Efficient Dipolar Double Quantum Filtering Under Magic Angle Spinning without a ^1H Decoupling Field

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ABSTRACT

We present a systematic study of dipolar double quantum (DQ) filtering in ^{13}C -labeled organic solids over a range of magic-angle spinning rates, using the SPC- n recoupling sequence element with a range of n symmetry values from 3 to 11. We find that efficient recoupling can be achieved for values $n \geq 7$, provided that the ^{13}C nutation frequency is on the order of 100 kHz or greater. The decoupling-field dependence was investigated and explicit heteronuclear decoupling interference conditions identified. The major determinant of DQ filtering efficiency is the decoupling interference between ^{13}C and ^1H fields. For ^{13}C nutation frequencies greater

1 than 75 kHz, optimal performance is observed without an applied ^1H field. At spinning rates
2 exceeding 20 kHz, symmetry conditions as low as $n=3$ were found to perform adequately.

4 INTRODUCTION

5 Pulse sequences that excite double-quantum (DQ) coherence give the experimenter
6 access to a wealth of structural information and techniques for simplifying the interpretation of
7 NMR spectra of biomolecules. In addition to the ubiquitous application of suppressing natural
8 abundance background signals, DQ coherence can be used to measure vector (torsion) angles in
9 peptide backbones [1,2,3,4,5] and polymers [6], study dynamics [2], and simplify chemical shift
10 assignment [7].

11 Homonuclear recoupling sequences that rely on zero-quantum mixing—such as dipolar-
12 assisted rotational resonance (DARR) [8], RFDR [9] or CORD-RFDR [10]—provide robust
13 methods for gaining information about spatial proximity but lack selectivity of individual spin
14 pairs and are prone to multi-spin sequential polarization transfers that complicate interpretation.
15 Adiabatic mixing schemes that rely on DQ excitation with low power carbon fields do not permit
16 the isolation of that DQ coherence [11,12]. Mixing schemes traditionally used for the excitation
17 and isolation of homonuclear DQ coherence—such as C7 [13], POST-C7 [14], CMR7 [15], and
18 SPC-5 [16]—have broad bandwidth but require ^1H decoupling fields during mixing that are at
19 least three times the ^{13}C field strength [17, 18]. The high power ^1H decoupling is necessary to
20 avoid the broad heteronuclear interference described in relation to C and R symmetry sequences
21 [19]. This requirement significantly limits the usage of C symmetry sequences not only for the
22 measurement of dipolar couplings, but chemical shift tensors, through the use of sequences like

1 ROCSA [34], which are valuable for developing extremely high resolution protein structures
2 [35].

3 At higher MAS rates low-power ^1H decoupling both during chemical shift evolution and
4 detection are common, and investigations have made clear that at high MAS rates low-power ^1H
5 decoupling during recoupling was feasible [18]. While high MAS solves many problems for both
6 resolution and sensitivity for ideal samples, many important systems such as amyloids exhibit
7 inhomogeneous broadening that negates the advantages of long transverse relaxation rates (T_2) at
8 high MAS rates requiring the use of larger capacity rotors to maintain sufficient sensitivity.
9 Additionally, at present all commercially available dynamic nuclear polarization instruments
10 require the use of rotors with maximum MAS frequency of 25 kHz [36]. These experimental
11 limitations motivate the investigation of low-power ^1H decoupling at moderate to high MAS
12 rates. While low power proton decoupling during dipolar recoupling has been reported
13 previously [14, 19, 20, 21, 31, 32, 33], double quantum filtering (DQF) has not been fully
14 explored in the context of difficult proteins with limited relaxation times and inhomogeneous
15 broadening. Here we present an exploration of the low power decoupling regime for the SPC-*n*
16 family of recoupling sequences that generalizes the SPC-5 recoupling sequence to arbitrary
17 symmetry conditions [16]. We confirm the feasibility of DQ spectroscopy with SPC-*n* double-
18 quantum filters in the absence of decoupling from 13 to 40 kHz MAS rates. We show
19 experimental results for $\text{U-}^{13}\text{C}, ^{15}\text{N}$ -N-acetylvaline and $\text{U-}^{13}\text{C}, ^{15}\text{N}$ - α -synuclein that demonstrate
20 DQ excitation over the full chemical shift range with high efficiency. These conditions are thus
21 well suited for the study of temperature-sensitive systems and for incorporation into pulse
22 sequences that make use of proton detection and other fast MAS techniques.

1 MATERIALS AND METHODS

2 Materials

3 Uniformly ^{13}C - ^{15}N -labeled wild-type α -synuclein was prepared following the method of
4 [22] and fibrils were prepared for solid-state NMR (SSNMR) as described in [23] and packed
5 into a 1.6 mm FastMAS rotor.

6 Solid-state NMR spectroscopy

7 All experiments were conducted on a 17.6 T (750 MHz) Varian VNMRS spectrometer
8 (Agilent Technologies, Santa Clara, CA) equipped with a FastMAS probe tuned to ^1H - ^{13}C
9 double resonance. The ^1H and ^{13}C $\pi/2$ pulse widths for N-acetyl-L-valine (NAV) were 1.80 and
10 1.80 μs , respectively, and were 1.75 and 1.70 μs for α -synuclein. MAS frequencies were
11 controlled with a Varian MAS controller to within ± 5 Hz at 13.333 kHz MAS and ± 15 Hz at 40
12 kHz. All experiments were carried out with the variable temperature gas at 0 $^\circ\text{C}$, which
13 corresponded to actual sample temperatures of 5 ± 3 $^\circ\text{C}$ at 13.333 kHz MAS, 15 ± 5 $^\circ\text{C}$ at 40 kHz
14 MAS and intermediate values at moderate spinning rates.

15 All data were collected using the pulse sequence shown in Fig. 1. In this work the mixing
16 sequence was tested in the context of a standard ^{13}C - ^{13}C , 2D experiment with an initial ^1H 90 $^\circ$
17 pulse followed by tangent-ramped ^1H - ^{13}C cross polarization before the indirect evolution period.
18 The carbon magnetization is then placed along the z-axis during a short z-filter, the SPC-n
19 recoupling is performed on the ^{13}C channel with or without constant wave ^1H decoupling, a
20 second z-filter is performed, the magnetization is placed back in the transverse plane, and is
21 detected with ^1H SPINAL decoupling. The SPC-n sequence consists of a repeated POST-C
22 element with phases determined by the symmetry condition as in Ref. [16]. The SPC-n element

is the repeated with its phase shifted by 90° in alternate scans, with a subsequent 180° phase shift of the receiver, to filter for DQ coherence.

One-dimensional spectra were A)

collected with the indirect evolution

time (t_1) set to zero. High power

SPINAL ^1H decoupling with an RF

field of 100 kHz was used during the t_1

and detection periods. The ^1H - ^{13}C cross

polarization was performed with a

tangent ramp on ^{13}C with ^1H and ^{13}C

nutration frequencies matched to modified Hartmann-Hahn conditions optimized at each spinning

rate. The SPC pulse width was set to an initial condition defined by the theoretical value

(according to the MAS frequency and desired symmetry condition) and then optimized over a

range of $\pm 5\%$ in order to maximize DQ filtered (DQF) signal intensity with a total excitation and

reconversion time of about 1 ms. All NMR data were processed with nmrglue [24] using

standard methods including zero-filling, Fourier transformation, and phase correction.

Integration and plotting of data were performed with scripts written in Python utilizing

matplotlib [25].

RESULTS AND DISCUSSION

Double quantum filtering efficiency over a range of MAS frequencies

Fig. 1. Pulse sequence utilized for the evaluation of DQ excitation with ^{13}C - ^{13}C SPC-n homonuclear mixing. The hatching on the second half of the SPC-n period indicates that the phase cycle is shifted by 90 degrees every other scan (with a 180 degree phase shift of the receiver), according to ref [16].

At low (<10 kHz) MAS rates, dipolar (as opposed to scalar) recoupling is the preferred approach to generating DQ coherence in organic solids, typically using POST-C7 for MAS rates from ~5 to ~7 kHz [14], SPC-5 for ~7 to ~10 kHz [16], and SPC-5₃ for ~9 to ~12 kHz [26]. These conditions result in ¹³C nutation frequencies ranging from ~30 to ~50 kHz. In this regime, optimal DQ filtering (DQF) efficiency is observed

Fig. 2. DQF efficiency as a function of mixing time for α -synuclein at a range of symmetry conditions and a constant MAS rate of 13.333 kHz. The ¹H decoupling field is off.

with the ¹H decoupling nutation frequency several times greater than for ¹³C: often >100 kHz and limited by the coil breakdown voltage. Although this condition avoids heteronuclear decoupling interference [19], it severely limits application of DQ sequences for higher magnetic field and/or MAS rates.

We explored solutions to this problem by comparing several values of symmetry number for SPC-*n* recoupling at 13 kHz MAS rate, with the ¹H field turned off during the ¹³C-¹³C recoupling period (**Fig. 2**). Using α -synuclein fibrils we observe DQF efficiencies up to 28% using the *n* = 9 condition with a ¹³C nutation frequency of 120 kHz, and similar results for *n* = 10 (133 kHz ¹³C nutation frequency). A slight reduction (to ~20-22%) in efficiency is observed for *n* = 8 (107 kHz ¹³C nutation frequency) and *n* = 11 (147 kHz ¹³C nutation frequency), and then

performance degrades considerably for lower n values (5, 6, and 7, with ^{13}C nutation frequencies of 67, 80, and 93 kHz, respectively). Optimal results are observed when the ^{13}C nutation frequency is in the range of ~100 to ~150 kHz.

These trends are also observed also at higher MAS rates in **Fig. 3**, where the DQF efficiency of the SPC- n recoupling sequence in the absence of ^1H decoupling is shown for spinning rates of 13.333, 16.667, 24.000, and 40.000 kHz and symmetry conditions $n = 3$ and $5 \leq n \leq 11$.

As described in the formalism of Edén and Levitt [36], SPC- n is a CN_2^1 symmetry sequence with an additional supercycle applied to suppress terms in the Hamiltonian with $\mu = \pm 1$. According to the selection rule specified in equation 1,

$$mn - \mu\nu \neq Nd \text{ for } d \in \mathbb{Z}$$

for $n \geq 5$ SPC- n recouples the $(m, \mu) = (-1, -2)$ and $(1, 2)$ terms. However, for $n = 4$, the additional terms $(m, \mu) = (-2, 0)$ and $(2, 0)$ are recoupled leading to ^{13}C CSA and heteronuclear dipolar recoupling, leading to poor DQF performance. For $n = 3$, the additional terms $(m, \mu) = (-2, -1)$ and $(2, 1)$ are allowed by the selection rule but suppressed by the supercycle. We observed maxima in DQF at approximately 1 ms with lower symmetry conditions tending towards

Fig. 3. DQ efficiency as a function of mixing time for α -synuclein MAS rates ranging from 16.667 to 40.000 kHz.

maxima at longer mixing times for all symmetry conditions which is consistent with the scaling factors for the POST-CN element given in [14] as:

$$\kappa_n = \frac{3in^3(1 - \exp\{\frac{4i\pi}{n}\})}{8\sqrt{2}\pi(4n^2 - 1)}$$

Values of this scaling factor for the symmetry conditions are tabulated in Table 1.

Fig. 4 shows that the maximum DQF efficiency for a given symmetry condition plateaus for values $n > 7$. While higher ^{13}C fields more effectively decouple the heteronuclear ^1H - ^{13}C interaction, shorter pulse lengths increase the relative impact of phase transients, decreasing the overall efficiency of recoupling [27,28]. Recent efforts to compensate for phase transients has shown an increase in performance for the C7 pulse sequence and could be applied to mitigate these effects [29,30].

Next we tested whether any experimentally achievable level of ^1H decoupling field would perform better than the ^{13}C field alone over the range of conditions studied (**Fig. 5**). DQF efficiency as a function of symmetry condition and ^1H power during decoupling exhibits a banded pattern that bears a striking resemblance to the theoretical predictions for CN_2^1 recoupling sequences presented in ref. [19] indicating that heteronuclear decoupling is the primary factor effecting the efficiency of SPC mixing. For all but the lowest symmetry conditions, the

Fig. 4. Double quantum efficiency as a function of SPC symmetry number.

1 maximum efficiency is attained when the decoupling field is absent. This high ^{13}C power, low ^1H
 2 power regime is advantageous as it lessens sample heating and facilitates experiments requiring

Fig. 5. Measured dependence of DQF efficiency as a function of the ^1H field strength and symmetry condition. For each sample the efficiency was normalized to the highest efficiency observed for that sample. Efficiency values were interpolated to generate a heat map. The three-fold symmetry condition was only tested at 40 kHz MAS frequency where 100 kHz ^1H power is only $2.5\omega_r$ resulting in the blank region in the lower right corner of each plot.

3 DQ coherence for temperature sensitive samples, such as membrane proteins, and for
 4 experiments utilizing probes with low ^1H power handling capabilities.

5 Finally, we evaluated the performance of DQF for acquiring 2D ^{13}C - ^{13}C correlation
 6 spectra. Such spectra are shown in **Fig. 6** with SPC-7 at 16.667 kHz, SPC-5 at 24.000 kHz and
 7 SPC-3 at 40.00 kHz. All spectra demonstrate reasonable, broadband excitation of DQ coherence
 8 and reconversion to single quantum coherence. At 1 ms mixing the DQ coherence arising from
 9 the dipolar couplings of neighboring ^{13}C nuclei is at a maximum; therefore, the two spectra
 10 primarily exhibit peaks corresponding to directly bonded carbons. In addition, in the SPC-7
 11 spectrum (Fig. 6a) there are some peaks corresponding to two-bond correlations (threonine C α -
 12 C γ region (60 ppm, 20 ppm)) on both sides of the diagonal. These two-bond correlations show

the opposite sign of the one-bond correlations, and at this mixing time three-bond correlations have negligible intensity. Therefore directly bonded carbons are unambiguously identified, making this mixing especially useful for chemical shift assignments where zero-quantum and spin-diffusion based mixing can crowd spectra with longer range correlations. Although the overall intensity of cross peaks in the SPC-3 spectrum is lower than those in the SPC-5 and SPC-7 spectra, the SPC-3 condition nevertheless generates useful correlations, especially in the $\text{C}\alpha\text{-C}'$ region. This observation is interesting, given that there is no explicit compensation for CSA and heteronuclear interactions [16]; we attribute this experimental observation to the combined effect of the 40 kHz MAS rate and the supercycled POST element.

CONCLUSIONS

In summary, the SPC- n recoupling sequence has been demonstrated to be effective under MAS frequencies up to 40

Fig. 6. 2D SPC ^{13}C - ^{13}C SQ-SQ correlations NMR spectra of $\text{U-}^{13}\text{C}$, ^{15}N - α synuclein at 17.63 T magnetic field. A) Three-fold symmetric SPC mixing for 1 ms at 40 kHz MAS frequency. B) Eight-fold symmetric SPC mixing for 1 ms at 16.667 kHz MAS frequency. Both spectra show broadband recoupling of dipolar couplings. At 1 ms mixing DQ coherence from dipolar couplings between neighboring carbons are at a maximum, thus primarily one-bond correlations are seen in these spectra. Some two-bond peaks appear in the Threonine $\text{C}\alpha\text{-C}\gamma$ region (60 ppm, 20 ppm) and mirrored across the diagonal.

1 kHz in the absence of ^1H decoupling. High-symmetry SPC- n recoupling is compatible with a
2 large range of MAS rates. The experimental results demonstrated here indicate that SPC- n
3 mixing is a robust and broadly applicable method for generating DQ coherence over a wide
4 range of MAS frequencies extending higher than previously reported. Additionally, high
5 symmetry SPC- n mixing does not require the high power ^1H decoupling required by SPC-5 and
6 POST-C7 mixing schemes opening it as a possibility for many more challenging systems. These
7 properties make SPC- n (with $n > 7$) a useful technique for general applicability from 13 to 40
8 kHz MAS rates, both as a longitudinal mixing scheme and for exciting DQ coherence for
9 filtering. This mixing element can be incorporated into pulse sequences for the measurement of
10 structural restraints and dynamics in proteins as well as other organic molecules at high magnetic
11 field and MAS rate conditions that are conducive to the study of complex macromolecules.

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