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Editing and diagonal peak suppression in three-dimensional HCCH protein NMR correlation experiments

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Abstract

A novel three-dimensional (3D) HCCH NMR experiment is introduced. It involves ^{13}C-^{13}C COSY or TOCSY coherence transfer plus two independent editing steps according to the number of protons attached to the individual carbons before and after the ^{13}C-^{13}C homonuclear mixing. This double editing leads to simplification of HCCH protein side chain spectra that otherwise are prone to spectral overlap. Another interesting feature is amino acid selectivity, i.e. that the presence of certain correlations in a doubly edited HCCH subspectrum gives a clue as to assignment to a particular subgroup of amino acids or segments thereof. Finally, the selection of two different multiplicities in the two editing steps leads to diagonal peak suppression in the ^{1}H-^{1}H (3D spectrum recorded with two ^{1}H and one ^{13}C dimension) or the ^{13}C-^{13}C (3D spectrum recorded with one ^{1}H and two ^{13}C dimensions) two-dimensional projection. The new experiment is demonstrated using a ^{13}C,^{15}N-labeled protein sample, chymotrypsin inhibitor 2, at 500 MHz.

Magnetization transfer between protons in large proteins, typically enriched in ^{13}C and ^{15}N, takes place via heteronuclear relays exploiting relatively large one-bond J coupling constants. When the two heteronuclei attached to a given pair of protons have well-separated chemical shift ranges, such as ^{13}C and ^{15}N, it is easy to eliminate the contributions that would lead to diagonal-type signals in a multidimensional NMR spectrum. This situation prevails for the triple-resonance protein NMR experiments involving the backbone nuclei but not for the HCCH experiments (Bax et al., 1990; Kay et al., 1990; Sørensen, 1990; Olejniczak et al., 1992) that are crucial for assignment in the side chains. In fact, three-dimensional (3D) protein HCCH NMR spectra involving the side chain spin systems can exhibit large diagonal-type signals and also otherwise suffer from signal overlap among cross peaks. This communication addresses both problems by proposing modified HCCH pulse sequences that edit into subspectra in two independent steps according to the numbers of protons attached to the two pertinent ^{13}C nuclei. First, this offers up to nine subspectra, which helps remove spectral overlap. Second, the edited subspectra representing correlations between CH_n and CH_m groups with $n \neq m$ are devoid of diagonal peaks.

While there are a number of methods for multiplicity editing, DEPT (Pegg et al., 1982) was chosen in the present context because it combines polarization transfer and editing with a good tolerance of a spread in the sizes of the J coupling constants. An even higher editing accuracy is possible (Sørensen et al., 1983), but that option was not considered in the pulse sequence outlined in Figure 1 because it would require a longer and more complex pulse sequence, which is undesirable considering the short relaxation times typical for proteins.

After a t_1 evolution period with proton magnetization, a DEPT-type transfer to the attached ^{13}C takes place and then a second evolution period, t_2. In order to keep losses by transverse ^{13}C relaxation small it is recommended to employ a constant-time evolution period of duration around $1/4J_{CC}$. (This is also

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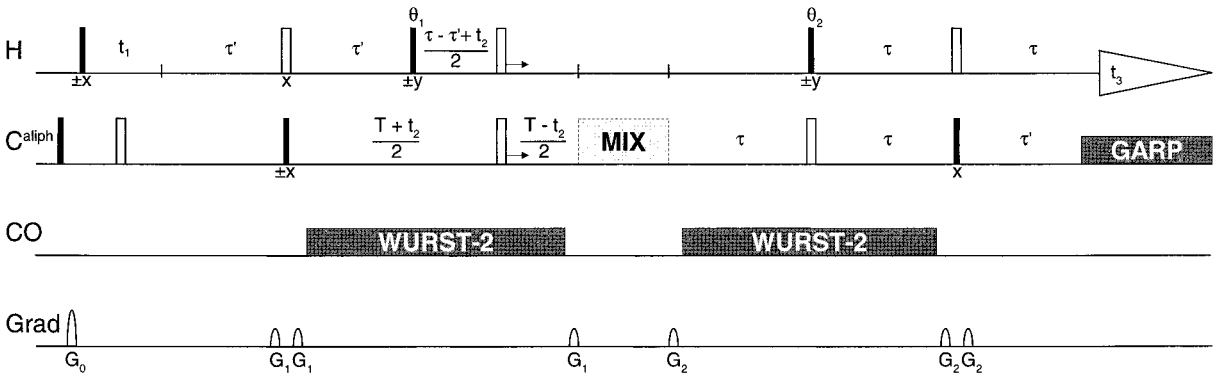

Figure 1. 3D double-DEPT HCCH pulse sequence with multiplicity editing. The dashed box indicates either a $(\pi/2)_x$ pulse or an isotropic mixing sequence preceded by a short x-phase spin-lock period. Filled and open bars represent $\pi/2$ and π pulses, respectively. The pulses θ_1 and θ_2 can also be implemented as two pairs of $\pi/2$ pulses with the phases $\{\pi,\theta_1\}$ and $\{\theta_2,\pi\}$, respectively. Pulsed field gradients are shown as open bell shapes. $\tau = \frac{1}{2}[J^{min} + 0.146(J^{max} - J^{min})]^{-1}$; $\tau' = \frac{1}{2}[J^{max} - 0.146(J^{max} - J^{min})]^{-1}$ (Nielsen et al., 1986); $T \approx (4J_{CC})^{-1}$ or $3(4J_{CC})^{-1}$. Pulse phases with the prefix $\pm$ represent independent two-step phase cycles with alternating receiver phase. States-TPPI is recommended for the $\pi/2$ pulses before t_1 and t_2, respectively. Nine data sets $\{\theta_1,\theta_2\} = \{\pi/4, \pi/4\}$, $\{\pi/4, \pi/2\}$, $\{\pi/4, 3\pi/4\}$, $\{\pi/2, \pi/4\}$, $\{\pi/2, \pi/2\}$, $\{\pi/2, 3\pi/4\}$, $\{3\pi/4, \pi/4\}$, $\{3\pi/4, \pi/2\}$, $\{3\pi/4, 3\pi/4\}$ are recorded and stored separately. Then the edited subspectra are generated by linear combination of these data sets. When equal numbers of scans are recorded for all θ angles the linear combinations are: CH-CH: $\{[\pi/2], [\pi/2]\}$; CH-CH_2: $\{[\pi/2], [\pi/4] - [3\pi/4]\}$; CH-$CH_3$: $\{[\pi/2], [\pi/4] + [3\pi/4] - [\pi/2]\sqrt{2}\}$; CH_2-CH: $\{[\pi/4] - [3\pi/4], [\pi/2]\}$; CH_2-CH_2: $\{[\pi/4] - [3\pi/4], [\pi/4] - [3\pi/4]\}$; CH_2-CH_3: $\{[\pi/4] - [3\pi/4], [\pi/4] + [3\pi/4] - [\pi/2]\sqrt{2}\}$; CH_3-CH: $\{[\pi/4] + [3\pi/4] - [\pi/2]\sqrt{2}, [\pi/2]\}$; CH_3-CH_2: $\{[\pi/4] + [3\pi/4] - [\pi/2]\sqrt{2}, [\pi/4] - [3\pi/4]\}$.

an acceptable compromise for keeping the ^{13}C magnetization in-phase with respect to J_{CC} in experiments with ^{13}C-^{13}C isotropic mixing.) Next, ^{13}C-^{13}C coherence transfer by either a single $\pi/2$ pulse or an isotropic mixing sequence takes place. In the case of small proteins a constant-time delay of $1/J_{CC}$ followed by isotropic mixing could be considered, which would eliminate the loss caused by passive J_{CC} coupling constants. After a ^{13}C-^{13}C COSY-type coherence transfer, the antiphase ^{13}C magnetization partly refocuses during a delay on the order of $1/4J_{CC}$ before a final DEPT-type transfer to the attached protons takes place. For TOCSY-type ^{13}C-^{13}C coherence transfer no refocusing is necessary, so the rest of the sequence only serves the needs of the final DEPT-type transfer from ^{13}C to ^{1}H. The two options for ^{13}C-^{13}C mixing are indicated by the dashed box in Figure 1. A delay $1/4J_{CC}$ is on the order of two $1/2J_{CH}$ delays in the ^{13}C-^{1}H DEPT elements. If a 3D spectrum with two ^{13}C dimensions is desirable one would delete the ^{1}H evolution period and employ ^{13}C evolution in the period after the ^{13}C-^{13}C transfer in a way that mirrors the scheme in the first ^{13}C evolution period.

As usual, a DEPT editing pulse θ_y can be replaced by two $\pi/2$ pulses of relative phase shift $\pi - \theta$. Specifically, the first and second $\theta_{\pm y}$ pulses should be replaced by $(\pi/2)_{-x}(\pi/2)_{\pm\theta}$ and $(\pi/2)_{\pm\theta}$ $(\pi/2)_{-x}$, respectively. For the editing into subspectra it is recommended to employ the original θ values $\pi/4$, $\pi/2$, and $3\pi/4$ (Pegg et al., 1982). This choice simultaneously optimizes the sensitivity of the CH and CH_2 subspectra while compromising the sensitivity of CH_3 a little (Sørensen, 1984). For the double editing it is necessary to record spectra with all nine combinations of the θ angles (i.e. $\theta_1 = \pi/4$, $\pi/2$, $3\pi/4$ and $\theta_2 = \pi/4$, $\pi/2$, $3\pi/4$). Further, better sensitivity is obtained when the number of scans is doubled in the combinations with a single $\pi/2$ angle and quadrupled in the combination where both θ angles are $\pi/2$. The sensitivity penalty for such a DEPT editing step compared to e.g. just employing $\theta \approx 55°$ is about 14% (Sørensen, 1984).

The first benefit of this scheme for double-DEPT HCCH editing is simplification of protein HCCH spectra because the peaks are edited into up to nine independent subspectra rather than all of them being in a single spectrum. Clearly, this is an attractive alternative to other amino acid-selective or editing approaches (Olejniczak et al., 1994; Dötsch et al., 1996; Rios et al., 1996; Löhr and Rüterjans, 1997; Muhandiram et al., 1997; Pellecchia et al., 1997; Bazzo et al., 1999; Schubert et al., 1999; Uhrín et al., 2000).

An additional benefit of double-DEPT HCCH editing is that diagonal peaks are suppressed in the subspectra whenever the numbers of attached protons selected by the first and second θ editing steps

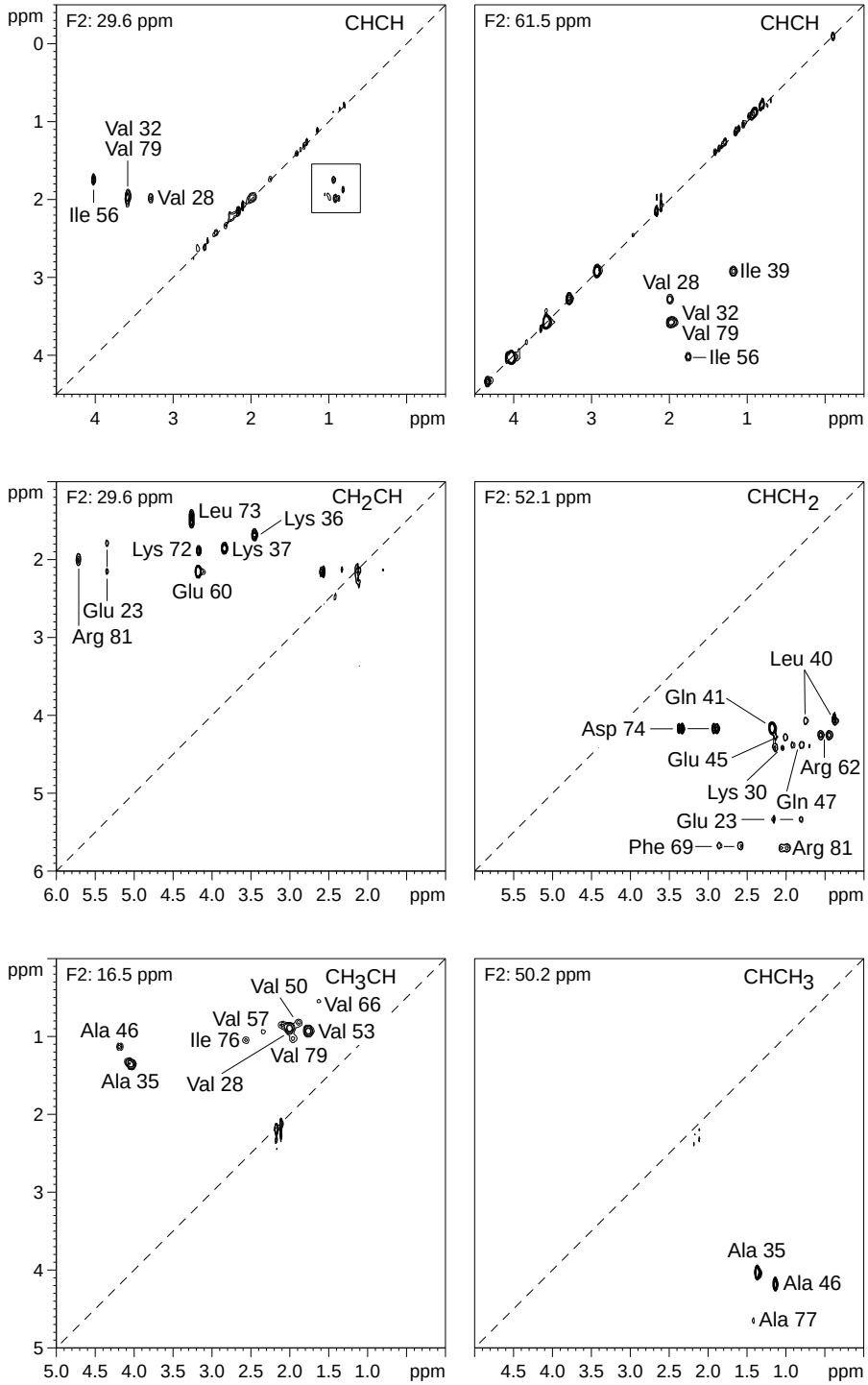

Figure 2. 2D sections from the aliphatic region of ^{15}N,^{13}C-labeled CI2 21–83 (90% H_2O/10% D_2O, 25 °C, pH = 4.2, 18 mg in 600 µl) recorded with the pulse sequence in Figure 1 using a $\pi/2$ pulse for ^{13}C-^{13}C mixing on a Varian Unity Inova 500 MHz spectrometer. Parameters: relaxation delay with 2.0 s presaturation of water resonance; $\tau = 4.09$ ms; $\tau' = 3.17$ ms; T = 7.26 ms; $t_1^{max} = 18.71$ ms; $t_2^{max} = 1.99$ ms; 2 scans (the phase cycle for the ^{13}C $\pi/2$ pulse indicated in Figure 1) for each data set. The ^{13}C offset was 39 ppm. Wurst-2 adiabatic decoupling of the carbonyl region was applied during the period with transverse ^{13}C magnetization while GARP was used for ^{13}C decoupling during acquisition. A data matrix of 264 × 32 × 1024 points covering 7000 × 7545 × 7000 Hz was recorded for each set of θ_1,θ_2 values in an interleaved manner. The time-domain data for the subspectra were zero-filled to 512 × 32 × 1024 points prior to Fourier transformation. The window functions were cosine in all three dimensions. The signals in the frame are cross talk and can be removed by appropriate linear combination with the corresponding section from the CH-CH_3 subspectrum as shown in Figure 3.

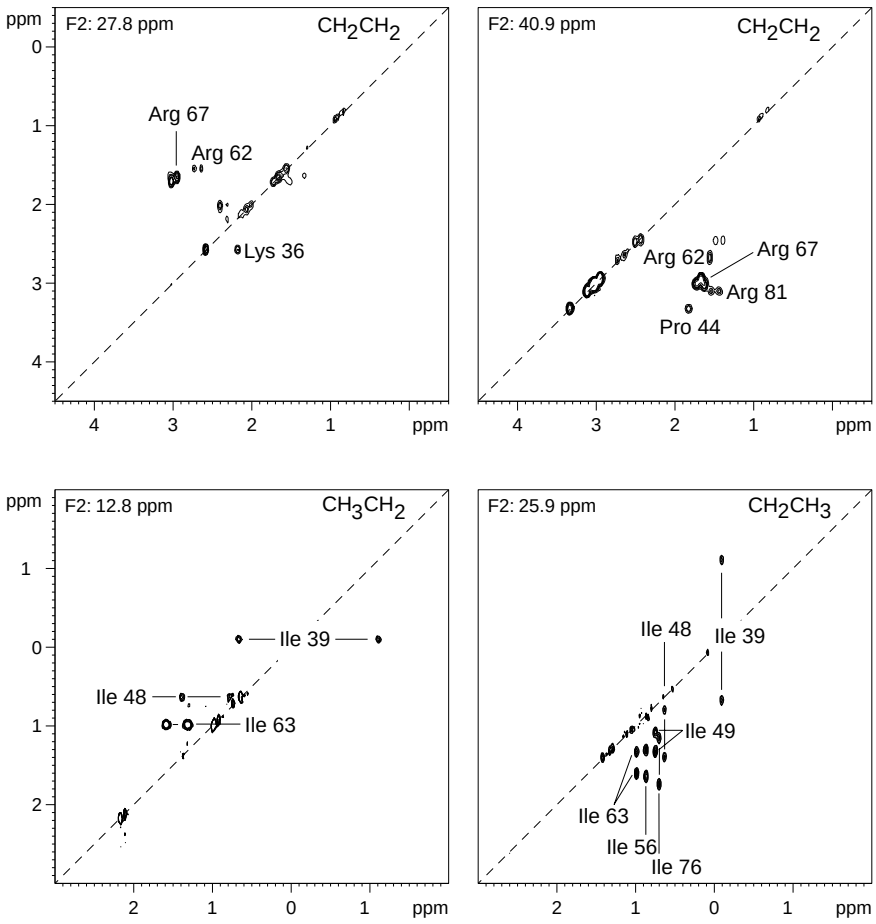

Figure 2. (continued).

are different. Obviously, this is handy for observation of cross peaks close to the diagonal. Hence, double-DEPT HCCH editing supplements the diagonal peak suppression schemes of TROSY-NOESY (Meissner and Sørensen, 2000a) for amide resonances and TROSY-HCCH for aromatic side chains (Meissner and Sørensen, 2000b).

An experimental test of the new experiment was performed on a sample of the $^{13}C,^{15}N$-labeled protein chymotrypsin inhibitor 2 (CI2) (Osmark et al., 1992; Madsen et al., 1993) at 500 MHz and the results are shown in Figure 2 in the form of sections taken at different ^{13}C frequencies in the F_2 dimension. The available assignment of CI2 is incomplete in the side chain regions and no attempt was made to complete the assignment in the present work. The double-DEPT editing results in good separation of cross peaks that would otherwise overlap at the coarse resolution employed in the ^{13}C dimension. This is in addition to the assignment clues provided by the editing.

In ^{13}C editing it is common to combine data obtained with different θ settings in ratios that deviate from the theoretical values in order to compensate for pulse imperfections and a spread in the J values; none of this was applied for the spectra presented in Figure 2. As a consequence, cross talk is observed in some of the subspectra particularly from large methyl signals. In Figure 3 an example is shown of a different linear combination leading to suppression of the CH-CH_3 cross talk in the CH-CH subspectra in Figure 2.

There is a simpler version of the proposed double-DEPT HCCH experiment if it is sufficient to edit only into four subspectra according to the number of attached protons being odd or even. That could be done in four experiments with the θ angles equal to $\pi/3$ and $2\pi/3$, and the diagonal peaks would be suppressed in

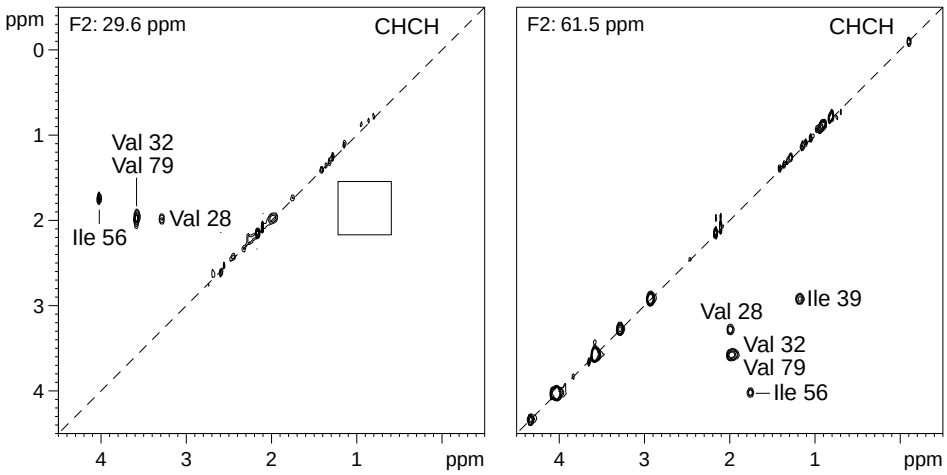

Figure 3. Same as the corresponding spectra in Figure 2 but with cross talk suppression by subtracting 28% of the CH-CH_3 subspectrum from the original CH-CH subspectrum.

two of the four subspectra. A similar approach has been employed for editing of INADEQUATE spectra (Sørensen et al., 1984, 1985).

In conclusion, we have introduced a new experiment, double-DEPT HCCH, for editing HCCH spectra into up to nine independent subspectra where the diagonal peaks are suppressed in six of these. Not only does this represent a considerable simplification of HCCH spectra, particularly those where ^{13}C-^{13}C TOCSY transfer is employed, but the patterns of peaks in the various subspectra also help identify resonances as belonging to certain types of amino acids.

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