Heteronuclear cross-relaxation effect modulated by the dynamics of N-functional groups in the solid state under $^{15}$N DP-MAS DNP

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**A B S T R A C T**

In a typical magic-angle spinning (MAS) dynamic nuclear polarization (DNP) nuclear magnetic resonance (NMR) experiment, several mechanisms are simultaneously involved when transferring much larger polarization of electron spins to NMR active nuclei of interest. Recently, specific cross-relaxation enhancement by active motions under DNP (SCREAM-DNP) [Daube et al. JACS 2016] has been reported as one of these mechanisms. Thereby $^{13}$C enhancement with inverted sign was observed in a direct polarization (DP) MAS DNP experiment, caused by reorientation dynamics of methyl that was not frozen out at 100 K. Here, we report on the spontaneous polarization transfer from hyperpolarized $^1$H to both primary amine and ammonium nitrogens, resulting in an additional positive signal enhancement in the $^{15}$N NMR spectra during $^{15}$N DP-MAS DNP. The cross-relaxation induced signal enhancement (CRE) for $^{15}$Ni opposite sign compared to that observed for $^{13}$C due to the negative sign of the gyromagnetic ratio of $^{15}$N. The influence on CRE efficiency caused by variation of the radical solution composition and by temperature was also investigated.

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1. Introduction

Dynamic Nuclear Polarization (DNP) is an emerging magnetic resonance technique to enhance the sensitivity of high-field magic-angle spinning (MAS) nuclear magnetic resonance (NMR) [1–4]. DNP enables signal enhancement, defined as the spin polarization achieved by DNP relative to the thermal equilibrium polarization of the respective nucleus, up to several orders of magnitude by transferring the large electron-spin polarization of paramagnetic polarizing agents to target NMR nuclei using microwave (MW) irradiation at specific frequencies. Alleviating the low sensitivity, which is the key challenge of traditional NMR spectroscopy, DNP has been successfully applied not only to the study of various biological samples [5–7] but also to solid samples in materials science [8–14]. However, despite substantial advances in DNP methodology [15,16] and instrumentation [17–19], NMR sensitivity is still a limiting factor in many applications [3]. Therefore, it is important to understand the different polarization transfer mechanisms occurring simultaneously during DNP in order to enable a systematic optimization of these experiments.

Molecular dynamics affecting the longitudinal relaxation time constant, $T_1$, which play a key role in the polarization transfer processes, are often neglected in MAS DNP measurements that are performed typically at 100 K, because most motional modes are effectively frozen out. However, some exceptional dynamics with small activation energies [20], such as the fast rotational motion of the methyl group [21], may show fast dynamical properties even at cryogenic temperatures. Recently, it has been reported that methyl group reorientation dynamics under DNP conditions can cause heteronuclear cross-relaxation mediated polarization transfer in the solid state [21–24]. Thereby, mobile methyl protons are hyperpolarized by DNP (Fig. 1a and b, pink arrow). Their magnetization is then spontaneously transferred to dipolarly coupled carbon atoms by cross-relaxation (Fig. 1a and b, red arrow), resulting in a negatively enhanced $^{13}$C resonance signal in a direct...

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The cross-relaxation mediated polarization transfer can be modelled by starting with the Solomon equations [22,29] for two dipole-coupled spins I, S. To empirically include solid-state DNP, the equations of motion for a closed two-spin system can be amended by a term that supplies non-equilibrium polarization from the outside. This corresponds to the coupling of the two-spin system to an external “DNP bath”, with the hypothetical steady-state polarization enhancement values $e_D$ and $e_S$ and corresponding enhancement rates $r_I^{DNP}$ and $r_S^{DNP}$ for the I and S spin, respectively (for details see electronic Supporting Information). $e_D$ and $e_S$ correspond to the DNP enhancement factors that would be achieved without coupling between I and S and with infinitely slow relaxation towards thermal equilibrium. For such a system, the total steady-state S polarization enhancement ($e_{tot}$) including cross-relaxation under DNP is

$$ e_{tot} = 1 + r_S^{DNP}(e_D - 1) / r_I^{DNP} - (e_I - 1) \frac{\gamma_I}{\gamma_S} \frac{\sigma_{IS}}{\gamma_S} $$

(1)

For the systems studied here, $e_{tot}$ is the $^{15}$N enhancement factor, or $^{15}$N spin polarization relative to its thermal equilibrium, and $e_I$ is the actual steady-state enhancement factor of $^1$H. $\gamma_I$ and $\gamma_S$ are the gyromagnetic ratios of $^1$H and $^{15}$N, respectively. $\sigma_{IS}$ is the $^1$H--$^{15}$N cross-relaxation rate and $r_S$ is the $^{15}$N longitudinal relaxation rate without coupling to the DNP bath. The first term on the right-hand side of this equation presents thermal equilibrium polarization ($TP$), the second term constitutes $^{15}$N direct enhancement ($\Delta DE$) achieved without the influence of cross-relaxation, and the third term represents the contribution from cross-relaxation effect ($ACRE$ or $\Delta NOE$). Notice the similarity of this third contribution with the enhancement in liquid-state Overhauser DNP, where the factor $\sigma_{IS}/(r_S + r_{DNP}^{iso})$ has been identified as the product of a coupling factor between the two spins and a leakage factor caused by relaxation back to thermal equilibrium [30].

The equation for the polarization enhancement may be simplified if it can be assumed that the DNP enhancement rate is much higher than the spin–lattice relaxation rate, i.e. $r_{DNP}^{iso} \gg r_S$, which often applies at cryogenic temperatures. Then we get

$$ e_{tot} \approx e_D - (e_I - 1) \frac{\gamma_I}{\gamma_S} \frac{\sigma_{IS}}{\gamma_S} $$

(2)

If, on the other hand, $^{15}$N direct enhancement ($\Delta DE$) on mechanisms other than cross-relaxation mediated from the I spin is very low, $r_{DNP}^{iso} \approx 0$, then the same expression as by Daube et al. is obtained [22],

$$ e_{tot} \approx e_{iso} = 1 - (e_I - 1) \frac{\gamma_I}{\gamma_S} \frac{\sigma_{IS}}{\gamma_S} $$

(3)

The gyromagnetic ratio of $^{15}$N (negative sign; $\gamma_N = -27.116 \times 10^6$ rad s$^{-1}$ T$^{-1}$) has an opposite sign compared to $^{13}$C (positive sign; $\gamma_C = 6.7262 \times 10^6$ rad s$^{-1}$ T$^{-1}$), so it is expected to show an opposite signal enhancement contribution in both CRE (the spontaneous Cross-Relaxation induced Enhancement under DNP) and the typical NOE. For example, if the substance of interest contains protonated nitrogen functional groups, which can rotate fast enough even at 100 K to facilitate cross-relaxation, hyperpolarization of $^1$H ($e_I > 1$) by DNP and the opposite signs of gyromagnetic ratios between $^1$H (positive, $\gamma = 26.7513 \times 10^6$ rad s$^{-1}$ T$^{-1}$) and $^{15}$N would cause a net positive enhancement ($e_{tot} > e_D$) by CRE in the $^{15}$N DP-MAS DNP NMR spectrum. On the other hand, saturation of $^1$H ($e_I = 0$) would induce a net negative enhancement ($e_{tot} < e_D$), a phenomenon that is traditionally called hetNOE [25,31].

In this paper, nomenclatures and symbols as shown in Table 1 are used to explain the spontaneous cross-relaxation induced...
enhancement (CRE) effect occurring during the DP-MAS DNP separately from the NOE. Direct hyperpolarization (DP) defines the set of effects affecting polarization of a particular nucleus under continuous MW irradiation without any rf manipulation.

3. Experimental

All chemicals were analytical grade. The $^{15}$N labeled benzyl ammonium sample was prepared by mixing $^{15}$N labeled benzyl amine (98 atom % $^{15}$N, Sigma Aldrich) and bis(trifluoromethysulfonyl)imide ($\geq 95.0\%$, Sigma Aldrich) in a 1:3 w/w ratio in an aprotic solvent consisting of a mixture of tetrachloroethane/DMSO $d_6$ DMSO in a ratio of 67/30/3 vol%. As polarizing agent, TEKPoliol [32] (Cortecnet) radical was added in a final concentration of 10 mM. Since the TEKPoliol radical is unstable in an acidic environment and at room temperature as can be seen in Fig. S1 in the supporting information, the TEKPoliol radical was added just before loading the sample into the MAS rotor and cooling down. The nitrogen spectrum exhibits a quartet splitting pattern in the $^{15}$N solution NMR spectrum (Fig. S2) without proton decoupling, indicating that the N-functional group exists as the primary ammonium ion.

The saturated $^{15}$N/$^{13}$C labeled glutamine (99 atom % $^{15}$N, 99 atom % $^{13}$C, Sigma Aldrich) samples were prepared using three different solvents with different $^{15}$N concentrations: glycerol-$d_6$/D$_2$O/ H$_2$O (60/30/10 vol%), glycerol-$d_6$/H$_2$O (60/40 vol%), glycerol/H$_2$O (60/40 vol%) mixture containing 10 mM AMUPoliol [33] (Cortecnet) radical. To confirm that the solubility of nitrogen is not affected by the different $^{15}$H/$^1$H isotope ratios in the solvent, the concentration of saturated glutamine in each solvent was determined by $^1$H NMR. The concentrations of glutamine were similar with 12.7, 12.2 and 12.2 mg/ml, respectively. The samples were then transferred into 3.2 mm sapphire rotors.

DNP experiments were performed on a Bruker (Karlsruhe, Germany) wide-bore Avance III HD 600 MHz spectrometer equipped with a triple resonance TCI ($^1$H, $^{13}$C, $^{15}$N) cryoprobe connected to a 395 GHz gyrotron with 60 mA of beam current as a continuous microwave source. Experiments were conducted at 100 K for all samples and additionally at 140 K for the glutamine sample in glycerol/H$_2$O (60/40 vol%) to confirm the temperature dependence of the cross-relaxation. For all experiments, excitation 90° pulses of 6.0 and 2.8 $\mu$s duration, corresponding to an rf field strength of 42 kHz and 89 kHz, were applied for $^{15}$N and $^1$H, respectively. SPINAL-64 decoupling was applied during acquisition with a $^1$H rf field of ca. 90 kHz. The number of scans was 8 at a MAS spinning frequency of 9 kHz. The pulse sequence [24] in Fig. S3 was used to identify the intimately linked the CRE effect and the NOE, depending on the presence or absence of the $^1$H saturation pulse train. A presaturation pulse-train with 16 90° pulses separated by 3 ms was applied to both $^1$H and $^{15}$N to destroy any transverse magnetization left. All direct DP spectra were measured using a single 90° pulse excitation of $^{15}$N without $^1$H saturation pulses. For the $^1$H saturation experiment (DP$_{sat}$), $^{1}$H saturation pulses with a pulse interval of 500 ms (Fig. S3, d21) were used to prevent further $^1$H polarization build-up. All DP and DP$_{sat}$ NMR experiments were performed using 6 variable polarization delays ranging from 10 s to 600 s.

4. Results and discussion

To estimate the CRE effect and the related NOE in the $^{15}$N DP-MAS DNP spectrum of the benzyl ammonium cation, $^{15}$N DP measurements were conducted with and without MW irradiation as well as with and without $^1$H saturation pulse trains as a function of polarization time. Comparisons of the different data sets according to different properties are shown in Fig. 3 and Fig. S4. As can be seen in Fig. 3(a) and S4(a), the signal intensities in the spectra and the build-up curves are drastically affected by $^1$H saturation pulse trains, which means that the CRE effect and the NOE play a significant role at 100 K. If the primary ammonium group of benzyl ammonium were not mobile at 100 K, a CRE effect and a NOE would not be expected, and the same intensity and build-up curves would be observable in Fig. 3(a,b) and S4(a,b), regardless if a $^1$H saturation pulse was applied or not. Likewise, $^1$H saturation also leads to a reduction of the signal intensity in the absence of DNP hyperpolarization, as shown in Fig. 3(b) and S4(b), which is a reflection of the NOE in the $^{15}$N spectrum. Comparing the effects observed in Fig. 3(a) and (b), the CRE effect contributes more strongly to the signal intensity of the $^{15}$N spectrum than the NOE and the DE in this sample. From Fig. 3(c) and (d), we can determine the contribution of the CRE in the $^{15}$N direct hyperpolarization effect (DP). Fig. 3(c) shows a larger difference than Fig. 3(d), which means that the CRE effect dominates the positive enhancement of the $^{15}$N signal in $^{15}$N DP-MAS DNP.

The full observable $^{15}$N signal enhancement factor ($I_{sat}$) in benzyl ammonium is strongly influenced by two different effects, the CRE (Fig. 1 a and b, red arrows), and the $^{15}$N DE (Fig. 1 a and b, blue and green arrows). The $^{15}$N MAS NMR spectrum recorded with $^1$H saturation and without MW, i.e. without any DNP effect, shows the smallest signal intensity due to the presence of the NOE which contributes a negative signal enhancement, while the highest signal intensity is found when both the $^{15}$N DE and the CRE effect contribute, which is achieved without $^1$H saturation and with MW irradiation (Figs 3, 4 and S4). From these results, it can be seen that ammonium ion rotational mobility, which enables CRE in $^{15}$N DP-MAS DNP, contributes significantly to the overall $^{15}$N polarization even at 100 K (Fig. 4, orange dashed line). As opposed to $^{12}$C, due to the negative sign of the $^{15}$N gyromagnetic ratio, an additional positive enhancement is found for the $^{15}$N polarization.

To determine the CRE effect in $^{15}$N DP-MAS DNP NMR spectra in a protic solvent, glutamine having one ammonium and one amide group ($N_a$ and $N_c$, respectively) with well-separated chemical shifts was used in a popular protic radical solution with a protonation degree of $\approx$10% i.e., glycerol-$d_6$/D$_2$O/H$_2$O (60/30/10 vol%) [34]. As seen in Fig. 5(a), $^1$H saturation has no impact on the signal intensity, which means the CRE effect and the NOE found in the previous benzyl amno-
A niunm sample are not significant in glutamine with this radical solution composition. This could be caused by two reasons: Either, the 1H–15N CRE effect induced by mobile protons under DNP conditions may be reduced because H/D exchange is expected for amine protons in protic solvent containing deuterium on the one hand (Figs. S5 and S6). Further, the amine dynamics of glutamine at 100 K could be insufficient for double quantum cross-relaxation between 1H and 15N on the other hand. In order to investigate this, the experiments were repeated with increasing 1H concentration in the radical solution. As shown in Fig. 5(a–c), an increasing signal intensity ratio (SIR) between the data with and without proton saturation can be identified at higher 1H concentration. This indicates that the protonation ratio of the amine of glutamine, which is altered by H/D exchange, can affect the efficiency of the 1H–15N CRE effect.

In general, deuterium in a radical solution is known to be beneficial for improving the diffusion of polarization from the sites of initial electron–nucleus polarization transfer to nuclei far away from the radical center and to increase the polarization transfer efficiency from protons to heteronuclei through relaxation–prolongating effects [35–37]. While some protons in the radical solution are needed to spread the polarization in the bulk, too many or too little of them can weaken the enhancement. In NMR of biological
samples, the radical solution composition used for the spectrum shown in Fig. 5 (a) has generally lead to the best performance \[38\]. However, when comparing the intensities of the plots in Fig. 5 (a–c), the 15N NMR signal intensity for glutamine increases with higher 1H concentration. The relative concentrations of the free proton in the radical solvents have a H/D (molar/molar) ratio of 16:84, 64:36, and 100:0. We further determined the DNP enhancement factor \(\varepsilon_{15N}^{CP}\) of 15N CPMAS (Fig. S6, Table S1) and with this the \(^1\)H polarization. A change in the protonation degree from 64% to 100% results in a decrease of the \(^1\)H polarization by a factor of 0.7, while in the DP experiment the additional signal by the CRE effect increases by a factor of about 2. Therefore, under the tested experimental conditions, for glutamine the \(^1\)H-15N CRE effect modulated by the amine protons appears to be more significant for the signal enhancement than the relaxation-prolongating effect of the deuterium.

To confirm the difference in CRE by the dynamic properties of the two nitrogen groups (\(N_a\) and \(N_e\)) in glutamine, the temperature was increased to 140 K. In Fig. 5 (c) and (d), the fully protonated sample is compared at different temperatures. The rate of change of the enhancement factor for \(N_a\) is more sensitive than that of \(N_e\). The structure of glutamine at neutral pH contains zwitterionic forms consisting of an \(\alpha\)-amino group (\(N_a\)) in the protonated \(-\text{NH}_3^+\) form and a carboxylic acid group in the deprotonated \(-\text{COO}^-\) form, and a simple amide (\(N_e\)) side chain. As can be seen in Fig. S7, the amide (\(N_e\)) side chain is stabilized due to the partial-double bond, resulting in slower dynamics. Therefore, the amide (\(N_e\)) side chain, which may have a smaller change in dynamics when the temperature is increased, experiences a smaller CRE effect on the spectrum.

5. Conclusions

We have shown evidence for the CRE effect, resulting in additional positive signal enhancement for the 15N NMR spectrum, by the spontaneous polarization transfer from hyperpolarized \(^1\)H to 15N during 15N DP-MAS DNP. A larger CRE effect can be achieved in an aprotic solvent due to the prevention of H/D exchange between solvent and substrate, as well as a more rapid reorientation dynamics of N-functional groups caused by weaker interactions.

Similar to a previous application \[24\] of a specific CRE by active motion under DNP (SCREAM-DNP) through the introduction of a 13CH3 labeled functional group as a probe into biomolecular systems, we expect that further surface signal enhancement using this effect could lead to more efficient and selective DNP-Surface enhanced NMR spectroscopy (DNP-SENS) in materials science. For example, surface structure information of nitrogen functionalized materials \[39,40\], which are prosperous as heterogeneous catalysis and energy materials, is critical for their further development. The surface signal can be selectively enhanced more strongly during DNP-SENS by binding molecules carrying rotatable nitrogen functional groups to the surface of the material. This would allow distinguishing clearly between surface and bulk signals due to a specific \(^1\)H-15N CRE effect.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmr.2020.106688.

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