

Measuring multiple carbon–nitrogen distances in natural abundant solids using R-RESPDOR NMR†

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Multiple carbon–nitrogen distances can be measured in natural abundant solids using Rotary Resonance Echo Saturation Pulse Double Resonance (R-RESPDOR) solid state NMR experiment.

The capability of measuring internuclear distances makes solid-state NMR a powerful method for structural characterization in both ordered and disordered states. The heteronuclear distance measurement was demonstrated in the early days by spin-echo double resonance which measures weak dipolar couplings selectively in the presence of other large spin interactions.¹ The principle has been extended to rotational echo double resonance (REDOR) under magic-angle spinning (MAS).² Enhanced sensitivity and resolution of MAS has made REDOR a robust method widely used for structural determination of complex systems such as protein, amyloid fiber, cell wall, glass, zeolite *etc.* The REDOR distance measurement has also been extended to quadrupolar spin systems with TRAPDOR,³ REAPDOR,⁴ DEAR,⁵ and RIDER.⁶

This Communication presents a robust solid state NMR method for simultaneously measuring multiple ¹³C/¹⁴N distances in natural abundant solids. ¹⁴N is a highly abundant (99.6%) spin-1 quadrupolar nucleus. Its distances with surrounding carbons provide useful information for molecular structure and spectral assignment. At ~1% ¹³C natural abundance, many ¹³C/¹⁴N pairs can be treated as isolated spin systems allowing simultaneous measurement of multiple ¹³C/¹⁴N distances without complications from ¹³C homonuclear dipolar coupling that often occur in uniformly ¹³C-labelled molecules. The rotary resonance echo saturation pulse double resonance (R-RESPDOR) method described in this Communication introduces two important changes to the rotational echo adiabatic passage double resonance (REAPDOR) previously developed for measuring distances between a spin-1/2 and a quadrupolar spin.⁴ First, REAPDOR applies a multiple-pulse recoupling sequence to the observed ¹³C spin and it requires an extremely stable MAS frequency (~0.1 Hz).^{6–8} R-RESPDOR uses the rotary resonance for dipolar recoupling, an analog of the rotary resonance recoupling^{9,10} by switching the recoupling rf to the observed ¹³C spin.^{11,12} The cw recoupling scheme is susceptible to spinning frequency fluctuation therefore applicable with most commercial NMR spectrometers. Second, R-RESPDOR uses ¹⁴N saturation instead of

adiabatic-passage pulse for measuring ¹³C/¹⁴N dipolar dephasing. A typical ¹⁴N adiabatic-passage pulse is restricted to about $\tau_r/3$ and requires a large adiabaticity parameter $\alpha = v_1^2/(v_d v_r) > 0.25$ for efficient dipolar dephasing and data interpretation using a semi-empirical universal curve.^{8,13–15} This adiabatic condition may not be met under fast MAS with large ¹⁴N quadrupolar couplings and insufficient rf field for the low- γ nucleus. In contrast, ¹⁴N saturation can be implemented easily with long enough pulses. The saturation not only achieves efficient dipolar dephasing but also smooths out the orientation and other parameter dependencies allowing simple data interpretation with a universal function.

Fig. 1a shows the R-RESPDOR pulse sequence for measuring ¹³C/¹⁴N dipolar coupling $D = \gamma_C \gamma_N \hbar / 4\pi r_{CN}^3$ under MAS. The ¹³C/¹⁴N dipolar interaction is recoupled when the cw rf field matches the MAS frequency $\omega_1 = \omega_r$.¹¹ The $\omega_1 = 2\omega_r$ rotary resonance also can be used but it has a smaller recoupling effect. Isotropic chemical shift, J and ¹³C/¹⁴N residual dipolar couplings are averaged by the rf. The rotary resonance also recovers the chemical shift anisotropy (CSA) of the observed ¹³C spin. The CSA is refocused by the middle π -pulse in the pulse sequence forming a rotary resonance echo.¹² A change of the ¹⁴N spin state by the saturation pulse interrupts the refocusing of the ¹³C/¹⁴N dipolar interaction causing modulations of the rotary resonance echo. The dipolar dephasing can be measured by two experiments, one with (S) and the other without (S_0) the ¹⁴N pulse. The signal fraction $\Delta S/S_0 = (S_0 - S)/S_0$ is compared with the simulation for the determination of the dipolar coupling constant (Fig. 1b).

Rotor-synchronization and MAS frequency stability are important for the echo and recoupling experiments. In REAPDOR, the CSA interaction is recovered under the REDOR multiple-pulse recoupling sequence and it depends on the timing of rotor position with respect to the π -pulses. The whole evolution and refocusing periods need be rotor-synchronized for a complete refocusing of the CSA occurring at the very end. Small MAS frequency fluctuations can accumulate timing errors over the long periods making the experiment extremely sensitive to MAS frequency control.^{6–8} Rotary resonance uses cw recoupling and the CSA refocusing requires only the middle spin-echo segment rotor-synchronized. Therefore, R-RESPDOR is much less sensitive to spinning frequency fluctuations.¹²

Dipolar modulation to the rotary resonance echo is the result of ¹⁴N spin state change induced by the ¹⁴N pulse. For a spin-1, the transitions can be of zero, single, or double quantum. The double-quantum $1 \leftrightarrow -1$ transition has a dipolar evolution twice as fast as the single-quantum, and no contribution takes place for the zero quantum part. Saturation of the three-level system implies 3:4:2 in probabilities for zero, single, and double-quantum transitions,

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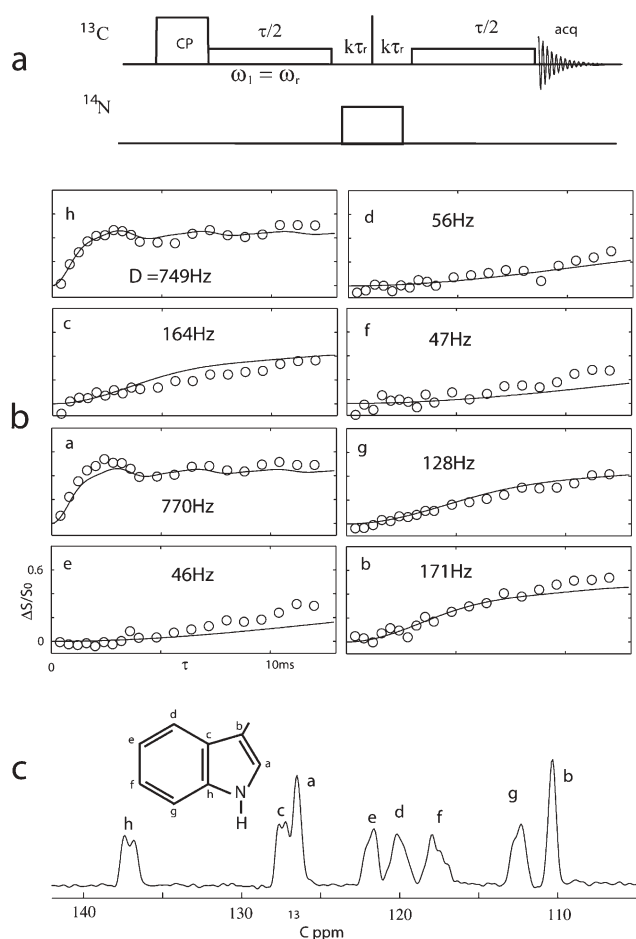


Fig. 1 (a) $^{13}\text{C}/^{14}\text{N}$ R-RESPDOR pulse sequence, (b) $\Delta S/S_0$ signal fractions and simulations using the listed dipolar coupling frequencies, and (c) MAS spectrum showing aromatic fragment and peak assignment of natural abundant L-tryptophan. The ^{13}C rf phase was kept constant through the pulse sequence and the carrier frequency was placed at 124 ppm. The multiple peak structures in the MAS spectrum are from crystallographically non-equivalent molecules in a unit cell. (14.1 T B_0 , 600 MHz Bruker-DRX console, 10 kHz MAS with 4 mm triple-resonance wide-bore probe, 512 scans with 4 s recycle delay for each spectrum, 150 μs ^{14}N pulse with ~ 40 kHz ν_1 , $k = 1$, SPINAL64¹⁶ ^1H decoupling with ~ 90 kHz ν_1).

respectively, leading to the following dipolar dephasing curve for the R-RESPDOR experiment.

$$\Delta S/S_0 = \frac{4}{9} \left[1 - S_d \left(\frac{\pi}{4} D\tau \right) \right] + \frac{2}{9} \left[1 - S_d \left(\frac{\pi}{2} D\tau \right) \right] \quad (1)$$

Here $1-S_d$ is the universal REDOR function for spin-1/2.^{2,8} The $\pi/4$ factor accounts for the scaling factor of the dipolar Hamiltonian between rotary resonance and REDOR recoupling under an assumption that the $^{13}\text{C}/^{14}\text{N}$ dipolar coupling is smaller than the ^{13}C CSA.¹⁷ The signal fraction $\Delta S/S_0$ is a function of the dimensionless parameter $D\cdot\tau$ arising from zero and flattening out at $\Delta S/S_0 \rightarrow 2/3$ with $D\cdot\tau \gg 1$.

Fig. 1b shows the R-RESPDOR measurement of eight aromatic carbons in natural abundant L-tryptophan with a single rotary resonance experiment. The measurements for the CO, C_α , C_β sites (not shown) require separate experiments due to the chemical shift

offsets for the rotary resonance. The two carbons next to indole ^{14}N (a and h) show dipolar oscillations from the one-bond $^{13}\text{C}/^{14}\text{N}$ coupling before reaching the equilibrium value about 0.45. This value differs from $\Delta S/S_0 \rightarrow 2/3$ expected for ideal situations. The difference mainly comes from a constant component that is always observed in CSA and dipolar rotary resonance experiment.^{9–12,17}

The constant component implies that a fraction of the signal does not contribute towards the dipolar evolution consequently lowering the equilibrium value. Incomplete ^{14}N saturation also contributes towards the difference because of higher probabilities for the non-contributing zero-quantum part in eqn (1). To account for these effects, a factor of 0.45/0.67, estimated from the $\Delta S/S_0$ curves of one-bond distance (a and h), is multiplied with eqn (1) for all carbon sites. After this correction, Fig. 1b shows good agreements between the R-RESPDOR simulations and the $\Delta S/S_0$ results for all sites. The dipolar coupling frequencies used in the simulations were obtained from a separate $^{13}\text{C}/^{15}\text{N}$ REDOR measurement of a selectively ^{15}N -labeled sample.¹⁷ The dipolar coupling frequencies $D = \gamma_{\text{C}/\text{N}}\hbar/4\pi^2r_{\text{CN}}^3$ can be used to extract carbon–nitrogen distances for one and two bond $^{13}\text{C}/^{14}\text{N}$ pairs. For $^{13}\text{C}/^{14}\text{N}$ pairs with three bonds and beyond (e, d, f), the determination of the distances must take the intermolecular contributions and the effect from the other ^{14}N spin (NH_3) into accounts. These contributions are evident from the faster dephasing curves than the two-spin simulations using the distances from $^{13}\text{C}/^{15}\text{N}$ REDOR measurement.

The R-RESPDOR method compliments with REAPDOR in cases of large CSA, fast MAS, and high magnetic fields. All three factors make REAPDOR experiments more difficult on MAS control, rf field requirement, and adiabatic-passage conditions. In contrast, these factors work favorably for R-RESPDOR. Large CSA and fast MAS drive the rotary resonance. The presence of a larger CSA makes the rotary resonance condition for heteronuclear recoupling wider and less dependent on rf field homogeneity. Furthermore, rotary resonance uses weak ^{13}C rf, therefore the interference with proton decoupling is less of a problem especially for protonated carbons sites (at the expense of reduced bandwidth for chemical shift). On the ^{14}N aspect, the saturation pulse length is not restricted by the spinning frequency and rapid quadrupolar frequency modulation helps to accelerate ^{14}N saturation by a pulse over several rotor periods.

It has been shown that the rotary resonance and ^{14}N saturation scheme make the R-RESPDOR a robust method for measuring $^{13}\text{C}/^{14}\text{N}$ distances in solids. The method can be applied to other quadrupolar nuclei. The capability of measuring multiple $^{13}\text{C}/^{14}\text{N}$ distances simultaneously at natural abundance makes R-RESPDOR a potential useful tool for spectral assignment and structural analysis of small to medium size molecules without the need of isotope labeling.

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