

## MLEV-17-Based Two-Dimensional Homonuclear Magnetization Transfer Spectroscopy

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Recently, we have shown that net magnetization transfer among scalar coupled homonuclear spins can be obtained by the application of a spin-lock field (1), or more effectively, by the application of a phase-alternated spin-lock field (2, 3). Analogous methods to accomplish net homonuclear magnetization transfer, based on different rf irradiation schemes have previously been reported by Braunschweiler and Ernst (4). As they and others (5-9) have pointed out, the key to net magnetization transfer between two coupled spins, A and X, is to remove the Zeeman contributions,  $H_{zA}$  and  $H_{zX}$ , from the Hamiltonian, or to make them identical, i.e.,  $H_{zA} = H_{zX}$ . This can be accomplished by suitable rf irradiation schemes or by zero-field NMR (10). In this communication we describe a new mixing scheme that is based on the MLEV-16 composite pulse decoupling cycle (11). We have modified this cycle to make it less sensitive to pulse imperfections and it will be shown that this type of MLEV mixing provides net magnetization transfer over a substantial bandwidth with only limited rf power. More importantly, the apparent decay constant of spin-locked magnetization can be prolonged by up to a factor of two (compared to  $T_{1\rho}$ ) by using this new type of mixing scheme.

If the Zeeman part of the Hamiltonian is eliminated, the spin system will evolve solely under the influence of scalar coupling. Magnetization can then propagate through the molecule in a way that is very similar to spin diffusion among protons in a rigid solid, where dipolar couplings are usually much larger than differences in chemical shift. For molecules consisting of only two coupled homonuclear spins, A and X, Braunschweiler and Ernst have shown that in the isotropic coupling limit there is an oscillatory exchange of the A and X magnetization, with period  $1/J_{AX}$ . Explicit results for the  $AX_2$  case have very recently been presented by Chandrakumar and Subramanian (12). For larger spin systems a computer simulation program appears to be the easiest way to predict the rate at which magnetization will propagate through the molecule. As demonstrated earlier (2), the net magnetization transfer obtained in this type of experiment permits the recording of phase-sensitive spectra, and gives in many cases enhanced resolution and sensitivity compared to the widely used COSY experiment (13-16). For short mixing times ( $<0.1/J$ ), only direct connectivities will be observed. For longer mixing times, magnetization that has been transferred from spin A to spin M during the first part of the mixing period can be relayed to spin X during the second

part, thus showing relayed connectivity between A and X in an AMX spin system where  $J_{AM}$  and  $J_{MX}$  have nonzero values. Multiple relays are also observed readily if long mixing periods are used (4, 2).

The methods we proposed earlier employ continuous rf irradiation to induce magnetization transfer and can be described conveniently as homonuclear analogs of the Hartmann–Hahn cross-polarization experiment (7–9). Results previously derived by Müller and Ernst (8) and Chingas *et al.* (9) can then be used directly to describe the effect of rf offset (1, 2). Although our original methods are very insensitive to pulse imperfections, in practice only a limited bandwidth can be covered. A second limitation is that magnetization decay occurs during the mixing period, with time constant  $T_{1\rho}$ . This prohibits the use of long mixing times for macromolecules that generally have short  $T_{1\rho}$  ( $\sim T_2$ ) values.

To overcome these limitations, we have introduced several changes. The resultant pulse scheme of the new 2D experiment is sketched in Fig. 1. The mixing period can be split into two parts: the composite pulse part and the two trim pulses at the beginning and end of the mixing period. The composite pulse cycle will be discussed first. We use an integer number of repetitions of the sequence: ABBA BBAA BAAB AAB  $180_x^\circ$ , where  $A$  is a composite  $180^\circ$  rotation about the  $x$  axis ( $90_y^\circ-180_x^\circ-90_y^\circ$ ) and  $B$  is the inverse of  $A$  (i.e.,  $90_y^\circ-180_x^\circ-90_y^\circ$ ). Hence, the composite pulse cycle consists of 16 composite pulses about the  $\pm x$  axis (the original MLEV-16) cycle, followed by a regular uncompensated  $180_x^\circ$  pulse. This final  $180_x^\circ$  pulse is very efficient at removing the overall effect of pulse imperfections that occurred during the preceding MLEV-16 cycle. Ideally, the MLEV-16 cycle rotates magnetization vectors that are initially parallel to the  $x$  axis through space in such a way that they end up along the  $x$  axis upon completion of the cycle. However, we found in our earlier experiments that if there is some pulse imperfection (imperfect phase shift or unbalanced power for  $x$ ,  $y$ ,  $-x$ , and  $-y$  channels) the magnetization vector will not completely come back to the  $x$  axis upon completion of the MLEV sequence. This error will accumulate if the MLEV cycle is repeated several times. The extra  $180_x^\circ$  pulse added at the end of each MLEV-16 cycle inverts the phase error, and after an even number of these “MLEV-17” cycles the magnetization will again be aligned along the  $x$  axis.

The two trim pulses (several ms each) at the beginning and end of the mixing period serve to defocus any magnetization that is not parallel to the  $x$  axis and ensure that the 2D spectrum can be phased easily to the absorption mode. During the composite

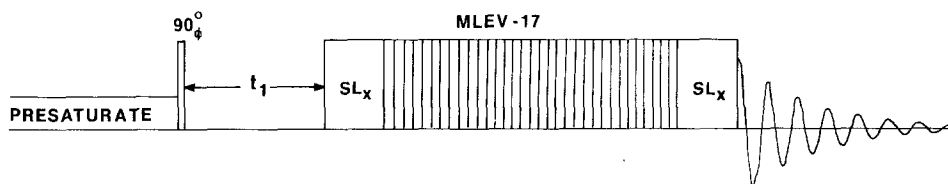


FIG. 1. Pulse scheme for obtaining 2D homonuclear Hartmann–Hahn spectra by using an extended MLEV-16 sequence, described in the text, preceded and followed by “trim pulses” that use rf inhomogeneity to defocus magnetization not parallel to the  $x$  axis. The phase of the  $90^\circ$  pulse is cycled along all four axes, as described in Ref. (1).

pulse cycle, magnetization that is initially along the  $x$  axis is rotated through space in such a way that effectively one-half of the time this magnetization is aligned along the static magnetic field. Therefore, the relaxation rate during the composite pulse cycle is determined by the average of  $1/T_1$  and  $1/T_{1\rho}$  (17). In the limit,  $T_1 \gg T_{1\rho}$ , one can therefore expect an apparent lengthening of the decay constant by up to 100%. Experimentally, we have observed a lengthening by 96% of the decay constant of the nonexchangeable amide protons in ribonuclease. In the case of interest, where there is periodic exchange of spin-locked magnetization between coupled spins, this exchange evolves through a state of antiphase components (4, 8, 9) for which the relaxation is not lengthened by the application of the MLEV sequence. This reduces somewhat the advantage of the MLEV sequence, giving an apparent lengthening of the decay constant of propagating magnetization by up to only 33% compared to earlier experiments (1-4). However, even this reduced gain in apparent  $T_{1\rho}$  can be crucial if the technique is applied to macromolecules.

Here, we demonstrate the method for the determination of connectivity in angiotensin-II, an octapeptide with the following structure: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe. A 32 mg sample was dissolved in 0.5 ml 80% H<sub>2</sub>O/20% D<sub>2</sub>O, at pH 3. Experiments were performed on a Nicolet 500 MHz spectrometer at 30°C. Four watts rf power was used to generate an effective rf field strength of 7.1 kHz, sufficient to cover a spectral width of  $\pm 2$  kHz (8 ppm). At offsets larger than 2 kHz from the transmitter the MLEV sequence still provides an effective spin-lock field but the cross-polarization rate for spins far off resonance starts dropping. Figure 2 shows the result obtained by using 32 MLEV-17 repetitions (total time 70 ms), flanked by two 2.5 ms trim pulses. For this relatively long mixing time, a significant number of direct and relay connectivities are observed. For example, intense amide-methyl cross peaks are observed for both the Val and Ile residues. Also, for this relatively long mixing time, intense cross peaks showing connectivity between the C $\alpha$ H and C $\beta$ H protons are observed in the proline residue. By taking slices through the 2D spectrum at the  $F_2$  frequencies of the amide protons, partial subspectra of the various amino acids are obtained (Fig. 3). Presaturation of the H<sub>2</sub>O resonance was used during the delay time between experiments only. Longitudinal H<sub>2</sub>O magnetization that builds up during the evolution period is effectively dephased by the two trim pulses. Hence, only a small amount of  $t_1$  noise caused by the residual H<sub>2</sub>O resonance is visible in Fig. 2.

The new Hartmann-Hahn based 2D experiments are very powerful for establishing connectivity and obtaining spectral assignment. The net magnetization transfer in this type of experiment yields high sensitivity, even for poorly resolved multiplets, and permits the recording of phase-sensitive 2D spectra, yielding improved resolution. For short mixing times (<20 ms) coherence transfer is mainly restricted to directly coupled protons; for longer mixing times relay connectivities are observed in a similar but often more sensitive way than in the homonuclear RELAY experiment (18-20). We have demonstrated that an MLEV-17 pulse cycle can be effectively used to transfer magnetization between homonuclear coupled spins. This sequence allows the use of modest rf field strengths for obtaining cross polarization over a wide spectral width. It is likely that some of the other sequences proposed for broad-band heteronuclear decoupling (21-25) can also be successfully used for this purpose.

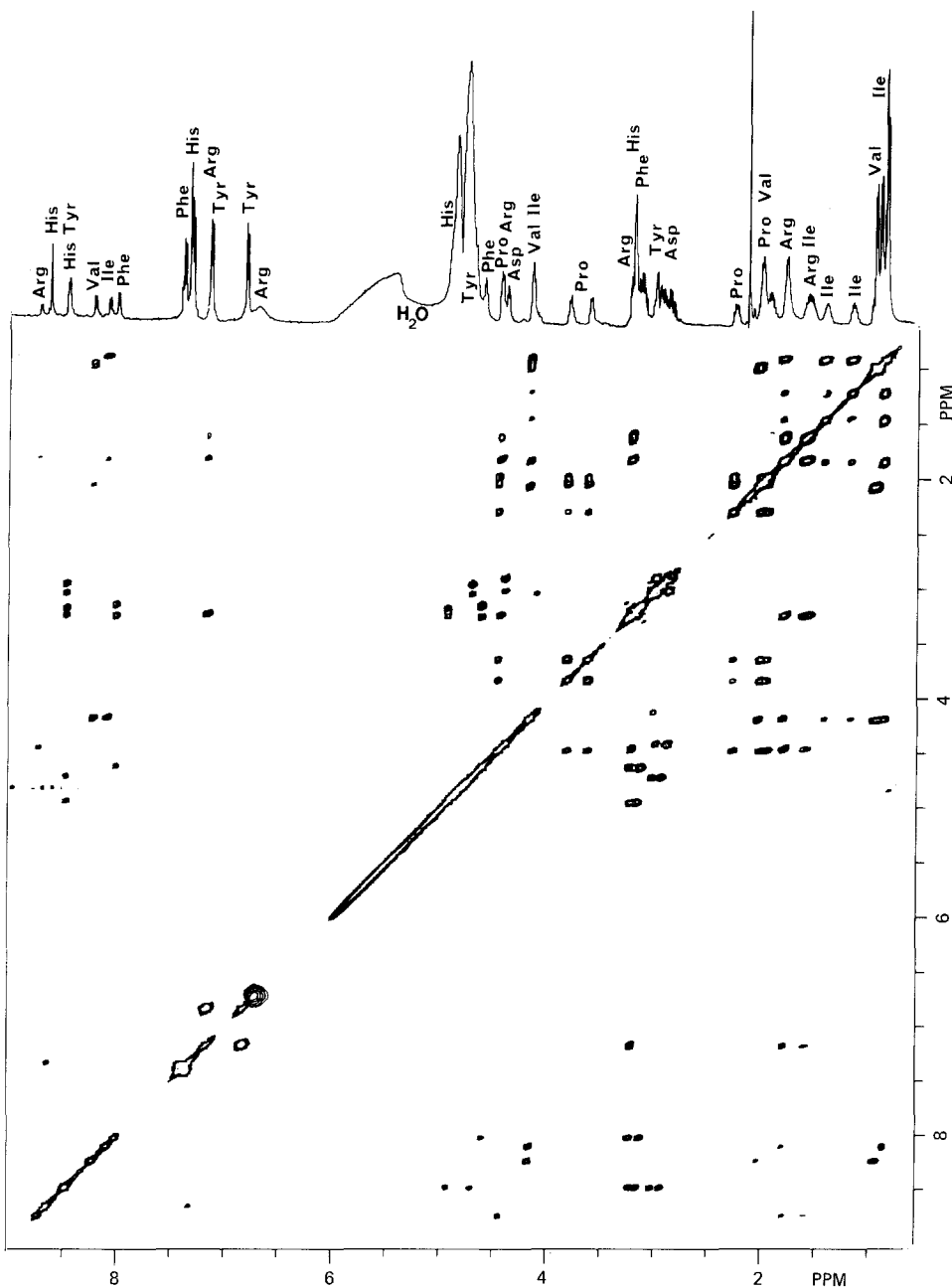


FIG. 2. Two-dimensional absorption-mode MLEV cross-polarization spectrum of a 70 mM solution of angiotensin-II in 80% H<sub>2</sub>O/20% D<sub>2</sub>O recorded at 500 MHz and at 30°C. The spectrum results from a 2 × 256 × 512 data matrix, and zero filling in both dimensions has been used to yield a 512 × 512 matrix for the absorptive part of the spectrum. In both dimensions an acquisition time of 51.2 ms was used. Sixteen scans were recorded for every value of  $t_1$ . In addition to the four-step phase cycle needed for quadrature detection, CYCLOPS phase cycling of the entire experiment was used. The total measuring time was 2 h, and the mixing time consisted of 32 MLEV-17 cycles (70 ms) plus two trim pulses of 2.5 ms each. Gaussian broadening was used in both dimensions to avoid truncation. No symmetrization or other beautifying routine was used to enhance spectral appearance.

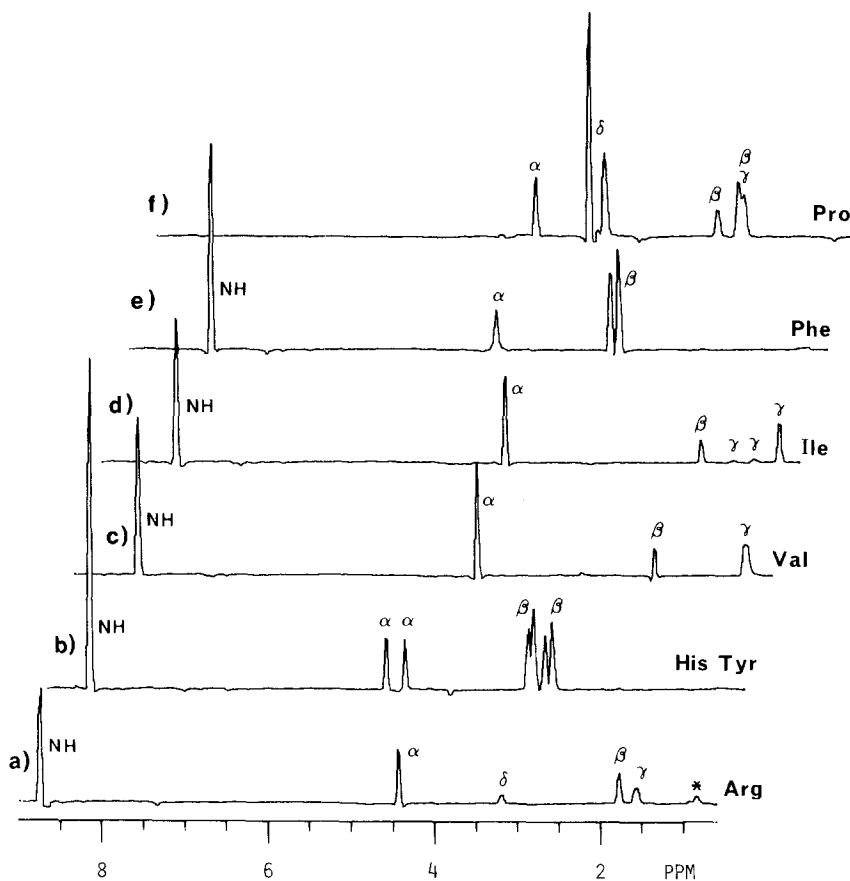


FIG. 3. Cross sections parallel to the  $F_1$  axis of the data matrix of Fig. 2 taken at the  $F_2$  frequencies of the various amide protons and at the low-field Pro  $C_\delta$  proton. The resonance in trace (a), indicated by an asterisk, is due to  $F_2$  baseline distortion caused by the intense methyl resonances.

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