Published in final edited form as:

J Magn Reson. 2018 November ; 296: 130–137. doi:10.1016/j.jmr.2018.09.004.

Refocusing CSA during magic angle spinning rotating-frame relaxation experiments

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Abstract

We examine coherent evolution of spin-locked magnetization during magic-angle spinning (MAS), in the context of relaxation experiments designed to probe chemical exchange (rotating-frame relaxation ($R_{1\rho}$)). Coherent evolution is expected in MAS based rotating-frame relaxation decay experiments if matching conditions are met (such as, $\omega_1 = n\omega_r$) and if the chemical shielding anisotropy (CSA) is substantial. We show here using numerical simulations and experiments that even when such matching requirements are avoided (e.g., $\omega_1 < 0.5\omega_r$, $\sim 1.5\omega_r$, $> 2.5\omega_r$), coherent evolution of spin-locked magnetization with large CSA is still considerable. The coherent evolution has important consequences on the analysis of relaxation decay and the ability to extract accurate information of interest about dynamics. We present a pulse sequence that employs rotary echoes and refocuses CSA contributions, allowing for more sensitive measurement of rotating-frame relaxation with less interference from coherent evolution. In practice, the proposed pulse sequence, REfocused CSA Rotating-frame Relaxation (RECRR) is robust to carrier frequency offset, B_1 -field inhomogeneity, and slight miscalibrations of the refocusing pulses.

Keywords

Chemical kinetics; CSA refocusing; Rotary echo

1. Introduction

NMR studies provide a sensitive means to study the conformational dynamics of biopolymers [1]. Many recent improvements in magic angle spinning based solid-state NMR of proteins have led to the possibility of site-specific information on dynamics for large and insoluble chemical and biochemical systems [2,3]. These experiments are inherently sensitive to conformational exchange processes wherein anisotropic molecular groups undergo large angular reorientations. Methods to probe molecular motions on a microsecond-to-millisecond timescale are particularly relevant to studying critical biological processes such as protein folding and allosteric coupling.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jmr.2018.09.004>.

Spin-lock based experiments to measure the rotating-frame relaxation rate, $R_{1\rho}$, are particularly popular [4–23]. Commonly these experiments employ moderate spinning frequencies (3000 to 25,000 Hz). Although ultrafast magic-angle spinning (MAS), above 60,000 Hz, has become commercially available, and has led to many spectroscopic benefits, the use of moderate spinning frequencies may be driven by practical considerations such as limited access to fast MAS, or limited detection sensitivity. In addition, $R_{1\rho}$ experiments at moderate spinning frequencies are more sensitive to intermediate time scale exchange processes [24]. However, in these experiments magnetization undergoes significant coherent evolution that depends on the details of MAS, the spin-lock field, and chemical shift anisotropy (CSA). As a consequence of the coherent evolution, the magnetization exhibits oscillations [25], with distorted spinning sideband intensities [26], leading to difficulties in the characterization of the motion by analyses of relaxation rates [5]. The effects of the coherent evolution of ^{15}N relaxation when $\omega_r > \delta$, (where δ describes the magnitude of the CSA), is typically not as prominent, which allows studies of ^{15}N relaxation to continue relatively unimpeded in contrast with ^{13}C relaxation for carbons with relatively large CSA. If the relaxation timescale is much longer than the timescale of the coherent oscillations the traditional analysis is reliable; this is the case when the CSA is small and the order parameter is relatively large. Therefore, there is interest in improving the MAS based $R_{1\rho}$ experiment and identifying experimental conditions that allow for sensitive and quantitative studies of dynamics without unwanted coherent evolution.

The pursuit of idealized spin-lock based experiments spans >50 years of literature. Solomon showed that the effect of B_1 -field inhomogeneity during spin-locks could be reduced by inverting the RF phase by 180° at time τ until time 2τ to perform a rotary echo [27], as depicted in Fig. 1b. This method is reminiscent of a Hahn-echo. Wells and Abramson inverted the B_0 -field at time τ and 3τ while continuously applying a B_1 -field to achieve a similar effect [28]. Solomon also demonstrated that the confounding effects of diffusion in liquids during a spin-lock could be reduced by further inverting the spin-lock RF phase at $(2n + 1)\tau$, analogous to the Carr-Purcell experiment [27,29] (Fig. 1c). In static solids, Rhim, Pines, and Waugh showed that the evolution of dipolar-coupled spins could be reversed using a spin-lock with alternating phase [30] (Fig. 1c). They were also able to extend their method to a “rotary solid echo” by inserting two $(\pi/2)$ pulses, reminiscent of a solid-echo or a magic echo. Gan, Grant, and Ernst demonstrated that a rotary echo could also be generated during MAS, by inserting a short delay between two spin-lock periods with opposite phases [25] as shown in Fig. 1d. A spin inversion due to the MAS modulated Hamiltonian is maximized at $2\tau + \frac{\pi}{\omega_1}$, when τ is set to $\tau_r/2n$ and $\omega_1 = n\omega_r$, for $n = 1$ or 2 . In the introduction of rotary resonance echo double resonance (R-REDOR), Gan also introduced a MAS rotary echo experiment (Fig. 1e) that uses a π -pulse between two spin-locking periods spaced by $2k'\tau_r$ [31]. Previously, Reddy and co-workers have incorporated spin echoes into rotating-frame relaxation filtered magnetic resonance imaging experiments to compensate for imperfections in the B_1 - and B_0 -fields [32,33]. These sequences explore useful properties in the spin physics of echoes, and led to exciting applications for dipolar couplings in solids. Here we review the distinct objective of measuring rotating-frame relaxation rates. We conclude that these particular sequences are not optimal because the amplitude of the echo

changes with time during MAS and spin-lock. Furthermore, we show in this paper that at least one related pulse sequence exhibits smooth decay during MAS and spin-lock.

Others have designed or used RF sequences that impose tighter control over which relaxation mechanisms are active during periods of transverse magnetizations. One such example from liquid state NMR is the use of CROP [34] and BB-CROP [35] pulses where optimal control and refocusing methods are used to optimize heteronuclear polarization transfer through J-couplings, by taking advantage of the relaxation mechanism. In MAS experiments, Schanda and co-workers use a ^1H π -pulse in the center of the spin-lock period to refocus the effect of ^{15}N CSA/ ^1H - ^{15}N dipole cross-correlated relaxation [13,15,16]. Lakomek and co-workers use two ^1H π -pulses at τ and 3τ (1/4 and 3/4 of the spin-lock time) for the same purpose [12]. Other groups have utilized spin-lock periods based on Lee-Goldberg type sequences to measure $R_{1\rho}$ experiments while aiming to remove effects of homonuclear couplings and reduce spin diffusion [20,36–39]. Together these experiments lay a strong foundation for controlling coherent and, to an extent, incoherent contributions during a spin-lock.

2. Results and discussion

2.1. Coherent contributions in $R_{1\rho}$ experiments

Coherent evolution is expected in MAS based rotating-frame relaxation decay experiment (Fig. 1a), if matching conditions are met (such as, $\omega_1 = n\omega_r$ [40–43]) and if the chemical shielding interaction is substantial, as was demonstrated previously [5,25,26]. More generally, even when such matching conditions are deliberately avoided ($\omega_1 < 0.5\omega_r$, $\sim 1.5\omega_r$, $> 2.5\omega_r$), the coherent evolution of the magnetization under the influence of the CSA is attenuated but not altogether avoided. As illustrated in Fig. 2a, when the CSA is comparable to the spinning frequency ($\omega_r \sim \delta$), the matching conditions have significant width, and coherent evolution during the experiment has consequences on the underlying analysis of the decay curves. The oscillation is not removed with the inclusion of large amplitude motions, as shown in Fig. 2b. The use of an effective “dead-time” can allow for the oscillations to be effectively ignored, as demonstrated by Krushelnitsky and co-workers [5]; however, this is not without reducing the amount of signal that is available. The coherent evolution has many obvious consequences for experimental decay curves during the spin-lock, including oscillation in the time decay of the signal, and distortions in the intensities and phases of spinning sidebands.

A general analytical description of the combined effects of coherent evolution and conformational dynamics during a spinlock and MAS remains lacking. At the rotary resonance condition ($\omega_1 = n\omega_r$) or very far off the condition, average Hamiltonian theory can be used to describe the coherent spin dynamics. For example, the importance of high-order effects in spin-locks was discussed in a study where average Hamiltonian theory was used to predict isotropic dipolar shifts in the rotating-frame [44]. Although the analysis of the coherent contributions during a general spin-lock pulse that is off (but not far off) the matching conditions has not been reported, closely related work has been published. Also, continuous-wave decoupling during MAS was analyzed using multimodal-Floquet theory [40–43]. These particular studies are not similar enough to our objective to serve as a

predictive tool in identifying improved pulse sequences or interpreting experimental data. Meanwhile, they highlight the complexity of the theory for these experiments. As an alternative to a general analytical description, numerical simulations can also be used to assess the contribution of coherent evolution in these experiments, and to guide the development of improved rotating-frame relaxation pulse sequences. Here we use the *Spinach* libraries for MATLAB [45–48] as a computational tool for numerical simulations and analysis of various rotating-frame relaxation pulse sequences. The use of analytical expressions derived from Redfield's relaxation theory to analyze the simulations demonstrated below is not advised and will yield erroneous results.

2.2. Oscillatory evolution in the rotating-frame relaxation decay curve; undersampling introduces errors in the rotating-frame relaxation decay curve

It is well known from Redfield's relaxation theory [49,50] that magnetization associated with a spin subject to weak and quickly fluctuating random fields decays exponentially. The spatial average for a powdered sample subject to the same fluctuations during MAS decays multi-exponentially [1]. However, the time constants for the two exponentials are often of the same order of magnitude, and the decay can sometimes still be described by a single decay constant, as is commonly performed in analyses of MAS based $R_{1\rho}$ experiments. For simplicity, we continue this tradition and in this study we describe relaxation effects by a simple decaying exponential whose decay constant is a phenomenological metric of the effect of chemical kinetics.

Extraction of the exponential decay constant is complicated by the evolution of the CSA during a traditional rotating-frame relaxation experiment, which is associated with large amplitude oscillations in the decay curves, as illustrated in recent studies [5] and Figs. 2 and 3. The coherent evolution and resulting oscillations in the decay curve have clear disadvantages for probing the relaxation decay times. The decay constant obtained from fitting this oscillatory curve is expected to depend on the coherent evolution as well as the exchange relaxation time. Moreover, because the function is not monotonic, the decay constant derived from the data will be dependent on the choice of experimental time points. Rotating-frame relaxation experiments on biological samples are most conveniently and efficiently performed by probing decays using only a few time points, and with time increments that are long in comparison to the rotor period. In this case, it is difficult to clearly distinguish coherent effects and decay behavior. Fitting such an undersampled oscillating curve with an exponential function results in deviations from the decay obtained by fitting an oversampled curve, and furthermore, the decay values are not stable with variation in the sampling schedule (Fig. 3a and S1). For moderate spinning frequencies, and typical anisotropic interactions strengths in biological solids, the fit values of rotating-frame relaxation decay curves are not stable with variation in the sampling schedule more infrequently than $\sim \tau_r/2$. We illustrate this by choosing several commonly used sampling schedules, sampling at multiples or near multiples of $n\tau_r$; the deviation in the decay constant $R_{1\rho} = (R_{1\rho,dense} - R_{1\rho,sparse})/R_{1\rho,dense}$ can be up to 60%. B_1 -field inhomogeneity is expected to damp coherent oscillations in experimental data [5], but this does not significantly alter the problem of systematic errors in the decay curve that is obtained as the oscillations are not significantly damped near the zero time point where the oscillations are

strongest. The distribution of extracted decay constants with respect to multiple different sampling schedules is shown in Fig. 3c.

These deviations in the fit decay constant have implications whenever these results are used to determine the energetics of the molecular motion that is being modeled. If the numerical simulations of equations that are being used to validate the frequency of the molecular motion do not include these errors, then the propagation of these deviations is carried to the energy values used to describe the motion. This would cause energy landscapes measured in such a way to suffer from large errors due to the underlying solid-state relaxation data that was used.

Moreover, when fitting the rotating-frame relaxation decay to an exponential, $A \exp(-R_{1\rho}t)$, the oscillation results in a loss of amplitude ($A < 1$). If the oscillations are avoided, and the decay is sampled beginning at later times (treating the early points that occur before the oscillations become damped effectively as a “dead-time” for the $R_{1\rho}$ experiment) [5], then sparse sampling schedules can perhaps be used, although the non-monotonic nature of the experimental decay curve yields a different decay constant when the initial portion of the decay is ignored. Moreover, there is a significant loss of data or of signal-to-noise ratio in this way of performing the experiment. When large amplitude motions are present, (i.e., when the order parameter (S^2) is much smaller than 1), much of the signal decays (>70%) during such a “dead-time”. Therefore, using an effective “dead-time” to reduce the effect of the unwanted coherent oscillation on the measured decay will reduce detection sensitivity.

2.3. Spinning sidebands in rotating-frame experiments

The evolution of the CSA during the rotating-frame relaxation experiment yields spectra that have spinning sideband intensities that deviate sharply from those expected from the CSA tensor parameters; in some instances, the sidebands even appear negative in the spectrum (Fig. S2). When fitting the rotating-frame relaxation decay to an exponential, $A \exp(-R_{1\rho}t)$ the integrated intensity of the center band is used, but the intensity of the spinning sidebands is often ignored due to signal-to-noise considerations. This causes a deviation,

$\Delta R_{1\rho}^* = (R_{1\rho, full} - R_{1\rho, center})/R_{1\rho, full}$ in the decay constant as compared to fitting the full magnetization decay (Fig. 3 and S1), in some cases up to 30%. One also has to be careful when comparing numerical simulations, which are often performed using the full spectrum magnetization that includes the spinning sideband intensities, to experimental rotating-frame relaxation decay of only the center bands. While these deviations are most significant when the spinning sidebands are most prominent ($\omega_r < \delta$), the distortions still exist and contribute to differences in the decays for the centerband vs. full spectra when the contribution of the spinning sidebands is apparently small.

2.4. Refocusing pulse sequence for measuring rotating-frame relaxation

The REfocused CSA Rotating-frame Relaxation (RECR) RF sequence shown in Fig. 1f adds two additional π -pulses phase shifted by 180° and also introduces a phase shift in the spin-locking field. By refocusing the CSA during the spin-lock, the stability of the decay curve to the sampling schedule is improved by a factor of approximately four as shown in Fig. 3. Also, the spinning sideband manifold more closely represents the expected CSA

pattern (Fig. S2). Thus the sequence has the advantage that the spinning sidebands can be effectively ignored (or included) for decay curves produced using RECRR with little consequence to the extracted decay constant (Fig. 3b). The distributions of the fit decay constants using the traditional and RECRR sequences (Fig. 3c) demonstrate the significant advantage of the RECRR sequence in terms of reproducing the decay constants independent of sampling schedule.

A model compound without dynamics can clearly show that the coherent contributions are reduced, without the distraction of decay caused by dynamics. Experimentally determined decays of RECRR on crystalline $^{13}\text{C}'$ -labeled L-alanine, shown in Fig. 4a, c, demonstrate that the refocusing of the CSA leads to less decay in the near absence of millisecond to microsecond molecular motions than using just a spin-lock (Fig. 4b, d). This is especially notable near the rotary resonance matching condition (Fig. 4c, d), where the center band signal is almost destroyed in the traditional sequence, but approximately half of the signal is retained with the RECRR sequence. It is essential to know the precise value of the spin-lock field strength to extract kinetic information from the decay curve (although in our experience the coherent contribution is not significantly affected by small deviations in the spinlock field strength). To ensure that our experiments and numerical simulations were matched, we carefully measured and fit nutation profiles near the $0.4\omega_r$ and $0.95\omega_r$ conditions, as shown in Fig. S3. Several other sequences intended to refocus the CSA during the spin-lock of the rotating-frame relaxation experiment were tested by numerical simulations, and some are shown in Fig. S4. These sequences did not perform as well as RECRR and are therefore not discussed further.

Numerical simulations are shown in Fig. 5 (matched to the experimental data in Fig. 4). The differences between the numerical simulations and the experimental data are small. The gray boxes in Figs. 4 and 5 indicate the range of values from the experimental RECRR decay for each condition. The numerically simulated decay curves are strikingly similar to the experimental curves, although for both RECRR and the traditional sequence they display slight amplitude differences and more persistent oscillatory behavior. We presume that this is mainly because the oscillations are damped in the experimental data due to B_1 -field inhomogeneity. The relative intensities of the spinning sidebands are similar comparing the experimental and numerically simulated data (Fig. 4e–h and Fig. 5e–h, noting the first two sidebands in either direction). In the traditional sequence, the simple spin-lock at the $\omega_1 = 0.4\omega_r$ condition, loss of the first set of spinning sidebands is predicted in numerical simulations and shown in experimental data. When $\omega_1 = 0.95\omega_r$, the loss of the center band signal in the traditional sequence is also shown in both the experimental and simulated spectra. Both the simulations and the experiments show that the spectra from the RECRR sequence does not show these losses of signal and instead displays a pattern similar to the $t = 0$ point (black traces). These similarities show the utility of numerical simulations to study the RECRR decay curves. However, comparing the difference in the simulated curves of the $\omega_1 = 0.95\omega_r$ and $\omega_1 = 1.05\omega_r$ condition, shown in Fig. S5, demonstrates that the coherent contributions due to the CSA do not display a very sharp dependence on the spin-lock field. The difference in the scaling factor for the spinning sidebands in the simulated vs experimental data is believed to be due to a combination of inaccurate CSA tensor parameters in the simulations and loss of the spinning sideband signal during the ^1H - ^{13}C CP

period of the experiments [26]. The relatively slow MAS spinning frequency was used to easily demonstrate the effects on the spinning sidebands where there are multiple spinning sidebands with large enough signal-to-noise to compare the shape between the RECRR and traditional experiments.

Near the matching conditions the RECRR sequence demonstrates a significant increase in signal intensity for the centerband. As discussed earlier the near matching (such as, $\omega_1 = n\omega_r$) conditions are particularly attractive for measuring relaxation in the rotating-frame; however, the traditional spin-lock experiment cannot be utilized to measure near the matching conditions due to the coherent contributions (Fig. S6a,b). As is shown in Fig. S6c,d, the RECRR sequence allows for the measurement of the relaxation decay near the matching conditions, where the traditional sequence shows little to no decay and the oscillations yield negative spectra during the decay.

While the advantage that the RECRR sequence presents of refocusing the CSA contributions during the rotating-frame relaxation experiment is significant, the RECRR sequence also displays a robustness to frequency offset, B_1 -field inhomogeneity, and miscalibrations of the refocusing pulses. Figs. S4b and S7 demonstrate the effect frequency offset and miscalibrations of the refocusing pulses, respectively. RECRR is based on the rotary echo sequences (discussed above) that were designed to reduce the influence of B_1 -field inhomogeneity.

2.5. The sensitivity of RECRR to molecular motions

The RECRR and traditional spin-lock experiment demonstrate analogous sensitivity to molecular motions on the timescale ~ 100 Hz to 10 kHz forward chemical exchange rate constant, k_f , as shown in Fig. 6. Despite the fact that the two experiments yield similar sensitivity in this range, the RECRR experiment shows a higher sensitivity when the exchange rate is slow (~ 10 to 100 Hz), yielding an order of magnitude increase in sensitivity for such motions. In addition to the increase in sensitivity for slower exchange rates, the RECRR sequence also has the advantage of greatly reducing the impact of the coherent evolution of the CSA and errors due to the oscillatory nature of the decay with coherent contributions, see above. It is interesting to note that the dependence of the sensitivity with respect to molecular motions of the RECRR sequence changes with spin-lock field strength, e.g., the slope of the magnetization decay constant as a function of the exchange rate constant curves changes with difference in spinlock field strength, as shown in Fig. 6. This is in contrast to the traditional spin-lock sequence, which just displays curves that have the maximum translated to different values of magnetization decay constant and exchange rate constant. Additionally, the powder average is performed here by averaging the decay constants from separate exponential fits of each crystallite. The exponentially fit decay constants from Fig. 3 were determined by fitting after powder average, which yields a similar result for the hopping frequency used in those simulations [24,51].

The amplitudes from the exponential fit are shown in the insets, showing the effect of the coherent contributions on the loss of signal in the traditional and RECRR sequences. An ideal pulse sequence would be characterized by a constant amplitude of 1; therefore deviations from a constant amplitude of unity are likely due to coherent contributions from

the CSA. Much of the improvement in the amplitude when using RECRR away from the match conditions is due to the spinning sideband intensity, as is shown in Figs. 4 and 5. Thus, the improved amplitude, far from the match conditions, shown in the simulations in Fig. 6, might not always translate directly to more detection sensitivity.

While increasing the MAS frequency reduces the effect of the coherent contributions, RECRR outperforms the traditional sequence even when spinning at 60 kHz concerning sensitivity to the underlying molecular motions. The vertical dashed lines in Fig. 6 help illustrate the improvement in sensitivity for RECRR in comparison to the traditional sequence. The improvement of the RECRR sequence is particularly considerable for conditions where the spin-lock frequency is lower, e.g., the $\omega_1 = 0.4$ and $1.5\omega_r$ conditions, the black and red curves in Fig. 6. In the range of practical measurability ($R_{1\rho} = 10$ Hz to 1 kHz), indicated as between the two horizontal dashed lines in Fig. 6, there is no discernable difference between the curves of the traditional sequence and RECRR at high spin-lock frequency. Therefore, the advantages of the RECRR sequence are more notable for low spin-lock field strengths and near the matching conditions.

3. Experimental

3.1. Numerical simulations

Numerical simulations were implemented using the *Spinach* libraries for MATLAB [45–48]. The ^{13}C single spin simulations of the sequences without molecular motions were performed with $\omega_{\text{OH}}/2\pi = 750$ or 900 MHz for ^1H , and using the chemical shift tensor parameters of the carbonyl carbon ($\delta = 80$ ppm, $\eta = 0.45$, and $\delta_{\text{iso}} = 180.96$ ppm) calculated using Gaussian 09 [52] and given in the *Spinach* libraries. The MAS frequency was 9360 Hz, and the spin-lock field strengths were 0.4 and $0.95\omega_r$ to match the experimental data. The ^{13}C two-site exchange simulations were performed with $\omega_{\text{OH}}/2\pi = 750$ MHz and chemical shift tensor parameters, $\delta = -120$ ppm, $\eta = 0.85$, and $\delta_{\text{iso}} = 135.1$ and 131.5 ppm, irradiation at 133.3 ppm. A single ^{13}C CSA tensor was changed to perform these simulations and therefore at any one moment in time during the simulations only one spin was present in the simulations. The MAS frequency was 16 kHz, corresponding to 85 ppm for ^{13}C , and 60 kHz depending on the simulation. The spin system that was used as a model for the numerical simulations with molecular motions was the delta carbons of the ring flip in phenylalanine, with $\alpha = 26^\circ$ and $\beta = 180^\circ$, and the forward exchange rate was 5000 s^{-1} for single exchange simulations. Example *Spinach* simulations can be found in the Supporting Information.

3.2. NMR experiments

NMR experiments were performed using a Bruker Avance II spectrometer operating at 21.1 T ($\omega_{\text{OH}}/2\pi = 900$ MHz). The spinning frequency, $\omega_r/2\pi$, was set to 9360 ± 2 Hz, this specific condition was used to avoid the $k = 2$ rotational resonance condition between the C' and $\text{C}\alpha$ carbon and for comparison with lower field data. The spin-lock field strengths were set near the 0.4 and $0.95\omega_r$ conditions with the exact value determined by fit nutation curves, as shown in Fig. S3. Data were processed in Topspin 3.6 and analyzed and plotted using the nmrglue [53] and matplotlib library [54].

4. Conclusions

Coherent evolution during the measurement of rotating-frame relaxation can cause strong oscillations in the decay curves. As a consequence of these coherent contributions, the decay constant obtained from fits of such experiments is unstable with respect to the choice of sampling schedule. The spinning sidebands of the spectra are distorted, particularly when $\omega_r < \delta$ and $\omega_1 < 2\omega_r$, and therefore the decay constant extracted from decays of the full spectrum magnetization deviates from the decay constant determined for just the center band. We demonstrate a pulse sequence, RECRR, to refocus the CSA contributions during the measurement of rotating-frame relaxation allowing for sensitive measurement of molecular motions without strong coherent evolution. Using numerical simulations and experimental results on a model compound, $^{13}\text{C}'$ -labeled L-alanine, the advantages of the RECRR sequence over the traditional sequence are demonstrated. Additional numerical simulations including molecular motions demonstrate the advantages of using the RECRR sequence over the traditional sequence to measure relaxation in the rotating-frame. The RECRR sequence was also found to be robust to frequency offset, B_1 -field inhomogeneity, and small miscalibrations of the refocusing pulses, allowing these measurements to be performed with relative ease. These advances are most advantageous for measuring rotating-frame relaxation rate at sites with relatively large chemical shift anisotropy, such as at the carbonyls in protein backbones in solid-state samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dr. Ilya Kuprov for helpful discussion of the Spinach simulation code. The authors thank Dr. Arthur Palmer for discussion of the solution NMR pulse sequences. The NMR data was collected at the New York Structural Biology Center (NYSBC) with support from the Center on Macromolecular Dynamics by NMR Spectroscopy (CoMD/NMR) a Biomedical Technology Research Resource (BTRR) supported by U.S. National Institutes of Health (NIH) through grant number: P41 GM118302. The NYSBC is also enabled by a grant from the Empire State Division of Science Technology and Innovation and Office of Research Infrastructure Programs/NIH Facility Improvement Grant CO6RR015495. A.E.M. is a member of the NYSBC. This work was supported by NIH Grant R01 GM088724 (to A.E.M).

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Fig. 1.

(a) The traditional sequence for measuring relaxation in the rotating-frame, (b–e) spin-lock sequences that were designed to offer more ideal properties: (b) Solomon rotary echo, (c) Carr-Purcell inspired rotary echo, (d) Gan, Grant, and Ernst MAS rotary echo, (e) R-REDOR echo. (f) The sequence used in this work, RECRR (REfocused CSA Rotating-frame Relaxation). Sections of each sequence with inverted pulse phases are shown as gray.

Fig. 2.

Numerically simulated decays for traditional rotating-frame relaxation sequence of ^{13}C with $\delta_{\text{CSA}} \sim \omega_r$ at three different spin-lock field strengths (a) without and (b) with molecular motions (described in experimental section). The oscillations are characteristic of coherent effects and are present regardless of the presence of molecular motions. The gray dashed lines indicate the extent of decay of the signal at the $t = 1$ ms point of the curves with molecular motions, to more clearly distinguish the two curves. Simulation parameters: $\gamma B_0/2\pi = 750$ MHz, CS: $\delta = -120$ ppm (22.5 kHz), $\eta = 0.85$, and $\delta_{\text{iso}} = 135.1$ and 131.5 (only motion) ppm, irradiation at 133.3 ppm. $\omega_r/2\pi = 16$ kHz (85 ppm for ^{13}C), and assuming a ring flip $k_f = 5$ kHz. The curves are normalized such that the $t = 0$ point is unity.

Fig. 3.

Simulated decay rates for the (a) traditional and (b) RECRR sequences (left) with fitted curves for various sampling schedules indicated. (Right) The bar charts demonstrate (top) the decay constant from a fit ($A \exp(-R_{1\rho}t)$), (middle) the deviation from the constant derived from the densest sampling schedule ($R_{1\rho}$), and (bottom) the deviation of the center band decay constant ($\Delta R_{1\rho}^*$) from the full spectrum magnetization decay (see Fig. S1 for more detail). (c) Histograms of the fit decay constant ($R_{1\rho}$) for the (left, gray) traditional and (right, red) RECRR sequences for various sampling schedules; the dashed vertical line indicates the decay constant for the densest sampling schedule. The decay constant, $R_{1\rho}$, was determined by fitting the powder average to a single exponential. Simulation parameters: $\gamma B_0/2\pi = 750$ MHz, CS: $\delta = -120$ ppm, $\eta = 0.85$, and $\delta_{\text{iso}} = 135.1$ and 131.5 ppm, irradiation at 133.3 ppm. $\omega_r/2\pi = 16$ kHz (85 ppm for ^{13}C), $\omega_1 = 0.4375\omega_r$, and assuming ring flip $k_f = 5$ kHz. The curves are normalized such that the $t = 0$ point is unity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4.

(a–d) Experimental ^{13}C $R_{1\rho}$ decays of $^{13}\text{C}'$ labeled L-alanine using RECRR (left) and traditional (right) rotating-frame relaxation sequences, showing the decay for the full spectrum (black) and only the center band (red). (e–h) Experimental ^{13}C spectra of the center band and sidebands of the RECRR and traditional experiments at various time points (black, $t = 0$ ms; colors, $t > 0$ ms). Experimental parameters: $\gamma B_0/2\pi = 900$ MHz, $\omega_r/2\pi = 9.36$ kHz, and irradiation at 179 ppm. The data was collected following ^{13}C (^1H) CP. The curves are normalized such that the $t = 0$ point is unity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5.

(a–d) Numerically simulated ^{13}C $R_{1\rho}$ decays of $^{13}\text{C}'$ labeled L -alanine using RECRR (left) and traditional (right) sequences demonstrating the decay of the full spectrum (black) and just the center band (red). (e–h) Simulated ^{13}C spectra of the center band and sidebands of the RECRR and traditional experiments at various time points (black, $t = 0$ ms; colors, $t > 0$ ms). Simulation parameters: $\gamma B_0/2\pi = 900$ MHz, CS: $\delta = 80$ ppm, $\eta = 0.45$, and $\delta_{\text{iso}} = 180.96$ ppm, $\omega_r/2\pi = 9.36$ kHz, and irradiation at 180.96 ppm. The curves are normalized such that the $t = 0$ point is unity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 6.

Sensitivity of relaxation rate to motion rate (described in experimental sections) for various applied field strengths, $\omega_1/2\pi$, for the (a, c) traditional spinlock, and (b, d) RECRR sequences at two different MAS spinning frequencies ($\omega_r/2\pi = 16$ kHz, top and $\omega_r/2\pi = 60$ kHz, bottom). Horizontal dashed lines indicate the limits of practical experimental measurements, and the vertical dashed lines indicate the slow-motion limit of the traditional sequence for detection. While both sequences are sensitive to exchange processes, at moderate spinning frequencies the refocused sequence is sensitive to motion over ~ 1 additional order of magnitude of flipping rate, vertical dashed line. The dispersion (ω_1 dependence of $R_{1\rho}$) would reveal k_f , though dispersion is more powerful for the refocused sequence. Notably, zero time asymptotes A (plotted in inset) are closer to an ideal value of 1.0 in the refocused sequence. The decay constant, $R_{1\rho}$, was determined by fitting each crystallite to a single exponential and determining the average. Simulation parameters: $\gamma B_0/2\pi = 750$ MHz, CS: $\delta = -120$ ppm, $\eta = 0.85$, and $\delta_{\text{iso}} = 135.1$ and 131.5 ppm, irradiation at 133.3 ppm. $\omega_r/2\pi = 16$ kHz (85 ppm for ^{13}C) and 60 kHz. The conditions that were used for the simulations were $\omega_1 = 0.4375\omega_r$, $\omega_1 = 1.5\omega_r$, $\omega_1 = 2.5625\omega_r$, $\omega_1 = 3.625\omega_r$, and $\omega_1 = 4.375\omega_r$.