Great Offset Difference Internuclear Selective Transfer

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Cite This: J. Phys. Chem. Lett. 2023, 14, 3939−3945

ABSTRACT: Carbon−carbon dipolar recoupling sequences are frequently used building blocks in routine magic-angle spinning NMR experiments. While broadband homonuclear first-order dipolar recoupling sequences mainly excite intra-residue correlations, selective methods can detect inter-residue transfers and long-range correlations. Here, we present the great offset difference internuclear selective transfer (GODIST) pulse sequence optimized for selective carbonyl or aliphatic recoupling at fast magic-angle spinning, here, 55 kHz. We observe a 3- to 5-fold increase in intensities compared with broadband RFDR recoupling for perdeuterated microcrystalline SH3 and for the membrane protein influenza A M2 in lipid bilayers. In 3D (H)COCO(N)H and (H)CO(CO)NH spectra, inter-residue carbonyl−carbonyl correlations up to about 5 Å are observed in uniformly 13C-labeled proteins.

Dipolar recoupling elements are the building blocks of various multidimensional proton-detected magic-angle spinning (MAS) NMR experiments that are crucial in both structure determination and in exploration of the dynamics of biological macromolecules. Homonuclear carbon−carbon dipolar recoupling sequences are crucial in amino-acid-typing during sequential assignment, as well as for distance measurements.

Dipolar recoupling sequences are characterized as either first-order or second-order sequences. Transfer between isolated two-spin systems can be observed with first-order sequences, since they recouple two-spin terms in the Hamiltonian, such as the dipolar coupling. For second-order sequences, the transfer dynamics involve at least three spins, since the relevant terms in the recoupled Hamiltonian depend on two dipolar couplings among three spins. First-order sequences have the potential advantage of relatively straightforward analysis, as the transfer depends on only two spins for isolated spin pairs. However, broadband first-order carbon−carbon recoupling applied to uniformly labeled proteins is subject to dipolar truncation, such that mostly intra-residue cross-peaks are observed. Second-order recoupling sequences, based on proton-driven spin diffusion or third spin assistance, can reduce the influence of dipolar truncation. While these methods result in increased intensities for long-distance correlations, the relationship between peak intensity and distance is less straightforward than in the case of first-order recoupling sequences since they depend on an additional spin interaction.

Selective methods have also been developed to overcome the aforementioned problems in order to more effectively measure weak, long-distance inter-residue correlations critical for structure determination. Specific spin-labeling provides an alternative solution that at the same time can yield exquisite line widths. Selective recoupling experiments based on band-selective pulses, zero- and double-quantum shift evolution, symmetry rules, and optimal control algorithms have been developed. For fast MAS, both double-quantum (DQ) and zero-quantum (ZQ) methods have been developed. DQ sequences are characterized by a Hamiltonian that induces simultaneous spin flips, while for ZQ sequences, spin flip-flops (no change in total spin angular momentum) are induced. Double-quantum sequences are not ideal for the detection of correlations that correspond to longer distances in uniformly labeled proteins, since relayed transfer can cancel direct transfer. They have, however, been successfully applied for quantitative distance measurement. MODIST, a selective method developed for proton recoupling, did not efficiently recouple 13C (Figure S7A). We therefore sought a new zero-quantum (ZQ) pulse sequence that achieves ë−z mixing with limited relaxation loss and is efficient only for spins with similar chemical shifts (e.g., among carbonyl or aliphatic spins).

Here, we present a first-order zero quantum recoupled method, the Great Offset Difference Internuclear Spin Transfer (GODIST) pulse sequence, which allows selective observation of aliphatic−aliphatic and carbonyl−carbonyl correlations at

Received: January 20, 2023
Accepted: March 24, 2023
Published: April 20, 2023
fast and ultra-fast MAS rates. Starting from the MODIST sequence\textsuperscript{69} we modified both the flip angles and the phase cycling to achieve a selective and efficient transfer between carbons of similar chemical shifts. Using numerical simulations, we restricted our simulated search space to 1–32 rotor periods, 0.125–16 pulses per rotor period, flip angles of 12.5°–360°, and phase steps of 90°. We optimized for minimal transfer between carbonyl and aliphatic spins, maximal transfer between carbonyl spins, maximal retention of the initial carbonyl signal (sum of the remaining and transferred signals), and a width of 6–7 kHz for the selective transfer (broad enough to cover the desired region).

Figure 1 shows simulations of the optimized sequence, GODIST, for a two-spin system (two carbonyl spins), as well as for a four-spin system (two carbonyl and two aliphatic spins). The GODIST sequence consists of 32 2π pulses with a γzxγzxγzxγzxγzxγzxγzx\textsuperscript{phase cycle (Figure 1A). The total length of the sequence is 64 rotor periods, which results in a carbon rf nutation frequency of half the MAS rate. The sequence is repeated as necessary to reach the desired mixing time.

Simulations (600 MHz proton Larmor frequency) in Figure 1C were carried out on a four-spin system and show the dependence of GODIST signals on the offset differences between carbonyl spins for several distances at a mixing time of 9.216 ms. As expected, the transfer efficiency between carbonyl groups decreases with increasing distance. We observed a plateau at ±40 ppm where the transfer reaches maximal efficiency, which is broad enough to cover the carbonyl region and nearly the whole aliphatic region. On the basis of these simulations, the width of the selective transfer (the offset difference for which the transferred signal is 50% of the maximal transfer) depends only slightly on the distance between the correlated spins and equals ~6 kHz. The transfer drops below 1% beyond a 100 ppm (15 kHz) offset difference.

Transfers are observed even in a two-spin system, thereby confirming that the sequence is a first-order zero quantum recoupling method. Simulated GODIST signals as a function of mixing time are shown in Figure 1D for the two-spin system and in Figure 1E for the four-spin system. While for the two-spin system (Figure 1D) the maximal transfer efficiency reaches ~50%, even for longer distances, for the four-spin system (Figure 1E), the maximal transfer efficiency decreases with distance, which can be considered as an attenuated dipolar truncation effect.\textsuperscript{39} In both cases, however, the total signal (the sum of the remaining signal of first carbonyl spin and the signal transferred to the second carbonyl) is well-retained (dashed lines in Figure 1D,E), and the transferred signal shows only small oscillations after reaching the plateau (Figure 1E, solid lines). These properties suggest that GODIST is an ideal sequence for selectively recoupling...
carbonyl or aliphatic moieties and detecting weak carbon–
carbon dipolar correlations.

The dependence of GODIST transfer efficiency on the
experimental conditions—MAS, external magnetic field, and
rf-field inhomogeneity—should be investigated. Simulations
show that with increasing MAS rates, higher external magnetic
fields are required for optimal GODIST performance: at 55
kHz MAS, a field of \( \sim 600\) MHz, at 83 kHz MAS, an \( \sim 800\)
MHz external field, while at 111 kHz MAS, a 1200 MHz
spectrometer would be ideal (Figure S1). The total initial
signal is preserved robustly across various MAS frequencies
and field strengths: \( \gtrsim 95\% \) of the initial signal is retained after
9.2 ms mixing. The one exception is measurement at a 1.2
GHz spectrometer in combination with 55 kHz MAS (Figure
S1C), in which case about 75% of the total signal is retained in
simulation. Even in this case, undesired carbonyl-aliphatic
transfers are negligible.

Figure S2A shows the simulated transferred GODIST signals
as a function of mixing time with flip angle deviations up to 6%
from the ideal flip angle value of \( \pi \). These simulations show
that this substantial mis-set of the rf-field power results in
retention of at least 50% of the ideal transfer, suggesting that
the sequence has a sufficient robustness against rf-field
inhomogeneity, which is an unavoidable feature of NMR
instrumentation. The transfer also has a relatively small
dependence on the orientation of the chemical shift
anisotropies (Figure S2B).

In order to demonstrate the selectivity of GODIST mixing,
we compared it with an efficient broadband recoupling method,
radio-frequency-driven recoupling (RFDR), with the
carrier frequency set to either the aliphatic (Figure 2A) or the
carbonyl region (Figure 2B). For proton–carbon (HC)
transfers, SPECIFIC–CP conditions \(^{70,71} \) were used. For both
methods, 2.304 and 25.344 ms mixing times were applied for
aliphatic (A) and carbonyl (B) regions.

Using a sample of perdeuterated microcrystalline SH3, we
found that broadband RFDR recoupling predictably mixes
signals between carbonyl and aliphatic protons, while GODIST
retains the signal inside the initial spectral regions, such that
aliphatic–aliphatic (Figure 2A) or carbonyl–carbonyl (Figure
2B) correlations are mostly observed.

Quantification of GODIST cross-peak intensities reveals a
multifold improvement in signal intensity over RFDR.
Aliphatic–aliphatic correlations in GODIST are observed with
a relatively modest improvement of up to 1.8-fold higher
intensity (Figure 2A). The transfer efficiency of GODIST is
reduced in comparison with RFDR for the largest offset
differences, as occurs for threonine \( \text{C}^\beta–\text{C}^\gamma \) correlations. The
similar efficiency for both methods is explained by the fact that
only carbonyl and aromatic spins lie outside the recoupling
bandwidth, while the majority of carbon spins in the protein
are aliphatic with strong, one-bond couplings to other aliphatic
moieties.

More strikingly, Figure 2B shows a dramatic improvement in
the number of observable correlations when GODIST is used for
carbonyl recoupling. While carbonyl–carbonyl RFDR
cross-peaks are at or below the noise level, GODIST cross-
peak intensities are up to 4.95-fold higher than the noise level.
At the same time, the diagonal in GODIST spectra is
significantly more intense, about 3-fold, than in RFDR. This
is a consequence of the carbonyl signal transfer to the aliphatic
region in the case of RFDR.

Using a lipid bilayer sample of uniformly \( ^{13}\text{C},^{15}\text{N} \)-labeled
influenza A M2, we performed additional 2D experiments to
evaluate the efficiency of GODIST for a nondeuterated sample
(Figure S3). Consistent with the deuterated sample, good
retention of the initial signal was observed, aliphatic–carbonyl
correlations were suppressed, and in this case, an increase in
intensity is observed for some aliphatic–aliphatic cross-peaks.
As with SH3, the total carbonyl signal in the GODIST spectra
of M2 is well preserved compared with RFDR (Figure S4).

In general, good agreement is observed between the
experimental results and the simulations. Aliphatic–aliphatic
transfers are not sensitive to the carrier frequency in the region
between 70 and 10 ppm (Figure S5), and aliphatic–carbonyl
transfers are well-suppressed. However, for carbon spins with
large offsets compared with the carrier frequency (\( \gtrsim 100\) ppm),
off-resonance effects \(^{13} \) decrease the efficiency of GODIST.
While at a carrier frequency of 140 ppm the aromatic–
aromatic correlations are readily detected (Figure S6),
aliphatic–aliphatic transfers are hardly observed, and some
carbonyl–aliphatic transfer occurs. Moreover, the large offset
distorts the diagonal of the aliphatic region, in particular for methyl groups.

We also acquired 2D experiments with three other selective methods—MODIST,69 DREAM,22,23 and SPR5 pulses75 (Figure S7A)—that show lower efficiency carbonyl–carbonyl correlations. Figure S7B shows GODIST efficiency at a 1200 MHz spectrometer. While GODIST's performance deteriorated under these conditions, we still were able to detect carbonyl–carbonyl correlations up to 3.2-fold higher than the noise level.

The additional dimension provided by proton-detected 3D spectra is essential for resolving unambiguous correlations that are used for protein structure determination.8 We, therefore, designed 3D, proton-detected versions of $^{13}$C–$^{13}$C correlation experiments. Figure 3A shows the $^{13}$C–$^{13}$C projection of the (H)COCO(N)H$^{g}$GODIST spectrum of perdeuterated microcrystalline SH3, with the assignment of selected peaks on the basis of previously determined chemical shifts.76,77 Most correlations belong to spins $\sim 3.4$ Å apart; however, the long mixing time of $\sim 25$ ms allowed the detection of 16 long-range and 3 medium-range carbonyl–carbonyl correlations up to about 5 Å (Table S1 and Figure S8), which arose due to a combination of relayed and direct transfers.

Figure 3B shows two strips from a 3D (H)CO(CO)NH$^{g}$GODIST spectrum. For T32 (left), we observed two carbonyl–carbonyl cross-peaks to neighboring residues. For F52 (right), a single neighboring residue, G51, was observed, and a second cross-peak to F52 was an ambiguous correlation that can be assigned to W42 and E22, both of which are long-range correlations (4.77 and 4.75 Å in the crystal structure, PDB ID 2NUZ). The F52–V53 correlation is not present in the strip, which is likely explained by lower initial intensity at residue V53 because of the neighboring residue, P54, lacking an amide proton.

The 3D (H)CO(CO)NH$^{g}$GODIST experiment performed similarly well for the influenza A M2 membrane protein (Figure 4). We normalized the intensities of the cross-peaks ($t_{\text{mix}} = 9.216$ ms) with peak intensities measured at 0 mixing. In each strip shown, only one correlation could be identified unambiguously, since the second one overlaps with the diagonal. On average, about 7% of the initial signal (zero mixing time) was transferred to the closest backbone carbonyl spin.

In summary, we introduced GODIST, a selective recoupling method suitable for systems with a large offset difference. This first-order recoupling sequence, designed with $^{13}$C resonances in mind, makes possible the detection of carbonyl–carbonyl correlations between spins up to about 5 Å in distance. The width of the selective transfer allows suppression of aliphatic–carbonyl correlations, while high retention of the initial signal allows the use of long mixing times, which is crucial for detecting longer carbon–carbon distances. We also demonstrated the efficiency and the robustness of the GODIST sequence against changes in carrier frequency position and flip angle. Comparison of GODIST and RFDR spectra showed a particular improvement for carbonyl–carbonyl cross-peaks, allowing us to identify 16 long-range correlations for SH3. We anticipate 3D (H)COCO(N)H$^{g}$GODIST and (H)CO(CO)-NH$^{g}$GODIST experiments to facilitate protein assignment and structure determination through the detection of both
sequential inter-residue carbonyl–carbonyl correlations, as well as long-range correlations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcl.lett.3c00194.

Numerical simulations of GODIST, additional experimental data using GODIST and RFDR for dipolar recoupling, experimental parameters (Figures S9–S15), and Bruker Topspin pulse programs implementing the GODIST sequence (PDF).

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Funding

Open access funded by Max Planck Society.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge financial support from the MPI for Biophysical Chemistry and from the Deutsche Forschungsgemeinschaft (Emmy Noether program Grant AN1316/1-1). We thank Dr. Dirk Bockelmann and Brigitta Angerstein for technical assistance.

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