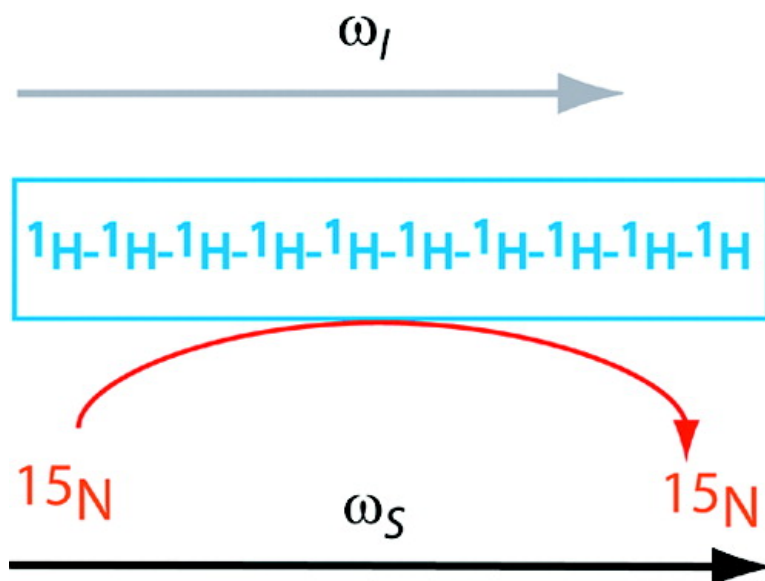


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Mismatched Hartmann–Hahn Conditions Cause Proton-Mediated Intermolecular Magnetization Transfer between Dilute Low-Spin Nuclei in NMR of Static Solids

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Since the discovery of cross polarization¹ (CP), magnetization transfer under the Hartmann–Hahn matching conditions remains the most popular means of both enhancing the sensitivity of dilute spins (e.g., ¹⁵N and ¹³C) and probing their interactions with the surrounding protons in NMR of biological solids. The proton network can also be used to probe the couplings between dilute spins themselves. For instance, proton-driven spin diffusion² represents a means of using the proton bath to induce the spin flips between chemically different dilute spin sites. While the dilute spin exchange experiment can be successfully applied in static solids³ to induce the correlations between spins separated by less than 3.5 Å, the mixing times to correlate more distant spins would become longer than 10 s, which would also result in additional loss in sensitivity due to relaxation. Very recently, cross-relaxation driven spin diffusion (CRDSD)⁴ somewhat related to the double cross polarization (DCP) experiment⁵ in the magic-angle spinning (MAS) NMR has been implemented to transfer polarization between ¹⁵N sites separated by 5 Å 1000 times faster than in a dilute-spin exchange experiment.³ When the ¹⁵N nuclei are irradiated, they become decoupled from the protons and exchange with each other primarily via their homonuclear interaction Hamiltonian. After some time, the ¹⁵N spin system attains nonergodic quasi-equilibrium (as was predicted theoretically by Brüschweiler and Ernst⁶) thus yielding cross peaks. Therefore, the CRDSD mechanism essentially relies on the presence of direct couplings between the dilute spins. On the other hand, polarization transfer over longer (>5 Å) distances, where the direct ¹⁵N–¹⁵N couplings become negligibly small, would be of great value not only for the spectral assignment of solid-state NMR spectra of oriented membrane proteins but also for establishing intermolecular contacts between the neighboring α-helices and the elucidation of their packing structure. Recently, third-spin assisted polarization transfer (TSAR) has been developed⁷ for the MAS conditions which does not require direct couplings between the dilute spins. It involves simultaneous irradiation of both rf channels with nearly the same amplitudes along the x-direction. In order to adapt this scheme to the static NMR case we note that in the nonspinning samples the proton- and low-side rf amplitudes would need to be different from each other. This is due to the fact that, to achieve the Hartmann–Hahn match under the MAS conditions, the difference between the irradiation amplitudes (in frequency units) must be equal to the rotor frequency to recouple the low-gamma spins and the protons.⁸ When the rf amplitudes are made nearly the same in the MAS case, this would result in a virtual offset term in the dipolar-recoupled Hamiltonian. It is this “mismatch” term that, as we shall demonstrate, causes proton-assisted transfer between the low spins. For now, we neglect the proton–proton interactions and consider just the couplings of the low spins to *N* protons (*I* spins). Numerical simulations with and without this assumption are given in the Supporting Informa-

tion. The direct interaction between the low spins *S*⁽¹⁾ and *S*⁽²⁾ is also neglected, as well as their chemical shift evolution. The total Hamiltonian operator in the doubly tilted rotating frame⁹ can be written as (setting ħ = 1):

$$H = \omega_S(S_z^{(1)} + S_z^{(2)}) + \omega_I I_z^{total} + \sum_{n=1}^N (a_{1n} S_x^{(1)} I_x^{(n)} + a_{2n} S_x^{(2)} I_x^{(n)}) \quad (1)$$

Here ω_S and ω_I are the rf amplitudes of the low and high spins, respectively. When ω_S and ω_I are larger than the heteronuclear couplings a_{1n} and a_{2n} , we use the truncated Hamiltonian (cf. ref 10):

$$H_T = -\Delta\omega_I I_z^{total} + \frac{1}{4} \sum_{n=1}^N a_{1n} (S_+^{(1)} I_-^{(n)} + S_-^{(1)} I_+^{(n)}) + a_{2n} (S_+^{(2)} I_-^{(n)} + S_-^{(2)} I_+^{(n)}) \quad (2)$$

where $\Delta\omega_I = \omega_S - \omega_I$ is the Hartmann–Hahn mismatch on the proton site. We are interested in finding the amplitude of the polarization transfer after the contact time *t*:

$$G(t) = \text{Trace}(S_z^{(2)} e^{-iH_T t} S_z^{(1)} e^{iH_T t}) \quad (3)$$

Next, the following transformation property valid for any noncommuting Hermitian matrices *A* and *B* is used:¹¹

$$e^{-i(A+B)t} = e^{-iAt} \exp\left(-i \int_0^t e^{iA t'} B e^{-iA t'} dt'\right) \quad (4)$$

where “O” stands for the Dyson time-ordering. Choosing $A \equiv -\Delta\omega_I I_z^{total}$ we then invoke the second-order Magnus expansion¹² using a stroboscopic observation of the periodic time-dependent Hamiltonian at times $t_n = 2\pi n/\Delta\omega_I$ to obtain an average time-independent Hamiltonian, which consists of three parts:

$$H_{\pm} = -\frac{1}{8\Delta\omega_I} \sum_{n=1}^N a_{1n} a_{2n} I_z^{(n)} (S_+^{(1)} S_-^{(2)} + S_-^{(1)} S_+^{(2)}) \quad (5a)$$

$$H_{II} = \frac{1}{8\Delta\omega_I} \sum_{i < j}^N (a_{1i} a_{1j} S_z^{(1)} + a_{2i} a_{2j} S_z^{(2)}) (I_+^{(i)} I_-^{(j)} + I_-^{(i)} I_+^{(j)}) \quad (5b)$$

$$H_{\Delta} = \frac{1}{16\Delta\omega_I} \sum_{n=1}^N a_{1n}^2 (S_z^{(1)} - I_z^{(n)}) + a_{2n}^2 (S_z^{(2)} - I_z^{(n)}) \quad (5c)$$

These expressions provide a clear meaning of the processes involved in the proton-mediated polarization transfer under mismatched Hartmann–Hahn conditions. The first term, H_{\pm} , corresponds to the effective recoupling between the low spins caused by the protons (even in the absence of their direct coupling); the second term, H_{II} , contains the equilibrating bath terms leading to a quasi-stationary equilibrium at longer times (cf. ref 13), whereas the third

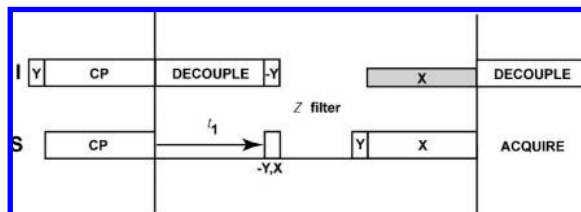


Figure 1. Pulse sequence used to measure the proton-mediated polarization transfer between ^{15}N nuclei from different NAL molecules in a single crystal. The cross-polarization part is applied to enhance sensitivity; next, the ^{15}N chemical shift evolves under the conditions of proton decoupling; the proton magnetization is flipped along the z -direction; the alternating $90^\circ -y/x$ pulse on the ^{15}N site selects either the real or imaginary component; a wait time of several seconds is applied to eliminate any residual proton magnetization along the x -axis; the proton-mediated transfer is achieved when the ^{15}N spins are brought along the x -direction followed by simultaneous rf irradiation on both channels with the rf amplitude of the ^1H site being slightly lower than the Hartmann–Hahn match; finally, the ^{15}N chemical shift is detected under the conditions of proton decoupling.

term, H_Δ , commutes with the first two and thus can be neglected if the following condition is met:

$$\sum_{n=1}^N a_{1n}^2 = \sum_{n=1}^N a_{2n}^2 \quad (6)$$

That is, the lattice sum of the first low spin is equal to that of the second spin. In this case the efficiency of the magnetization transfer is expected to be maximal by analogy with the Hartmann–Hahn cross-polarization transfer mechanism.¹³ The last condition can be satisfied statistically if one considers an infinitely large proton bath with all possible couplings present. This is especially important for NMR of static solids, where the couplings of the ^{15}N spins to their nearest protons could be quite different for various amide sites. Equation 6 implies that magnetization transfer now depends on the magnitudes of the couplings to all protons at the same time thus making the matching condition somewhat less stringent.

An NMR experiment implementing dilute-spin magnetization transfer via the proton bath is diagrammed in Figure 1. This experiment was performed on a single crystal of *n*-acetyl leucine (NAL) having intermolecular distances between the closest sites of 6.5–6.7 Å (ACLEU01, deposition 624793).¹⁴ The mismatched rf amplitude on the proton side was set to 90% of the exact Hartmann–Hahn match amplitude. Figure 2 shows that all 12 cross peaks are observed as a result of the proton-mediated transfer using the sequence of Figure 1. These peaks arise only after a 7 ms mixing time (or shorter) and can be only due to the intermolecular ^{15}N spin exchange since there is a single amide site per NAL molecule. When the proton rf field was set back to the Hartmann–Hahn matching amplitude, much of the ^{15}N magnetization was transferred back to the proton bath resulting in little or no intensity. Changing the proton mismatched amplitude to 95% of the match also resulted in decreased intensity, whereas changing it to 85% and 80% increased the intensity of the main diagonal peaks while decreasing the intensity of cross peaks (results not shown). No cross peaks could be observed by using the dilute spin-exchange experiment even after a 15 s mixing time (see Supporting Information).

In conclusion, a quantum-mechanical description of the proton-mediated polarization transfer between dilute spin nuclei is proposed for the static NMR case. It is shown both theoretically and experimentally that mismatched (by about $\pm 10\%$) Hartmann–Hahn conditions provide effective recoupling of dilute spins via the proton bath even in the absence of direct interactions between the low-

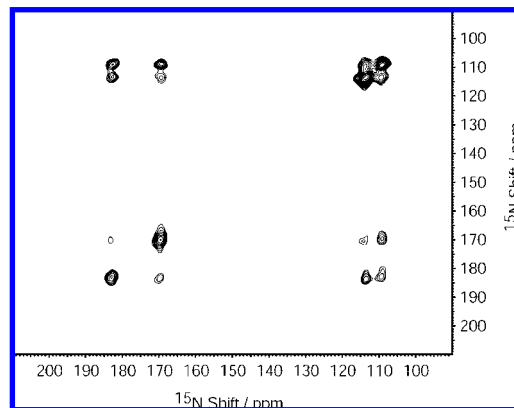


Figure 2. Solid-state ^{15}N NMR spectrum showing the cross peaks among all four distinct molecules of a small (0.7 mg) NAL single crystal separated by distances greater than 6.5 Å. The pulse sequence described in Figure 1 was used with the following parameters: CP time 1 ms; rf amplitude 57 kHz; t_1 dwell time 80 μs ; z -filter time 3 s; ^1H rf amplitude during the proton-mediated transfer was set to 90% of the exact Hartmann–Hahn match (~ 51 kHz); ^{15}N contact time 7 ms; 256 scans; 8 s recycle delay. The experiment was performed on a 500 MHz Bruker Avance II instrument with a triple-resonance E-Free probe equipped with a 5 mm solenoid coil.

gamma nuclei. The Magnus expansion provides a description of this mechanism by means of the three-species spin-flip Hamiltonian which contains proton-mediated spin-flip terms for the dilute spin pair. This treatment is only approximate, and more rigorous mean-field statistical theories of the many-body spin dynamics may be required. The magnetization transfer between dilute spins was experimentally demonstrated using a single crystal of *n*-acetyl leucine with intermolecular distances between the ^{15}N – ^{15}N pairs exceeding 6.5 Å. Therefore, this technique should be of major interest for studying inter- and intramolecular interactions in solid-state NMR of oriented membrane proteins and assignment of their spectra.

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Supporting Information Available: Detailed many-spin numerical simulations of the proton-mediated ^{15}N – ^{15}N magnetization transfer using the full and average Hamiltonians and an X-ray structure of the NAL crystal. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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