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Triple Resonance Solid State NMR Experiments with Reduced Dimensionality Evolution Periods

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Two solid state NMR triple resonance experiments which utilize the simultaneous incrementation of two chemical shift evolution periods to obtain a spectrum with reduced dimensionality are described. The CO N CA experiment establishes the correlation of $^{13}C_{i-1}$ to $^{13}C\alpha_i$ and $^{15}N_i$ by simultaneously encoding the $^{13}CO_{i-1}$ and $^{15}N_i$ chemical shifts. The CA N COCA experiment establishes the correlation $^{13}Ca_i$ and $^{15}CO_i$ to $^{13}C\alpha_{i-1}$ and $^{15}N_{i-1}$ within a single experiment by simultaneous encoding of the $^{13}C\alpha_i$ and $^{15}N_i$ chemical shifts. This experiment establishes sequential amino acid correlations in close analogy to the solution state HNCA experiment. Reduced dimensionality 2D experiments are a practical alternative to recording multiple 3D data sets for the purpose of obtaining sequence-specific resonance assignments of peptides and proteins in the solid state.

The experimental characterization of biomolecular structure and dynamics by NMR requires, as its inceptive step, the determination of sequence specific resonance assignments. A panoply of resonance assignment experiments has been developed in liquid state NMR (*1*) and recent advances in homonuclear (*2*) and heteronuclear (*3*) dipolar recoupling schemes have yielded similar results in high resolution solid state NMR (ssNMR). Partial or complete resonance assignments have been reported for four proteins in the solid state: human ubiquitin (*4, 5*), Bovine Pancreatic Trypsin Inhibitor (BPTI) (*6*), the SH3 domain of human α-spectrin (*7*), and the Light Harvesting Complex LHII from *Rhodopseudomonas acidophilia*-10050 (*8*).

The resolution and information content of the NMR experiment is improved by the use of additional frequency labeling periods (i.e., 3D and 4D NMR) (*1, 5, 9, 10*). Sun and coworkers developed the initial three dimensional solid state heteronuclear correlation (HETCOR) experiments and demonstrated their utility in obtaining resonance assignments in small biomolecules (*9*). Subsequently, more sophisticated NCACX and NCOCACX sequences, which employ band selective homonuclear and heteronuclear transfer, have been introduced (*3–5, 10*).

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The advantages associated with improved resolution and information content of higher dimensionality experiments are offset partially by the corresponding increase in the experimental acquisition time. In a conventional multidimensional HETCOR experiment, each of the indirect evolution periods ($t_1, t_2, \ldots$) associated with a chemical shift dimension ($\omega_1, \omega_2, \ldots$) is incremented *independently*. The number of independent experiments that must be performed to obtain a phase-sensitive lineshape increases geometrically with the number of dimensions in the experiment (*11*). Consequently, a method that retains the increased resolution and information content of higher dimensionality experiments without the associated cost in acquisition time is of paramount importance.

The use of reduced dimensionality (or projection) evolution periods in solution NMR triple resonance experiments is well known (*12–19*). In reduced dimensionality triple resonance experiments, two or more indirect evolution periods ($t_1, t_2, \ldots$) which sample chemical shifts ($\mathbf{\Omega}_1, \mathbf{\Omega}_2, \ldots$) are incremented *simultaneously*; frequency discrimination is obtained on only one of the simultaneously incremented chemical shift evolution periods (i.e., t_1) and $t_2 = \kappa t_1$. If $\kappa = 0$ (i.e., the evolution time period t_2 is not incremented) a single peak appears in ω_1 at $\mathbf{\Omega}_1$ as in the conventional HETCOR experiment. However, if $\kappa > 0$, the single resonance in ω_1 is split into two peaks, centered on $\mathbf{\Omega}_1$ and located at the sum and difference of the chemical shift offset of the two spins ($\mathbf{\Omega}_1 + \kappa\mathbf{\Omega}_2$ and $\mathbf{\Omega}_1 - \kappa\mathbf{\Omega}_2$) (*vide infra*). To avoid a corresponding *decrease* in resolution it is necessary to choose κ and the rf-carrier position such that the upfield ($\mathbf{\Omega}_1 - \kappa\mathbf{\Omega}_2$) and downfield ($\mathbf{\Omega}_1 + \kappa\mathbf{\Omega}_2$) components of the doublet are shifted to distinct regions of the spectrum. In general, for any two *n* spin systems ζ ($\zeta = \mathbf{a}, \mathbf{b}$) with chemical shifts ($\mathbf{\Omega}_{1\zeta}, \mathbf{\Omega}_{2\zeta}, \ldots \mathbf{\Omega}_{n\zeta}$) at least one member of the doublet will be resolved as long as there is one unique chemical shift ($\mathbf{\Omega}_1, \mathbf{\Omega}_2, \ldots \mathbf{\Omega}_n$). Ambiguities that arise due to coincidental degeneracy are resolved either by consideration of the known symmetry properties of the experiment or by recording a second 2D spectrum with a different value for κ and/or the rf-carrier position.

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Reduced dimensionality periods may also be incorporated into ssNMR experiments. Takegoshi and coworkers combined narrowband excitation with reduced dimensionality evolution periods in an experiment to determine the peptide torsion angle ϕ (*20*). To date, however, all ssNMR triple resonance experiments utilize conventional sampling in the indirect dimension. The pulse sequences for two new 3D ssNMR triple resonance experiments that incorporate reduced dimensionality evolution periods are presented in Figs. 1a and 1b and consist of the standard repertoire of building blocks familiar to ssNMR assignment techniques.

The projection of $\boldsymbol{\Omega}(^{13}\mathrm{CO})$ onto a $[\boldsymbol{\Omega}(^{15}\mathrm{N}), \boldsymbol{\Omega}(^{13}\mathrm{C}\alpha)]$ dimension is employed in several solution NMR experiments to facilitate the resolution of ^{15}N, $^{13}\mathrm{C}\alpha$ correlation peaks (*16–19*). An analogous solid state NMR experiment, CO N CA (Fig. 1a) establishes the correlations $^{13}\mathrm{CO}_{i-1}$ to $^{13}\mathrm{C}\alpha_i$ and $^{15}\mathrm{N}_i$ (underlined letters signify chemical shifts which evolve simultaneously as single-quantum coherences) (*13*). The experiment is designed to complement the NCA/NCOCA based solid state NMR protein resonance assignment strategy. The magnetization pathway of this experiment is given by

$$^{13}\mathrm{CO}_{i-1} \rightarrow {}^{15}\mathrm{N}_i \rightarrow {}^{13}\mathrm{C}\alpha_i. \qquad [1]$$

Initial ^{13}C magnetization is created by ramped cross-polarization (CP) from ^{1}H (*21, 22*). ^{13}C chemical shifts evolve during the t_1 evolution period. Subsequently, band selective CP from $^{13}\mathrm{CO}_{i-1}$ to $^{15}\mathrm{N}_i$ is obtained by setting the appropriate spin lock field strength on the ^{15}N and ramping through the SPECIFIC CP match condition on the ^{13}C channel (*3*). Transferred magnetization evolves at the nitrogen chemical shift during t_2 (magnetization not transferred by the CP is removed by the phase cycle). Following ^{15}N chemical shift evolution, the magnetization is transferred to the $^{13}\mathrm{C}\alpha_i$ and detected giving

$$\exp\left[i\boldsymbol{\Omega}\left(^{13}\mathrm{C}\alpha_i\right)\mathrm{t}_3\right]\cos\left[\boldsymbol{\Omega}\left(^{13}\mathrm{CO}_{i-1}\right)\mathrm{t}_1\right]\cos\left[\boldsymbol{\Omega}\left(^{15}\mathrm{N}_i\right)\mathrm{t}_2\right]. \qquad [2]$$

A three-dimensional data set (spectrum) which correlates $\boldsymbol{\Omega}(^{13}\mathrm{CO}_{i-1})$ to $\boldsymbol{\Omega}(^{15}\mathrm{N}_i)$ and $\boldsymbol{\Omega}(^{13}\mathrm{C}\alpha_i)$ is obtained if the data is acquired with t_1 and t_2 incremented independently and frequency discrimination is applied to both indirect chemical shift evolution periods (*11*). Alternatively, if $t_2 = t_1$ [*2*] rearranges to

$$\exp\left[i\boldsymbol{\Omega}\left(^{13}\mathrm{C}\alpha_i\right)\mathrm{t}_3\right]\cos\left\{\left[\boldsymbol{\Omega}\left(^{13}\mathrm{CO}_{i-1}\right)+\boldsymbol{\Omega}\left(^{15}\mathrm{N}_i\right)\right]\mathrm{t}_1\right\} + \cos\left\{\left[\boldsymbol{\Omega}\left(^{13}\mathrm{CO}_{i-1}\right)-\boldsymbol{\Omega}\left(^{15}\mathrm{N}_i\right)\right]\mathrm{t}_1\right\}. \qquad [3]$$

To facilitate comparison with conventional triple resonance experiments (i.e., NCA) quadrature detection is obtained on the ^{15}N channel (ϕ_5) giving

$$\exp\left[i\boldsymbol{\Omega}\left(^{13}\mathrm{C}\alpha_i\right)\mathrm{t}_3\right]\cos\left[\boldsymbol{\Omega}\left(^{13}\mathrm{CO}_{i-1}\right)\mathrm{t}_1\right]\sin\left[\boldsymbol{\Omega}\left(^{15}\mathrm{N}_i\right)\mathrm{t}_2\right] \qquad [4]$$

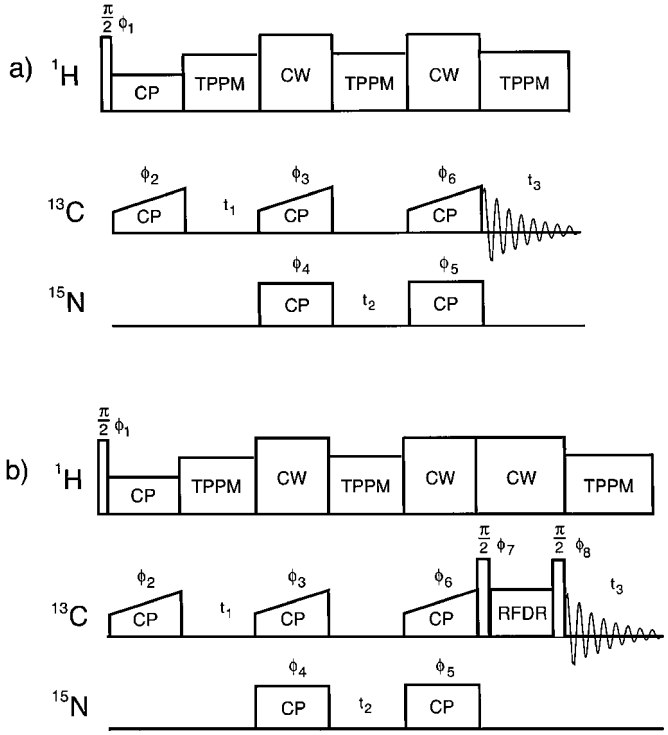

FIG. 1. (a) Pulse sequence for the CO N CA experiment. The narrow rectangle represents a $\pi/2$ pulse (100 kHz). CW (100 kHz) and TPPM (24) (90 kHz, 15° phase difference) ^{1}H decoupling are applied during mixing and evolution, respectively. The first cross polarization (CP) is a broadband ramped Hartman–Hahn ^{1}H to ^{13}C transfer step (*22*) with constant ^{1}H (50 kHz) and ramped ^{13}C fields (50–60 kHz). Heteronuclear (^{13}CO to ^{15}N and ^{15}N to $^{13}\mathrm{C}\alpha$) transfers are obtained with SPECIFIC CP (*3*) using a constant ^{15}N (30 kHz) and ramped ^{13}C rf-fields (1.25 ms mixing time). Phase cycling: $\phi_1 = 4[4(x), 4(-x)]$; $\phi_2 = 8(x, -x, x, -x)$; $\phi_3 = x$; $\phi_4 = 8(x, x, -x, -x)$; $\phi_5 = x$; $\phi_6 = 8(x), 8(y), 8(-x), 8(-y)$; $\phi_{\mathrm{rec}} = (x, -x, -x, x), (-x, x, x, -x)$, $(y, -y, -y, y), (-y, y, y, -y), (-x, x, x, -x), (x, -x, -x, x)(-y, y, y, -y)$, $(y, -y, -y, y)$. Quadrature detection is obtained incrementing $\phi_5 = (x, y)$ according to States *et al.* (*11*). (b) Pulse sequence for the CA N COCA experiment. The narrow rectangles represent $\pi/2$ pulses (100 kHz ^{1}H, 50 kHz ^{13}C). CW (100 kHz) and TPPM (24) (90 kHz, 15° phase difference) ^{1}H decoupling are applied during mixing and evolution periods, respectively. The first cross polarization (CP) is a broadband ramped Hartman–Hahn ^{1}H to ^{13}C transfer step with constant ^{1}H (50 kHz) and ramped ^{13}C fields (50–60 kHz) (*22*). Heteronuclear (^{13}CO to ^{15}N and ^{15}N to $^{13}\mathrm{C}\alpha$) transfers are obtained with SPECIFIC CP (*3*) using a constant ^{15}N (30 kHz) and ramped ^{13}C rf-fields (1.25 ms mixing time). Homonuclear polarization transfer is via RFDR (*23*) (50 kHz field, 1.79 msec. mixing time). Phase cycling: $\phi_1 = 4[4(x), 4(-x)]$; $\phi_2 = 8(x, -x, x, -x)$; $\phi_3 = x$; $\phi_4 = 8(x, x, -x, -x)$; $\phi_5 = x$; $\phi_6 = 4[4(x), 4(-x)]$; $\phi_7 = 2[(y, -y, -y, y)\ 2[(-y, y, y, -y), (y, -y, -y, y)]$; $\phi_7 = 2[(x, -x, -x, x), 2(-x, x, x, -x)(x, -x, -x, x)]$; $\phi_{\mathrm{rec}} = 4(x, -x, -x, x), 4(-x, x, x, -x)$. Quadrature detection is obtained by incrementing $\phi_3 = (x, y)$ according to States *et al.* (*11*).

that rearranges to

$$\exp\left[i\boldsymbol{\Omega}\left(^{13}\mathrm{C}\alpha_i\right)\mathrm{t}_3\right]\sin\left\{\left[\boldsymbol{\Omega}\left(^{13}\mathrm{CO}_{i-1}\right)+\boldsymbol{\Omega}\left(^{15}\mathrm{N}_i\right)\right]\mathrm{t}_1\right\} - \sin\left\{\left[\boldsymbol{\Omega}\left(^{13}\mathrm{CO}_{i-1}\right)-\boldsymbol{\Omega}\left(^{15}\mathrm{N}_i\right)\right]\mathrm{t}_1\right\}. \qquad [5]$$

The resulting two-dimensional spectrum has $\omega_3 = \mathbf{\Omega}(^{13}C\alpha_i)$, $\omega_1 = \{\mathbf{\Omega}(^{15}N_i) \pm \mathbf{\Omega}(^{13}CO_{i-1})\}$. The chemical shift offset (from the ^{13}C rf-carrier) of the $^{13}CO_{i-1}$ resonance has been encoded as a splitting into two peaks centered on $\mathbf{\Omega}(^{15}N_i)$.

The protocol for assigning protein NMR resonances in the solid state requires two experiments. An NCA-type experiment is used to determine the correlation of $\mathbf{\Omega}(^{13}C\alpha_i)$ and $\mathbf{\Omega}(^{15}N_i)$ and an NCOCA to obtain the correlation between $\mathbf{\Omega}(^{13}C\alpha_{i-1})$ and $\mathbf{\Omega}(^{15}N_i)$ (*4*, *5*). The identification of the common $\mathbf{\Omega}(^{15}N_i)$ is used to obtain sequential assignments. Ideally, both inter- and intraresidue correlations would be obtained within a single experiment (as is common in many solution NMR triple resonance experiments) (*1*).

The direct correlation of $\mathbf{\Omega}(^{13}C\alpha_{i-1})$ to $\mathbf{\Omega}(^{13}C\alpha_i)$, for example, links sequential amino acids in the protein sequence; projecting $\mathbf{\Omega}(^{15}N_i)$ onto $\mathbf{\Omega}(^{13}C\alpha_i)$ may further disperse the coincidental overlap of correlation peaks. A schematic of this CA N COCA pulse sequence is shown in Fig. 1b. The experiment establishes correlations between $^{13}C\alpha_i$ and $^{15}N_i$ to $^{13}CO_{i-1}$ and $^{13}C\alpha_{i-1}$. The signal follows two (overlapping) pathways,

$$^{13}C\alpha_i \rightarrow {}^{15}N_i \rightarrow {}^{13}CO_{i-1} \qquad [6]$$

for the downfield region and

$$^{13}C\alpha_i \rightarrow {}^{15}N_i \rightarrow {}^{13}CO_{i-1} \rightarrow {}^{13}C\alpha_{i-1} \qquad [7]$$

for the upfield region. Initial ^{13}C magnetization is created by ramped CP from ^1H to ^{13}C (*21*, *22*); chemical shifts evolve during the t_1 evolution period. Subsequently, polarization is transferred from $^{13}C\alpha_i$ to $^{15}N_i$ via SPECIFIC CP (*3*). The SPECIFIC condition is obtained by applying a constant spin lock field on the ^{15}N channel and ramping through the match condition on ^{13}C. The transferred magnetization evolves during t_2 at $\mathbf{\Omega}(^{15}N_i)$. Following chemical shift evolution, the magnetization is transferred to $^{13}CO_{i-1}$ by a second SPECIFIC CP transfer. The magnetization is stored along the $\pm z$ axis where ^{13}C magnetization exchange between ^{13}CO and $^{13}C\alpha$ is obtained via Radio Frequency Driven Recoupling (RFDR) (*23*). The magnetization is subsequently returned to the transverse plane by a second $\pi/2$ pulse and detected. As with the CO N CA, the data can be acquired as a 3D experiment. However, if acquired with simultaneous evolution of t_1 and t_2, the signal of interest is described by

$$\{\exp[i\mathbf{\Omega}(^{13}C\alpha_{i-1})t_3] + \exp[i\mathbf{\Omega}(^{13}CO_{i-1})t_3]\}\cos\{[\mathbf{\Omega}(^{13}C\alpha_i) + \mathbf{\Omega}(^{15}N_i)]t_1\} + \cos\{[\mathbf{\Omega}(^{13}C\alpha_i) - \mathbf{\Omega}(^{15}N_i)]t_1\}. \qquad [8]$$

The two-dimensional spectrum has $\omega_3 = \{\mathbf{\Omega}(^{13}C\alpha_{i-1}), \mathbf{\Omega}(^{13}CO_{i-1})\}$, $\omega_1 = [\mathbf{\Omega}(^{13}C\alpha_i) \pm \mathbf{\Omega}(^{15}N_i)]$. The chemical shift offset of the $^{15}N_i$ resonance from the ^{15}N rf-carrier has been encoded as a splitting into two peaks centered on $\mathbf{\Omega}(^{13}C\alpha_i)$.

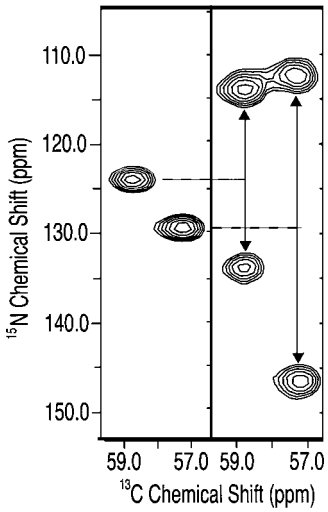

FIG. 2. Contour plot of CO N CA experiment. Both CO N CA and CA N COCA experiments were demonstrated on a polycrystalline sample of 100%-U-[^{13}C,^{15}N]-N-Aceyl-Valine-Leucine custom synthesized by SynPep Corporation (Dublin, CA) with U-[^{13}C,^{15}N]-labeled amino acids purchased from Cambridge Isotope Labs (Andover, MA). The sample was recrystallized from 1 : 1 (v/v) H_2O: acetone solution and center packed in a 4-mm zirconia Chemagnetics rotor (Fort Collins, CO). NMR spectra were acquired at 11.7 T (500 MHz ^1H, 125.8 MHz ^{13}C, 50.7 MHz ^{15}N), using a custom designed spectrometer (courtesy of David J. Ruben) with a Chemagnetics (Fort Collins, CO) triple resonance probe equipped with a 4-mm Chemagnetics spinning module. The spinning frequency was set to 8.950 kHz and regulated to ± 5 Hz with a Doty Scientific (Columbia, SC) spinning frequency controller. One hundred twenty-eight scans were acquired per t_1 point. The ^{13}C and ^{15}N carrier were placed at 167.57 and 126.04 ppm, respectively; 1024 points were acquired in ω_3 (20 ms evolution time) and 64 points in ω_1 (14.3 ms evolution time). The data was processed in FELIX (Molecular Simulations, Ventura, CA) in ω_3 by linear predicting back the 1st point, applying an exponential line broadening of 20 Hz, zero filling by a factor of two and fourier transform. In ω_1, the data was processed using a 90° phase shifted sine bell followed by zero filling and fourier transform. The final size of the matrix was 2048 by 512 points. The data was indirectly referenced to an external sample of solid U-[^{13}C,^{15}N]-glycine assuming a ^{13}CO resonance of 176.0 ppm. In (a) $t_1 = 0$; (b) $t_1 = t_2$. Vertical arrows mark the splitting due to simultaneous evolution of $\mathbf{\Omega}(^{13}CO)$ and $\mathbf{\Omega}(^{15}N)$. The split resonances are centered on $\mathbf{\Omega}(^{15}N_i)$ which is indicated by horizontal (dashed) lines.

Figure 2 (left) shows a contour plot of the CO N CA experiment performed on a sample of the peptide U-[^{15}N,^{13}C]-N-Acetyl-Valine-Leucine (VL) acquired with $t_2 = 0$. Two peaks appear in the spectrum with $\omega_1 = \mathbf{\Omega}(^{15}N_i)$ and $\omega_3 = \mathbf{\Omega}(^{13}C\alpha_i)$- the valine at $\omega_1 = 123.9$ ppm, $\omega_3 = 58.7$ ppm and the leucine at $\omega_1 = 129.1$, $\omega_3 = 57.1$ ppm. The peaks are located at the same frequencies as in a conventional band-selective NCA experiment (data not shown). The spectrum in Fig. 2 (right) corresponds to the same experiment recorded with simultaneous incrementation of the t_1 and t_2 evolution periods. Each peak has been split into a doublet centered on $\mathbf{\Omega}(^{15}N_i)$ (marked with a dashed line). The splitting ($\Delta\omega_3$), marked by the solid arrows, is equal to twice the chemical shift offset of the preceding carbonyl resonance from the ^{13}C carrier at 167.57 ppm. For valine, the preceding carbonyl is derived from

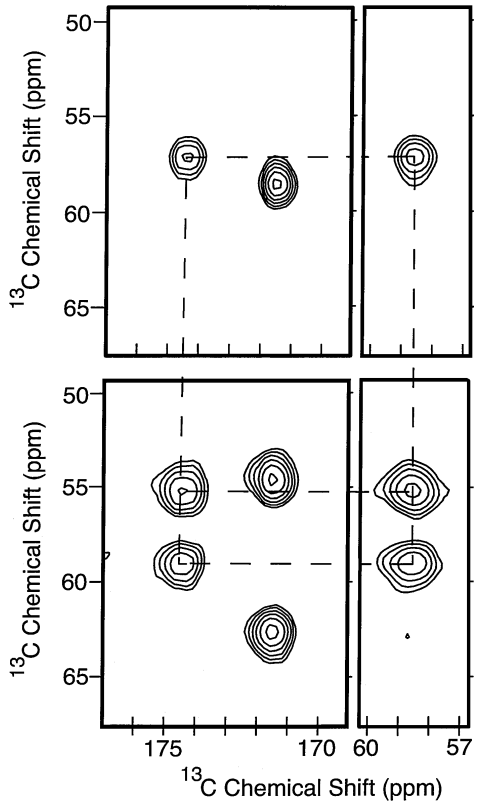

FIG. 3. Contour plots of the CA N COCA experiment. Data were acquired on the same spectrometer and identical conditions as in Fig. 2 except the ^{13}C and ^{15}N carriers were placed at 58.77 and 133.9 ppm, respectively. In the directly detected dimension 1024 points were acquired in ω_3 (20 ms evolution time) and 32 points were acquired in ω_1 (7.2 ms evolution time). The data were processed in FELIX as described in Fig. 2. The final size of the matrix was 2048 by 512 points. In (top) $t_2 = 0$; (bottom) $t_1 = t_2$. Horizontal (dashed) lines mark resonances that evolved at identical shifts in ω_1, vertical dashed lines connect identical ω_3 resonance frequencies. The splitting in (bottom) is given by $[\mathbf{\Omega}(^{13}\mathrm{C}\alpha_i) \pm \mathbf{\Omega}(^{15}\mathrm{N}_i)]$.

the acetyl moiety; $\Delta\omega_3 = 1012.64$ Hz or 4.03 ppm from the carrier, corresponding to $\mathbf{\Omega}(^{13}\mathrm{CO}) = 171.6$ ppm. The leucine peak has $\Delta\omega_3 = 1712.6$ Hz or 6.81 ppm from the carrier giving $\mathbf{\Omega}(^{13}\mathrm{CO}) = 174.4$ ppm. These values are in agreement with those obtained from a band selective NCO experiment on the same sample (data not shown).

A contour plot of the CA N COCA experiment acquired with (top) $t_2 = 0$ and (bottom) $t_2 = t_1$ appears in Fig. 3. Upfield $^{13}C\alpha$ (right panel) and downfield ^{13}CO (left panel) are displayed separately. The two sets of resonances correspond to magnetization that has followed the trajectory described in Eqs. [6] and [7], respectively. The vertical (dashed) line marks peaks that have evolved at the same chemical shift in ω_1 (57.1 ppm) and are assigned to magnetization that originated on the leucine $^{13}C\alpha$. The two peaks appear in ω_3 on the valine ^{13}CO (pathway 1, 174.4 ppm) and the valine $^{13}C\alpha$ (pathway 2, 58.6 ppm). A second resonance appears in the downfield region; it is assigned to magnetization that originates on the valine $^{13}C\alpha$. In the absence of a one bond ^{13}CO–$^{13}C\alpha$ dipolar coupling the magnetization pathway is terminated on the ^{13}CO of the acetyl moiety.

A spectrum acquired with $t_1 = t_2$ (simultaneous evolution of $^{13}C\alpha$ and ^{15}N) is presented in Fig. 3 (bottom). While the peaks appear at identical positions in ω_3 (vertical dashed lines), the peaks have been split into doublets in ω_1. The magnitude of the splitting ($\Delta\omega_1$) is equal to twice the chemical shift offset of the amide nitrogen resonance from the ^{15}N carrier position at 133.9 ppm. As in Fig. 3 (top) resonances which have evolved at the same ^{15}N and $^{13}C\alpha$ chemical shifts are marked by horizontal dashed lines and $\Delta\omega_1 = 488$ Hz. This is equivalent to a ^{15}N chemical shift of 129.1 ppm, in agreement with the previously determined value for the leucine ^{15}N. The second downfield resonance has $\Delta\omega_1 = 1010$ Hz which corresponds to the previously determined ^{15}N chemical shift of valine at 123.9 ppm.

In conclusion, we have demonstrated two new ssNMR triple resonance experiments and have shown how the 3D spectrum may be acquired as a 2D experiment by the simultaneous incrementation of two evolution periods. The utility of 3D HETCOR experiments in obtaining peptide and protein assignments in the solid state has been previously demonstrated (*5, 9, 10*). Reduced dimensionality ssNMR experiments complement conventional 2D HETCOR without the additional time associated with 3D acquisition (*12–15*). Also, the detection of peak pairs (and their associated symmetry) versus single peaks may enhance the confidence in the assignment of weaker resonances, a feature essential for automating the resonance assignment process. The fidelity of the assignments may be further validated by recording a second spectrum, varying either the carrier position or the value of κ. There is no comparable means of verifying the assignments obtained in a 3D spectrum other than recording multiple experiments with complementary coherence transfer pathways. The projection approach also offers the significant advantage of enhanced resolution associated with recording spectra that maximize the evolution period in the indirect dimensions. We anticipate that these straightforward extensions of assignment experiments may prove to be of considerable utility in obtaining resonance assignments for peptides and proteins in the solid state.

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