

REDOR 3D: Heteronuclear Distance Measurements in Uniformly Labeled and Natural Abundance Solids

Carl A. Michal and Lynn W. Jelinski*

Center for Advanced Technology in Biotechnology
Cornell University, Ithaca, New York 14853

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One of the most powerful capabilities of solid-state NMR is the measurement of internuclear distances from dipolar couplings in solids. The spin-echo double resonance (SEDOR)¹ experiment demonstrated the principles by which small dipolar couplings could be accurately measured in the presence of other, stronger interactions. With the advent of routine magic-angle spinning to enhance sensitivity and average chemical shift anisotropies, an analogous technique, rotational-echo double resonance (REDOR),^{2,3} was developed for measuring weak heteronuclear dipolar couplings in rotating solids. This technique has now been applied to a variety of systems^{4–7} and has spawned a plethora of related experiments including TEDOR,⁸ TRAPDOR,⁹ and REAPDOR.¹⁰ A similar variety of experiments exists for measuring homonuclear couplings including DRAMA¹¹ and CEDRA¹² along with the polarization transfer experiments MELODRAMA¹³ and rotational resonance.^{14,15}

Although each of these experiments has its own strengths, they share the common limitation that all quantitative heteronuclear distance measurements rely on the presence of isolated spin pairs. In this work, we present a new experiment which combines the resolution enhancement of a second chemical shift dimension with the quantitative distance measurement capability of REDOR. Although it bears a superficial resemblance to a version of TEDOR first performed by Fyfe et al.¹⁶ and further explored by van Eck and Veeman,¹⁷ this new experiment has the advantage of providing quantitative distance results, even in cases of complicated spin groupings. There is also some similarity to double TEDOR,¹⁸ in which DANTE inversions are used for *S*-spin chemical-shift specificity. In this paper, we demonstrate the experiment with the model compound L-histidine-U-¹³C₆-U-¹⁵N₃·HCl·H₂O. From a single 3D data set, all

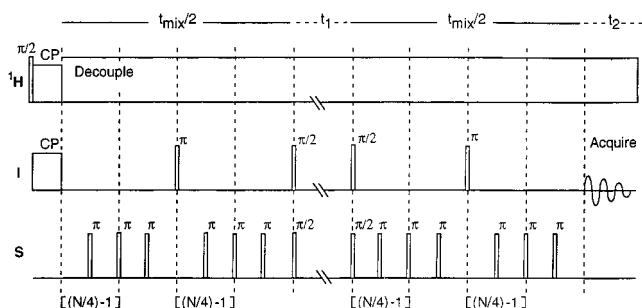


Figure 1. REDOR 3D pulse sequence for heteronuclear dipolar correlation distance measurements. The vertical dashed lines indicate the completion of a rotor cycle.

10 distances are $< 3 \text{ \AA}$ are successfully extracted and found to agree with the neutron diffraction structure.

The REDOR 3D pulse sequence (Figure 1) consists of two identical REDOR “mixing” periods separated by a t_1 evolution period which begins and ends with $\pi/2$ pulses on both *I* and *S* spins. The first REDOR portion generates coherence proportional to $I_y S_z$ from the initial I_x term. The $\pi/2$ pulse preceding the t_1 period transforms this coherence to $I_z S_x$, which then evolves under the *S* spin chemical shift. The final $\pi/2$ pulses select one component from the *S* spin x – y plane and convert it back to $I_y S_z$, which the final REDOR period reconverts into observable I_x . Extraneous terms in the density matrix are eliminated with appropriate phase cycling. High-power proton decoupling is applied throughout the mixing, evolution, and detection periods. *I* spin chemical shifts are detected in the direct (t_2) dimension, *S* spin chemical shifts are encoded in the t_1 period, and dipolar couplings may be extracted from the universal curves produced by plotting cross-peak intensities versus total mixing time. The 2D planes are collected phase-sensitively and are processed accordingly.

Peak amplitudes are corrected for T_2 effects by multiplying by $\exp(-t_{\text{mix}}/T_2)$ where t_{mix} is the total REDOR mixing time. Appropriate T_2 values are determined from *I*-spin spin-echo spectra.

¹³C–¹⁵N correlation spectra of L-histidine with four and 20 rotor cycles total mixing time, along with the ¹³C 1D spectrum are displayed in Figure 2. Because the spectral width in the indirect dimension is the same as the rotor frequency, the N1 peak is folded back into the spectral window.

The four-cycle plane clearly shows all one-bond correlations, while longer range couplings (e.g., the two-bond C1–N1 cross peak) are visible in the 20-cycle plane. Internuclear distances are extracted from T_2 -corrected peak integrals as a function of mixing time by nonlinear least-squares fitting to REDOR difference curves. The fitting program is aided by the fast algorithm for calculating REDOR universal curves by Mueller.^{19,20} The distances are extracted here with no special consideration for the multiple couplings which act simultaneously.

The correlation of the extracted distances with those expected from the neutron diffraction structure²¹ (Figure 3C) is remarkable. This sample is a network of nine directly bonded dipole moments, and all distances which could be faithfully represented ($w_D t_{\text{mix}} \geq 2$) in the data set are found to be in reasonable agreement with the expected values. In the case of an IS_1S_2 grouping, standard REDOR tends to reveal only the stronger I – S_n coupling if the two differ substantially. The 2D correlation version of TEDOR explored by van Eck and Veeman¹⁷ has

* To whom correspondence should be addressed.

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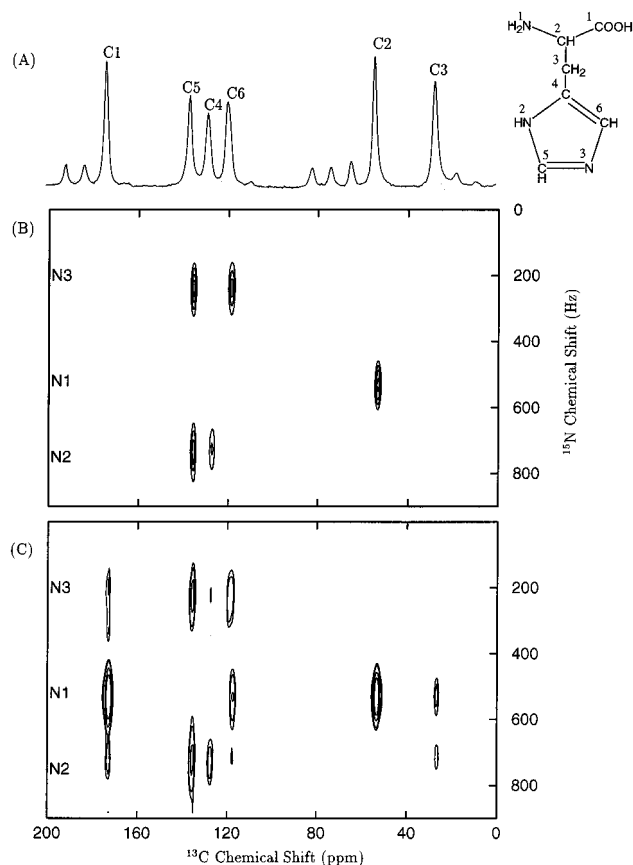


Figure 2. Spectra of L-histidine- $U\text{-}^{13}\text{C}_6\text{-}U\text{-}^{15}\text{N}_3\cdot\text{HCl}\cdot\text{H}_2\text{O}$ (Cambridge Isotopes Labs, Andover, MA) acquired on a home-built spectrometer with a Doty Scientific narrow bore triple-channel probe at a proton resonance frequency of 360.16 MHz. A total of eight planes of 32 t_1 points each were collected in a total acquisition time of 7 h: (A) 1D ^{13}C spectrum; (B) 2D $^{13}\text{C}\text{-}^{15}\text{N}$ correlation spectrum with four rotor cycle REDOR mixing period; (C) as in B with 20-cycle mixing period.

much more difficulty with this situation, as magnetization is "leaked" away from the transfer by the multiple couplings. In fact, the TEDOR version has difficulties with any spin grouping larger than pairs because of this problem. In this experiment, however, simulations reveal only minimal effects of multiple couplings. This suggests that the experiment may be compatible with distance extractions through transform operations.^{4,20}

To evaluate the effects of multiple couplings, simulations of three-spin (IS_1S_2) systems were performed. The simulations are based on numerical integration of the exact expressions for the density matrix at $t_2 = 0$. The calculated dephasing curve for the $I\text{-}S_1$ cross-peak thus depends on the magnitudes of both the $I\text{-}S_1$ and $I\text{-}S_2$ couplings as well as on the angle between the two internuclear vectors. Possible effects of chemical shift tensors were ignored. Each of the simulations includes an overall scaling parameter used to best fit the experimental data. The simulations (Figure 3A,B) indicate that distance extractions using two-spin universal REDOR curves give adequate results as long as data are recorded such that $w_{IS}t_{\text{mix}} \geq 2$. There is some distortion from the ideal two-spin curves, but for the case

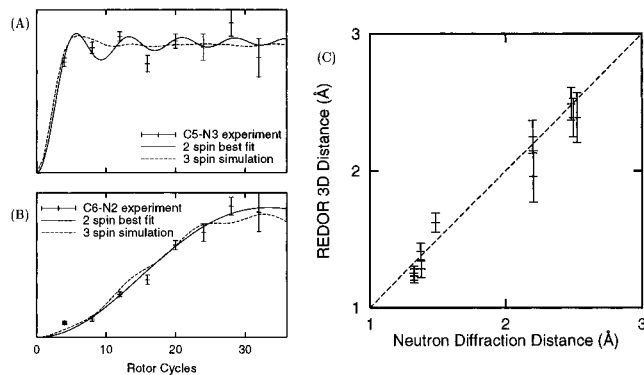


Figure 3. REDOR 3D distance extractions. Parts A and B compare cross peak intensities to three-spin simulations made using neutron diffraction geometry of (C5, N2, N3) and (C6, N2, N3), best fits calculated assuming isolated spin pairs. C shows the correlation of measured distances with neutron diffraction structure. Error bars are derived from the two-spin universal curve fitting routine and do not include effects of multiple couplings.

of a ^{13}C nucleus with two directly bonded ^{15}N neighbors, this distortion leads to an apparent distance of 1.24 Å compared to the expected 1.33 Å. These distortions are comparable to those observed in standard REDOR with multiple couplings, where the largest effect is from the nearest neighbor. The advantage here is that the separation by S chemical shift unmasks the weaker couplings. Further simulations reveal the following general trends: in cases where the two dipolar couplings differ substantially in magnitude, the buildup curve corresponding to the weaker coupling is faithfully represented, while that of the stronger coupling is distorted on a time scale set by the buildup of the weaker coupling. If the two couplings are of similar magnitude, the distortion depends in detail upon the geometry. In all cases, the initial buildup and first-maximum appear to provide a good estimate of the actual coupling.

REDOR 3D was applied to *O*-phospho-L-tyrosine to test its utility in improving resolution and distances in unlabeled systems. Although there is substantial overlap in the 1D ^{13}C spectrum, a single plane obtained with 16 t_1 steps and eight rotor cycles total mixing time (data not shown) displays 12 resolved peaks from six carbon sites in the two inequivalent molecules per unit cell.

In summary, REDOR 3D shows great promise in increasing the information content of solid-state NMR structural studies. Where previous methods limited the number of resolvable distances and required isolated spin pairs, this method appears to provide quantitative distances even with complicated spin groupings. Further simulations are necessary to fully understand the effects of such groupings, but initial results on three-spin groups are favorable. These results suggest that REDOR 3D will be useful in the study of a wide range of systems, including protein-membrane and drug-receptor interactions with a wide variety of labeling strategies.

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