Zero-quantum stochastic dipolar recoupling in solid state nuclear magnetic resonance

Wei Qiang and Robert Tycko^{a)}

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0520, USA

(Received 29 June 2012; accepted 17 August 2012; published online 11 September 2012)

We present the theoretical description and experimental demonstration of a zero-quantum stochastic dipolar recoupling (ZQ-SDR) technique for solid state nuclear magnetic resonance (NMR) studies of ^{13}C -labeled molecules, including proteins, under magic-angle spinning (MAS). The ZQ-SDR technique combines zero-quantum recoupling pulse sequence blocks with randomly varying chemical shift precession periods to create randomly amplitude- and phase-modulated effective homonuclear magnetic dipole-dipole couplings. To a good approximation, couplings between different ^{13}C spin pairs become uncorrelated under ZQ-SDR, leading to spin dynamics (averaged over many repetitions of the ZQ-SDR sequence) that are fully described by an orientation-dependent $N \times N$ polarization transfer rate matrix for an N -spin system, with rates that are inversely proportional to the sixth power of internuclear distances. Suppression of polarization transfers due to non-commutivity of pairwise couplings (i.e., dipolar truncation) does not occur under ZQ-SDR, as we show both analytically and numerically. Experimental demonstrations are reported for uniformly ^{13}C -labeled L-valine powder (at 14.1 T and 28.00 kHz MAS), uniformly ^{13}C -labeled protein GB1 in microcrystalline form (at 17.6 T and 40.00 kHz MAS), and partially labeled ^{13}C -labeled protein GB1 (at 14.1 T and 40.00 kHz MAS). The experimental results verify that spin dynamics under ZQ-SDR are described accurately by rate matrices and suggest the utility of ZQ-SDR in structural studies of ^{13}C -labeled solids. [<http://dx.doi.org/10.1063/1.4749258>]

I. INTRODUCTION

In nuclear magnetic resonance (NMR) spectroscopy of solids, distances between nuclei can be determined from measurements of nuclear magnetic dipole-dipole couplings, as the strength of these couplings is proportional to the inverse cube of the internuclear distances.¹ Solid state NMR studies in chemistry and biochemistry usually require magic-angle spinning (MAS) to enhance the resolution and sensitivity of the measurements.² Since MAS by itself averages out dipole-dipole couplings, specific radio-frequency (rf) pulse sequences are applied in synchrony with the sample rotation of MAS to restore dipole-dipole couplings. Rotation-synchronized pulse sequences of this type were first developed over twenty years ago,^{3–6} and are now called “dipolar recoupling sequences.”^{7–15} Homonuclear or heteronuclear dipolar recoupling sequences restore dipole-dipole couplings among identical or different nuclear isotopes, respectively. When a dipolar recoupling sequence with variable duration τ_d is incorporated into an appropriate one-dimensional or multidimensional NMR technique, dipole-dipole coupling strengths can be determined either from the decay of the NMR signal from a given nuclear spin with increasing τ_d due to couplings to other spins (i.e., a dipolar dephasing curve) or from the growth of the NMR signal from a given spin with increasing τ_d due to transfers of nuclear spin polarization from

other spins (i.e., a crosspeak build-up curve in the case of a multidimensional experiment).

When applied to a many-spin system, a homonuclear recoupling sequence creates an effective dipole-dipole coupling Hamiltonian that is a sum of terms, one for each pair of nuclear spins. The individual terms generally do not commute with one another, leading to quantum mechanical interferences between the terms. In particular, the weaker terms, which correspond to couplings between pairs of nuclei with relatively long internuclear distances, act like small perturbations on the stronger terms. If the different terms do not commute with one another, the weaker terms can effectively vanish to lowest order, making the long-range couplings undetectable. This phenomenon can be called “dipolar truncation.”^{16,17} Dipolar truncation is an important problem in applications of homonuclear recoupling sequences in structural studies of molecular solids, because information about molecular conformations or intermolecular contacts is contained in distances between atoms that are separated by multiple bonds or are not chemically bonded.

To give one practical example of dipolar truncation, two-dimensional ^{13}C - ^{13}C solid state NMR spectra of uniformly ^{13}C -labeled proteins, obtained with 1.5–3.0 ms dipolar recoupling sequences in the mixing period, typically show strong crosspeaks that connect ^{13}C NMR signals of all carbon sites within each amino acid residue that are separated by one bond. However, crosspeaks between the CO site of residue k and the C_α site of residue $k+1$ are vanishingly small, as are crosspeaks between C_γ and C_ϵ sites of Met residues, even

^{a)} Author to whom correspondence should be addressed. Electronic mail: robertty@mail.nih.gov. Phone: 301-402-8272. Fax: 301-496-0825.

FIG. 1. Numerical simulations of ^{13}C spin polarization transfers in a three-spin system under coherent (a) and stochastic (b) recoupling. The distance from spin 1 to spin 2 is fixed at 4.0 Å, while the distance x from spin 2 to spin 3 equals 8.0 Å (red lines), 4.0 Å (green lines), or 2.0 Å (blue lines). Simulations are averaged over orientations of the three-spin system in the MAS rotor. Zero-quantum recoupling is accomplished with the fpRFDR sequence, using $8.333\ \mu\text{s}$ ^{13}C π pulses, a MAS rotation period $\tau_R = 25.00\ \mu\text{s}$, and a $64\tau_R$ fpRFDR block length. Spins 1, 2, and 3 have resonance offsets of 3013 Hz, -753 Hz, and -2260 Hz from the rf carrier frequency, respectively. Spin polarizations on spin 2 (solid lines) and spin 3 (dashed lines) are plotted as a function of the total fpRFDR period, assuming an initial polarization of 1.0 on spin 1. Chemical shift precession periods are $m' = 0$ for coherent and $m' = 3$ for stochastic recoupling. Stochastic recoupling simulations are averaged over 64 sets of random delays τ_n for each combination of α and β . Symbols in panel b are rate matrix simulations for $x = 8.0$ Å (circles), 4.0 Å (squares), and 2.0 Å (triangles).

though the two-bond $\text{CO}-\text{C}_\alpha$ and $\text{C}_\gamma-\text{C}_\varepsilon$ distances are only 1.6–1.8 times greater than the one-bond carbon-carbon distances. The two-bond couplings are truncated by the one-bond couplings, although the one-bond couplings are only 4–6 times stronger than the two-bond couplings. Simulations of polarization transfer in a three-spin system in Fig. 1(a) further illustrate the dipolar truncation effect. In these simulations, polarization transfer from spin 1 to spin 2 under the finite-pulse radio-frequency-driven recoupling (fpRFDR) sequence^{14,18} is suppressed as spin 3 approaches spin 2, even though the distance from spin 1 to spin 2 is always less than the distance from spin 1 to spin 3.

Several approaches to overcoming the dipolar truncation problem in recoupling experiments have been proposed. One approach is to use “spin diffusion” or “second-order recoupling” processes to drive polarization transfers among nu-

clear spins.^{16,19–23} Although this approach leads to detectable long-range crosspeaks in multidimensional solid state NMR spectra, this approach may not fully eliminate dipolar truncation (since the effective dipole-dipole couplings between different spin pairs still do not commute with one another). Enhanced long-range crosspeak intensities can be attributed to the longer mixing periods that are used in spin diffusion and second-order recoupling experiments, and to the reduction in effective one-bond coupling strengths. It is unclear whether internuclear distances in many-spin systems can be determined quantitatively from data acquired with spin diffusion or second-order recoupling techniques, although valuable qualitative information about molecular structure can certainly be obtained.^{24,25} A second approach is to use techniques that recouple only pairs of nuclei that have certain chemical shifts, thus converting a many-spin system to a two-spin system. Such frequency-selective recoupling techniques use weak rf irradiation,²⁶ simultaneous recoupling of dipole-dipole couplings and chemical shifts,^{27–29} or alternating periods of dipolar (or scalar) recoupling and free precession under chemical shifts^{30–32} to introduce dependences on NMR frequencies. Distances between non-bonded pairs of ^{13}C - ^{13}C nuclei in uniformly ^{13}C -labeled compounds have been measured accurately with frequency-selective recoupling techniques.^{28,30,31,33}

We have recently introduced a third approach to overcoming dipolar truncation, called “stochastic dipolar recoupling” (SDR).^{34,35} In SDR, random variables, changing from scan to scan, are introduced into the dipolar recoupling sequence in such a way that each pairwise coupling term fluctuates and averages to zero. Random fluctuations of different terms are (ideally) uncorrelated as long as different spin pairs have different chemical shifts. When averaged over many scans and in the limit of short correlation times, spin polarization transfers under SDR are well described by pairwise rates that are inversely proportional to the sixth power of the internuclear distance and proportional to the correlation time, without quantum mechanical interference due to non-commutativity of different pairwise coupling terms. The polarization transfer dynamics of a many-spin system under SDR in solid state NMR are closely analogous to the dynamics due to nuclear Overhauser effects in liquid state NMR.

Our original demonstrations of SDR used the double-quantum (DQ) recoupling sequence POST-C7¹¹ to create an effective Hamiltonian² of the form:

$$H_{\text{DQ}} = \sum_{j>k} (d_{jk} S_{j+} S_{k+} + d_{jk}^* S_{j-} S_{k-}), \quad (1)$$

where d_{jk} is the effective coupling constant for spins j and k , S_{jq} is the q^{th} component of the spin angular momentum operator for spin j , and $S_{j\pm} = S_{jx} \pm iS_{jy}$. Periods of free precession under isotropic chemical shifts were inserted between DQ recoupling blocks, as in the SEASHORE (Shift-Evolution-Assisted Selective HOMonuclear REcoupling) frequency-selective recoupling technique.³¹ The rf carrier frequency during each free precession period was chosen randomly, leading to an effective Hamiltonian for the n^{th} recoupling block of the

form:

$$\tilde{H}_{DQ}(n) = \sum_{j>k} (d_{jk} e^{-i\phi_{jk}(n)} S_{j+} S_{k+} + d_{jk}^* e^{i\phi_{jk}(n)} S_{j-} S_{k-}), \quad (2)$$

where $\phi_{jk}(n)$ are random variables that depend on the choice of carrier frequencies in the first $n-1$ precession periods and on the sum of the chemical shifts of spins j and k . Experimental demonstrations of DQ-SDR validated the theory, but were limited to systems with relatively small chemical shift ranges.^{34,35} This limitation arose because DQ-SDR requires the basic DQ recoupling sequence to produce the Hamiltonian in Eq. (1) to high accuracy and because the POST-C7 sequence produces a pure DQ effective Hamiltonian over relatively small ranges of chemical shifts.

In this paper, we describe a more robust version of SDR that is based on zero-quantum (ZQ) dipolar recoupling, which can be implemented with greater accuracy over larger chemical shift ranges using known recoupling sequences.^{10,14,36} In place of random variations in rf carrier frequency, we use variations in the synchronization of ZQ recoupling blocks with MAS (i.e., variations in the “rotor phase”) to create randomly fluctuating pairwise dipole-dipole couplings. We present the general theory of ZQ-SDR in Sec. II below. Section III describes experimental and numerical methods. Section IV presents experimental results for ^{13}C -labeled L-valine powder and ^{13}C -labeled protein GB1³⁷ that substantiate the theory and demonstrate the possible utility of ZQ-SDR in structural studies. Section V summarizes the results and briefly discusses potential applications.

II. THEORY OF ZERO-QUANTUM STOCHASTIC DIPOLAR RECOUPLING

The ZQ-SDR sequence consists of N repetitions of the block shown in Fig. 2(a). In Fig. 2(a), ZQ represents a dipolar recoupling sequence of length $m\tau_R$, where τ_R is the MAS rotation period, that produces the effective Hamiltonian

$$H_{ZQ} = \sum_{j>k} d_{jk} \left[2S_{jz}S_{kz} - \frac{1}{2}(S_{j+}S_{k-} + S_{j-}S_{k+}) \right] \quad (3)$$

when the recoupling block starts at the beginning of a MAS rotation period (i.e., when $\tau_n = 0$), where d_{jk} is a real number that can be expressed as

$$d_{jk} = \sum_{q=-2}^2 c_{qjk}(\alpha, \beta) e^{iq\gamma}. \quad (4)$$

In Eq. (4), α , β , and γ are Euler angles that specify the orientation of a molecule or crystallite in the MAS rotor, with γ being the angle about the MAS rotation axis, c_{qjk} is identically zero, and $c_{qjk} = c_{-qjk}^*$. In Fig. 2(a), each ZQ recoupling block is embedded in a period of length $M\tau_R$ with a randomly chosen displacement τ_n from the beginning of this period, so that $\tau_n' = m\tau_R - \tau_n$, where $m' = M - m$. As shown previously,¹⁵ such a displacement in time changes Eq. (4) to

$$\tilde{d}_{njk} = \sum_{q=-2}^2 c_{qjk}(\alpha, \beta) \exp[iq(\gamma - \omega_R \tau_n)]. \quad (5)$$

FIG. 2. Rf pulse sequence for zero-quantum stochastic dipolar recoupling. (a) One ZQ-SDR block consists of a ZQ recoupling block of length $m\tau_R$ and chemical shift precession periods τ_n and τ_n' within a total period $M\tau_R$, where τ_n varies randomly from block to block and from scan to scan. In experiments and simulations in this paper, ZQ is a fpRFDR pulse sequence. (b) Double-resonance pulse sequence for one-dimensional experiments. After ^1H - ^{13}C cross-polarization with a ramped ^{13}C rf amplitude, ^{13}C polarization is stored longitudinally. A weak, Gaussian-shaped pulse (dashed line) is applied and alternate scans and signals are alternately added and subtracted to select the polarization of specific ^{13}C sites. N ZQ-SDR blocks are then applied before ^{13}C free-induction decay signals are observed in the t_2 period. DCPL represents ^1H decoupling, either by continuous wave (white) or pulsed (diagonal pattern) irradiation. (c) Pulse sequence for two-dimensional experiments. The x or y component of transverse ^{13}C polarization is selected by the rf phase η (either $\pm y$ to select the x component or $\pm x$ to select the y component, with alternate addition and subtraction of signals).

During the τ_n and τ_n' periods, we assume that the nuclear spin Hamiltonian contains only isotropic chemical shift terms, so that $H = \sum_j \omega_j S_{jz}$. It is safe to ignore anisotropic chemical shifts, which average to zero over τ_R , when τ_R^{-1} is large compared with the chemical shift anisotropies and when $(m\tau_R)^{-1}$ is large compared with the dipole-dipole couplings. The evolution operator of the n^{th} ZQ-SDR block alone is then

$$U_n = \exp \left(-i \sum_{j'} \omega_{j'} \tau_n' S_{j'z} \right) \exp \left\{ -i \sum_{j>k} \tilde{d}_{njk} \left[2S_{jz}S_{kz} - \frac{1}{2}(S_{j+}S_{k-} + S_{j-}S_{k+}) \right] m\tau_R \right\} \exp \left(-i \sum_{j''} \omega_{j''} \tau_n S_{j''z} \right). \quad (6)$$

The evolution operator for the entire ZQ-SDR sequence is an ordered product of terms of the form in Eq. (6). Separating chemical shift evolution terms from dipole-dipole

evolution terms, this product can be transformed to

$$U_N = U_{CS} \prod_{n=1}^N \exp[-i m \tilde{H}_{ZQ}(n) \tau_R], \quad (7a)$$

$$\tilde{H}_{ZQ}(n) = \sum_{j>k} \tilde{d}_{nj k} \left[2S_{jz} S_{kz} - \frac{1}{2} (e^{i\phi_{nj k}} S_{j+} S_{k-} + e^{-i\phi_{nj k}} S_{j-} S_{k+}) \right], \quad (7b)$$

$$\phi_{nj k} = \omega_{jk} [nm' \tau_R - \tau'_n], \quad (7c)$$

where the product in Eq. (7a) is in order of increasing n from right to left, $\omega_{jk} = \omega_j - \omega_k$, and

$$U_{CS} = \exp[-i \sum_{j'} N m' \omega_{j'} \tau_R S_{j'z}]. \quad (8)$$

According to Eq. (7), the effective dipole-dipole coupling Hamiltonian during the ZQ-SDR sequence is constant during each block of length $m\tau_R$, but varies from one block to the next due to both the random amplitude modulations in Eq. (5) and the random phase modulations in Eq. (7c). The effective Hamiltonian in each block also varies from scan to scan if different sets of τ_n values are chosen in each scan. Fig. 3 shows an example of fluctuating couplings under ZQ-SDR in a three-spin system.

The average over many scans of a single term in the effective Hamiltonian for the n^{th} block is

$$\langle \tilde{H}_{ZQ}^{jk}(n) \rangle = 2 \langle \tilde{d}_{nj k} \rangle S_{jz} S_{kz} - \frac{1}{2} \langle \tilde{d}_{nj k} e^{i\phi_{nj k}} \rangle S_{j+} S_{k-} - \frac{1}{2} \langle \tilde{d}_{nj k} e^{-i\phi_{nj k}} \rangle S_{j-} S_{k+}. \quad (9)$$

FIG. 3. Simulations of effective ^{13}C - ^{13}C coupling constants [see Eq. (5)] in a three-spin system under ZQ-SDR in a single scan (solid symbols) and averaged over 10^6 scans (hollow symbols). Lines are guides to the eye. The simulations use fpRFDR with $8.333 \mu\text{s}$ π pulses, $\tau_R = 25.00 \mu\text{s}$, $m = 4$, $M = 7$, $\alpha = 70^\circ$, $\beta = 110^\circ$, and $\gamma = 215^\circ$. The three spins have coordinates (1,0,0), (0,1,0), and (0,0,1) and resonance offsets of 5.0 kHz, 2.0 kHz, and -7.0 kHz. Effective couplings between spins 1 and 2 (squares), spins 1 and 3 (circles), and spins 2 and 3 (triangles) vary randomly in an apparently uncorrelated manner, and average to zero over many scans.

From Eq. (5), $\langle \tilde{d}_{nj k} \rangle$ vanishes if τ_n varies uniformly over an integer multiple of τ_R . From Eqs. (5) and (7c),

$$\langle \tilde{d}_{nj k} e^{i\phi_{nj k}} \rangle = \sum_{q=-2}^2 c_{qjk}(\alpha, \beta) e^{i(q\gamma + nm' \omega_{jk} \tau_R)} \langle e^{-i[(q\omega_R + \omega_{jk})\tau_n]} \rangle \quad (10a)$$

$$= i \sum_{q=-2}^2 c_{qjk}(\alpha, \beta) e^{i(q\gamma + nm' \omega_{jk} \tau_R)} \times (e^{-i\omega_{jk} m' \tau_R} - 1) / (2\pi m' q + m' \omega_{jk} \tau_R), \quad (10b)$$

where Eq. (10b) follows from averaging over values of τ_n in the interval from 0 to $(m'-m)\tau_R$. Evidently, $\langle \tilde{d}_{nj k} e^{i\phi_{nj k}} \rangle$ does not vanish identically when $\omega_{jk} m' \tau_R$ is not a multiple of 2π . However, phase modulation as a function of n from the $e^{i(q\gamma + nm' \omega_{jk} \tau_R)}$ factors in Eq. (10b) tends to average these terms towards zero over successive blocks within the ZQ-SDR sequence.

Averages of products of terms (i.e., correlation functions) are given by

$$\langle \tilde{H}_{ZQ}^{jk}(n) \tilde{H}_{ZQ}^{uv}(n') \rangle = \left\langle \tilde{d}_{nj k} [2S_{jz} S_{kz} - \frac{1}{2} (\tilde{d}_{nj k} e^{i\phi_{nj k}} S_{j+} S_{k-} + \tilde{d}_{nj k} e^{-i\phi_{nj k}} S_{j-} S_{k+})] \times \tilde{d}_{n' uv} [2S_{uz} S_{vz} - \frac{1}{2} (\tilde{d}_{n' uv} e^{i\phi_{n' uv}} S_{u+} S_{v-} + \tilde{d}_{n' uv} e^{-i\phi_{n' uv}} S_{u-} S_{v+})] \right\rangle. \quad (11)$$

The right side of Eq. (11) includes the following expressions:

$$\langle \tilde{d}_{nj k} \tilde{d}_{n' uv} \rangle = \delta_{nn'} \sum_{q=-2}^2 c_{qjk} c_{-quv}, \quad (12a)$$

$$\langle \tilde{d}_{nj k} \tilde{d}_{n' uv} e^{\pm i\phi_{nj k}} \rangle = \delta_{nn'} e^{\pm i n m' \omega_{jk} \tau_R} \sum_{q, q'=-2}^2 c_{qjk} c_{q'uv} e^{i(q+q')\gamma} \times \langle e^{-i[(q+q')\omega_R \pm \omega_{jk}] \tau_n} \rangle, \quad (12b)$$

$$\langle \tilde{d}_{nj k} \tilde{d}_{n' uv} e^{\pm i\phi_{nj k}} e^{\pm i\phi_{n' uv}} \rangle = e^{\pm i(n\omega_{jk} + n'\omega_{uv})m' \tau_R} \sum_{q, q'=-2}^2 c_{qjk} c_{q'uv} e^{i(q+q')\gamma} \times \langle e^{-i[(q\omega_R \pm \omega_{jk})\tau_n e^{-i[(q'\omega_R \pm \omega_{uv})\tau_{n'}]}] \rangle}, \quad (12c)$$

$$\langle \tilde{d}_{nj k} \tilde{d}_{n' uv} e^{\pm i\phi_{nj k}} e^{\mp i\phi_{n' uv}} \rangle = e^{\pm i(n\omega_{jk} - n'\omega_{uv})m' \tau_R} \sum_{q, q'=-2}^2 c_{qjk} c_{q'uv} e^{i(q+q')\gamma} \times \langle e^{-i[(q\omega_R \pm \omega_{jk})\tau_n e^{-i[(q'\omega_R \mp \omega_{uv})\tau_{n'}]}] \rangle}. \quad (12d)$$

As proven in the Appendix, the spin dynamics under ZQ-SDR can be accurately described by a polarization transfer

rate matrix $\mathbf{W}$ with off-diagonal elements $W_{jk} = \langle \tilde{d}_{jk}^2 \rangle m \tau_R / 4$ if the following conditions are satisfied: (i) $(\langle \tilde{d}_{jk}^2 \rangle)^{1/2} m \tau_R$ is small; (ii) $\langle \tilde{H}_{ZQ}^{jk}(n) \rangle$ vanishes; (iii) correlation functions in Eqs. (12) vanish except when $n = n'$ and $j, k = u, v$. In fact, Eqs. (12) show that different pairwise coupling terms in $\tilde{H}_{ZQ}(n)$ are not fully uncorrelated with one another. Nonetheless, numerical simulations show that the correlations are typically small. Fig. S1 of the supplementary material³⁸ shows numerical calculations of the correlation functions for a three-spin system.

Fig. 1(b) shows simulations of polarization transfers under ZQ-SDR in a three-spin system, comparing results from numerical calculations of the full quantum mechanical dynamics (i.e., density matrix calculations, including the full time-dependent Hamiltonian under MAS and the fpRFDR sequence and including averaging over random choices of τ_n values) with results from a rate matrix calculation of spin polarization transfers. Agreement between the quantum mechanical calculations and the rate matrix calculations is good, but not perfect. Deviations are attributable to effects of the residual correlations discussed above. Experimental results described below provide additional verification that the spin dynamics under ZQ-SDR can be described by a polarization transfer rate matrix with a useful level of accuracy.

III. EXPERIMENTAL AND NUMERICAL METHODS

Numerical simulations of the full quantum mechanical dynamics under fpRFDR and ZQ-SDR in Fig. 1 were performed with FORTRAN programs that were written specifically for this purpose (available upon request to robertty@mail.nih.gov). These programs explicitly include the time-dependence of dipole-dipole couplings under MAS, the rf pulses, and isotropic chemical shifts in the many-spin Hamiltonian during the fpRFDR blocks. Average Hamiltonian approximations are not used. Evolution during τ_n and τ_n' periods includes only the isotropic chemical shifts. Polarization transfers are averaged over random choices of τ_n and over values of the Euler angles α , β , and γ that specify the orientation of the spin system in the MAS rotor.

Rate matrix simulations were performed by evaluating the rate matrix $\mathbf{W}(\alpha, \beta)$ for given values of α and β , calculating polarization transfers after N fpRFDR blocks from the equation $p_k(N) = \sum_j m^N \tau_R^N (\mathbf{W}^N)_{jk} p_j(0)$, and averaging over α and β . The rate matrix elements $W_{jk} = \langle \tilde{d}_{jk}^2 \rangle m \tau_R / 4$ were evaluated from Eq. (12a) and the following expressions, which are derived from an average Hamiltonian theory calculation² for the fpRFDR sequence:

$$c_{\pm 2jk} = \pm \frac{\gamma_n^2 \hbar}{R_{jk}^3} \frac{3i[\exp(\pm 2i\omega_R \tau_p) - 1]}{256\pi(1 - \omega_R^2/\omega_1^2)} [\sin^2 \Theta_{jk} e^{2i\Phi_{jk}} \\ \times (1 \mp \cos \beta)^2 e^{-2i\alpha} + \sin^2 \Theta_{jk} e^{-2i\Phi_{jk}} (1 \pm \cos \beta)^2 e^{2i\alpha} \\ \mp 2 \sin 2\Theta_{jk} e^{i\Phi_{jk}} \sin \beta (1 \mp \cos \beta) e^{-i\alpha} \\ \pm 2 \sin 2\Theta_{jk} e^{-i\Phi_{jk}} \sin \beta (1 \pm \cos \beta) e^{i\alpha} \\ + 2(3 \cos^2 \Theta_{jk} - 1) \sin^2 \beta], \quad (13a)$$

$$c_{\pm 1jk} = -\frac{\gamma_n^2 \hbar}{R_{jk}^3} \frac{3i[\exp(\pm i\omega_R \tau_p) - 1]}{8\sqrt{2}\pi(4 - \omega_R^2/\omega_1^2)} [-\sin^2 \Theta_{jk} e^{2i\Phi_{jk}} \\ \times \sin \beta (1 \mp \cos \beta) e^{-2i\alpha} + \sin^2 \Theta_{jk} e^{-2i\Phi_{jk}} \\ \times \sin \beta (1 \pm \cos \beta) e^{2i\alpha} + \sin 2\Theta_{jk} e^{i\Phi_{jk}} \\ \times \sin \beta (1 \mp \cos \beta) (2 \cos \beta \pm 1) e^{-i\alpha} - \sin 2\Theta_{jk} e^{-i\Phi_{jk}} \\ \times \sin \beta (1 \pm \cos \beta) (2 \cos \beta \mp 1) e^{i\alpha} \\ \pm (3 \cos^2 \Theta_{jk} - 1) \sin 2\beta], \quad (13b)$$

where $\omega_R = 2\pi/\tau_R$, $\omega_1 = \pi/\tau_p$, τ_p is the π pulse length in the fpRFDR sequence, γ_n is the nuclear gyromagnetic ratio, R_{jk} is the distance between spins j and k , and Θ_{jk} and Φ_{jk} are polar and azimuthal angles for the internuclear vector in a “molecule-fixed” frame that is related to the “rotor-fixed” frame by Euler angles α , β , and γ . Rate matrix simulations of protein GB1 data were performed with programs written in C++ (available upon request from wei.qiang@nih.gov), using atomic coordinates in Protein Data Bank (PDB) file 2GI9 for calculations of R_{jk} , Θ_{jk} , and Φ_{jk} .

Experiments were performed in 14.1 T and 17.6 T fields (150.7 MHz and 187.9 MHz ^{13}C NMR frequencies, respectively), using MAS NMR probes with 1.8 mm diameter rotors obtained from the group of Dr. Ago Samoson (Tallinn University of Technology, Estonia) and Varian InfinityPlus and Infinity spectrometer consoles, respectively. Rf pulse sequences for one-dimensional (1D) and two-dimensional (2D) ZQ-SDR measurements are shown in Fig. 2. In our experiments, the ZQ-SDR sequence in Figs. 2(b) and 2(c) consisted of N repetitions of the block in Fig. 2(a), using fpRFDR as the ZQ element. Random values of τ_n were calculated within the pulse program by a simple random number generation algorithm, initialized with a seed integer that was supplied externally as a pulse sequence parameter. In Figs. 2(b) and 2(c), transverse ^{13}C polarization is first created by standard ^1H - ^{13}C cross-polarization. In 1D measurements (Fig. 2(b)), ^{13}C polarization is then stored longitudinally by a $\pi/2$ pulse. A frequency-selective, Gaussian-shaped π pulse (1.0 ms) is applied on alternating scans to select the polarization of a single ^{13}C site, prior to application of the ZQ-SDR sequence. Free-induction decay (FID) signals are detected (with alternate addition and subtraction) after another ^{13}C $\pi/2$ pulse. Continuous-wave (cw) ^1H decoupling is applied during the frequency-selective π pulse and the ZQ-SDR sequence. Two-pulse phase-modulated (TPPM) decoupling³⁹ is applied during the FID. In 2D measurements (Fig. 2(c)), either TPPM decoupling or rotor-synchronized ^1H π pulse decoupling (with XY8 phases⁴⁰) is applied in the t_1 and t_2 periods. Either the real or imaginary (x or y) component of transverse ^{13}C polarization is selected by varying the phase η of the ^{13}C $\pi/2$ pulse at the end of the t_1 period. In 1D measurements, each set of random τ_n values is used twice, corresponding to scans in which the frequency-selective π pulse is either present or absent. In 2D measurements, the same sets of random τ_n values are used in each t_1 point. Apart from these conditions, different sets of τ_n values are used in each scan. Phases of ^{13}C π pulses in fpRFDR blocks follow XY8, XY16, or XY32 patterns,⁴⁰ depending on the value of m . Other details of

experimental conditions are given below and in the figure captions. Data were analyzed and plotted with VARIAN SPINSIGHT software and NMRPIPE⁴¹ software.

IV. EXPERIMENTAL RESULTS

A. Uniformly ^{13}C -labeled L-valine

Fig. 4(a) shows a series of 1D spectra of uniformly ^{13}C -labeled L-valine powder, obtained at 14.1 T with the pulse sequence in Fig. 2(b) at a MAS frequency of 28.00 kHz. ^{13}C π pulse lengths in fpRFDR blocks were 10.0 μs , applied with the carrier frequency at 81.5 ppm. ^1H decoupling fields were 120 kHz during the FID and 145 kHz during the ZQ-SDR sequence. The frequency-selective π pulse was applied on-resonance with methyl signals at 20 ppm, initially selecting the polarization of the two C_γ sites (with a small contribution from the C_β site at 31 ppm). With increasing stochastic recoupling time $Nm\tau_R$, the C_γ signals decrease and the signals from C_β , C_α , and CO sites increase as polarization transfers occur under the ZQ-SDR sequence. Fig. 4(b) shows

FIG. 4. (a) Experimental spectra of uniformly ^{13}C -labeled L-valine powder at 14.1 T obtained with the pulse sequence in Fig. 2(b), with 28.00 kHz MAS, $m = 16$, $M = 17$, 10.0 μs ^{13}C π pulses in fpRFDR blocks with XY16 phases, ^{13}C carrier frequency at 81.5 ppm during the ZQ-SDR period, 128 scans per spectrum, and $N = 0, 4, 8, \dots, 32$. Polarization of the two C_γ methyl sites was selected by applying the Gaussian-shaped π pulse in Fig. 2(b) at 20 ppm. ^1H decoupling fields were 145 kHz during the ZQ-SDR period and 120 kHz during acquisition of free-induction decay signals. (b) Dependence of NMR peak areas on the recoupling time $Nm\tau_R$ for the indicate sites. At each value of the recoupling time, the total area is normalized to 1.0 to compensate for gradual signal loss with increasing recoupling time (35% loss at $Nm\tau_R = 18.29$ ms). Data are shown for $M-m = 1$ (solid symbols) and $M-m = 4$ (hollow symbols).

that the polarization transfer curves have a smooth, monotonic, exponential-like appearance, approaching a steady state in which polarization is divided equally over all five ^{13}C sites of L-valine. (Note that there are two C_γ sites, so that the combined C_γ polarization should approach 40% of the total if the polarization is divided equally.) Interestingly, the polarization transfer curves are nearly identical for $m' = 1$ and $m' = 4$, as expected if zero-quantum (or other) coherences that are created by a given fpRFDR block do not survive to the next block after averaging over many scans with different random values of τ_n .

Fig. 5 shows additional polarization transfer curves, obtained with various values of m . When plotted as a function of the recoupling time $Nm\tau_R$ (Figs. 5(a) and 5(c)), the data show that the polarization transfer rates increase with increasing m . When plotted as a function of $Nm^2\tau_R$ (Figs. 5(b) and 5(d)), the curves are nearly independent of m , showing that the rates are directly proportional to m . This behavior is expected if polarization transfers are driven by randomly fluctuating dipole-dipole couplings, with correlation times equal to the length of an individual fpRFDR block (i.e., $m\tau_R$), as discussed above and in the Appendix. Of course, polarization transfer curves under conventional dipolar recoupling sequences would depend on $Nm\tau_R$ but not on the value of m alone.

B. Uniformly ^{13}C -labeled protein GB1

Fig. 6 shows examples of 2D ^{13}C - ^{13}C NMR spectra of protein GB1, a 56-residue model protein³⁷ that has been the subject of many previous solid state NMR studies,^{42–47} obtained with the pulse sequence in Fig. 2(c). For these measurements, GB1 was uniformly ^{13}C -labeled by bacterial expression on uniformly ^{13}C -labeled glucose and prepared in microcrystalline form as previously described.⁴⁷ Based on ultraviolet absorption of the GB1 solution prior to crystallization, the NMR sample contained approximately 9 mg of GB1. ^{13}C chemical shift assignments for this sample have been reported previously.⁴⁷ Measurements were performed at 17.6 T with $\omega_R/2\pi = 40.00$ kHz. To minimize sample heating from rf irradiation, ^1H π pulse decoupling with one 3.0 μs pulse per τ_R period was used instead of high-power TPPM decoupling during t_1 and t_2 . ^{13}C π pulse lengths in fpRFDR block were 10.0 μs , $m = 16$, and $m' = 1$. The large ^{13}C chemical shift range at 17.6 T precluded uniform excitation of the entire ^{13}C spectrum. Therefore, the ^{13}C carrier frequency was set to 47 ppm during the ZQ-SDR period, and polarization transfers were limited primarily to aliphatic carbons.

2D spectra as in Fig. 6 were recorded with $N = 2, 4, 8, 14, 20, 26$, and 32. For eight residues with well-resolved crosspeaks, volumes of intraresidue crosspeaks that connect methyl signals to other aliphatic signals were measured. These crosspeak volumes represent polarization transfers from the methyl sites to other carbon sites within the same residue. The experimental crosspeak volumes were then compared with results from two sets of rate matrix simulations. In the first set of simulations, polarization transfer rates between ^{13}C sites j and k of a given residue were assumed to equal W_0/R_{jk}^6 , where R_{jk} is the internuclear

FIG. 5. (a,b) Dependence of NMR peak areas for uniformly ^{13}C -labeled L-valine powder on the recoupling time $Nm\tau_R$ or on $Nm^2\tau_R$ for the indicated values of m , the number of ^{13}C π pulses in each fpRFDR block. Polarization of the C_β site was selected by applying the Gaussian-shaped π pulse at 31 ppm. Other experimental conditions were the same as in Fig. 4, except that $M-m = 2$. Lines are guides to the eye. (c,d) Same, but with the Gaussian-shaped π pulse at 20 ppm to select C_γ polarization.

distance and W_0 is a fitting parameter that is independent of j and k . Values of R_{jk} were determined from atomic coordinates in PDB 2GI9, a GB1 crystal structure reported by Rienstra and coworkers.⁴⁸ Additional fitting parameters were the overall scale factor of the experimental data (i.e., the initial methyl ^{13}C spin polarization), called P , and an exponential decay time T_2^* for the total crosspeak volume, invoked to account for signal loss due to spin relaxation, pulse imperfections, and imperfect ^1H - ^{13}C decoupling during the ZQ-SDR period. Experimental data and best-fit simulations are shown in Fig. 7. Best-fit parameters and uncertainties are given in Table I, with uncertainties defined by variations in individual parameters that increase the χ^2 deviation between experiments and simulations by $\sqrt{2\chi_{\min}^2}$, where $\chi_{\min}^2$ is the minimum deviation. (To be explicit, $\chi^2 \equiv \sum_{i=1}^K [E_i - P \cdot C_i(W_0) \cdot \exp(-N_i m \tau_R / T_2^*)]^2 / \sigma_i^2$, where K is the number of data points, E_i and C_i are the i^{th} experimental and calculated points, N_i is the i^{th} value of N , and σ_i is the uncertainty in E_i .) Contour plots of χ^2 as a function of W_0 and P are shown in Fig. S2 of the supplementary material.³⁸ The overall agreement between these experimental data and rate matrix simulations for intraresidue polarization transfers under ZQ-SDR, assuming that polarization transfer rates are inversely proportional to R_{jk} , is satisfactory (i.e., $\chi_{\min}^2$ is less than or roughly equal to the number of experimental data points). Best-fit values of W_0 determined independently for the eight residues agree with the average value of $926 \pm 170 \text{ \AA}^6\text{s}^{-1}$ to within the uncertainties in these values.

In the second set of rate matrix simulations, polarization transfer rates $W_{jk}(\alpha, \beta)$ were calculated with Eq. (13), again using coordinates in PDB 2GI9, and the simulations were averaged over α and β . The only adjustable parameters were P and T_2^* , as defined above. Experimental data and best-fit simulations are shown in Fig. 8. Best-fit parameters and uncertainties are given in Table II. Contour plots of χ^2 as a function of P and T_2^* are shown in Fig. S3 of the supplementary material.³⁸ Agreement between experimental data and simulations is again satisfactory.

TABLE I. Results of fitting ZQ-SDR polarization transfer data for uniformly ^{13}C -labeled protein GB1 with a model in which polarization transfer rates between ^{13}C sites j and k are equal to W_0/R_{jk} , P is the overall scale factor, and T_2^* is the decay time of the total polarization. Best-fit simulations are compared with experimental data in Fig. 7.

Residue	$\chi_{\min}^2$	W_0 ($\text{\AA}^6/\text{s}$)	P (arb. units)	T_2^* (ms)
Met1	1.0	1300 ± 450	4.3 ± 0.4	8.2
Ile6	10.8	1030 ± 230	8.0 ± 0.8	10.8
Thr18	15.8	850 ± 130	18.8 ± 1.2	7.2
Glu19	3.4	780 ± 350	7.0 ± 0.6	9.1
Val29	7.0	880 ± 150	14.0 ± 0.9	8.5
Val39	9.5	930 ± 150	16.8 ± 1.0	8.1
Thr44	8.9	700 ± 100	15.7 ± 1.4	8.0
Thr49	18.9	950 ± 200	14.8 ± 1.1	9.2

FIG. 6. Aliphatic regions of 2D ^{13}C - ^{13}C NMR spectra of uniformly ^{13}C -labeled protein GB1 in microcrystalline form, obtained at 17.6 T with the pulse sequence in Fig. 2(c), with 40.00 kHz MAS, $m = 16$, $M = 17$, $10.0 \mu\text{s}$ ^{13}C π pulses in fpRFDR blocks with XY16 phases, ^{13}C carrier frequency at 47 ppm during the ZQ-SDR period. 1D slices at ^{13}C chemical shifts of I6 C_δ , T18 C_γ , V29 $\text{C}_{\gamma 1}$, and E19 C_γ (color-coded dashed lines in 2D spectra) are shown at the left. (a) $N = 4$, 275 complex t_1 points, $25 \mu\text{s}$ t_1 increment, total of 17 600 scans with a 1.8 s recycle delay. (b) $N = 32$, total of 140 800 scans.

FIG. 7. Dependence of crosspeak volumes on ZQ-SDR recoupling time $Nm\tau_R$ in 2D ^{13}C - ^{13}C NMR spectra of uniformly ^{13}C -labeled protein GB1, as in Fig. 6. Crosspeak volumes are corrected for differences in the numbers scans in each 2D spectrum. Data are shown for intraresidue crosspeaks from Met1, Ile6, Thr18, Glu19, Val19, Val39, Thr44, and Thr49 (a-h, respectively). Error bars indicate the root-mean-squared noise in the volume measurements. Color-coded lines are best-fit rate matrix simulations, assuming that ^{13}C - ^{13}C polarization transfer rates are proportional to $1/R^6$ and with the constant of proportionality being a fitting parameter. For valines, $\text{C}_{\gamma 1}$ is taken to be the methyl site with the larger of the two methyl chemical shift values.

FIG. 8. Same as Fig. 7, but showing best-fit rate matrix simulations in which ^{13}C - ^{13}C polarization transfer rates are calculated from theory, with an orientation dependence that follows from Eq. (13).

By considering a spin pair with $\Phi_{jk} = 0$ in Eq. (13), the orientational average of $W_{jk}(\alpha, \beta)$ can be calculated to be

$$W_{jk}^{\text{ave}} = \left[\frac{9(1 - \cos \omega_R \tau_p)}{30\pi^3(4 - \omega_R^2/\omega_1^2)^2} + \frac{3(1 - \cos 2\omega_R \tau_p)}{1280\pi^3(1 - \omega_R^2/\omega_1^2)^2} \right] \times \frac{m\tau_R\gamma_n^4\hbar^2}{R_{jk}^6}. \quad (14)$$

For ^{13}C nuclei, $\gamma_n^2\hbar = 47.71 \times 10^3 \text{ Å}^3\text{s}^{-1}$. Under the experimental conditions for Figs. 6–8 described above, Eq. (14) implies $W_{jk}^{\text{ave}} = 1778 \text{ Å}^6\text{s}^{-1}/R_{jk}^6$. As described above, the value of W_0 extracted from the experimental data is smaller by a factor of two. We attribute this apparent discrepancy to the fact that polarization transfer rates under ZQ-SDR are orientation-dependent. Fitting the data with orientation-independent rates leads to a systematic underestimation of the rates and to concomitantly smaller values of T_2^* .

As shown in Fig. S4 of the supplementary material,³⁸ the polarization transfer data can also be fit with the calculated rates $W_{jk}(\alpha, \beta)$, but including only the one-bond ^{13}C - ^{13}C rates.

TABLE II. Results of fitting ZQ-SDR polarization transfer data for uniformly ^{13}C -labeled protein GB1 with a model in which orientation-dependent polarization transfer rates between ^{13}C sites j and k are calculated from Eq. (13), P is the unitless overall scale factor, and T_2^* is the decay time of the total polarization. Best-fit simulations are compared with experimental data in Fig. 8.

Residue	χ^2_{min}	P (arb. units)	T_2^* (ms)
Met1	0.9	3.0 ± 0.5	10.5 ± 1.7
Ile6	20.2	5.1 ± 1.1	15.0 ± 7.5
Thr18	10.4	9.0 ± 1.2	11.8 ± 2.2
Glu19	9.1	4.3 ± 0.8	16.0 ± 8.7
Val29	8.6	8.0 ± 1.0	14.0 ± 3.3
Val39	15.1	10.8 ± 1.1	10.2 ± 1.8
Thr44	12.4	6.8 ± 1.2	12.0 ± 2.8
Thr49	24.3	8.5 ± 0.8	13.5 ± 2.2

Thus, in uniformly ^{13}C -labeled GB1, two-bond or multiple-bond polarization transfers within a given residue result primarily from series of one-bond transfers. This result is expected, since one-bond rates are about 16 times greater than two-bond rates, given the $1/R^6$ dependence.

2D spectra of microcrystalline, uniformly ^{13}C -labeled GB1 were also recorded with $\tau_n = \tau_n' = 0.5\tau_R$ (i.e., no random variations in the dipolar recoupling sequence), but with all other conditions identical to those in Figs. 6–8. As shown in Fig. 9 and Table III, attempts to fit the resulting polarization transfer data with rate matrix simulations were unsuccessful, yielding best-fit χ^2 values that were significantly larger than those in Tables I and II for data sets with high signal-to-noise. Interestingly, as shown in Fig. S5 of the supplementary material,³⁸ 2D spectra obtained without random variations in τ_n exhibit significantly smaller intensities in multiple-bond crosspeaks for the same value of $Nm\tau_R$, suggesting that the ZQ-SDR technique can be a useful tool for generating multiple-bond crosspeaks in uniformly ^{13}C -labeled samples.

TABLE III. Results of fitting polarization transfer data for uniformly ^{13}C -labeled protein GB1 under non-stochastic ZQ recoupling with a model in which polarization transfer rates between ^{13}C sites j and k are equal to W_0/R_{jk}^6 , P is the unitless overall scale factor, and T_2^* is the decay time of the total polarization. Best-fit simulations are compared with experimental data in Fig. 9.

Residue	χ^2_{min}	W_0 ($\text{Å}^6/\text{s}$)	P (arb. units)	T_2^* (ms)
Met1	13.5	1400 ± 380	5.8 ± 1.2	8.5
Ile6	510.0	990 ± 200	8.8 ± 3.8	9.0
Thr18	22.8	2260 ± 480	6.3 ± 3.3	2.5
Glu19	31.1	1390 ± 280	5.5 ± 1.5	15.0
Val29	220.0	1360 ± 230	13.0 ± 2.3	7.0
Val39	221.5	1390 ± 250	13.0 ± 2.5	7.5
Thr44	23.0	2230 ± 450	5.5 ± 2.6	2.5
Thr49	26.5	2200 ± 450	8.0 ± 2.5	2.0

FIG. 9. Same as Fig. 7, but for data obtained from non-stochastic ZQ dipolar recoupling. Compared with ZQ-SDR data in Figs. 7 and 8, these data are not well described by polarization transfer rates proportional to $1/R^6$.

C. Partially ^{13}C -labeled protein GB1

A microcrystalline GB1 sample was also prepared by bacterial expression on $1,3\text{-}^{13}\text{C}_2$ -glycerol, resulting in selective ^{13}C labeling of certain amino acids according to patterns described by LeMaster and Kushlan.^{25,49,50} In particular, as verified by the 2D ^{13}C - ^{13}C spectra, valine residues were significantly ^{13}C -labeled only at CO and methyl (both $\text{C}_{\gamma 1}$ and $\text{C}_{\gamma 2}$) sites, and leucine residues were significantly ^{13}C -labeled

only at C_{α} and methyl (both $\text{C}_{\delta 1}$ and $\text{C}_{\delta 2}$) sites. With these labeling patterns, polarization transfers from methyl to CO (for valine) or methyl to C_{α} (for leucine) sites must occur directly, rather than through a series of one-bond transfers as described above. The corresponding polarization transfer rates should then depend on $\text{CO}-\text{C}_{\gamma}$ or $\text{C}_{\alpha}-\text{C}_{\delta}$ distances, and hence on the conformations of valine or leucine sidechains.

Fig. 10 shows examples of 2D ^{13}C - ^{13}C NMR spectra of partially ^{13}C -labeled GB1, obtained at 14.1 T and 40.00 kHz

FIG. 10. Carbonyl and aliphatic regions of 2D ^{13}C - ^{13}C NMR spectra of partially ^{13}C -labeled protein GB1 in microcrystalline form, obtained at 14.1 T with the pulse sequence in Fig. 2(c), with 40.00 kHz MAS, $m = 16$, $M = 17$, $10.0\ \mu\text{s}$ ^{13}C π pulses in fpRFDR blocks with XY16 phases, ^{13}C carrier frequency at 94 ppm during the ZQ-SDR period. 1D slices at ^{13}C chemical shifts of V54 $\text{C}_{\gamma 2}$ and L12 C_{α} (dashed lines in 2D spectra) are shown at the left. Arrows indicate $\text{CO}/\text{C}_{\gamma 1}$ and $\text{CO}/\text{C}_{\gamma 2}$ crosspeaks of V54, and $\text{C}_{\alpha}/\text{C}_{\delta 1}$ and $\text{C}_{\alpha}/\text{C}_{\delta 2}$ crosspeaks of L12. (a) $N = 12$, 275 complex t_1 points, $25\ \mu\text{s}$ t_1 increment, total of 35 200 scans with a 2.0 s recycle delay. (b) $N = 42$, total of 105 600 scans. In the 1D slices, vertical scales are adjusted so that diagonal peaks have equal heights at both values of N , then multiplied by an additional factor of 10 to make crosspeaks readily visible.

FIG. 11. (a-d) Dependence of crosspeak volumes on ZQ-SDR recoupling time $Nm\tau_R$ in 2D ^{13}C - ^{13}C NMR spectra of partially ^{13}C -labeled protein GB1, as in Fig. 10. Data are shown for three-bond intrasidue crosspeaks from Leu5, Leu12, Val29, and Val54, respectively. (e-h) Dependence of the minimized χ^2 deviation between experimental data and rate matrix simulations on the sidechain torsion angle ρ assumed in the simulations (see text for definition of ρ), with fitting parameters P and T_2^* . Best-fit simulations are shown as solid and dotted lines in panels a-d. Fits were performed with $C_{\delta 1}$ and $C_{\gamma 1}$ assigned as the methyl sites with larger chemical shift values (green squares), and with the opposite assignments (red circles). Dashed vertical lines indicate values of ρ extracted from PDB 2GI9.

MAS with the pulse sequence in Fig. 2(c). Based on ultraviolet absorption of the GB1 solution prior to crystallization, the NMR sample contained approximately 5 mg of GB1. Other conditions were nearly identical to those used in experiments on uniformly ^{13}C -labeled GB1 at 17.6 T described above, except that the ^{13}C carrier frequency was set to 94 ppm during the ZQ-SDR period. At the lower field, these conditions allowed uniform excitation of the full ^{13}C spectrum, including both carbonyl and methyl signals. As shown in Fig. 10, weak three-bond crosspeaks between valine methyl and CO signals and between leucine methyl and C_α signals can be detected at relatively long recoupling times. Figs. 11(a)–11(d) show the crosspeak build-up curves for L5, L12, V29, and V54.

Rate-matrix simulations of the corresponding polarization transfers in isolated, partially ^{13}C -labeled valine and leucine residues were performed as described above, using the theoretical expression for $W_{jk}(\alpha, \beta)$. One sidechain torsion angle, called ρ , was varied in these simulations. For valine, ρ was defined to be the angle about the C_α - C_β bond determined by the $\text{CO}-C_\alpha-C_\beta-C_{\gamma 2}$ group; for leucine, ρ was defined to be the angle about the C_β - C_γ bond determined by the $C_\alpha-C_\beta-C_\gamma-C_{\delta 1}$ group. Simulated data were compared with the experimental data, and $\chi^2_{\min}$ was calculated as a function of ρ , using P and T_2^* as adjustable parameters. In order to define the value of P , the experimental data included one diagonal peak volume (CO for valines, C_α for leucines, at $Nm\tau_R = 0.8$ ms) with a 10% uncertainty, in addition to the crosspeak volumes plotted in Figs. 11(a)–11(d). In the absence of stereospecific assignments of the valine and leucine methyl ^{13}C chemical shifts, both possible assignments were tested (e.g., $^{13}\text{C}_{\delta 1}$ chemical shift $>$ $^{13}\text{C}_{\delta 2}$ chemical shift, or vice versa). Results are shown in Figs. 11(e)–11(h) and compared with values of ρ extracted from PDB 2GI9. In all cases,

$\chi^2_{\min}$ depends strongly on ρ , demonstrating that the ZQ-SDR data contain information about sidechain conformation, and the value of ρ extracted from PDB 2GI9 corresponds to a relatively small value of $\chi^2_{\min}$ for one of the two possible assignments of methyl chemical shifts.

V. DISCUSSION

Analytical and numerical results presented above and in the Appendix show that the ZQ-SDR technique depicted in Fig. 2(a) produces spin dynamics in a dipole-coupled many-spin system that are qualitatively different from the dynamics under conventional coherent dipolar recoupling techniques. In particular, the dynamics in an N -spin system under ZQ-SDR are well described by an $N \times N$ rate matrix, with rates proportional to $1/r^6$. The rate of polarization transfer between spins j and k is independent of the distances and directions to other spins. The dipolar truncation effect illustrated in Fig. 1(a) is avoided. As shown in Fig. 1(b), the net polarization transfer from j to k at a given recoupling time still depends on the location of other spins, but this is due to the interactions of various pairwise rates (as contained in the rate matrix), not to dipolar truncation.

As discussed above, individual pairwise coupling terms do not average completely to zero over many scans under ZQ-SDR, due to interference between amplitude modulations in Eq. (5) and phase modulations in Eq. (7). Correlations between different pairwise coupling terms also do not average completely to zero. Nonetheless, in typical spin systems, it appears that residual average couplings and residual correlations do not have large effects. This conclusion is based on both the numerical simulations and the experimental results.

The rate matrix for a given spin system depends on the overall orientation of the spin system in the MAS rotor (i.e., the α and β angles). Thus, polarization transfer curves for a powder sample have a “stretched exponential” character that complicates the interpretation of ZQ-SDR data in terms of internuclear distances and molecular structure. However, results for uniformly ^{13}C -labeled GB1 in Figs. 7 and 8 show that comparably good fits to the experimental data can be achieved either with rate matrix simulations that ignore the orientation dependence and use an empirically determined constant of proportionality between rates and $1/R^6$, or with simulations that use the orientation-dependent theoretical expression for the rates. It is worth emphasizing that rate matrix simulations (involving $N \times N$ matrices) are inherently faster than full quantum mechanical simulations (involving $2^N \times 2^N$ matrices for spin-1/2 nuclei). It is not computationally prohibitive to vary multiple structural parameters in orientationally-averaged simulations of ZQ-SDR measurements on a many-spin system. It is also worth emphasizing that the theoretical expression for rate matrix elements, calculated from Eq. (13), appears to agree with experimental ZQ-SDR data. In contrast, efforts to fit ^{13}C - ^{13}C spin diffusion data with similar rate matrices require empirical calibration of the rates.²⁴

In uniformly ^{13}C -labeled samples, polarization transfers are dominated by one-bond couplings, which correspond to the largest rates. Therefore, ZQ-SDR data from uniformly labeled samples are not sufficiently sensitive to molecular conformation to allow parameters such as torsion angles to be determined realistically. However, as shown in Fig. 11, partial labeling to eliminate certain one-bond couplings renders the data conformationally sensitive. Since partial ^{13}C labeling is already widely used in structural studies of proteins by solid state NMR, both to simplify the task of resonance assignment and to facilitate the detection of long-range inter-residue contacts,^{25,51,52} ZQ-SDR measurements may become a useful additional tool for obtaining quantitative constraints on sidechain conformations or other structural features.

In principle, ZQ-SDR measurements may allow quantitative measurements of inter-residue ^{13}C - ^{13}C distances in proteins. Such measurements will most likely require sparse labeling, long recoupling times, and high signal-to-noise, because the relevant distances generally exceed 3.5 Å, implying $W_{jk}^{\text{ave}} \sim 1 \text{ s}^{-1}$ in Eq. (14).

As shown in Fig. 6, ZQ-SDR produces efficient polarization transfers and hence strong crosspeaks in 2D ^{13}C - ^{13}C spectra of uniformly ^{13}C -labeled proteins at 40.00 kHz MAS. The fpRFDR sequence is compatible (and improves) with higher MAS frequencies, making the ZQ-SDR technique also compatible with higher MAS frequencies, where high-power ^1H decoupling may become unnecessary.^{14,53} Results in Fig. S5 of the supplementary material³⁸ show that stochastic recoupling can enhance multiple-bond crosspeak intensities, compared with non-stochastic recoupling. Thus, ZQ-SDR may become a preferred method for obtaining 2D spectra with strong crosspeaks at very high MAS frequencies, for resonance assignments or for other purposes, even when quantitative distance measurements are not the goal.

Finally, although the experimental results in this paper are limited to systems of dipole-coupled ^{13}C nuclei, the ZQ-SDR technique can certainly be applied to other nuclei. Particularly at high MAS frequencies, applications to systems of coupled ^{31}P or ^1H nuclei should be feasible. In such systems, the absence of one-bond couplings may make the ZQ-SDR data especially sensitive to important structural features. Proton-mediated 2D ^{13}C - ^{13}C , ^{15}N - ^{15}N , or ^{15}N - ^{13}C spectra^{54–56} (i.e., CHHC, NHHN, or NHHc spectra), using ^1H - ^1H ZQ-SDR in the mixing periods, may contain valuable quantitative or semi-quantitative structural information for proteins or other systems.

ACKNOWLEDGMENTS

This work was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health.

APPENDIX: DERIVATION OF THE POLARIZATION TRANSFER RATE MATRIX FOR ZQ-SDR

Consider the evolution of a system of dipole-coupled spin-1/2 nuclei under a stochastic recoupling sequence that produces a randomly fluctuating but piecewise-constant effective Hamiltonian, with the following form in the n^{th} time period:

$$\tilde{H}_{\text{ZQ}}(n) = \sum_{j>k} H_{jk}(n), \quad (\text{A1a})$$

$$H_{jk}(n) = \tilde{d}_{\text{nj}k} [2S_{jz}S_{kz} - \frac{1}{2}(e^{i\phi_{\text{nj}k}}S_{j+}S_{k-} + e^{-i\phi_{\text{nj}k}}S_{j-}S_{k+})]. \quad (\text{A1b})$$

All time periods during which $\tilde{H}_{\text{ZQ}}(n)$ is constant have the same length τ (equal to $m\tau_R$ in ZQ-SDR experiments). Assume that the coupling constants $\tilde{d}_{\text{nj}k}$ (which are real numbers, with units of rad/s) and phases $\phi_{\text{nj}k}$ vary from scan to scan in such a way that the following conditions are satisfied:

$$\langle \tilde{d}_{\text{nj}k} \rangle = 0, \quad (\text{A2a})$$

$$\langle \tilde{d}_{\text{nj}k} e^{i\phi_{\text{nj}k}} \rangle = 0, \quad (\text{A2b})$$

$$\langle \tilde{d}_{\text{nj}k} \tilde{d}_{\text{n}'j'k'} \rangle = \delta_{n,n'} \delta_{j,j'} \delta_{k,k'} \langle \tilde{d}_{jk}^2 \rangle, \quad (\text{A2c})$$

$$\langle \tilde{d}_{\text{nj}k} \tilde{d}_{\text{n}'j'k'} e^{i\phi_{\text{nj}k}} \rangle = 0, \quad (\text{A2d})$$

$$\langle \tilde{d}_{\text{nj}k} \tilde{d}_{\text{n}'j'k'} e^{i\phi_{\text{nj}k}} e^{i\phi_{\text{n}'j'k'}} \rangle = 0, \quad (\text{A2e})$$

$$\langle \tilde{d}_{\text{nj}k} \tilde{d}_{\text{n}'j'k'} e^{i\phi_{\text{nj}k}} e^{-i\phi_{\text{n}'j'k'}} \rangle = 0. \quad (\text{A2f})$$

The angular brackets indicate an average over scans. $\langle \tilde{d}_{jk}^2 \rangle$ is independent of n because the same basic pulse sequence block is used in each time period. Note that Eqs. (A2) are satisfied approximately in ZQ-SDR experiments, as discussed in Sec. I of the main text and illustrated in Fig. S1 of the supplementary material.³⁸ Additionally assume that the

initial state of the system is described by a density operator of the form:

$$\rho(0) = \sum_j p_j(0) S_{jz}, \quad (\text{A3})$$

where $p_j(0)$ is the initial polarization of spin j . The average density operator after N time periods is

$$\langle \rho(N) \rangle = \langle \hat{U}_T(N) \hat{U}_T(N-1) \dots \hat{U}_T(2) \hat{U}_T(1) \rangle \rho(0), \quad (\text{A4})$$

where $\hat{U}_T(n)$ is the evolution superoperator for the spin system under the Hamiltonian in Eqs. (A1) in the n^{th} time period. Under the assumptions in Eqs. (A2), Eq. (A4) can be rewritten

$$\langle \rho(N) \rangle = \langle \hat{U}_T(N) \rangle \langle \hat{U}_T(N-1) \rangle \dots \langle \hat{U}_T(2) \rangle \langle \hat{U}_T(1) \rangle \rho(0), \quad (\text{A5a})$$

$$= \langle \hat{U}_T \rangle^N \rho(0). \quad (\text{A5b})$$

The time period index is omitted in Eq. (A5b) and where appropriate in subsequent equations because all time periods have the same averages.

Using the relations $\hat{U}_T \rho(0) = [U_T, \rho(0)]$ and $U_T = \exp(-i\tilde{H}_{ZQ}\tau)$, the averaged evolution over one time period can be expressed to second order in τ as

$$\begin{aligned} \langle \hat{U}_T \rangle \rho(0) &\approx \rho(0) - i\tau \langle [\tilde{H}_{ZQ}, \rho(0)] \rangle - \frac{\tau^2}{2} \langle \tilde{H}_{ZQ}^2 \rangle \rho(0) \\ &\quad - \frac{\tau^2}{2} \rho(0) \langle \tilde{H}_{ZQ}^2 \rangle \\ &\quad + \tau^2 \langle \tilde{H}_{ZQ} \rho(0) \tilde{H}_{ZQ} \rangle \end{aligned} \quad (\text{A6a})$$

$$= \sum_j p_j(0) \left\{ S_{jz} - \frac{\tau^2}{2} \sum_{k \neq j} \left[\langle H_{jk}^2 \rangle S_{jz} + S_{jz} \langle H_{jk}^2 \rangle - 2 \langle H_{jk} S_{jz} H_{jk} \rangle \right] \right\} \quad (\text{A6b})$$

$$\approx \sum_j p_j(0) \left\{ \prod_{k \neq j} \langle \hat{U}_{jk} \rangle S_{jz} \right\}. \quad (\text{A6c})$$

Eqs. (A2) ensure that the first-order term in Eq. (A6a) and second-order cross terms between different pairwise couplings (i.e., H_{jk} and $H_{j'k'}$ with $k' \neq k$ or $j' \neq j$) vanish in Eq. (A6b). In Eq. (A6c), $\langle \hat{U}_{jk} \rangle$ is the average evolution superoperator for one time period under H_{jk} alone. Equation (A6c) shows that the evolution of polarization from spin j is a product of the evolution due to each coupling H_{jk} , without interference between different couplings.

Initial polarization on spin j corresponds to an initial density operator component proportional to S_{jz} . Since H_{jk} is a zero-quantum operator, evolution under H_{jk} can only transform S_{jz} to a linear combination of S_{jz} , S_{kz} , $S_{jz}S_{kz}$, $S_{j+}S_{k-}$, and $S_{j-}S_{k+}$, because these are the only zero-quantum operators for a pair of spin-1/2 nuclei. It can be shown that $\text{Tr}\{A[\langle H_{jk}^2 \rangle S_{jz} + S_{jz} \langle H_{jk}^2 \rangle - 2 \langle H_{jk} S_{jz} H_{jk} \rangle]\} = 0$ for $A = S_{jz}S_{kz}$, $A = S_{j+}S_{k-}$, and $A = S_{j-}S_{k+}$. Therefore,

$\langle \hat{U}_{jk} \rangle$ can only transform S_{jz} to a linear combination of S_{jz} and S_{kz} . Consequently, if $\rho(0)$ satisfies Eq. (A3), the averaged dynamics of the many-spin system are limited to polarization transfers, implying that

$$\langle \rho(N) \rangle = \sum_k p_k(N) S_{kz} \quad (\text{A7})$$

and

$$p_k(N) = \frac{1}{\text{Tr}\{S_{kz}^2\}} \sum_j \text{Tr}\{S_{kz} \langle \hat{U}_T \rangle^N S_{jz}\} p_j(0) \quad (\text{A8a})$$

$$= \sum_j \tau^N (\mathbf{W}^N)_{jk} p_j(0), \quad (\text{A8b})$$

where $\mathbf{W}$ is the polarization transfer rate matrix with off-diagonal elements

$$W_{jk} = \frac{1}{\tau} \text{Tr}\{S_{kz} \langle \hat{U}_{kj} \rangle S_{jz}\} / \text{Tr}\{S_{kz}^2\} \quad (\text{A9a})$$

$$= \langle \tilde{d}_{kj}^2 \rangle \tau / 4. \quad (\text{A9b})$$

Equation (A9b) follows by explicit calculation, using Eqs. (A1), (A2) and (A6). Conservation of the total polarization implies that diagonal elements are $W_{jj} = 1 - \sum_{k \neq j} W_{kj}$. The factors of τ^N and $1/\tau$ are included in Eqs. (A8b) and (A9a) to give W_{jk} the units of inverse time, as expected for rate matrix elements.

¹G. E. Pake, *J. Chem. Phys.* **16**, 327 (1948).

²U. Haeberlen, *High Resolution NMR in Solids: Selective Averaging* (Academic, New York, 1976), Vol. Supplement 1, Advances in Magnetic Resonance.

³B. H. Meier and W. L. Earl, *J. Chem. Phys.* **85**, 4905 (1986).

⁴T. G. Oas, R. G. Griffin, and M. H. Levitt, *J. Chem. Phys.* **89**, 692 (1988).

⁵T. Gullion and J. Schaefer, *J. Magn. Reson.* **81**, 196 (1989).

⁶R. Tycko and G. Dabbagh, *Chem. Phys. Lett.* **173**, 461 (1990).

⁷T. Gullion and S. Vega, *Chem. Phys. Lett.* **194**, 423 (1992).

⁸D. M. Gregory, D. J. Mitchell, J. A. Stringer, S. Kiihne, J. C. Shiels, J. Callahan, M. A. Mehta, and G. P. Drobny, *Chem. Phys. Lett.* **246**, 654 (1995).

⁹Y. K. Lee, N. D. Kurur, M. Helmle, O. G. Johannessen, N. C. Nielsen, and M. H. Levitt, *Chem. Phys. Lett.* **242**, 304 (1995).

¹⁰A. E. Bennett, C. M. Rienstra, J. M. Griffiths, W. G. Zhen, P. T. Lansbury, and R. G. Griffin, *J. Chem. Phys.* **108**, 9463 (1998).

¹¹M. Hohwy, H. J. Jakobsen, M. Eden, M. H. Levitt, and N. C. Nielsen, *J. Chem. Phys.* **108**, 2686 (1998).

¹²A. Brinkmann, M. Eden, and M. H. Levitt, *J. Chem. Phys.* **112**, 8539 (2000).

¹³M. Carravetta, M. Eden, X. Zhao, A. Brinkmann, and M. H. Levitt, *Chem. Phys. Lett.* **321**, 205 (2000).

¹⁴Y. Ishii, *J. Chem. Phys.* **114**, 8473 (2001).

¹⁵R. Tycko, *J. Chem. Phys.* **126**, 064506 (2007).

¹⁶A. Grommek, B. H. Meier, and M. Ernst, *Chem. Phys. Lett.* **427**, 404 (2006).

¹⁷M. J. Bayro, M. Huber, R. Ramachandran, T. C. Davenport, B. H. Meier, M. Ernst, and R. G. Griffin, *J. Chem. Phys.* **130**, 114506 (2009).

¹⁸Y. Ishii, J. J. Balbach, and R. Tycko, *Chem. Phys.* **266**, 231 (2001).

¹⁹I. Scholz, M. Huber, T. Manolikas, B. H. Meier, and M. Ernst, *Chem. Phys. Lett.* **460**, 278 (2008).

²⁰M. Weingarth, D. E. Demco, G. Bodenhausen, and P. Tekely, *Chem. Phys. Lett.* **469**, 342 (2009).

²¹G. De Paep, J. R. Lewandowski, A. Loquet, A. Bockmann, and R. G. Griffin, *J. Chem. Phys.* **129**, 245101 (2008).

²²C. R. Morcombe, V. Gaponenko, R. A. Byrd, and K. W. Zilm, *J. Am. Chem. Soc.* **126**, 7196 (2004).

- ²³K. Takegoshi, S. Nakamura, and T. Terao, *Chem. Phys. Lett.* **344**, 631 (2001).
- ²⁴T. Manolikas, T. Herrmann, and B. H. Meier, *J. Am. Chem. Soc.* **130**, 3959 (2008).
- ²⁵F. Castellani, B. J. van Rossum, A. Diehl, K. Rehbein, and H. Oschkinat, *Biochemistry* **42**, 11476 (2003).
- ²⁶K. Takegoshi, K. Nomura, and T. Terao, *J. Magn. Reson.* **127**, 206 (1997).
- ²⁷I. Marin-Montesinos, G. Mollica, M. Carravetta, A. Gansmuller, G. Pilelo, M. Bechmann, A. Sebald, and M. H. Levitt, *Chem. Phys. Lett.* **432**, 572 (2006).
- ²⁸L. A. Straaso, M. Bjerring, N. Khaneja, and N. C. Nielsen, *J. Chem. Phys.* **130**, 225103 (2009).
- ²⁹N. Khaneja and N. C. Nielsen, *J. Chem. Phys.* **128**, 015103 (2008).
- ³⁰K. N. Hu and R. Tycko, *J. Chem. Phys.* **131**, 045101 (2009).
- ³¹A. K. Paravastu and R. Tycko, *J. Chem. Phys.* **124**, 194303 (2006).
- ³²Y. Mou and J. C. C. Chan, *Chem. Phys. Lett.* **419**, 144 (2006).
- ³³K. Nomura, K. Takegoshi, T. Terao, K. Uchida, and M. Kainosho, *J. Biomol. NMR* **17**, 111 (2000).
- ³⁴R. Tycko, *J. Phys. Chem. B* **112**, 6114 (2008).
- ³⁵R. Tycko, *Phys. Rev. Lett.* **99**, 187601 (2007).
- ³⁶L. A. Straaso and N. C. Nielsen, *J. Chem. Phys.* **133**, 064501 (2010).
- ³⁷A. M. Gronenborn, D. R. Filpula, N. Z. Essig, A. Achari, M. Whitlow, P. T. Wingfield, and G. M. Clore, *Science* **253**, 657 (1991).
- ³⁸See supplementary material at <http://dx.doi.org/10.1063/1.4749258> for Figures S1-S5.
- ³⁹A. E. Bennett, C. M. Rienstra, M. Auger, K. V. Lakshmi, and R. G. Griffin, *J. Chem. Phys.* **103**, 6951 (1995).
- ⁴⁰T. Gullion, D. B. Baker, and M. S. Conradi, *J. Magn. Reson.* **89**, 479 (1990).
- ⁴¹F. Delaglio, S. Grzesiek, G. W. Vuister, G. Zhu, J. Pfeifer, and A. Bax, *J. Biomol. NMR* **6**, 277 (1995).
- ⁴²H. L. F. Schmidt, L. J. Sperling, Y. G. Gao, B. J. Wylie, J. M. Boettcher, S. R. Wilson, and C. A. Rienstra, *J. Phys. Chem. B* **111**, 14362 (2007).
- ⁴³W. T. Franks, B. J. Wylie, H. L. F. Schmidt, A. J. Nieuwkoop, R. M. Mayrhofer, G. J. Shah, D. T. Graesser, and C. M. Rienstra, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 4621 (2008).
- ⁴⁴A. J. Nieuwkoop, B. J. Wylie, W. T. Franks, G. J. Shah, and C. M. Rienstra, *J. Chem. Phys.* **131**, 095101 (2009).
- ⁴⁵P. S. Nadaud, I. Sengupta, J. J. Helmus, and C. P. Jaroniec, *J. Biomol. NMR* **51**, 293 (2011).
- ⁴⁶B. J. Wylie, L. J. Sperling, A. J. Nieuwkoop, W. T. Franks, E. Oldfield, and C. M. Rienstra, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 16974 (2011).
- ⁴⁷K. N. Hu, W. Qiang, G. A. Bermejo, C. D. Schwieters, and R. Tycko, *J. Magn. Reson.* **218**, 115 (2012).
- ⁴⁸W. T. Franks, B. J. Wylie, S. A. Stellfox, and C. M. Rienstra, *J. Am. Chem. Soc.* **128**, 3154 (2006).
- ⁴⁹D. M. LeMaster and D. M. Kushlan, *J. Am. Chem. Soc.* **118**, 9255 (1996).
- ⁵⁰M. Hong and K. Jakes, *J. Biomol. NMR* **14**, 71 (1999).
- ⁵¹V. A. Higman, J. Flinders, M. Hiller, S. Jehle, S. Markovic, S. Fiedler, B. J. van Rossum, and H. Oschkinat, *J. Biomol. NMR* **44**, 245 (2009).
- ⁵²H. Van Melckebeke, C. Wasmer, A. Lange, A. B. Eiso, A. Loquet, A. Bockmann, and B. H. Meier, *J. Am. Chem. Soc.* **132**, 13765 (2010).
- ⁵³M. J. Bayro, R. Ramachandran, M. A. Caporini, M. T. Eddy, and R. G. Griffin, *J. Chem. Phys.* **128**, 052321 (2008).
- ⁵⁴R. Tycko and Y. Ishii, *J. Am. Chem. Soc.* **125**, 6606 (2003).
- ⁵⁵A. Lange, K. Seidel, L. Verdier, S. Luca, and M. Baldus, *J. Am. Chem. Soc.* **125**, 12640 (2003).
- ⁵⁶A. Lange, S. Luca, and M. Baldus, *J. Am. Chem. Soc.* **124**, 9704 (2002).