-obtained intensities are plotted in Fig. 9. Following the above arguments, the experimental points corresponding to $t_{\text{exc}} = 1, 2$, or $3\tau_R$ were then fitted to REDOR curves for spin pairs, which are given by single $\sin^2$ term in Eq. (14), without $\cos^2$ modulation factors ($\sin^2 \Phi = \frac{1}{2} - \frac{1}{4} \cos 2\Phi$ might be a more familiar representation of a REDOR curve). Note that a “fit” to a REDOR curve is a very simple procedure: it merely means the adjustment of the $x$-scaling of a REDOR curve.

**FIG. 7.** Structure of l-tyrosine based on neutron diffraction data [57]. The indicated intra- and intermolecular distances may be compared with our NMR results (Table 2).

**FIG. 8.** Slices from 2D $^1$H-$^{13}$C DIP-HSQC spectra performed on U-$^{13}$C l-tyrosine at 30 kHz MAS, taken at the indicated carbon positions (see Fig. 7 for the numbering) and recoupling times ($N_{\text{exc}} = N_{\text{rec}}$). Dotted lines show the deconvolution into single Lorentzians; the gray background traces are the sums of the deconvolutions.

The so-obtained intensities are plotted in Fig. 9. Following the above arguments, the experimental points corresponding to $t_{\text{exc}} = 1, 2$, or $3\tau_R$ were then fitted to REDOR curves for spin pairs, which are given by single $\sin^2$ term in Eq. (14), without $\cos^2$ modulation factors ($\sin^2 \Phi = \frac{1}{2} - \frac{1}{4} \cos 2\Phi$ might be a more familiar representation of a REDOR curve). Note that a “fit” to a REDOR curve is a very simple procedure: it merely means the adjustment of the $x$-scaling of a REDOR curve.
master curve, which is calculated once and used for all fits by numerical interpolation. The plateau value for the absolute intensity is scaled according to our findings from the simulations (0.25 for CH carbons, 0.5 for quaternary ones).

Generally, the observed deviations from the theoretical curves correspond to what is expected from simulations. In particular, data taken at higher \( t_{\text{exc}} \) (excluded from the fit) fall below the fit curves. Considering the magnitude of these deviations, and the dominance of expected systematic errors in this type of analysis, we refrained from any closer treatment of the experimental errors. The results are to be considered as estimations, and it is the purpose of this work to qualitatively demonstrate the general degree of accuracy of the approach. This can be inferred from Table 2 in which distances obtained from selected dipolar coupling constants, as estimated from the experiments, and the distances taken from the neutron structure [57] are compiled.

As expected, most of the longer distances are overestimated by NMR; only the distances next to the CH carbons are underestimated. Noting this, it might even be possible to derive further empirical rules which correct for the largest deviations. Generally, most of the distances are reproduced within 10% from the neutron diffraction distances. The approach is thus certainly not feasible for precision structure determination, but provides valuable insight into the molecular structure on the lengthscale of medium-range \( ^{13}\text{C} \ldots ^{1}\text{H} \) distances.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>CH₂</th>
<th>CH</th>
<th>H8</th>
<th>H5</th>
<th>H6</th>
<th>H7</th>
<th>NH₃</th>
<th>OH(9)</th>
<th>COOH(1)</th>
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<td>4.72</td>
<td>5.06</td>
<td>6.60</td>
<td>7.42</td>
<td>7.52</td>
<td>9.88</td>
<td>12.4</td>
</tr>
<tr>
<td>FWHM (Hz)</td>
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<td>1340</td>
<td>1150</td>
<td>1370</td>
<td>1600</td>
<td>1540</td>
<td>1110</td>
<td>770</td>
<td>620</td>
</tr>
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</table>

**FIG. 9.** Analysis of experimental data from 2D \( ^{1}\text{H}^{-^{13}\text{C}} \) DIP-HSQC spectra performed on \( \text{U}^{-^{13}\text{C}} \)-l-tyrosine at 30 kHz MAS. The experimental points correspond to individual antiphase magnetization intensities associated with the carbons C6, C8, and C9 ((a), (b), and (c), respectively) and the coupled protons determined by deconvolution. The coupling constants were determined by a fit to spin-pair build-up (REDOR) curves, the plateaus of which were scaled to 0.25 for (a) and (b), and to 0.5 for (c). Points in brackets were excluded from the fit.
4.3. Angle Determination Using Asymmetric Recoupling Schemes

In the last sections, heteronuclear dipolar recoupling approaches were presented which allow strong primary couplings to be precisely measured (Section 4.1) and provide an estimate for weaker secondary couplings (Section 4.2). In this way, internuclear distances on a short-to-medium range are accessible. In the following, we turn to a class of experiments which sensitively combine information on two individual distances with the information on the angle between the two internuclear vectors.

In this context, the concept of directed recoupling plays a central role (see Section 2.4). For demonstration purposes, consider first a simple methylene group, CH₂. Performing an II-type recoupling experiment, as depicted in Fig. 2(b), on this I₂S three-spin system results in a MAS sideband pattern in which the two IS couplings are rotor-encoded independent of each other (according to Eq. (9)), and the resulting pattern is simply a superposition of the IS spin-pair patterns. In Fig. 10(a), the experimental pattern (obtained on U–¹³C laz-tyrosine) is shown together with a pattern calculated for a two-spin system with a CH distance of 115 pm. Practically, the same pattern is observed when the experiment is performed on a CH group (in U–¹³C laz-alanine), which is a “real” two-spin system without further strong CH couplings that could interfere (see Fig. 10(b)). This demonstrates that, for two strong CH couplings, full separation can be accomplished and mutual interference is avoided by applying a directed I→S, i.e., H→C→H, recoupling scheme. In contrast, when SS-type recoupling, i.e., H→C→H, is applied to a CH₂ group by performing the experiment depicted in Fig. 2(a), the sideband pattern characteristically deviates from the two-spin patterns (see Fig. 10(c)). As has been discussed for Eq. (8), the additional higher-order sidebands arise from cosmodulations of the leading two-spin IS term, involving the second I-spin. Hence, these modulations and, consequently, the deviations in the sideband pattern depend on the relative orientation of the two coupling tensors. For comparison, in

<table>
<thead>
<tr>
<th>( D_{CH}/2\pi ) (Hz)</th>
<th>( r_{CH} ) (pm)</th>
<th>( r_{CH} ) (pm)</th>
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<tbody>
<tr>
<td>C6-H5</td>
<td>4.8</td>
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</tr>
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<td>C6-H9</td>
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</tr>
<tr>
<td>C8-H7</td>
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<td>200</td>
</tr>
<tr>
<td>C8-H1</td>
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<td>C9-H9</td>
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<td>C9-H8</td>
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<td>C9-H6</td>
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</tr>
<tr>
<td>C9-H1</td>
<td>1.7</td>
<td>261</td>
</tr>
</tbody>
</table>
a two-spin CH group, there is no such interference effect. Then, the direction of recoupling does not matter, and all recoupling schemes (II, IS, SI and SS) yield the same sideband pattern (see Fig. 10(d) for SS-type recoupling).

The interference of two dipole–dipole couplings should be avoided as far as internuclear distance measurements for individual pairs of nuclei are concerned. On the other hand, the sideband pattern shown in Fig. 10(c) contains the information on the H–C–H angle, such that in general SS-type, i.e., $S \rightarrow I$; recoupling schemes could be used for $I \rightarrow S$ angle determinations and, conversely, II-type, i.e., $I \rightarrow S$; recoupling schemes for $S \rightarrow I$ angle determinations. To ensure practical applicability, however, the angular dependence of the pattern needs to be more pronounced than a creation of a few higher-order sidebands, which could well be lost in the noise when the signal quality is a bit poorer.

The required increase in angle sensitivity can simply be achieved by applying asymmetric recoupling schemes in which the recoupling times used for excitation and reconversion of the dipolar IS correlations or dipolar-ordered states are no longer equal. For excitation/reconversion times of $2/4\tau_R$ (at 30 kHz MAS), the resulting angular sensitivity is illustrated in Fig. 11. The patterns are calculated for a CH$_2$ three-spin system with two identical CH distances of 115 pm and an H–C–H distance ranging from 90$^\circ$ to 180$^\circ$. Due to the symmetry properties of the dipolar tensors, the patterns are sensitive to an angle range covering 90$^\circ$, before becoming redundant. For CH$_2$ groups, an H–C–H angle of the order of 110$^\circ$ is expected, and the respective range of angles is expanded in Fig. 11. The obvious differences between the patterns observed for angle changes of only 2$^\circ$ emphasize the marked sensitivity of the
method. Comparing the calculated patterns with the one obtained in an experiment on the CH₂ group in L-tyrosine yields an angle of 116°.

It should be mentioned that the method is not suited at all for a precision investigation of CH₂ geometry. The similar magnitude of the two CH dipolar coupling tensors in connection with asymmetric recoupling times leads to a substantial signal loss (reduction to about 10% as compared to $N_{exc} = N_{rec}$). Furthermore, owing to the very strong $^1$H–$^1$H homonuclear coupling, finite-pulse effects and effects due to fast bond librations (leading to slightly asymmetric coupling tensors) on the patterns are rather large. The above example should thus be taken with a grain of salt; it mainly served to illustrate the basic principle and the surprisingly high sensitivity to the angle.

After having demonstrated the potential of directed asymmetric recoupling schemes, we now turn to an N–H⋯N hydrogen bond, i.e., an $S_2L$ three-spin system, in which two N–H distances as well as the N–H–N angle is to be determined. The N–H⋯N hydrogen bond investigated here is part of an array of four hydrogen bonds formed by N-butylaminocarbonyl-6-tridecyl-isocytosine. The material adopted the “enol” form with a pyrimidine-4-ol unit, as shown in Fig. 12. The proton thus forms

**FIG. 11.** Angular dependence of sideband patterns resulting from asymmetric recoupling schemes. The patterns are calculated for a CH₂ spin system with two identical CH distances of 115 pm. Recoupling times were $t_{exc} = 2\tau_R$ and $t_{rec} = 4\tau_R$ at 30 kHz MAS. In the top right corner, the experimental pattern (dotted line) of the CH₂ group in L-tyrosine is compared to the best-fit calculated pattern.
an N–H⋯N hydrogen bond between a urea–nitrogen and a pyrimidine–nitrogen. In regard of this structure, a shorter and a longer N–H distance can be expected, corresponding to a (slightly) extended urea–N–H chemical bond and a pyrimidine-N–H hydrogen bond, respectively. Due to the difference in the two NH dipolar coupling constants and the weak 1H homonuclear effects, the loss of spectral intensity due to asymmetric recoupling and the influence of experimental imperfections, respectively, are minor. The results to be detailed in the following can therefore be considered quantitative within the given limits of accuracy.

Since the same proton is involved in both types of bonds, an H → N recoupling scheme allows the N–H–N angle to be measured, while an N → H recoupling scheme is the method of choice for determining the N–H distances individually. For the latter purpose, SS-type recoupling is provided by the DIP-HDOR pulse sequence depicted in Fig. 2(a), in combination with 15N chemical-shift resolution in the direct spectral dimension. At the resonance frequency of the urea–nitrogen, the experimental sideband pattern corresponds to an N–H distance of 118 ± 3 pm (shown in Fig. 13(a)). For the pyrimidine–nitrogen, however, an accurate measurement of the N⋯H distance is difficult because during recoupling the nitrogen does not only experience the dipole–dipole coupling of the proton in the hydrogen bond, but also two intramolecular couplings of the neighboring NH and OH protons. Unfortunately, with respect to the pyrimidine–nitrogen, these two other protons are within the same distance range as the proton of interest (150…250 pm). Consequently, the sideband pattern observed at the pyrimidine–nitrogen is not

FIG. 12. Hydrogen-bonded structure of N-butilaminocarbonyl-6-tridecyl-isocytosine (in the “enol” form with a pyrimidin-4-ol unit), which is isotopically enriched with 15N on all three nitrogens of the guanidine unit. The N–H⋯N angle determination was carried out on the two central hydrogen bonds highlighted in the figure. Perturbing interactions hindering the direct determination of the N⋯H coupling are indicated by arrows.
sufficiently dominated by the N⋯H coupling of interest, but rather contains a superposition of three couplings. In Fig. 12, the perturbing interactions are indicated by arrows. In many cases, such problems can be circumvented by using HSQC experiments, which combine the coupling information of sideband patterns with 1H chemical-shift resolution in the indirect dimension (as also discussed in Section 4.2). In our case, however, a HSQC experiment would only allow us to exclude the OH proton because the other perturbing proton belongs to an N–H⋯N hydrogen bond as well and has therefore exactly the same chemical shift as the proton of interest. The same degree of separation is also provided by H → N recoupling schemes (see Fig. 2(b)), in which the proton in the N–H⋯N hydrogen bond acts as a probe for heteronuclear dipole–dipole couplings to the nitrogen nuclei in its proximity. Overall, the so-obtained sideband patterns depend on two N–H distances as well as the N–H–N angle. The smaller of the two N–H distances is known from the SS-type HDOR experiment discussed above, such that two unknown parameters remain to

FIG. 13. Calculated (solid) and experimental (dotted) sideband patterns for the N–H⋯N moieties in N-butylaminocarbonyl-6-tridecyl-isocytosine (see Fig. 12): (a) symmetric N → H recoupling (SS-type) with $t_{\text{exc}} = t_{\text{rec}} = 8\tau_R$, (b) asymmetric H → N recoupling (II-type) with $t_{\text{exc}} = 3\tau_R$ and $t_{\text{rec}} = 6\tau_R$, (c) asymmetric H → N recoupling (II-type) with $t_{\text{exc}} = 4\tau_R$ and $t_{\text{rec}} = 8\tau_R$. The NH distances and N–H–N angles used for the calculations are given with the patterns. In all cases, the MAS frequency was 30 kHz.
be derived from the experimental patterns, i.e., the N⋯H distance and the N–H–N angle. With respect to potential perturbations, the OH proton is a priori not involved, such that only one intramolecular interaction with the pyridine–nitrogen might lead to distortions in the sideband pattern.

In Figs. 13(b) and 13(c), spinning-sideband patterns are shown, which were recorded, in the indirect dimension of the II-type recoupling sequence depicted in Fig. 2(b). The $^1$H chemical-shift resolution in the direct dimension was used to select the pattern of the proton in the N–H⋯N hydrogen bond. Asymmetric recoupling schemes with excitation/reconversion times of $3/6\tau_R$ and $4/8\tau_R$ (in Figs. 13(b) and 13(c), respectively) ensure a pronounced sensitivity on the N–H–N angle as well as on the N–H distances. For both patterns, best agreement between a three-spin calculation and the experiments is achieved for distances of $(115 \pm 2)$ pm for N–H and $(175 \pm 5)$ pm for N⋯H as well as $(168 \pm 5)$° for the N–H–N angle. The patterns depend most strongly on the shortest N–H distance, corresponding to the strongest N–H coupling, and the value of 115 pm agrees, within the experimental accuracy, with the value obtained from the SS-type HDOR experiments. The other N–H distance and the N–H–N angle can be determined independently and without ambiguity from each of the patterns in Figs. 13(b) and 13(c). The agreement of the distances and angles determined from the two patterns is remarkable and further confirms the reliability of the results; in particular, no perturbing effect of the $^{15}$N nucleus in the neighboring hydrogen bond is noticeable. To give an idea about the sensitivity of the method, a set of sideband patterns is shown in Fig. 14 for N⋯H distances and N–H–N angles within the range of interest. Thus, the geometry of the N–H⋯N hydrogen bond can be fully and precisely elucidated by asymmetric directed HDOR recoupling experiments.

4.4. Natural Abundance $^{15}$N–$^1$H Shift Correlation Using Inverse $^1$H-Detected TEDOR

The low isotopic abundance of $^{15}$N ($\sim 0.37\%$) and its low magnetogyric ratio ($\gamma_N/\gamma_H \approx 10$) renders directly detected $^{15}$N 2D spectroscopy essentially hopeless in solid-state NMR. A prominent exception was presented by Lesage et al., who demonstrated the feasibility of CP-based $^1$H–$^{15}$N shift correlation, where very narrow $^1$H lines as provided by Lee–Goldburg homodecoupling in the indirect dimension partially compensate for the low sensitivity of $^{15}$N [58]. Indirect $^1$H detection, on the other hand, bears a large potential for a substantial gain in sensitivity [13, 59–62]. Following this idea, one of the variants of REDOR-based correlation experiments presented in this publication is applicable to $^{15}$N in natural abundance.

For $^1$H-detected spectroscopy on systems with X nuclei in low abundance, the major experimental problem is posed by the large signal contribution of unwanted $^1$H magnetization arising from protons without X nuclei in their vicinity. The removal of such signals is usually left to the phase cycle, which, however, lowers the dynamic range of the receiver by more than two orders of magnitude. Receiver noise and remaining contamination of the final signal with spurious $^1$H signal lead to a prohibitively large amount of $t_1$ noise if no further precautions are taken. The most
preferable way to solve this problem is the destruction of the unwanted proton signals before detection, thus allowing the use of an increased receiver gain.

In solution-state NMR, unwanted signals are nowadays routinely removed using pulsed-field gradients (PFGs) [63], and PFG probe equipment is commercially available. In the solid state, fast MAS techniques are gradually approaching a regime of dipolar decoupling, where such gradient-based strategies become applicable as well [64]. Apart from PFGs, however, the familiar presence of strong dipole–dipole couplings provides another efficient way for the removal of unwanted magnetization in the solid state. Ishii et al. have just recently presented an approach based on the application of two long (400 μs) $^1$H dephasing pulses applied during the z-filter period of a double-CP $^{13}$C–$^1$H correlation experiment. This trick can only be adopted if a pure magnetization state of the heteronucleus is present at one point during the pulse sequence. In combination with $^1$H detection, this criterion can only be met when two polarization transfer schemes are catenated in terms of an $^1$H → X → $^1$H experiment. Note that the $^1$H-detected II-type recoupling experiment (Fig. 2(b)) does not suit the purpose because it creates only mixed $^1$H–X coherence states.

For our approach to $^{15}$N–$^1$H shift correlation in naturally abundant samples, we propose a combination of a CP-step for initial $^1$H → $^{15}$N transfer with a subsequent recoupled $^{15}$N → $^1$H transfer. The pulse sequence is shown in Fig. 15. The $^{15}$N $t_1$ dimension is inserted directly after the initial CP step, and followed by a z-filter. During this filter, uncoupled $^1$H signal is removed by two $^1$H dephasing pulses (each of 400 μs duration) or, alternatively, by a spoil gradient pulse (of 100 μs duration).
The second $^{15}\text{N} \rightarrow ^1\text{H}$ transfer is accomplished by a dipolar recoupling scheme, whose two recoupling periods are kept together in a TEDOR fashion [49]. Note that in all previously presented REPT experiments a $t_1$ period is inserted between the two recoupling blocks in order to achieve rotor encoding or allow for a chemical-shift evolution. For distinction, we restrict the use of the acronym “REPT” to the latter type of experiments, while “TEDOR” stands for the transfer scheme with the $t_1$ spectral dimension before the recoupling sequence, in reference to the earliest publication of this particular concept of polarization transfer by Hing et al. [49].

Figure 16 shows three $^{15}\text{N} \rightarrow ^1\text{H}$ shift correlation spectra of L-histidine · HCl · H$_2$O with $^{15}\text{N}$ in natural abundance, recorded using the pulse sequence depicted in Fig. 15. The spectral resolution of the $^1\text{H}$ lines is provided by fast MAS (at 30 kHz) alone, while additional continuous-wave RF decoupling is applied during the $^{15}\text{N} t_1$ dimension. A noteworthy feature of the pulse sequences is that no $t_1$-noise was observed. After a total of only 8192 signal accumulations, 2D $^{15}\text{N} \rightarrow ^1\text{H}$ correlation spectra can be obtained with a satisfactory signal-to-noise ratio. Thus, the time requirements for such experiments are on the order of a few hours up to 1 day, which makes $^{15}\text{N}$ natural-abundance NMR spectroscopy feasible in the solid state.

Comparing Figs. 16(a) and 16(b), it is apparent that longer TEDOR recoupling times ($> 2\tau_R$) are required to ensure an efficient transfer of polarization from $^{15}\text{N}$ to $^1\text{H}$ in the NH$_3^+$ group, in which the N–H dipole–dipole couplings are reduced due to the rotation of the group (in analogy with C–H couplings in rotating methyl groups as discussed in Section 4.1). For short recoupling times ($\leq 2\tau_R$), practically only signals of the more strongly coupled N–H species are observed, which belong to the (N-protonated) imidazole ring of the histidine. In this way, the recoupling conditions chosen for the TEDOR part of the experiment provide a simple means of distinction.
and/or selection of signals with respect to N–H couplings. Concerning the efficiency of ¹H dephasing and signal suppression, the overall signal quality achievable by ¹H RF pulses or spoil gradients was found to be practically identical. The lower signal to noise of the spectrum shown in Fig. 16(c) as compared to the spectra in Figs. 16(a) and 16(b) is mainly due to the differences in the $B_0$ field (500 and 700 MHz $¹H$ frequency, respectively).

5. SUMMARY AND CONCLUSIONS

In this paper, we have put a variety of recently developed REDOR-type heteronuclear dipolar recoupling techniques into a common context and presented the concept of directed recoupling as well as the features of asymmetric recoupling schemes. Using these ideas, we introduce four novel experiments for fast-MAS applications:

- Rotor-encoded REDOR (REREDOR).
- Analysis of spectral intensities using X-detected DIP-HSQC.
- Asymmetric HDOR recoupling schemes.
- Inverse $¹H$-detected TEDOR correlation spectroscopy.

REREDOR represents the conceptually simplest combination of heteronuclear dipolar recoupling with the generation of spinning-sideband patterns via rotor encoding. In this way, heteronuclear dipolar coupling constants can be sensitively measured for all typical spin geometries, such as XH, XH₂ or XH₃. Since even weak
couplings give rise to meaningful sideband patterns, typical molecular units, such as CH, CH$_2$ and CH$_3$, can readily serve as probes for local molecular dynamics. Moreover, using the chemical-shift information of the $^{13}$C resonances, the information on the dynamics can be directly assigned to individual sites in the structure. Due to its applicability to samples naturally abundant in $^{13}$C, REREDOR has large potential as a powerful tool for routine solid-state material characterization and can replace $^2$H NMR experiments in a variety of applications. Demanding syntheses of specifically $^2$H-labeled materials providing information for only one particular site are then unnecessary. The site-specific information from REREDOR spectra is, however, gained at the expense of detailed geometric information inherent in static $^2$H spectra [65].

On the technical side, the insertion of short incremented delays into the REDOR $\pi$-pulse train, corresponding to an indirect $t_1$ dimension, represents a minimal modification of the pulse sequence, which avoids additional experimental imperfections. The sideband patterns generated by means of $t_1$ rotor-encoding allow us to measure dipole–dipole couplings for a range of different recoupling times, which can provide a consistency check with respect to the influence of multi-spin effects and other sources of error. In comparison with the more traditional MAS SLF experiments, REREDOR poses less experimental challenges, since SLF approaches require either high spinning frequencies or homodecoupling schemes (including an additional scaling factor for the heteronuclear coupling) for an efficient suppression of perturbing homonuclear couplings. In the context of the variety of REDOR-based techniques, it should be emphasized that REREDOR is the only option for sideband patterns associated with (mobile) CH$_2$ groups with two differently oriented (averaged) dipolar coupling tensors. In REPT experiments, the CH$_2$ coupling geometry leads to destructive interference and a complete loss of spectral intensity at rather short recoupling times [11], whereas the SLF character of REREDOR permits an efficient measurement.

The analysis of spectral intensities observed in X-detected DIP-HSQC experiments provides a means to estimate medium-range distances of 200–300 pm in H$_n$C spin-systems with spectrally resolvable proton sites. In this way, this DIP-HSQC approach widens the range of accessible distances and brings intermolecular contacts into the reach of heteronuclear dipolar recoupling experiments. This aspect is of particular interest for investigations of larger molecules and supramolecular structures and has already been addressed in terms of the MELODI approach by Yao et al. [66]. The quality of the medium- to long-range data does certainly not allow dipole–dipole couplings and, hence, distances to be measured at the same level of accuracy as is achievable for strong short-range couplings by sideband-pattern analysis. Nevertheless, such limited data quality is often more than sufficient when just distance constraints, to be used in further refinement procedures based on molecular modeling, are needed. Also, when different conformational models of systems of known connectivity (such as a protein) or a qualitative idea about the geometry of an intermolecular contact (e.g., a protein–ligand interaction) is to be tested, the REDOR-based correlation experiment might serve the purpose. On the plus-side, it features advantages such as experimental simplicity (no elaborate set-up is needed, $^1$H chemical shifts are not scaled by a multi-pulse sequence) and a simple
protocol for data analysis (no complex simulations). Most importantly, all correlations are present in a spectrum measured with a uniformly labeled sample. This is in stark contrast to recently developed modern CP-based techniques [6], which yield very precise medium-range distances (up to 250 pm), but suffer signal loss when the proton to which the distance is to be established is bound to $^{13}$C.

Note that all the limitations of the X-detected DIP-HSQC approach, which are posed by the multiple angular dependences, are removed when inverse-detected (II) experiments are performed on a system with dilute $S$ spins. The spin system response will then consist of a sum of pair couplings (as given by Eq. (9) in Section 4.2), and the analysis based on the spin-pair formula is valid over a much wider range of recoupling times. The problems to be encountered then are of a different nature: the reference intensity needed to determine the intensities on an absolute scale will be dominated by non-bonded $I$ spins and is therefore not accessible. Build-up curves will thus have to be measured until a plateau value for the intensity is reached (requiring a multitude of 2D spectra), or a sideband experiment with chemical-shift resolution in the indirect dimension has to be performed. If this very promising approach is really feasible remains to be investigated.

Returning to short-range internuclear distances, but sticking to multiple-spin geometries, we have further presented the application of asymmetric recoupling schemes and the analysis of the so-obtained sideband patterns to determine internuclear distances and angles in three-spin systems. Interference effects of multiple couplings are known for REPT approaches and have, for example, been used to identify the signals of rigid CH$_2$ groups in 1D $^{13}$C spectra by a selective loss of signal. On the other hand, this lack of CH$_2$ signal encountered in TEDOR and REPT experiments under certain conditions has stimulated the development of the REREDOR technique described above, which provides uniform and efficient recoupling of all CH$_n$ entities. The interference effects can be drastically amplified in a constructive and, hence, useful manner by applying recoupling periods of different lengths before and after the $t_1$ interval. Then, the sideband pattern generated by the $t_1$ rotor-encoding exhibits a pronounced sensitivity on the geometry of the spin system. In the XH$_2$ and X$_2$H cases considered here, the shape of the patterns is naturally dominated by the strongest X–H coupling, but even in the presence of a dominating coupling a second weaker coupling as well as the X–H⋯X (or H–X⋯H) angle can be determined to high precision. The concept of directed recoupling, i.e., the use of II- or SS-type recoupling experiments, allows the experiment to be straightforwardly adapted to an X–H⋯X or H–X⋯H situation. Moreover, symmetric recoupling schemes can also be applied in this directed fashion to supplement the three-spin geometry investigations with selective spin-pair information on X–H or X⋯H distances. These approaches bear significant potential for the investigation of hydrogen bonds, as has been demonstrated here for an N–H⋯N bond.

Finally, we have presented a pulse sequence employing inverse, i.e., $^1$H, detection via double $^1$H $\rightarrow$ X $\rightarrow$ $^1$H polarization transfer. Due to the sensitivity enhancement associated with $^1$H detection, natural-abundance $^{15}$N–$^1$H correlation spectroscopy becomes feasible in solid-state NMR. In contrast to the inverse-detection scheme proposed by Ishii et al. [59,62], which is based on a double CP, our approach
includes a TEDOR step for the second X →¹H transfer. The use of a recoupling technique not only ensures an efficient polarization transfer for weaker couplings associated with low-γ nuclei under fast MAS conditions, but it also opens up the possibility to discriminate between X–H correlation signals depending on the strength of the respective X–H coupling. Moreover, the correlation spectra presented here can be extended in terms of rotor encoding which then again allows for a precise determination of couplings by means of spinning-sideband patterns [67].

In view of the multitude of possible applications in chemistry, materials science and biology, the large potential of ¹⁵N–¹H shift correlation combined with precision distance determination [67] in low or even naturally abundant systems without the need for dedicated hardware cannot be emphasized enough. Using PFGs for suppression of unwanted ¹H magnetization places an additional demand on the equipment, but bears potential for the application of the II experiment in natural abundance. This experiment features the much more favorable signal composition from spin pairs, as expected from the PDLF configuration, and a potentially much higher sensitivity enhancement factor [13] due to the lack of an initial CP. The II experiment as applied to the determination of N–H hydrogen bonds in ¹⁵N labeled systems [19] has already proven its high potential. Further investigations on its possible extension to samples naturally abundant in ¹⁵N are under way.

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