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Supporting Information

Long-Range Aliphatic Correlations in Protein MAS NMR

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This supplement contains:

1. A discussion on finite-pulse effects in band selective RFDR, Figure S1

2. A discussion on heteronuclear contributions to band selective RFDR recoupling, Figure S2

3. A broadband RFDR spectrum for comparison to BASE RFDR, Figure S3

4. Overlaid full spectral views of BASE RFDR and DARR spectra, Figure S4

Finite-pulse effects in RFDR-type recoupling pulse sequences

The original theoretical description of radio frequency-driven recoupling (RFDR) considered infinitely short π pulses, yielding a recoupled dipolar Hamiltonian of purely zero-quantum nature with an effective coupling constant that vanishes for small isotropic chemical shift differences between the interacting pair of spins.[1, 2] In practice, it was observed that RFDR correlation spectra exhibit intense crosspeaks between nuclei with small chemical shift differences.[1-4]. This effect, also observed in a related experiment[5], was attributed to the finite duration of the recoupling π pulses. The RFDR experiment was analyzed theoretically in great detail by Boender, *et al.*[6], showing that the experimental observations that RFDR sequences recouple both closely and widely separated resonances were consistent with their treatment based on Floquet theory. In a subsequent study[7], the effect of finite pulses was shown to become increasingly relevant at high spinning frequencies ($\geq$ 20 kHz). In general, the optimal rf settings for RFDR-type experiments thus depend on chemical shift differences, desired bandwidth, spinning frequency, and minimizing heteronuclear interference[8]. While fast spinning ($\geq$ 20 kHz) offers a number of advantages[8], many solid-state NMR studies require flexibility in the range of MAS frequencies employed. We therefore investigated the effect of finite pulses in RFDR at moderate spinning rates (8 to 16 kHz), and found that in the case of small chemical shift dispersion it is possible to achieve efficient polarization transfer by employing long-pulse or band-selective conditions, specifically those in the regime where the recoupling irradiation occupies between half and the full rotor period, as shown in Figure S1. In the present manuscript we refer to this regime, depicted in the pulse

sequence in Figure 1(a) of the main text, as band selective radio frequency-driven recoupling (BASE RFDR). Polarization is efficiently preserved by the recoupling π pulses while heteronuclear interference during the mixing period is easily avoided with a large ratio between ^{1}H and ^{13}C irradiation, making the approach ideally suited for long-range polarization transfer.

The effect of heteronuclear interactions

One of the practical advantages of BASE RFDR is that heteronuclear decoupling can be achieved with moderately strong ^{1}H fields during mixing (70 to 100 kHz) with minimal losses from heteronuclear interference, which is generally of significant concern[8]. Figure S2 compares the influence of various dipolar couplings during a BASE RFDR polarization transfer experiment consisting of 12.5 kHz ^{13}C rf field and 80 kHz ^{1}H rf field at 12.5 kHz spinning frequency via numerical simulations with a CH-CH spin system. As can be observed, the ^{13}C-^{13}C coupling alone leads to highly efficient recoupling over the 3.8 Å internuclear distance. In an all-couplings simulation, the efficiency decreases slightly by an amount that is consistent with depolarization of ^{13}C magnetization by heteronuclear interference in a lone ^{1}H-^{13}C spin pair under similar rf and MAS settings (simulations not shown). Therefore, we can attribute the slight decrease in efficiency to incomplete suppression of first-order heteronuclear interactions. However, we can see that removing the ^{13}C-^{13}C coupling still results in a small extent of polarization transfer, likely due to the effect of higher-order interactions involving heteronuclear dipolar couplings and cross-terms between them, as occurs in third spin assisted recoupling (TSAR)[9]. While the relative TSAR effect in these simulations appears to be minor compared to the direct ^{13}C-^{13}C coupling we must note that (1) TSAR simulations are highly dependent on spin-system geometry, (2) the selected spin system geometry (which is of interest in the present study) is roughly 30% less efficient than the collinear C-H-C geometry that is optimal for TSAR transfer, and (3) in real spin systems the influence of geometry is attenuated by the presence of many other couplings making the TSAR mechanism more general. While the recoupling mechanism in BASE RFDR is clearly distinct from that of TSAR, the latter may be simultaneously active, leading to enhanced recoupling efficiency in a BASE RFDR experiment. Indeed, in BASE RFDR simulations including all couplings, the ^{1}H field can be chosen to maximize the TSAR effect (around 60 kHz in these settings), although at the expense of overall recoupling efficiency due to heteronuclear depolarization. On the other hand, with ^{1}H fields above 100 kHz we observe first-order direct ^{13}C-^{13}C recoupling, as expected. Numerical simulations in Figures S1 and S2 were carried out with the SPINEVOLUTION[10] package.

Broadband RFDR of 2-PI3-SH3

PI3-SH3 fibrils prepared with protein produced with 2-^{13}C glycerol as the sole carbon source (2-PI3-SH3) were examined in this study. This type of labeling simplifies the spin dynamics as explained in the main text. Figure S3 presents a broadband RFDR[1, 2] spectrum of this sample, acquired with a 24.0 ms mixing period. RFDR mixing is well suited for long mixing periods thanks to its robustness agaisnt rf inhomogeneity and pulse and phase imperfections. The tolerance of the π pulse train with respect to rf imperfections is due in part to XY-16 phase cycling[11]. We found that appending XY-16 with Y$\overline{X}$-16 to form a 32-step phase cycle further preserves ^{13}C polarization over long mixing periods, in particular for weak ^{13}C rf fields. The 32-step cycle is thus XYXY YXYX $\overline{X}\overline{Y}\overline{X}\overline{Y}$ $\overline{Y}\overline{X}\overline{Y}\overline{X}$ Y$\overline{X}$Y$\overline{X}$ $\overline{X}$Y$\overline{X}$Y $\overline{Y}$X$\overline{Y}$X X$\overline{Y}$X$\overline{Y}$. Despite the robust performance of broadband RFDR, evidenced by many medium and long-range carbonyl-to-aliphatic contacts in Figure S3, few correlations are observed in the aliphatic-aliphatic region of the spectrum. From a technical point of view, this shortcoming is the result of the small chemical shift difference between the aliphatic resonances and also possible interference effects between ^{13}C and ^{1}H rf fields that often lead to incomplete heteronuclear decoupling[8]. Additionally, examining the spin systems generated by alternating labeling (see for example references [12] and [13]) one notices that carbonyl and Cα nuclei, although mostly not labeled in the same residue, are often the closest labeled spins (when sequential), separated by two bonds and leading to considerable dipolar truncation[14]. Other aliphatic nuclei also tend to be in close proximity to labeled carbonyl nuclei and thus carbonyl-aliphatic crosspeaks are the strongest in broadband RFDR spectra of 2-PI3-SH3.

Comparison between BASE RFDR and DARR

Figure 1(b) in the main text demonstrates that LP RFDR mixing produces many long-range aliphatic-aliphatic correlations in 2-PI3-SH3. The majority of these crosspeaks arise from sequential Cα-Cα, sequential Cα-Cβ, and other distant pairs of nuclei. The assignments shown in the inset of Figure 1(b) are part of ongoing work by the same authors (in preparation). The performance of LP RFDR is highlighted in Figure 2 of the main text with a comparison to DARR/RAD[15, 16] mixing, illustrating that long-range aliphatic-aliphatic recoupling via LP RFDR is generally superior to spin diffusion methods. Figure S4 shows an overlay of the LP RFDR and DARR spectra from Figure 2, in full view, excluding the empty right side of the spectra. It can be observed that carbonyl-aliphatic correlations are suppressed in LP RFDR, while long-range aliphatic-aliphatic correlations are enhanced with respect to broadband RFDR. Correlation crosspeaks between distant aliphatic ^{13}C nuclei in LP RFDR are more generally more sensitive that those obtained with DARR. Exceptions to this trend (some of which are noted in Figure 2 in the main text) can be expected to arise from remaining one-bond dipolar truncation in at least

one of the interacting nuclei. Alternating labeling[12] with 2-^{13}C glycerol produces a small number of directly bonded pairs, such as Val Cα-Cβ, Leu Cβ-Cγ, and Ile Cα-Cβ.[13] For such spin systems, dipolar truncation can be expected to decrease the efficiency of LP RFDR transfer to distant spins, while mixing schemes that rely mainly on higher-order interactions as TSAR and DARR are generally robust to such multi-spin effects.

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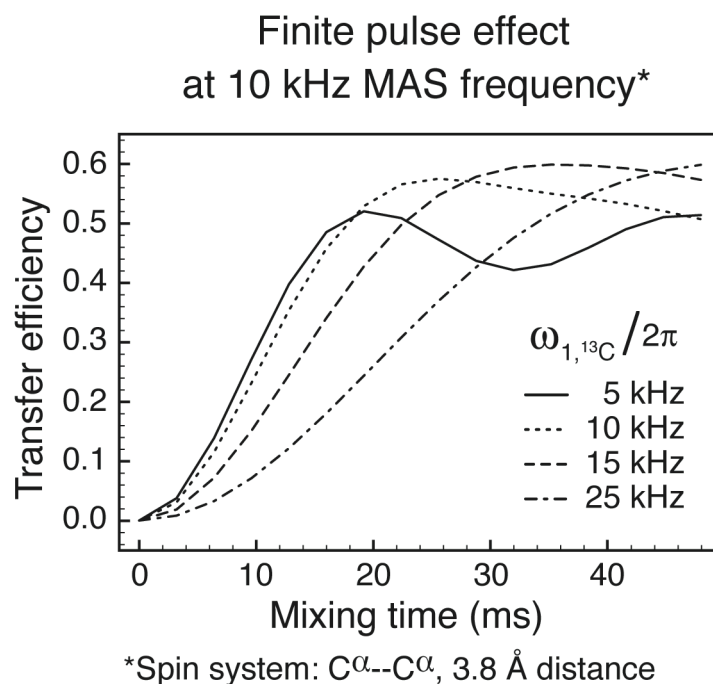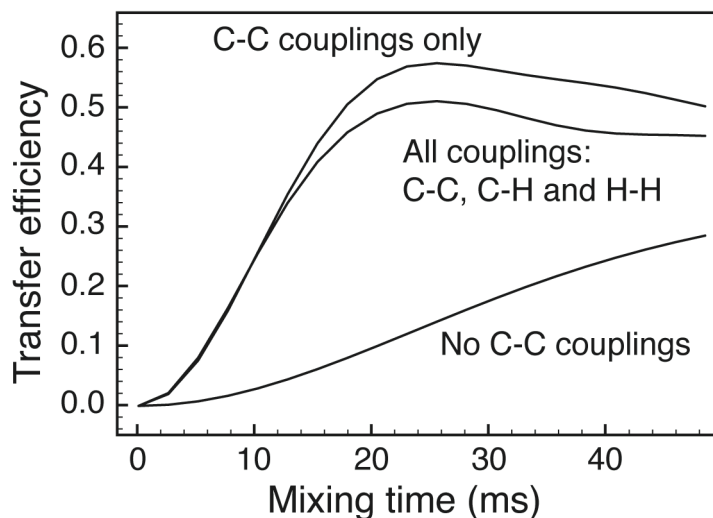[16] C. R. Morcombe, V. Gaponenko, R. A. Byrd, K. W. Zilm, *J. Am. Chem. Soc.* **2004**, *126*, 7196.

Finite pulse effect at 10 kHz MAS frequency*

*Spin system: C^α--C^α, 3.8 Å distance

Figure S1. Numerical simulations of polarization transfer via RFDR-type longitudinal mixing at 10 kHz spinning frequency for various ^{13}C recoupling π-pulse nutation frequencies ($\omega_{1,^{13}C}/2\pi$). The simulated spin system was a [13]C-^{13}C pair with an internuclear distance of 3.8 Å (typical of sequential C^α- C^α nuclei) and a chemical shift separation of 2 kHz at 16.4 T magnetic field. Polarization buildup rates increase with the weakest irradiation levels, and the overall optimal transfer is achieved with ^{13}C rf fields between 5 and 10 kHz (in which the recoupling pulse occupies the full and half the rotor period, respectively), indicating that the finite-pulse mechanism of RFDR-type pulse sequences persists even at moderate spinning frequencies. The efficiency of the finite-pulse effect is the result of the low chemical shift separation between the recoupled resonances, as is found in aliphatic ^{13}C regions of protein NMR spectra.

Dipolar interactions in BASE RFDR*

C-C couplings only

All couplings:
C-C, C-H and H-H

No C-C couplings

Transfer efficiency

0.6
0.5
0.4
0.3
0.2
0.1
0.0

Mixing time (ms)

0   10   20   30   40

*Spin system: $C^\alpha H^\alpha$--$C^\alpha H^\alpha$. Settings:

$$\frac{\omega_{rotor}}{2\pi} = 12.5 \text{ kHz}, \quad \frac{\omega_{1,^{13}C}}{2\pi} = 12.5 \text{ kHz}, \quad \frac{\omega_{1,^{1}H}}{2\pi} = 80 \text{ kHz}$$

Figure S2. Numerical simulations of band selective RFDR polarization transfer mediated by different dipolar interactions in three cases: ^{13}C-^{13}C only, no ^{13}C-^{13}C couplings (only ^{1}H-^{13}C, ^{1}H-^{1}H, and their cross-terms), and all dipolar couplings. The simulated 4-spin system consisted of two ^{13}C-^{1}H pairs in a geometry corresponding to sequential $C^\alpha H^\alpha$- $C^\alpha H^\alpha$ nuclei (3.8 Å ^{13}C-^{13}C inter-nuclear distance) in a nearly ideal β-strand configuration, with a H-C-C-H dihedral angle of 179.8°, and C-H-C angles of 70° and 74°.
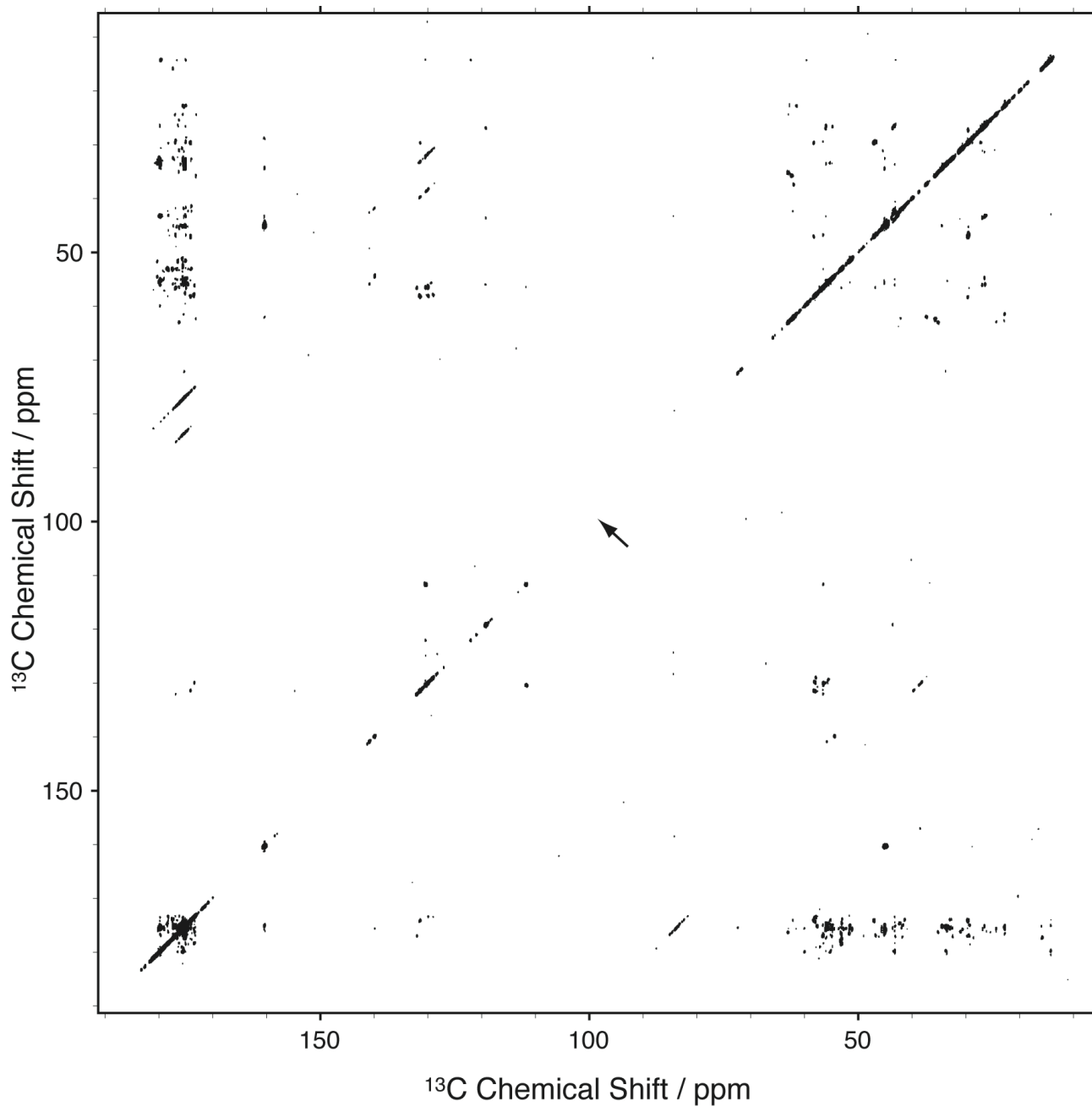
Figure S3. Broadband RFDR spectrum of PI3-SH3 fibrils acquired at 16.0 kHz spinning frequency and 16.4 T (700 MHz [1]H Larmor frequency) using 40 kHz ^{13}C recoupling pulses and a 100 kHz ^{1}H decoupling field during a 24.0 ms mixing period. The arrow indicates the carrier position (98 ppm). 2048 and 512 points with dwell times of 12 us and 30 us were acquired in the direct and indirect dimensions, respectively (total t_2=24.576 ms and t_1=15.36 ms), with 32 scans averaged per t_1 increment, for a total acquisition time of 27 hrs. 100 kHz TPPM decoupling was applied during chemical shift evolution periods.
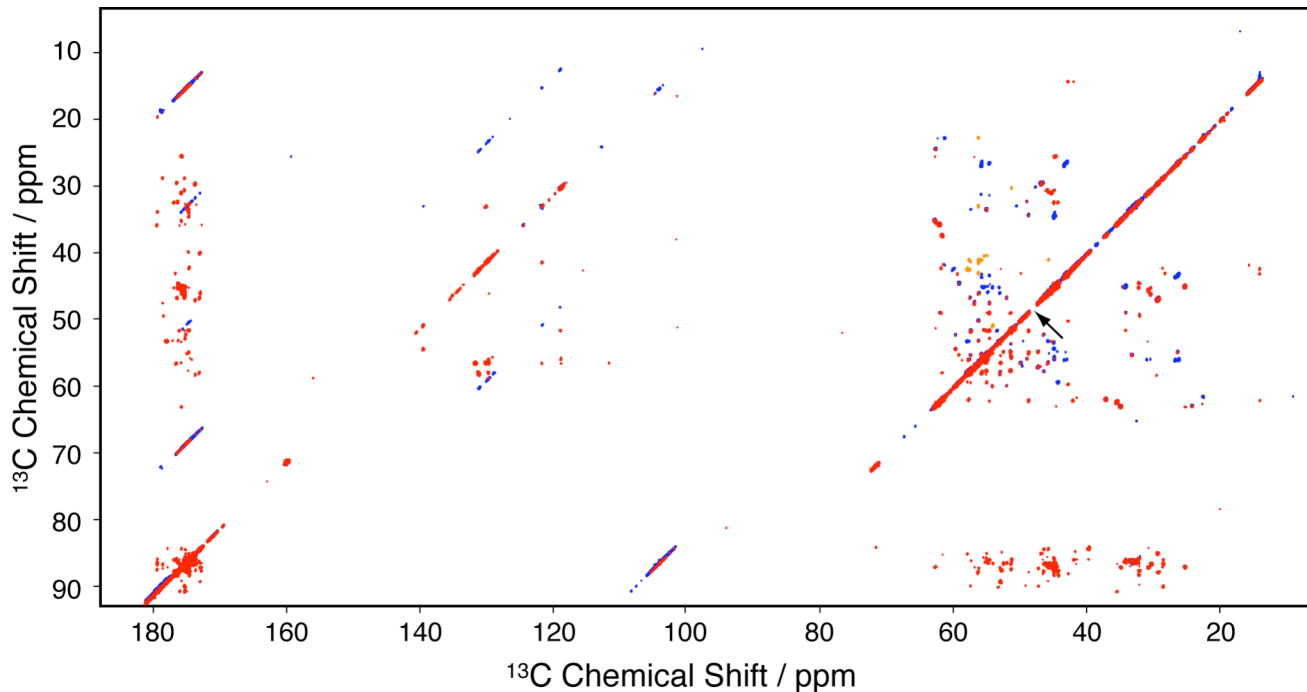
Figure S4. Overlay of BASE RFDR (blue) and DARR (red and orange) spectra of PI3-SH3 fibrils. The spectra are full views of those in Figure 2 in the main text and were acquired at 12.5 kHz spinning frequency and 16.4 T (700 MHz [1]H Larmor frequency). The BASE RFDR mixing period consisted of 12.5 kHz ^{13}C rotor-synchronized π pulses with a 32-step phase cycle for total duration of 17.92 ms, while the DARR mixing period consisted of ~12 kHz ^{1}H irradiation with a 500 ms duration. The arrow indicates the carrier position (48 ppm) for both experiments. DARR crosspeaks folded into the aliphatic region are shown in orange contours for clarity. For each 2D correlation experiment 2048 and 256 points with dwell times of 12 us and 60 us were acquired in the direct and indirect dimensions, respectively (total t_2=24.576 ms and t_1=15.36 ms), with 16 scans averaged per t_1 increment. The total experimental time was 6.8 hrs per 2D. 100 kHz TPPM decoupling was applied during chemical shift evolution periods. The BASE RFDR experiment was performed four times and averaged together to yield the spectrum shown in Figure 1(b) of the main text.