Measurement of $^{13}$C–$^{15}$N Distances in Uniformly $^{13}$C Labeled Biomolecules: J-Decoupled REDOR

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Measurement of distances between pairs of heteronuclei is important for constraining the conformation of biomolecules in the solid state. In particular, rotational-echo double-resonance (REDOR) has been extensively applied to problems of biological interest. Most of these experiments have been performed on isolated heteronuclear spin pairs, where the interpretation of experimental results is relatively straightforward. It is preferable to perform REDOR in multi-spin systems, because of the possibility of measuring several distances in a single sample. However, in REDOR experiments on multiply $^{13}$C labeled samples the dephasing profiles are complicated by additional interactions. While placing all REDOR dephasing pulses on the $^{15}$N channel avoids the recoupling of $^{13}$C spins by the $\pi$-pulse train, the homonuclear $^{13}$C–$^{13}$C dipole and $J$ couplings can still contribute to the dephasing of $^{13}$C coherences. Here we discuss an approach to $^{13}$C observe REDOR, which relies on the selective excitation of the $^{13}$C spectrum that removes the coherent evolution of the spin system under homonuclear $^{13}$C–$^{13}$C $J$ couplings.

Several recently proposed techniques address the problem of heteronuclear distance measurements in spin systems consisting of multiple $^{13}$C and $^{15}$N nuclei. The multiple pulse decoupled REDOR sequence was designed to attenuate the effects of residual homonuclear $^{13}$C–$^{13}$C dipole couplings on the dephasing of $^{13}$C coherences. However, it does not explicitly account for $^{13}$C–$^{13}$C $J$ couplings, which compromise the accurate measurement of weak heteronuclear dipole couplings. In peptides, $J$ couplings between directly bonded $^{13}$C nuclei are ~30–60 Hz, and the most informative $^{13}$C–$^{13}$C dipolar couplings are often of similar magnitude (e.g., 25 Hz for a 5.0 Å C–N distance).

For a spin system consisting of $n$ $^{13}$C nuclei and a single $^{15}$N spin, the effective Hamiltonian for the REDOR pulse sequence (Figure 1a) in the rapid spinning regime is:

$$H = \sum_{i=1}^{n} \Phi_{CN} \cos(2\pi_{i}^{N}C_{i}^{N}N_{i}) + \sum_{i<j} \pi J_{i}^{C_{i}}C_{j}^{C_{j}}$$

Here $J_{CC}$ is the $C_{i}$–$C_{j}$ scalar coupling constant, $\Phi_{CN} = \cos(2\pi_{i}^{N}C_{i}^{N}N_{i})$.

Figure 1. Pulse sequences for conventional REDOR (a) and J-decoupled REDOR (b). The $^{15}$N pulse length was 10 $\mu$s and the pulses were phased according to the xy-16 scheme. For the sequence in part a, the $^{13}$C $\pi$ pulse length was 10 $\mu$s, and for experiments on uniformly $^{13}$C labeled samples, the coherence filter ($\pi/2$–$z$–$z$–$\pi/2$) was inserted prior to signal acquisition. For J-decoupled REDOR (b) the Gaussian $\pi$ pulse parameters were $\tau_{cusp} = 0.6$ ms, 64 increments, and 5% truncation. $\pi$ $^{1}$H decoupling at 100 kHz was applied during the evolution period, and 83 kHz TPPM was used during acquisition. The phase cycle used, $\phi_{cusp} = \pi/2$ and $\phi_{cusp} = \pi/4$, ensures that the only $^{13}$C spins contributing to the observable signal are those inverted by the selective pulse.

$-\sqrt{2}b_{CN} \sin(2\beta) \sin(\gamma)$ is the effective $C_{i}$–$N$ dipolar coupling, with the constant coupling $b_{CN} \propto r_{CN}^{-3}$ ($r_{CN}$ is the internuclear distance), and the Euler angles $\beta$ and $\gamma$ relate the principal axis system of the interaction to the rotor-fixed reference frame. Equation 1 assumes that (i) $J$ couplings can be treated in the weak coupling limit and (ii) coherent evolution of $^{13}$C signals under ~2 kHz dipolar couplings between directly bonded $^{13}$C nuclei is refocused by rapid spinning ($\omega/2\pi \sim 10$ kHz) for integer multiples of the rotor period. For a coupled three-spin system ($C_{1}–C_{2}–N$) the initial density operator $\rho(0) = C_{1} + C_{2} \nu$ evolves under the effective Hamiltonian into observable coherences $C_{1} \nu \nu \nu \cos(\pi J_{1}C_{1}^{C_{1}}C_{2}^{C_{2}})$ and $C_{2} \nu \nu \nu \cos(\pi J_{1}C_{1}^{C_{1}}C_{2}^{C_{2}})$, and antiphase coherences $2C_{1}C_{2} \nu \nu \nu \sin(\pi J_{1}C_{1}^{C_{1}}C_{2}^{C_{2}})$ and $2C_{1}C_{2} \nu \nu \nu \sin(\pi J_{1}C_{1}^{C_{1}}C_{2}^{C_{2}})$. The antiphase coherences can evolve into observable magnetization under $J_{CE}$ during the detection period, leading to phase-twisted spectra (Figure 2a). This problem can be overcome by filtering the coherence prior to detection (see Figure 1 caption). Figure 2a shows slices from spin–echo experiments for [1,2-13C,15N]glycine for 8.4 ms of $J_{CE}$ evolution acquired with and without the coherence filter. The spectrum obtained with a simple spin–echo experiment displays phase-twisted line shapes. In contrast, the spin–echo experiment followed by the coherence filter results in purely absorptive signals.

The problem of coherent evolution under $^{13}$C–$^{13}$C $J$ couplings during dipolar dephasing ($S_{x}$) and reference ($S_{y}$) experiments is addressed by replacing the hard $\pi$ pulse with a rotor-synchronized, frequency-selective Gaussian $\pi$ pulse applied to one $^{13}$C spin (Figure 1b). For all evolution times the dipole interaction between this $^{13}$C and the $^{15}$N spin is retained as in conventional REDOR, while the $J$ couplings to the remaining $^{13}$C nuclei are refocused (the signs of all spin terms having the form $2C_{i}C_{j}; j = 1, 2, ..., n \neq i$, are reversed following the selective inversion of the $C_{i}$ spin). This type of homonuclear $J$-decoupling was used previously to enhance resolution in two-dimensional solution spectra and solids-state spectra. With the assumption that the selective pulse on

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C_i can be treated as an ideal pulse, the effective Hamiltonian for the pulse sequence in Figure 1b is given by the two-spin REDOR Hamiltonian, $H = \Phi_{C_N} 2J_{CN}$, and the observable signal averaged over the crystallite ensemble is then $s(t) = \int \bar{S}/S_0 = \Delta S_\text{S} \delta(t)$. Since the selective pulse removes all $^{13}$C resonances, it is a constant-time element present in all $S_0$ and $S_i$ experiments and any effects due to the pulse can be taken into account by calculating $S_0 = S_i/S_0 = \Delta S_\text{S} \delta(t)$. For $[1,2-^{13}$C,$^{15}$N]$\text{glycine}$ at $\omega_0/2\pi = 10.0$ kHz and $500$ MHz $^1$Hz frequency (data not shown), $S_0$ and $S_i$ curves experience only an overall scaling ($\sim 25\%$ loss in signal intensity) for $0.6$ ms Gaussian pulse relative to a hard pulse.

Figure 2b compares $S_0$ curves for $[1,2-^{13}$C,$^{15}$N]$\text{glycine}$ and $[1,2-^{13}$C,$^{15}$N]$\text{threonine}$ obtained with conventional and J-decoupled REDOR. However, for multiply $^{13}$C labeled systems the quality of experimental data is compromised due to multiple zero-crossings and low signal intensities in $S$ and $S_0$ curves. This is demonstrated in Figure 3 for the case of $\sim 50$ Hz $C^\text{a}$-$N$ dipole coupling in $[U-^{13}$C,$^{15}$N]$\text{threonine}$, where $\Delta S_\text{S}$ curves obtained with conventional and J-decoupled REDOR are compared. Accurate determination of weak couplings requires evolution times on the order of $30$ ms. However in the presence of homonuclear $J$ couplings, conventional REDOR $\Delta S_\text{S}$ curves can account for $J$ coupling effects only up to $\sim 10$ ms (depending on the exact value of $J_{CJ}$). On the other hand, J-decoupled REDOR has the ability to provide useful experimental data for the entire evolution period because the $S_0$ curve for $[1-^{13}$C,$^{15}$N]$\text{glycine}$ ($\bullet$). All experiments were performed at $500$ MHz $^1$Hz frequency and $\omega_0/2\pi = 10.0$ kHz ($\pm 5.0$ Hz).

**Figure 2.** Comparison of conventional REDOR and J-decoupled REDOR for samples with single and multiple $^{13}$C labels. (a) Slices through $S_0$ curves acquired for $[1,2-^{13}$C,$^{15}$N]$\text{glycine}$ (only $C^\text{a}$ resonance is shown) with the following pulse sequences on $^{13}$C: (i) CP-$\pi-\pi-\pi$-acquire; (ii) CP-$\pi-\pi-\pi$-coherence filter- acquire; (iii) CP-$\pi-\pi$ (Gauss)-$\pi$-acquire; $r = 4.2$ ms. (b) $S_0$ curves for the $C^\text{a}$ resonance in $[1,2-^{13}$C,$^{15}$N]$\text{glycine}$ ($\Phi = $ conventional REDOR; $\square = $ J-decoupled REDOR) and the $C^\text{a}$ resonance in $[U-^{13}$C,$^{15}$N]$\text{threonine}$ ($\Phi =$ conventional REDOR; $\bigcirc =$ J-decoupled REDOR). Also shown is the $S_0$ curve for $[1-^{13}$C,$^{15}$N]$\text{glycine}$ ($\bigcirc$). (c) $\Delta S_\text{S}$/$S_0$ curves obtained with conventional REDOR for $C^\text{a}$ resonance in $[1-^{13}$C,$^{15}$N]$\text{glycine}$ ($\bigcirc$) and J-decoupled REDOR in $[1,2-^{13}$C,$^{15}$N]$\text{glycine}$ ($\bullet$). All experiments were performed at $500$ MHz $^1$Hz frequency and $\omega_0/2\pi = 10.0$ kHz ($\pm 5.0$ Hz).

**Figure 3.** $\Delta S_\text{S}/S_0$ curves for the $C^\text{a}$ resonance in $[U-^{13}$C,$^{15}$N]$\text{threonine}$ obtained with conventional REDOR ($\bigcirc$) and J-decoupled REDOR ($\bullet$). $\Delta S_\text{S}/S_0$ curve ($\bigcirc$) simulated according to the analytical expression (see text) for the $C^\text{a}$-$N$ coupling of $54$ Hz obtained from the neutron diffraction structure and curves ($\bigcirc$) for $44$ and $64$ Hz couplings are shown for comparison.

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