Supporting Information

Proton Assisted Insensitive Nuclei Cross Polarization

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This supporting information is divided into five sections.

1. MODEL SPIN SYSTEM

All of the simulations shown in the manuscript and in the Supporting Information (with the exception of those in Section 6) utilized a model seven spin system composed of $^{15}NH^{13}C_{\alpha}H^{13}C_{\beta}H_2$. The spatial coordinates, isotropic and anisotropic shifts, asymmetry parameter and orientation of the CSA for each shift tensor for each spin are included in Table S.I.-1 below. The same parameters were used for the simulations in Figure 2 of the text.

Table S.I.-1: Spin system used in the simulations reported in Figure 2 of the text. *Homonuclear* and *heteronuclear* indirect *J* couplings are not included in the simulations. Their effects are small (as suggested by a simulation using 60 Hz $J_{C\text{-}C}$ and 10 Hz $J_{N\text{-}C}$; not shown here), but could be included in quantitative studies.

Index	Nuc.	X (Å)	Y (Å)	Z (Å)	σ_{iso} (ppm)	σ_{aniso} (ppm)	η	CSA orientation
1	^{13}C	1.246	-0.783	-0.894	-16.3	25.3	0.0	(0, 45, 0)
2	^{13}C	0.718	0.033	0.320	0	23.99	0.92	(-80, 15, 3)
3	^{15}N	0.678	-2.128	-0.894	0	100	0.8	(0, 30, 0)
4	^{1}H	1.267	-2.967	-0.894	0	0	0	-
5	^{1}H	0.953	-0.280	-1.833	0	0	0	-
6	^{1}H	1.188	1.036	0.300	0	0	0	-
7	^{1}H	1.097	-0.432	1.252	0	0	0	-

2. PAIN-CP MECHANISM

Figure S.I.-1 shows the dependence of the n=0 PAIN-CP $(\omega_{1C}/2\pi)=(\omega_{1N}/2\pi)=55$ kHz and $(\omega_{1H}/2\pi)= 49$ kHz magnetization transfer from ^{15}N to $^{13}C_{\beta}$ on the various interactions present in the seven spin system.

1. <u>^{13}C-^{15}N or $^{13}C_{\alpha}$-$^{13}C_{\beta}$ couplings absent:</u> When the ^{13}C-^{15}N or $^{13}C_{\alpha}$-$^{13}C_{\beta}$ couplings are separately removed, we observe approximately the same buildup curve as with all the couplings present. This indicates that the transfer does not involve a DCP type mechanism, and illustrates that the ^{13}C-^{13}C couplings are not involved in the long distance transfer observed in PAIN-CP.

2. ^{1}H-^{1}H couplings off : When the ^{1}H-^{1}H couplings are removed the transfer is even slightly more efficient and faster. This indicates that PAIN-CP mechanism does not rely on ^{1}H-^{1}H couplings.
3. ^{1}H-$^{13}C_{\alpha}$ couplings off: When all the couplings from the ^{1}H to the $^{13}C_{\alpha}$ are quenched, we observe a decrease in the efficiency and rate of the transfer. This clearly shows that a part of the ^{15}N to ^{13}C long range transfer also involves a homonuclear ^{13}C TSAR effect.

This figure illustrates that the complex mechanism involved in PAIN-CP is completely different from DCP in spite of the similarity of the two pulse sequences. A detailed quantitative analysis of the process is currently under progress.

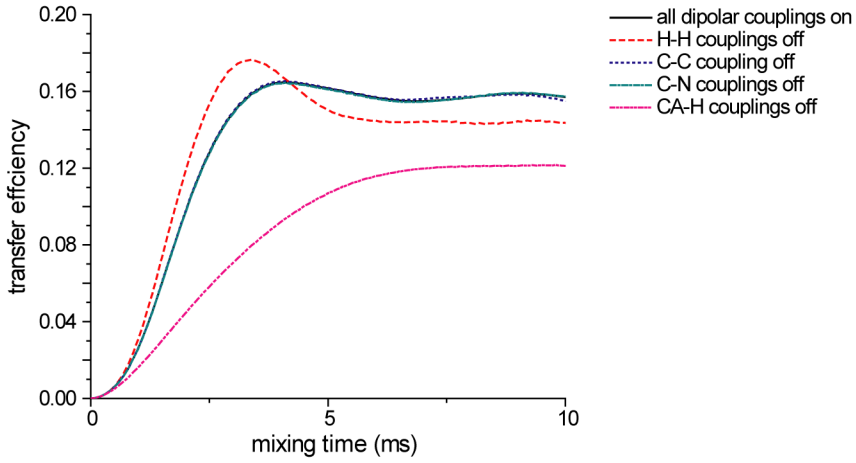

Figure S.I.-1: PAIN-CP pathways. The simulated curves correspond to N-C_{β} transfer (two-bond transfer) with identical settings as for the PAIN-CP simulations in Figure 2. Note that the simulations with all dipolar couplings included, C-C coupling removed and C-N coupling removed yield essentially the same curve.

The PAIN-CP effect presented in this communication corresponds to the n=0 matching condition, meaning that the ^{15}N and ^{13}C r.f. fields are identical. It is worth noting that a substantial PAIN-CP transfer can be achieved even when the ^{13}C and ^{15}N irradiation are mismatched by a multiple of MAS frequency.
For example, in the n=1 case, the choice of ^{1}H irradiation power determines the relative importance of the DCP and PAIN-CP mechanisms during the transfer. Under high ^{1}H power irradiation (i.e. >100 kHz r.f.), the ^{15}N-^{13}C polarization transfer is mediated primarily by DCP (see Figure S.I.-2) because the scaling factor of PAIN-CP term becomes too small. With a reduced ^{1}H irradiation (39 kHz) that avoids ^{1}H-X recoupling, we recover a substantial PAIN-CP effect, though smaller than in the n=0 case. Note that when we vary the ^{1}H power from 0-100 kHz in the n=1 case, we found several optima, with a global maximum at 39 kHz. Each of these optima leads to a polarization transfer that is 2-6 times larger than polarization transfer for DCP with same ^{15}N, ^{13}C powers (25 kHz, 45 kHz respectively) and 100 kHz ^{1}H CW decoupling

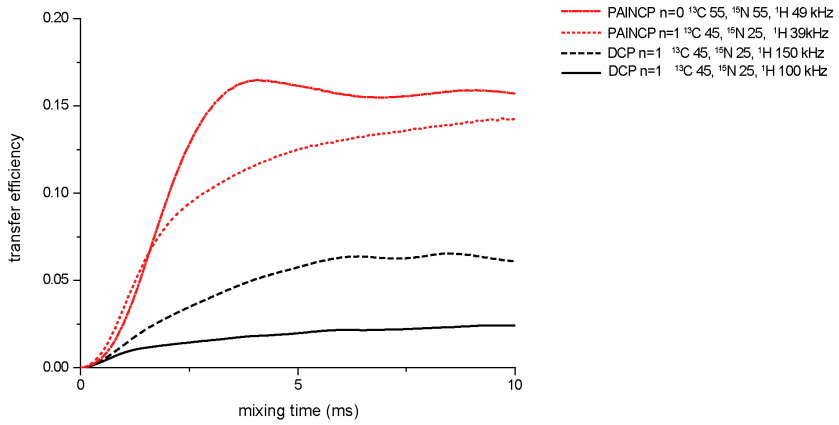

Figure S.I.-2: Simulations of ^{15}N-$^{13}C_\beta$ two-bond transfer for PAIN-CP (red), DCP (black) sequences. The solid, dotted red curves correspond to n=0 and n=1 PAIN-CP matching respectively. For the DCP simulations, the ^{1}H decoupling was chosen to be 100 (solid) and 150 (dotted) kHz. Except for n=1 PAIN-CP, the three other simulations are similar to Figure 2.

3. COMPLEMENTARY INFORMATION ON ONE BOND TRANSFER

Figure 2 of the text shows simulations of a *two-bond* ^{15}N to C_β magnetization transfer at $\omega_r/2\pi$=20 kHz (using the model seven spin system described above), and illustrates the superior performance of the PAIN-CP sequence in this regime.

Figure S.I.-3 shows the *one-bond* ^{15}N to C_α transfer efficiency (using the same spin system and simulation parameters as in Figure 2) and highlights the differences between PAIN-CP, DCP, TEDOR, REPT and GATE with respect to their performance at high MAS and Larmor frequencies. The figure illustrates the following important points.

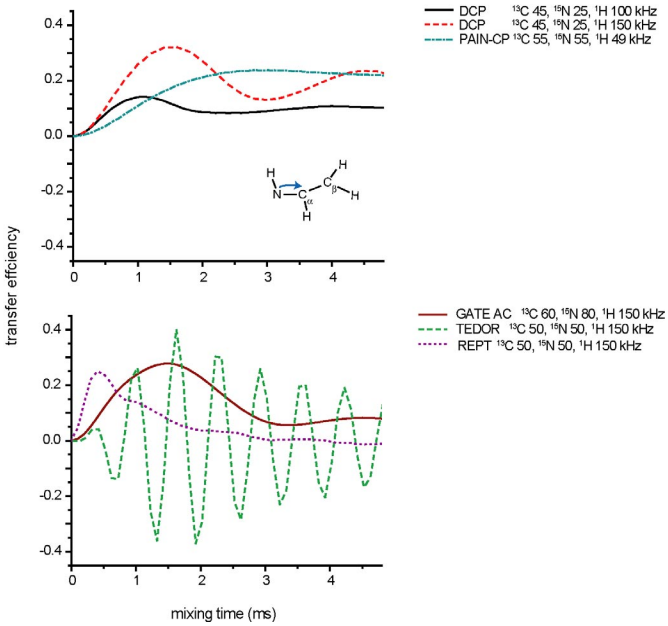

Figure S.I.3: N- C_α transfer (one-bond transfer) using the same spin system and simulation conditions as in Figure 2 of the text.

1. DCP transfer efficiency dependence on the ^{1}H r.f. field: DCP is an efficient technique for one-bond transfer, provided that the ^{1}H r.f. field is at least 3 times higher than ^{13}C and ^{15}N r.f. fields, a condition that is difficult to fulfill at $\omega_r/2\pi \geq 20$ kHz either due to hardware or r.f. heating leading to imperfect ^{1}H decoupling. Under such constraint PAIN-CP provides comparable or better transfer efficiency compared to DCP.
2. PAIN-CP one-bond transfer efficiencies: PAIN-CP can be used efficiently for one-bond transfer experiments at high MAS frequencies – and is often the best compromise in between performance and experimental requirements. This method is especially well suited for biological samples which may be sensitive to excessive r.f. heating. Moreover, PAIN-CP buildups reach an equilibrium plateau, which relaxes the constraint on the precise optimal mixing time, making the experimental setup much more straightforward.
3. TEDOR/REPT: The classical version of TEDOR displays oscillations due to the isotropic chemical shift, which greatly complicates the choice of the mixing time for a real sample with a distribution of chemical shifts. The chemical shift evolution can be refocused by applying a strong π pulse in between the REDOR periods (a sequence referred to as REPT or dipolar INEPT). However, the compensating pulse has a side effect of interfering with the ^{1}H decoupling. The stronger the pulse the greater is the requirement for the ^{1}H decoupling field. Therefore a compromise between efficient refocusing and decoupling is required. In the simulations the ^{1}H decoupling field is 150 kHz except during the 100 kHz ^{13}C/^{15}N refocusing pulses where no decoupling is applied (these settings yield the best result for 50-100 kHz refocusing and 0-150 kHz decoupling powers).
4. GATE: The longitudinal recoupling sequence, GATE, has a large scaling factor but requires very demanding conditions at MAS frequencies >15 kHz.

These simulations show that PAIN-CP should be considered as an alternative to the usual one bond polarization schemes (DCP, TEDOR/REPT) in the high $\omega_r/2\pi$ regime. Moreover, our experiments show that for DCP a decoupling mismatch of 2.5 is not sufficient. As a result PAIN-CP performed better for one-bond transfers than DCP, given our experimental constraints. The PAIN-CP sequence should be widely used since it is a reasonable option for performing efficient heteronuclear ^{15}N-^{13}C transfer at high Larmor and spinning frequencies.

4. TRANSFERS TO A WEAKLY COUPLED SPIN IN THE PRESENCE OF A STRONGLY COUPLED SPIN

Figure S.I.-4 below illustrates the influence of a directly bonded ^{13}C on the polarization transfer from ^{15}N to a remote ^{13}C spin for DCP, TEDOR and PAIN-CP. In order to focus on the effect of the C_α spin, the spin system for the DCP and TEDOR simulations do not contain protons. On the other hand, we include 3 protons in the PAIN-CP spin system, as its mechanism relies on surrounding protons. Isotropic chemical shifts and CSAs were neglected in all the simulations.

The simulation shows a transfer over 3 Å distance in the presence (N-C_2, in red) and in the absence (N-C_2, in blue) of the C_α spin directly bonded to the nitrogen. Although DCP yields the most efficient transfer in the absence of the C_α spin, almost no magnetization can be transferred when C_α is added. In the TEDOR case, although there is no dipolar truncation due to the longitudinal form of the recoupled Hamiltonian, the transfer to the remote spin C_2 in the presence of a strongly coupled C_α is almost absent. In contrast, PAIN-CP appears to be the only mechanism able to provide significant long distance transfer in both situations. In fact, the transferred magnetization is even larger in the presence of a strongly coupled C_α spin and can be attributed to an additional transfer pathway relying on homonuclear TSAR mechanism. Details of the mechanism are currently under investigation. Note, that the vertical axis is reduced for the simulations in the second row of the figure.

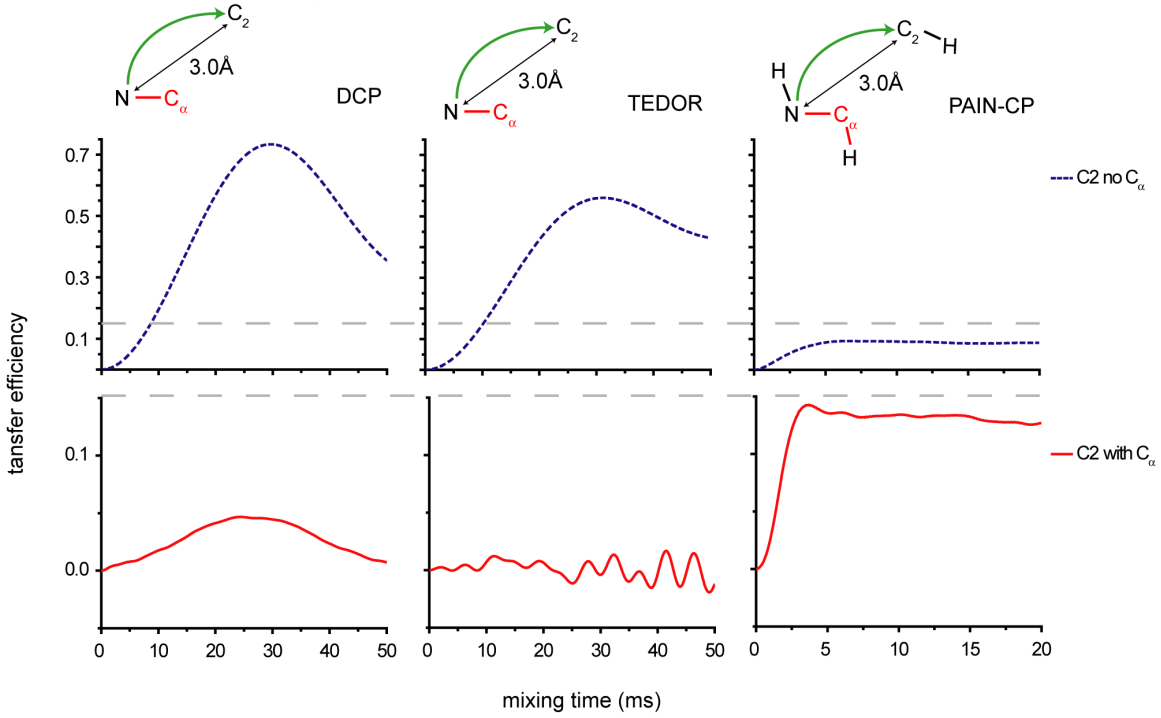

Figure S.I.-4: Influence of a nearby carbon (C_α) in the polarization transfer from ^{15}N to remote spin (C_2). The dashed line represents 0.15 normalized intensity.

5. DCP vs. PAIN-CP – OVERALL POLARIZATION TRANSFER (EXPERIMENT)

Figure S.I.-5 shows an experimental comparison of the total ^{15}N-^{13}C transfer (i.e. sum over all cross peak integrals above the noise level) between PAIN-CP and DCP with high power decoupling (set to 112 kHz, i.e. 2.5 times ^{13}C r.f. field). The r.f. field strengths were respectively 45, 25 kHz on the ^{13}C, ^{15}N channel. 1D data were recorded on a 750 MHz spectrometer with $\omega_r/2\pi$=20 kHz using [U-^{13}C,^{15}N]-N-*f*-MLF-OH.

Direct ^{1}H-^{13}C transfer was eliminated by flipping the remaining locked ^{1}H magnetization (after the first to ^{1}H-^{15}N CP pulses) to the Z axis before the PAIN-CP mixing period (see S.I.-7 for details).

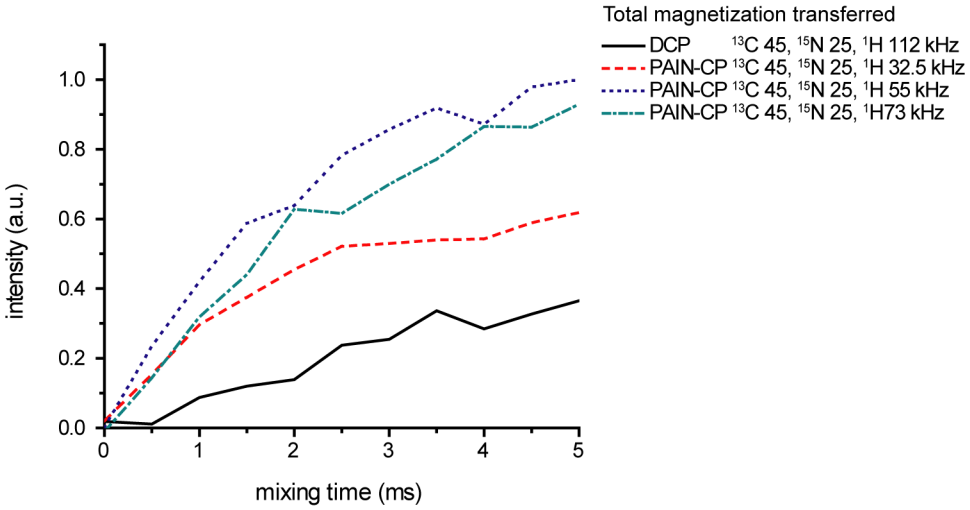

Figure S.I.-5: Experimental comparison of the total magnetization N-C transfer (i.e. sum over all cross peak integrals above the noise level) between DCP and three different n=1 PAIN-CP variants.

At a mixing time of 3 ms, the transferred magnetization is approximately four times larger for PAIN-CP (^{13}C 45/ ^{15}N 25/ ^{1}H 55 kHz) than for DCP, an observation that is in good agreement with simulations and cross peaks volume ratios extracted from 2Ds obtained under identical conditions (data not shown).

6. PULSE SEQUENCE

The pulse sequence used in the simulations and experiments included in this section on supporting information is depicted in Figure 1 of the manuscript. While we employed constant amplitude r.f. fields on each channel, extensions involving adiabatic passages can be considered.

7. ELIMINATION OF ^{1}H-^{13}C TRANSFER DURING PAIN-CP MIXING

This section addresses the problem of ^{1}H-^{13}C transfer during the ^{15}N-^{13}C PAIN-CP step. This is not a concern for the simulations as the initial ^{1}H magnetization was always set to 0. However, during the experiments there may remain some ^{1}H magnetization along the locking axis after the first ^{1}H-^{15}N CP pulses. The application of a ^{1}H pulse that stores the magnetization along the Z-axis prior to the PAIN-CP mixing is a convenient way to avoid any ^{1}H-^{13}C transfer since the residual ^{1}H magnetization along the Z axis commutes with the effective PAIN-CP Hamiltonian.

This is illustrated in Figure S.I.-6 where the black/red curves correspond to C_{α}/C_{β} buildups depending on the state of the initial density matrix: the squares correspond to initial polarizations on both ^{1}H and ^{15}N, the solid lines on ^{15}N only.

Another way to eliminate the ^{1}H-^{13}C transfer is to phase shift the ^{1}H r.f. by π halfway through the PAIN-CP mixing (data not shown).

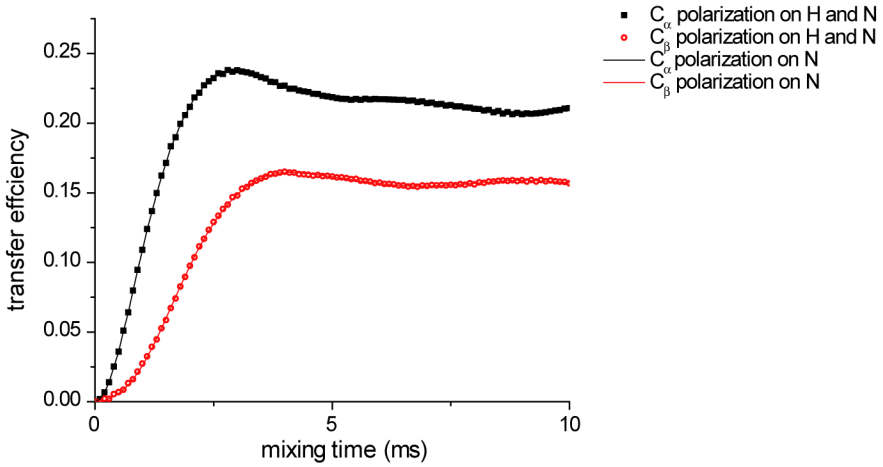

Figure S.I.-6: N- C_β transfer (two-bond transfer) using the same spin system and settings as in Figure 2 of the manuscript. The application of a ^1H $\pi/2$ flip pulse before the PAIN-CP mixing period eliminates ^1H-^{13}C transfer.