Pulse induced half rotational resonance with angular dependent total enhancement of multi-dimensional solid-state NMR correlation spectra

Orr Simon Lusky, Amir Goldbourt

School of Chemistry, Faculty of Exact sciences, Tel Aviv University, Tel Aviv, Israel.

amirgo@tauex.tau.ac.il

Abstract

We demonstrate a new resonance condition that obeys the relation $\Delta \delta = n \nu_R / 2$, where $\Delta \delta$ is the chemical shift difference between two homonuclear-coupled spins, $\nu_R$ is the magic-angle spinning speed and $n$ is an integer. This ‘half rotational resonance’ recoupling condition is obtained by the application of rotor-synchronous $^1$H pulses when protons are dipolar-coupled to the homonuclear spins. We suggest a new experimental scheme entitled ‘pulse induced resonance with angular dependent total enhancement’ (PIRATE) that can enhance proton-driven spin diffusion by the application of a single $^1$H pulse every rotor period. This scheme can be utilised to obtain homonuclear correlations in multidimensional solid state NMR experiments. Experimental evidence is demonstrated on the two carbon spins of glycine and is supported by numerical simulations.

Introduction

Solid state NMR (ssNMR) has become a common tool in structural biology studies. Its various merits include the ability to study biomolecules such as proteins, nucleic acids, polysaccharides, and their complexes [1–9]. Moreover, ssNMR methods are able to characterise the dynamics of molecules on a wide range of time scales [10,11]. Such studies rely heavily on efficient magnetisation transfer methods that utilise spin-spin interaction. Experiments such as INADEQUATE [12] and TOBSY [13] utilise scalar couplings to transfer polarisation through bonds between homonuclear spins. However, these interactions are relatively weak, in the order of Hz, and therefore require long mixing times and sufficiently long relaxation times as well. Anisotropic interactions, and particularly the dipolar interaction, are much stronger at short distances (a few ångstroms): however, they are averaged with the sample spinning. At low spinning rates, the anisotropic interactions are only partially averaged, and may be utilised for magnetisation transfer through space. The
‘proton driven spin diffusion’ (PDSD) [14] experiment is an example of how such transfer can be used, and was successfully demonstrated in many occasions, including the determination of the first protein structure by NMR [15]. Spin diffusion itself can be utilised very efficiently to transfer polarisation between protons in experiments based on the X-H-H-X scheme, where X can be $^{31}$P, $^{13}$C, $^{15}$N, or other nuclei [16,17].

Since PDSD relies on residual dipolar couplings that are not averaged by spinning, its performance deteriorates at elevated spinning frequencies. Further enhancement of magnetisation transfer can be obtained by recoupling; generating an average interaction Hamiltonian that is time independent during the evolution time, cancelling (at least partially) the effect of the MAS. Numerous such pulse sequences have been proposed [18], with most methods relying on the generation of a particular resonance condition that nullifies the MAS time averaging. The resonance conditions can be met by setting the right experimental parameters, as in the simplest case of rotational resonance ($\mathcal{R}2$) [19]. This condition requires fixing the MAS rate according to $\Delta\delta = n\nu_R$, where $\Delta\delta$ is the isotropic chemical shift difference between two homonuclear coupled spins, $\nu_R$ is the MAS rate and $n$ is an integer. When this condition is met, a spinning sideband of one spin overlaps with the isotropic shift of the other spin, and recoupling of the homonuclear dipolar interaction is achieved without the application of any external radio-frequency (rf) pulses.

Rf pulses can also be used for recoupling. Various approaches utilise direct homonuclear polarisation transfer. For example radio frequency driven recoupling (RFDR) [20] utilises rotor-synchronous hard $\pi$ pulses on the homonuclear-coupled spins to increase the bandwidth of the $\mathcal{R}2$ condition. Narrow-band RFDR[22], SEDRA [23], and finite-pulse RFDR [21] can further control this bandwidth. In the ‘homonuclear rotary resonance’ (HORROR) experiment the spins are irradiated at an intensity ($\nu_1$) that equals half the rotation speed, $\nu_1 = \nu_R/2$ [24]. This experiment was further improved by sweeping adiabatically through this condition in an experiment termed DREAM, dipolar recoupling enhancement through amplitude modulation [25]. Many homonuclear recoupling experiments can be classified under the symmetry rules of C and R sequences [26], including their supercycled versions [27].

Another approach for achieving homonuclear transfer utilises the coupled proton bath and is based on the aforementioned PDSD technique. In PDSD, the heteronuclear interaction between the protons and the homonuclear coupled X spins (X can be $^{13}$C, $^{15}$N etc.) is only partially averaged by sample spinning, promoting transfer between the X spins. However,
active recoupling of the $^1$H-X heteronuclear interaction further enhances polarisation transfer between the X spins. For example, such recoupling was achieved by irradiating on one of the spins with an rf power that equals to an integer of the MAS rate ($v_{1H} = n\nu_r, n = 1,2$) [28]. This resonance condition is known as ‘rotary resonance recoupling’, $\beta3$, and has been used in the ‘dipolar assisted rotational resonance’ (DARR) experiment to enhance proton driven spin diffusion [29]. It was further shown that supercycling of the continuous $^1$H recoupling pulse increases the bandwidth and can improve recoupling [30,31]. Further adjustments to spin rates beyond 25-30 kHz have been proposed. In the mixed rotational and rotary resonance’ (MIRROR) [32] experiment the rf power applied to the $^1$H pulse is matched to the sum of the spinning speed and the chemical shift offset difference, $v_{1H} = n\nu_r + \Delta\delta$. Another approach, COmbined R2n(v)-Driven broadband homonuclear recoupling (CORD) [33], utilises a combination of the rotary resonance condition and the HORROR condition thereby increasing the bandwidth of $\beta3$-based recoupling. Another mechanism was proposed that enables transfer of magnetisation between homonuclear spins. In the proton-assisted recoupling (PAR) experiment, a single $^1$H spin coupled to two homonuclear coupled spins is sufficient to promote magnetisation transfer if all three spins are irradiated simultaneously at proper conditions [34]. The mechanism was earlier proposed to promote heteronuclear spin transfer in an experiment entitled TSAR, third-spin assisted recoupling [35]. In both cases, Hartman-Hahn ($v_{1H} = v_{1X} \pm \nu_r$) and $\beta3$ ($v_1 = n\nu_r$) conditions should be avoided, and the experiments are well suited for spinning rates beyond 30 kHz.

Recently we have shown that by applying rotor synchronous $\pi$ pulses on $^1$H during $^{15}$N-15N PDSD transfer, an enhancement is obtained at relatively low spinning speeds (8-10 kHz) [36]. This enhancement allowed us to detect hydrogen-bonds in the full-length RNA extracted directly from MS2 bacteriophage. Particularly, the low gyromagnetic ratio of $^{15}$N, and the dynamic nature of the 3569-bases-long genome required the application of low MAS rates and very long mixing times (up to 16 sec). In such cases continuous irradiation (as in DARR, or PAR) may be prohibited by the hardware and extending the mixing time, and adding the proton pulses were essential for observing inter-nucleotide contacts.

In this paper we explore the effect of $^1$H pulses during proton-driven spin-diffusion. We show that the application of a single $^1$H $\pi$ pulse every rotor period during PDSD enhances polarisation transfer and in addition, generates a new resonance condition that is satisfied when the difference between the chemical shifts of coupled spins equals half integers of the
spinning speed ($\Delta \delta = n\nu_R/2$). We term this new condition ‘half rotational resonance’ ($\mathcal{HR}2$), as it appears to be a generalisation of the $\mathcal{R}2$ condition. We demonstrate that the mechanism relies on a single proton that is dipolar coupled to at least one of two homonuclear coupled spins (e.g. $^{13}\text{C}$, $^{15}\text{N}$). We also show that the usage of different flip angles for the rotor-synchronous pulses creates a trade-off between enhancement of the proton-driven spin-diffusion mechanism and the fulfilment of the $\mathcal{HR}2$ condition. Using numerical simulations and experiments on fully $^{13}\text{C}$ labelled glycine as a two-spin model system, we show the potential of this experiment in the design of solid-state NMR experiments in low to moderate MAS rates.

Materials and Methods

Sample. Fully labelled $^{13}\text{C}$ glycine was purchased from Cambridge Isotope Laboratories, Inc., and was packed into a 4mm ZrO$_2$ MAS rotor.

NMR methods. NMR experiments were carried out on Bruker Avance III spectrometer operating at 14.1T, equipped with a MAS 4mm probe. A complete list of experimental parameters appears in the supporting information (SI). The chemical shifts of $^{13}\text{C}$ were externally referenced to Adamantane at 40.48ppm [37].

Data Analysis. NMR data were processed using TopSpin3.5. Analysis was performed using TopSpin3.5 and SPARKY version 3.134 [38].

Numerical Simulations. The NMR simulation package SIMPSON [39] was used to simulate the application of the pulse schemes on an $X_2H_3$ spin system (X=$^{13}\text{C}$ in this case, and represents a low-$\gamma$ spin $\frac{1}{2}$). The starting operator was $I_{x1}I_{x2}$, and the detection operator was a normalised $I_{x2}$ for all the simulations. The script and simulation parameters appear in the SI.

Results and Discussion

Simulations

Magnetisation transfer with $^1\text{H}$ $\pi$ pulses: We chose to simulate an $X_2H_3$ dipolar-coupled spin system, where X spins are insensitive nuclei (e.g. $^{13}\text{C}$, $^{15}\text{N}$). Such a system allows us to explore three different transfer mechanisms, namely proton driven spin diffusion, direct dipolar transfer, and third-spin assisted recoupling. The total Hamiltonian for such a system in the rotating frame (Eq. 1) contains the chemical shifts ($\mathcal{H}_{\text{CSA}}$) of spins X and H, the homonuclear
($\hat{R}_{d}^{II}$, $\hat{R}_{d}^{SS}$) and heteronuclear ($\hat{R}_{d}^{IS}$) dipolar interactions with I and S representing X and $^1$H spins respectively, and the time dependent radiofrequency Hamiltonian, $\hat{H}_{rf}(t)$.

\[
(1) \hat{H}_{X_2H_3}(t) = \hat{H}_{CSA}(t) + \hat{H}_{CSA}(t) + \hat{H}_{d}^{SS}(t) + \hat{H}_{d}^{II}(t) + \hat{H}_{d}^{IS}(t) + \hat{H}_{rf}(t).
\]

The density operator of the two X spins is set to an initial state of $I_{z1}$-$I_{z2}$ and magnetisation transfer is monitored by the detection operator $I_{z2}$, calculated after evolution times that are whole integers of the rotor period. In order to apply the pulse scheme, ideal pulses are applied to the proton channel in the middle of each rotor period. Changing the position of the pulse to the beginning or end of the rotor period had no visible effects on the results.

We initially compared the effect of $^1$H 180° pulses by comparing such a scheme to PDSD. Two typical build-up curves are shown in figure 1a, one with the application of the pulses, and one without. Clear enhancement is observed at the chosen conditions, MAS rate of 6 kHz and a chemical shift difference of 5 kHz. We then modified the chemical shift difference between the two X spins and extracted the values of $I_{z2}$ that correspond to maximal magnetisation transfer in the build-up curves. Figures 1b shows the maximal transfer efficiency in absence and presence of 180° pulses. In all recorded spectra the $\mathcal{R}2$ condition was apparent. However, in all cases where 180° pulses were applied, additional maxima were spotted. Those maxima correspond to a new resonance condition obeying the following relation:

\[
(2) \Delta \delta = \frac{n}{2} \nu_R.
\]

It generalises the $\mathcal{R}2$ condition by allowing recoupling at MAS rates that equal the chemical shift difference also by half integers, or 'half-rotational resonance', $\mathcal{H}\mathcal{R}\mathcal{R}2$.

In addition to the enhancement resulting from the $\mathcal{H}\mathcal{R}\mathcal{R}2$ condition, it can be seen that the maximal magnetisation transfer is enhanced for all values of $\Delta \delta$ due to the application of the pulses. This suggests an enhancement of the PDSD effect in addition to the $\mathcal{H}\mathcal{R}\mathcal{R}2$ condition and is therefore applicable to homonuclear correlation experiments. We already demonstrated this effect on $^{15}$N-$^{15}$N correlation spectra of full-length RNA extracted from the MS2 bacteriophage, where the $^1$H pulses enhanced the sensitivity of the experiment allowing us to observe many inter-nucleotide cross-peaks [36].
Figure 1: (a) Simulations of build-up curves showing the magnetisation of a selectively inverted $^{13}\text{C}$ spin in a fully dipolar-coupled $^{13}\text{C}_2\text{H}_3$ spin system. The MAS rate was 6 kHz and the chemical shift difference between the two $^{13}\text{C}$ spins was 5 kHz. The y-axis is normalised so that $I_z(0)=-1$. (b) Maximal magnetisation transfer as a function of the chemical shift difference $\Delta\delta$ between the two $^{13}\text{C}$ spins at a MAS rate of 6 kHz. In both plots the blue line represents magnetisation transfer in the absence of pulses (PDSD experiment), whereas the orange line represents the transfer following the application of $^1\text{H}$ 180° pulses (PIRATE experiment).

We can identify three different effects in figure 1b: rotational resonance, half-rotational resonance, and proton-driven spin diffusion, all of which exist in the presence of $^1\text{H}$ π pulses. In order to identify the interactions that contribute to the enhancement of PDSD and the appearance of the $\mathcal{H}\mathcal{R}^2_2$ resonance, we compared in figure 2 the maximal expectation values of $I_{z2}$ that are obtained by a fully coupled $X_2\text{H}_3$ spin system (blue curves) to conditions, in which we selectively eliminate or change different parts of the Hamiltonian. The expectation values were extracted from the maximum of the build-up curves, such as those shown figure S1 of the SI. Initially, the homonuclear proton dipolar interaction term $\mathcal{H}_{\text{dH}}^{HH}$ was nullified, significantly reducing the PDSD effect (fig. 2a). Yet both $\mathcal{R}^2_2$ and the new $\mathcal{H}\mathcal{R}^2_2$ were still present. We can conclude that the $^1\text{H}^\text{-}^1\text{H}$ dipolar couplings are not required for generating the new resonance condition. When $\mathcal{H}_{\text{dH}}^{H\text{X}}$, the heteronuclear dipolar Hamiltonian, was nullified as well (practically removing the protons from the system) recoupling only appeared at $\mathcal{R}^2_2$, with a negligible magnetisation transfer in any other value of $\Delta\delta$, as seen in figure 2b.

In figure 2c $\mathcal{H}_{\text{dH}}^{HH}$ was reintroduced and $\mathcal{H}_{\text{dX}}^{H\text{X}}$ was limited to the closest $X$ spins only. This results in recoupling that is very similar to a fully coupled system. In figures 2d and 2e we search for a minimal set of interactions that still retains the $\mathcal{H}\mathcal{R}^2_2$ effect. We find that a single $^1\text{H}-^{13}\text{C}$ interaction in the presence of $^{13}\text{C}^{13}\text{C}$ coupling is sufficient to introduce the half-rotational resonance transfer condition. We then modified the interaction strengths. When the $^{13}\text{C}^{13}\text{C}$ coupling was reduced, the contribution of proton driven spin-diffusion was
reduced for all $\Delta \delta$ but $\mathcal{HR}_2$ remained as efficient as before, as seen in figure 2f. The only difference was that the build-up time was longer (see figures S1a and S1f in the SI). When the orientation of the dipolar couplings between two spin-pairs was matched (indicated in figure 2g as thick coloured lines), the results showed a negligible change in the total PDSD effect. Finally the effect of $\mathcal{R}_\text{CSA}$ was examined. The results shown in figure 2h show a very small decrease in the total magnetisation transfer only in the presence of very large CSA values (200-500 ppm, atypical for carbons) but no effect on $\mathcal{HR}_2$.

The simulations suggest that in order to obtain transfer at the half-rotational resonance condition, a minimal set of interactions is required defined by a single heteronuclear and a single homonuclear coupling term, that is, an H-X-X system.

**Figure 2**: Comparisons of the maximal polarisation transfer efficiency from a selectively inverted $^{13}$C spin to another $^{13}$C spin in a $^{13}$C$_2$H$_3$ spin system ($^{13}$C in blue, $^1$H in green) as a function of the chemical shift difference.
between the $^{13}$C spins. The MAS rate is 6 kHz in all cases, a single 180° pulse was applied to $^1$H spins every rotor period, and the coupling values are varied. The maximal transfer was obtained by taking the maximum from build-up curves such as those shown in figure S1 of the SI for particular cases. The blue curve in all graphs represent a fully coupled system with the following dipolar coupling constants: $D_{^{13}C-^{13}C} = 2$ kHz, $D_{^{1}H-^{13}C} = 21.94$ kHz, $D_{^{1}H-^{1}H} = 25.04$ kHz twice and 55.04 kHz once. The orange curve is for (a) $D_{^{1}H-^{1}H} = 0$ kHz; (b) no protons: $D_{^{1}H-^{1}H} = D_{^{1}H-^{13}C} = 0$ kHz; (c) Four $D_{^{1}H-^{13}C}$ couplings removed (d) $^{13}$C$_2^1$H$_1$ spin system with both $D_{^{1}H-^{13}C}$ couplings present; (e) $^{13}$C$_2^1$H$_1$ spin system with a single $D_{^{1}H-^{13}C}$ coupling present; (f) fully coupled system, $D_{^{13}C-^{13}C} = 1$ kHz; (g) fully-coupled system with orientation dependence ($\beta$ angles of two X-H pairs and two H-H pairs (both $D_{^{1}H-^{1}H} = 25.04$ kHz) dipolar tensors were set to 60° and 40° respectively) with the same angles of the dipolar interaction tensor; (h) fully coupled system, CSA of $^{13}$C are 200 ppm/300 ppm in orange, and 200 ppm/500 ppm in yellow. The y-axes are normalised; $I_{z2}(0) = -1$.

**Magnetisation transfer with variable flip angle $^1$H pulses:** A unique observation was the dependence of the $\mathcal{HR2}$ condition and proton-driven spin-diffusion efficiency on the flip angle of the pulses applied on the protons during the mixing time. Flip angles between 90° and 180° were simulated. Figure 3a shows the effects of the flip angles on the magnetisation transfer at a spinning frequency of 6 kHz. In figures 3b-d, the maximal magnetisation transfer is presented as a function of both the spinning frequency $v_R$ and the chemical shift difference $\Delta \delta$. The results show a trade-off between fulfilment of the $\mathcal{HR2}$ condition and a better broad-band enhancement with respect to $\Delta \delta$. Generally, flip angles between 90°-120° exhibited the best total enhancement. However, these angles attenuate the new $\mathcal{HR2}$ condition. The heat-maps (c) and (d) demonstrate that up to ~10 kHz, an enhancement in the polarisation transfer via proton-driven spin diffusion is gained by the application of pulses, with a clear advantage to the application of 90° pulse. This enhancement is accompanied by the disappearance of the new $\mathcal{HR2}$ condition.
Figure 3: (a) Comparison of the maximal magnetisation transfer ($I_{z2}$) as a function of the chemical shift difference $\Delta\delta$ ($^{13}$C) for different flip angles at $v_R = 6$ kHz. The maximal transfer was taken from the corresponding build-up curves. (b)-(d) are heat-maps showing the maximal magnetisation transfer as a function of $\Delta\delta$ ($^{13}$C) and $v_R$ in the absence of $^1$H pulses (b), and in the presence of pulses with flip angles of 90° (c) and 180° (d). The colormap is normalised such that $I_{z2}(0) = -1$ (dark blue).

Since the new resonance condition and the enhancement originate from the pulses, we term this new scheme ‘Pulse Induced Resonance with Angular-dependent Total Enhancement’ (PIRATE).

NMR experiments

In order to experimentally demonstrate the effects shown in the simulations, a fully labelled $^{13}$C glycine sample was used as a model system having two $^{13}$C spins. In our 14.1T magnet, the chemical shift difference between the carbonyl ($I_{z1}$) and the alpha carbon ($I_{z2}$) is 20.054 kHz. Therefore, following Eq. 2, the recoupling at the $\mathcal{R}2$ condition is given by ($n$ integer)

$$\nu_R = \frac{40.108}{n}[kHz].$$

The $\mathcal{R}2$ condition in this case is fulfilled for even values of $n$. At first, a set of 1D experiments were conducted without any $^1$H pulses, as shown in figure 4a, in order to demonstrate the $\mathcal{R}2$ condition as shown before [19]. A selective inversion pulse was applied to $C\alpha$, and $^{13}$C spectra
were collected at a longitudinal evolution time $\tau_m = 5$ ms. A typical spectrum appears in figure 5a. The magnetisation transfer was quantified by subtracting the integral of the carbonyl signal from that of Ca, giving a value that reports on the expectation value $<I_{z1} - I_{z2}>$, as followed in the simulations. This value was then plotted as a function of the MAS rate, as shown in figure 5b (and simulated in figure 2). Then, similar experiments and data analysis were performed by applying a single $^1$H pulse every rotor period as shown in figure 4b.

As expected, in the absence of $^1$H pulses a significant rise in the magnetisation transfer was observed at MAS rates of 10 kHz and 6.5 kHz ($n=4,6$ in Eq. 3). Additionally, the transfer was more efficient at lower MAS rates, in accordance with decreased averaging of the heteronuclear dipolar interactions. When rotor-synchronous pulses were applied (according to the scheme in figure 4b) at three different flip angles, 90°, 109°, and 180°, transfer efficiencies varied and the new resonance emerged. The results show that the application of 180° pulses on the proton channel during the evolution time created two additional maxima of transfer at 13.369 kHz and at 8 kHz ($n=3,5$ in equation 3, $\mathcal{R}2$ condition) that were absent without the application of pulses. When 90° pulses were applied only the $\mathcal{R}2$ condition is met however the enhancement in the PDSD mechanism is visible off-$\mathcal{R}2$ condition, and in

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**Figure 4:** The pulse sequences used for experiments on $^{13}$C$_2$-glycine. (a) $^1$H-$^{13}$C Cross-polarization experiment followed by a selective inversion pulse (on Ca). (b) As in (a), with rotor-synchronous $^1$H pulses having a flip angle $\alpha$ applied during the mixing time (PIRATE scheme). (c) A 2D PIRATE experiment. 'n' indicates the number of rotor periods.
particular at the higher regime of MAS rates above 11 kHz, where the efficiency of proton-driven spin diffusion in the absence of pulses is significantly smaller. Applying 109° pulses shows enhancement in PDSD compared to the 180° pulses, and the \( \mathcal{HR}2 \) condition is still visible although at reduced intensity. This result agrees with the simulations above showing the dependence of the resonance on the pulse flip angle.

**Figure 5:** (a) A typical spectrum of \(^{13}\text{C}\)\(_2\)-glycine taken after inverting \(\text{C}_\alpha\) and allowing magnetisation to transfer for 5 ms using the PIRATE pulse scheme with flip angles of 180° and a MAS rate of 6 kHz. The spectrum was processed with a line broadening of 200 Hz. (b) Magnetisation transfer as a function of the MAS rate. The transfer was calculated by subtracting the intensity of the carbonyl carbon from that of \(\text{C}_\alpha\) for all spectra acquired as in (a) but using different flip angles or without pulses.

The PIRATE scheme can be applied to multi-dimensional experiments to allow magnetisation transfer. In systems containing spins with dispersed chemical shift differences the \( \mathcal{HR}2 \) condition can be used to selectively increase the magnetisation transfer. This approach is similar to prior studies utilising the spinning frequency dependence of the \( \mathcal{R}2 \) condition to perform rotational-resonance width (\( \mathcal{R}2_w \)) based distance measurements [40] or constant-time frequency-dependent narrow-band radio frequency driven recoupling [22]. In such cases, 180° pulses are highly useful since they enhance the \( \mathcal{HR}2 \) condition. However, for obtaining broad-band enhancement of magnetisation transfer that is independent of \( \Delta\delta \), 90°-120° pulses are superior.

A set 2D PIRATE experiments, as depicted in figure 4c, was conducted to demonstrate the enhancement at multidimensional experiments. The experiments were conducted on \(^{13}\text{C}\)\(_2\)-glycine at the \( \mathcal{HR}2 \) condition (\( \nu_R = 13.369 \) kHz) and at off resonance conditions (\( \nu_R = 11.5 \) kHz)
with different flip angles. The signal to noise ratios (SNR) of the diagonal and correlation signals were used for comparison. The normalised values appear in table 1.

Figure 6a shows a comparison of on- and off-$\\mathcal{H}R2$ resonance 2D experiments using 180° pulses. A clear enhancement in the intensity of the cross-peaks at half-rotational-resonance conditions is depicted in the plot. The enhancement in transfer efficiency off-resonance due to the different flip angles in the PIRATE scheme is indicated by an increase in the SNR when 90° pulses are applied instead of 180° pulses, as demonstrated in figure 6b. On-resonance, as shown in figure 6c, the spectra from 90° pulses exhibit a decrease in the SNR values with respect to 180°, demonstrating the dependence of the $\\mathcal{H}R2$ condition on the flip angle. Moreover, this also shows the applicability of this experiment for moderate MAS rates where the PDSD experiment is less efficient.

**Figure 6**: Comparison of 2D $^{13}$C-$^{13}$C correlation experiments using the PIRATE scheme. (a) $^1$H pulses of 180° pulses were applied on-resonance (13,369 Hz, black) and off-resonance (11,500 Hz, red). The correlation cross-peak $\alpha$-C is enlarged to demonstrate the enhancement in the intensity due to the $\\mathcal{H}R2$ condition. (b) $\alpha$-C cross-peak intensity at off-resonance conditions with 180° (black) and 90° pulses (blue). (c) $\alpha$-C cross-peak at on-resonance with 180° (black) and 90° pulses (blue). All spectra were processed with line broadening of 500Hz on both dimensions and are drawn using an identical contour level scale.
| Cross-peak | Flip angle | Off-resonance (11,500Hz) | On-resonance (13,369Hz) |
|------------|------------|--------------------------|-------------------------|
| C·C        | 90°        | 0.78                     | 0.77                    |
|            | 180°       | 0.63                     | 0.59                    |
| Ca·Ca      | 90°        | 1.00                     | 0.87                    |
|            | 180°       | 0.80                     | 0.67                    |
| Ca·C       | 90°        | 0.35                     | 0.30                    |
|            | 180°       | 0.24                     | 0.36                    |
| C·Ca       | 90°        | 0.35                     | 0.31                    |
|            | 180°       | 0.24                     | 0.38                    |

**Table 1**: SNR values of cross-peaks from PIRATE 2D spectra collected with flip angles of 90° and 180° at MAS rates on- and off- half-rotational-resonance. The SNR values were normalised with respect to the Ca-Ca diagonal peak derived from the experiment with α=90°.

**Summary and Conclusions**

We have demonstrated the existence of a “half rotational resonance” condition in solid-state NMR experiments involving homonuclear spins coupled to protons. When the MAS rate is fixed to half (and full) integers of the chemical shift difference between the two coupled spins, a significant enhancement in the transfer occurs if rotor-synchronous pulses are applied to the proton channel. In addition to the enhancement at this new resonance condition, an improvement in polarisation transfer was observed across all chemical shift difference values (broad-band enhancement) in comparison to free evolution. The flip angle of the pulses was shown to have an impact on both effects, where for an angle of 180° the new resonance condition is maximised and for angles closer to 90° the PDSD effect is superior across a broad range of chemical shift difference values. This versatility allows designing various experiments for insensitive nuclei at moderate MAS rates and seconds-long mixing times where continuous irradiation is not desired, for example for obtaining 15N-15N correlations in proteins, DNA and RNA.

From simulations we could reveal the key terms in the spin Hamiltonian that contribute to the effect. Half-rotational resonance was observed for a minimal H-X-X system containing a single proton coupled to some other spin X via heteronuclear dipolar coupling, whereas the X spin is homonuclear-coupled to another X spin. The 1H-1H homonuclear interactions are partially recoupled by the application of the pulses providing broad-band increase of the PDSD effect. We find that X-spin CSA has a very small effect on transfer efficiency at values relevant to 13C and 15N spins.
Through experiments on glycine we have demonstrated the enhancement at the resonance condition when using PIRATE with 180° pulses, and broad-band enhancement when using 90° pulses. The scheme has been proven superior to PDSD and can therefore be used for diverse applications, including distance measurements and studies of biological samples that require long mixing times and slow spinning.

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Supporting Information

Figure S1. Build-up curves at $\nu_R = 6 \text{ kHz}$ showing the normalised intensity of an inverted spin as a function of mixing time at rotational-resonance (blue, ‘R2’, $\Delta\delta = n\nu_R = 6\text{ kHz}$), half-rotational resonance (red, ‘HR2’, $\Delta\delta = 0.5n\nu_R = 3\text{ kHz}$), and off resonance (yellow, ‘off’, $\Delta\delta = 5\text{ kHz}$). The dipolar coupling networks are shown by the connectivities between the $^1\text{H}$ spins (green circles) and $^{13}\text{C}$ spins (blue circles). Plot ‘0’ is the fully coupled system and was used to generate the corresponding three points in the blue curves of figure 2. Similarly other build-up curves were used to generate figure 2a-h. The actual couplings were as follows: (0, a-e) $D_{13\text{C}-13\text{C}} = 2 \text{ kHz}$, $D_{1\text{H}-13\text{C}} = 21.94 \text{ kHz}$, $D_{1\text{H}-1\text{H}} = 25.04 \text{ kHz}$ twice and 55.04 kHz once; (f) $D_{13\text{C}-13\text{C}} = 1 \text{ kHz}$; (g) the $\beta$ angles of the dipolar tensors were changed (pairs of different $X-^1\text{H}$ spins both set to 60°, two pairs of $^1\text{H}-^1\text{H}$, both $D_{1\text{H}-1\text{H}} = 25.04 \text{ kHz}$, set to 40°); (h) CSA of $^{13}\text{C}$ are 200 ppm/300 ppm.

Simpson script for PIRATE: in red – parameters that were varied in simulations

```
spinsys {
channels 13C 1H
nuclei 13C 13C 1H 1H 1H
shift 1 -1000 2p 0.0 -29.6 91.2 -19.4
shift 2 5000 10p 0.0 29.6 21.2 -19.4
dipole 1 2 -2000 0 0 0
  dipole 1 3 -21940 0 80 0
  dipole 1 4 -21940 0 70 0
  dipole 1 5 -21940 0 60 0
```
dipole 2 3 -21940 0 10 0
dipole 2 4 -21940 0 30 0
dipole 2 5 -21940 0 20 0
dipole 3 4 -25040 0 50 0
dipole 3 5 -25040 0 150 0
dipole 4 5 -55040 0 40 0

par {
    start_operator I1z-I2z
    detect_operator I2z
    spin_rate
    gamma_angles 20
    sw spin_rate
    crystal_file rep100
    np 128
    verbose 1101
    proton_frequency 400e6
    conjugate_fid true
}

proc pulseq {} {
    global par
    set tr [expr 1.0e6/$par(spin_rate)]
    set tr2 [expr 0.5e6/$par(spin_rate)]

delay $tr2
delay $tr2
pulseid 5 0000 0 100000 0
store 1
reset
    for {set i 0} {$i<$par(np)} {incr i} {
        acq
        prop 1
    }
}

proc main {} {
    global par
    set f [fsimpson]
    fsave $f $par(name).fid -xreim
}

Experimental parameters:

Table S1a: Parameters for 1D $^{13}$C-$^{13}$C CP-selective inverse experiments

| Experiment group | PDSD | PIRATE 90° | PIRATE 109.5° | PIRATE 180° |
|------------------|------|------------|---------------|-------------|
| Field [T]        | 14.1 | 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 9.7, 10, 10.2, 10.5, 11, 11.5, 12, 12.5, 13, 13.4, 13.5, 14, 14.5, 15 | 14.1 | 3988 |
| Spinning frequency ($\nu_R$) [kHz] | 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 9.7, 10, 10.2, 10.5, 11, 11.5, 12, 12.5, 13, 13.4, 13.5, 14, 14.5, 15 | 20 |
| Acquisition points | 3988 |
| Acquisition time [ms] | 20 |
| Carrier frequency [ppm] | 111 |
| Frequency jump (for selective inverse pulse) [Hz] | -9680 |
| 1H 90° pulse length [µs] | 2.5 |
| 13C 90° pulse length [µs] | 4 |
| 1H PIRATE pulses length [µs] | No pulses | 2.5 | 3.04 | 5 |
| 13C 180° inversion pulse length [µs] | 100 |
| CP power ($\nu_H$) [kHz] | 77.5 |
| CP power ($\nu_C$) [kHz] | 62.5 |
| CP contact time [ms] | 2 |
| Build-up time [ms] | 5 |
| 1H decoupling power [kHz] | 100 |
| swf+tppm decoupling pulse [µs] | 5 |
| Relaxation delay [s] | 2.4 |
| Scans | 16 |
| SW [kHz] | 100 |

**Processing parameters**

| Processing software | TopSpin 3.5 |
| Zero fill | 8192 |
| Apodization | Line broadening 200Hz |

**Table S1b:** Parameters for 2D $^{13}$C-$^{13}$C PIRATE experiments

| Experiment | Off-resonance PIRATE 90° | Off-resonance PIRATE 180° | On-resonance PIRATE 90° | On-resonance PIRATE 180° |
|---|---|---|---|---|
| Field [T] | 14.1 |
| Spinning frequency ($\nu_R$) [kHz] | 11.5 | 11.5 | 13.369 | 13.369 |
| Acquisition points (t1/t2) | 3988/450 |
| Acquisition time [ms] (t1/t2) | 0.02/0.0045 |
| Carrier frequency [ppm] | 111 |
| 1H 90° Pulse length [µs] | 2.5 |
| 15N 90° Pulse length [µs] | 4.5 |
| CP power ($\nu_H$) [kHz] | 74 |
| CP power ($\nu_C$) [kHz] | 55.5 |
| CP contact time [ms] | 2 |
| PDSD mixing time [ms] | 5 |
| 1H decoupling power [kHz] | 100 |
| swf+tppm decoupling pulse [µs] | 5 |
| Relaxation delay [s] | 2.4 |
| Scans | 16 |
| SW F1/F2 [kHz] | 100/50 |

**Processing parameters F1/F2**

| Processing software | TopSpin 3.5 |
| Zero fill (t1/t2) | 8192/1024 |
| Apodization | Line broadening 500Hz on both dimensions |