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# Adiabatic TOCSY for C,C and H,H J-transfer

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**Abstract**

Adiabatic pulses have been widely used for broadband decoupling and spin inversion at high magnetic fields. In this paper we propose adiabatic pulses and supercycles that can be used at high magnetic fields like 800 or 900 MHz to obtain broadband TOCSY sequences with C,C or H,H J-transfer. The new mixing sequences are equal or even superior to the well known DIPSI-2,3 experiments with respect to bandwidth. They prove robust against pulse miscalibration and $B_1$ inhomogeneity and are therefore attractive for fully automated spectrometer environments. These adiabatic mixing sequences have been incorporated in a novel z-filter HCCH-TOCSY experiment.

## Introduction

The TOCSY building block (Braunschweiler and Ernst, 1983; Glaser and Quant, 1996) is one of the most important building blocks used in biomolecular NMR either for backbone or for side chain assignment experiments (Sattler et al., 1999). TOCSY achieves the fastest magnetization transfer between coupled spins and relies on the removal of chemical shifts from the Hamiltonian, which for a given bandwidth in ppm is getting more and more difficult as magnetic field strength increases. Therefore new TOCSY sequences need to be developed where the higher $B_0$ field is not compensated for by an increase in $B_1$ field, which would result in a quadratic increase of RF power. Here we emphasize on the design of building blocks and phase cycles, which require low power, have a broad bandwidth and show a square shaped coherence transfer efficiency diagram.

Adiabatic pulses (Tannus and Garwood, 1997) are used in high resolution liquid NMR for decoupling and spin inversion over large frequency bandwidths (Bendall, 1995; Fu and Bodenhausen, 1995; Kupce and Freeman, 1995; Kupce et al., 1998; Starcuk et al., 1994) with a reasonable amount of RF-field strength. Additionally these pulses are also extremely robust against pulse miscalibration and $B_1$ field inhomogeneities. Therefore it is of obvious interest whether these adiabatic pulses are suitable to be used in TOCSY experiments for C,C and H,H J-transfer. Kupce et al. (1998) have shown that adiabatic pulses can be used in H,H-TOCSY experiments. However the published sequence, based on ca-WURST pulses (constant adiabaticity WURST-n, n = 2 or 8 (Kupce and Freeman, 1995)) expanded by the supercycle *P5M4* (Tycko et al., 1985), is far less broad banded than any of the traditional TOCSY sequences like MLEV-17, DIPSI-2,3 and FLOPSY-16. In this sequence an excellent transfer between resonances close to the diagonal is achieved, but the transfer efficiency diagram is far from being square shaped. Therefore we set out to find sequences with a more square shaped mixing profile that will also be suitable for C,C J-transfer. We will present pulses and supercycles that can be used in adiabatic TOCSYs for C,C and H,H J-transfer that perform better than the well known DIPSI-2,3 (for H,H and C,C J-transfer) (Shaka et al., 1988) and FLOPSY-16 (Kadkhodaie et al., 1991) (for H,H J-transfer) sequence.

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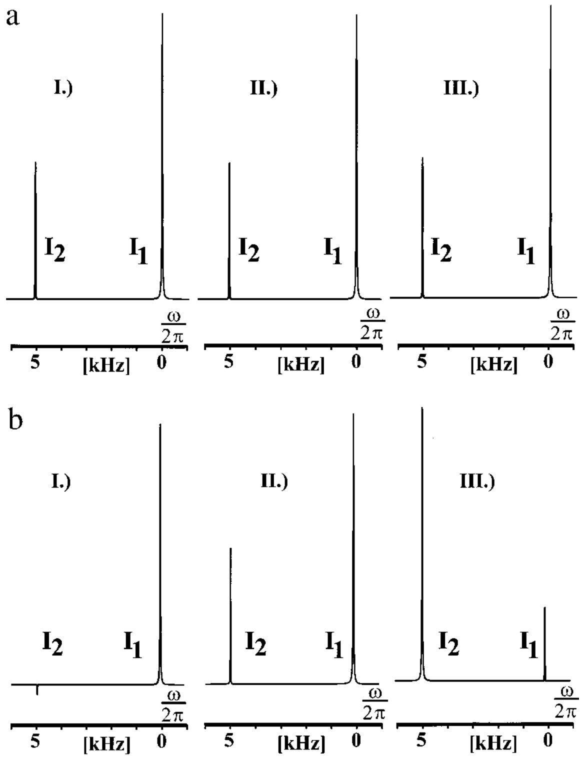


*Figure 1.* Efficiency of the adiabatic TOCSY transfer as a function of different parameters. All spectra were simulated as an ideal two spin system in which spin 1 was selectively excited and the magnetization was subsequently transferred to spin 2. In all simulations a ca-WURST-8 with a *P5M4* supercycle is used for magnetization transfer. In (a) can be seen that the time difference for the inversion of the two coupled spins $\Delta$t is not a critical parameter in TOCSY transfer. The following pulses were used to establish the magnetization transfer: (I) ca-WURST-8, total sweep width = 800 kHz, duration $T_p$ = 100 μs, $\gamma B_1/2\pi$ = 62605 Hz, time difference between the inversion of the two spins $\Delta t$ = 0.4 μs; (II) ca-WURST-8, total sweep width = 200 kHz, $T_p$ = 100 μs, $\gamma B_1/2\pi$ = 31303 Hz, $\Delta t$ = 1.6 μs; (III) ca-WURST-8, total sweep width = 50 kHz, $T_p$ = 100 μs, $\gamma B_1/2\pi$ = 15651 Hz, $\Delta t$ = 6.5 μs. As it can be seen in (b) the most important parameter for the transfer efficiency in adiabatic TOCSY schemes is the duration of the adiabatic pulses. The following pulses were used to establish the magnetization transfer: (I) ca-WURST-8, total sweep width = 400 kHz, $T_p$ = 200 μs, $\gamma B_1/2\pi$ = 31303 Hz, $\Delta t$ = 1.6 μs; (II) ca-WURST-8, total sweep width = 200 kHz, $T_p$ = 100 μs, $\gamma B_1/2\pi$ = 31303 Hz, $\Delta t$ = 1.6 μs; (III) ca-WURST-8, total sweep width = 100 kHz, $T_p$ = 50 μs, $\gamma B_1/2\pi$ = 31303 Hz, $\Delta t$ = 1.6 μs.

## Materials and methods

Adiabatic pulses invert spins by a ‘slow’ passage of a chirped pulse through resonance. So spins having a different resonance frequency will be inverted at different times. This differs from the behavior during a square pulse, where all spins are inverted simultaneously. In order to assess the relevance of this feature of adiabatic pulses for the efficiency of magnetization transfer, we carried out simulations on an ideal two spin system. The spins $I_1$ and $I_2$ are coupled to each other with a coupling constant J. After selectively exciting $I_1$, magnetization is transferred by a TOCSY sequence to $I_2$. The TOCSY sequence consists of a pulse with a WURST-8 shape subjected to a *P5M4* supercycle expansion (P5: 0°, 150°, 60°, 150°, 0°, expanded by MLEV4, details are given in the caption of Figures 1a and 1b) (Tycko et al., 1985). This is the same type of pulse and supercycle as has been used by Kupce et al. for establishing the H,H transfer. The frequency covered by the sweep nevertheless is significantly different in order to establish a truly adiabatic behavior. The resulting large RF field strength is of no concern in the context of the simulation. Figure 1a demonstrates that the time difference between the inversion of the two spins in an adiabatic passage is not a critical parameter for Hartmann-Hahn transfer. The most important parameter for the transfer, as is shown in Figure 1b, is the duration of the adiabatic pulse. So the ideal pulse should operate at a reasonable RF field strength (ca. 10 kHz) and be as short as possible, but still fulfilling the adiabatic condition. The key to the experiment is to find a pulse having these characteristics. Unfortunately this is not a trivial task, since the adiabatic behavior will suffer severely once the pulse length is getting too short. The pulse, which eventually worked best in our hands, is a tanh/tan pulse described in the context of the BIR pulse family (BIR - $B_1$ insensitive rotation) (Staewen et al., 1990; Garwood and Ke, 1991; Hwang et al., 1998), which performs a fast sweep at affordable RF power and an acceptable adiabaticity level. Even though short pulses can be constructed with other types of shapes as well, their performance with respect to the adiabatic behavior or the required peak power was not as good as for the tanh/tan pulse. This tanh/tan pulse can be constructed from the following adiabatic half passage and its time reversed half passage:

$$\gamma B_1(t) = \gamma B_1^o f_B(t),$$
$$\Delta\omega(t) = \Delta\omega^o f_\omega(t).$$

$f_B(t)$ and $f_\omega(t)$ are dimensionless, time dependent modulation functions described by the hyperbolic tangent (tanh) and tangent (tan) function respectively, with $0 \leq t \leq T_p/2$ (Hwang et al., 1998):

$$f_B(t) = \tanh[\xi 2t/T_p],$$
$$f_\omega(t) = \tan[\kappa(1 - 2t/T_p)]/\tan[\kappa].$$

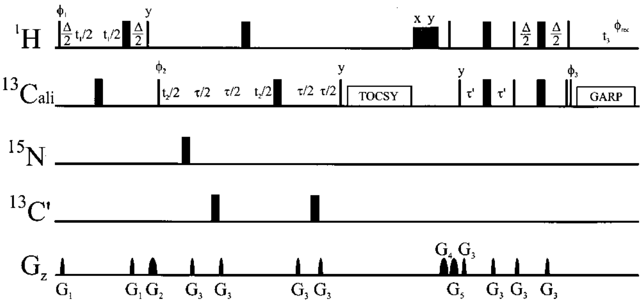


*Figure 2.* Pulse sequence of the z-filter HCCH-TOCSY experiment. Narrow and thick bars represent 90° and 180° pulses. Unless otherwise indicated the default phase for pulses is x. Phase cycling: $\phi_1 = x, -x$, $\phi_2 = 2(x), 2(-x)$, $\phi_3 = 4(x), 4(-x)$, $\phi_{rec} = x, 2(-x), x$. Quadrature detection in both dimensions is obtained by altering $\phi_1$ and $\phi_2$ respectively in the States-TPPI manner (Marion et al., 1989). Proton trim pulses of 1 ms were used. Delay durations: $\Delta = 3.2$ ms, $\tau = 475$ µs and $\tau' = 1.1$ ms. Carrier positions: $^1$H = 4.65 ppm, $^{13}$C = 42 ppm, $^{15}$N = 114.5 ppm. Proton pulses are applied using a 38.5 kHz rf field. $^{13}C_{ali}$ pulses are hard pulses with a field of 20.8 kHz. Off resonance Q3 shaped pulses with duration of 256 µs were used for $^{13}$C′ decoupling pulses. $^{15}$N pulses are at a field of 2.6 kHz and $^{13}$C GARP decoupling (Shaka et al., 1985) during acquisition is applied with a 2.5 kHz field. Gradients (sine bell shaped): $G_1$ = (500 µs, 16 G/cm), $G_2$ = (1 ms, 30 G/cm), $G_3$ = (300 µs, 16 G/cm), $G_4$ = (5 ms, 60 G/cm), $G_5$ = (4.4 ms, 40 G/cm). For the 2D version of the experiment 8 scans per $t_2$ (256 complex points, spectral width 12374.02 Hz) experiment were recorded with 2048 complex points in $t_3$ (spectral width 10000 Hz). A repetition delay of 2 s was used between scans, giving rise to a total measurement time of approximately 1.5 h for the 2D version of the experiment. Every TOCSY sequence explained in the text can be used with this z-filter HCCH-TOCSY sequence.

As seen in Figure 1b III nearly complete TOCSY transfer of magnetization from spin $I_1$ to spin $I_2$ can be achieved with a pulse duration of 50 µs. A tanh/tan pulse of 50 µs duration (tanh/tan: $\Delta\omega^0 = 150$ kHz, $\gamma B_1/2\pi^{avr} = 9750$ Hz, $\gamma B_1/2\pi^{max} = 10504$ Hz, $\zeta = 10$, $\tan\kappa = 20$, Q = 2 (Staewen et al., 1990; Garwood and Ke, 1991; Hwang et al., 1998)) has a comparable RF field strength to commonly used square pulses (duration of 90° pulse = 25 µs, $\gamma B_1/2\pi = 10000$ Hz). We have also used BIR-4 rotations (Staewen et al., 1990; Garwood and Ke, 1991), that are composed of two tanh/tan units, for C,C and H,H J-transfer (BIR-4: $\Delta\omega^0 = 150$ kHz, duration = 100 µs, $\gamma B_1/2\pi^{avr} = 9750$ Hz, $\gamma B_1/2\pi^{max} = 10504$ Hz, $\zeta = 10$, $\tan\kappa = 20$, Q = 2). The advantage of BIR-4 when compared to a tanh/tan rotation is its broader inversion profile that correlates with a higher adiabaticity. The double duration of BIR-4 compared to tanh/tan has no impact on the transfer performance. However, this will require a doubling of the mixing time in order to fully complete one expansion cycle. In the case of larger and more complicated expansions this makes the sequence less flexible for adaptation to an optimum mixing time dictated by the coupling topology of the spin system. The scheme of expanding the tanh/tan pulse to the BIR-4 pulse is generally applicable to other shapes as well.

To obtain a square shaped offset dependent mixing profile the basic building block of the TOCSY sequence needs to be expanded into supercycles. The supercycles most successfully used with adiabatic pulses are composed of P5 (P5: 0°, 150°, 60°,150°, 0°) and P9 (P9: 0°, 15°, 180°, 165°, 270°, 165°, 180°, 15°, 0°) (Cho et al., 1986; Skinner and Bendall, 1997; Tycko et al., 1985) expanded by MLEV-16 ($RR\bar{R}\bar{R}$, $\bar{R}RR\bar{R}$, $\bar{R}\bar{R}RR$, $R\bar{R}\bar{R}R$) (Jacobs et al., 1982; Levitt et al., 1982; Shaka et al., 1983). We found that multiple variants of these supercycles like *P5P9M16* perform well in simulation and experiment. Long cycle times make these expansions ideally suited for H,H TOCSY experiments (Figure 6). All H,H TOCSY experiments can also be performed in a sensitivity enhanced manner yielding a gain of about 1.2.

In our study all C,C TOCSY experiments were performed as a z-filter version (Rance, 1987). Whereas in the original version (Kay et al., 1993) transverse magnetization is spin locked and then rotated to the z-axis before applying the $^1$H-pulses/gradients for water suppression, in the version described here magnetization is brought to the z-axis immediately before the spinlock sequence. This way the TOCSY operates on z-magnetization. Simulation showed that TOCSY mixing sequences perform more robust (Glaser and Kramer, 2000) when applied in a z-filter. The novel HCCH-TOCSY version where the C,C TOCSY is performed in a z-filter is shown in Figure 2. Details of the experiment are given in the caption of Figure 2.

All simulations were done using NMRSIM 2.9.1.b software (Bruker Analytik GmbH, Rheinstetten, Germany), which is part of the Bruker NMR-SUITE. An ideal two spin system with a J-coupling of 7 Hz was

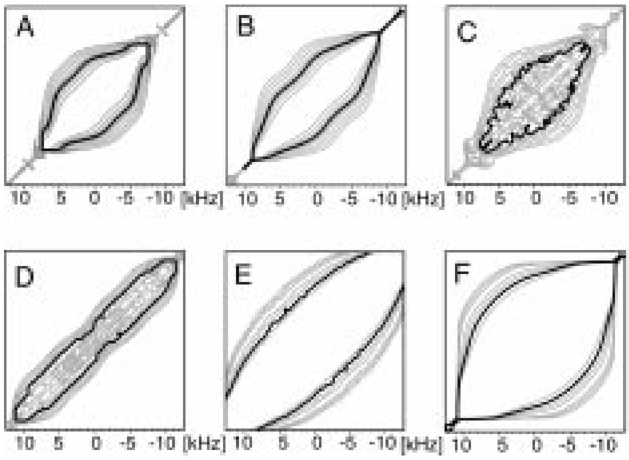


*Figure 3.* Comparison of different TOCSY schemes: The 80% transfer efficiency contour line is emphasized in black: (A) DIPSI-2 with $\gamma B_1/2\pi = 10$ kHz. (B) DIPSI-3 with $\gamma B_1/2\pi = 10$ kHz. (C) tanh/tan sequence: tanh/tan, *P9M16*: $T_p = 50$ µs, $\Delta\omega^0 = 150$ kHz, $\gamma B_1/2\pi^{max} = 11960$ Hz, $\zeta = 10$, tan $\kappa = 20$. (D) ca-WURST-8, *P5M4*: $T_p = 150$ µs, total sweep width = 35 kHz, $\gamma B_1/2\pi^{max} = 10692$ Hz. (E) Using the *P9M16* supercycle with a BIR-4 rotation (BIR-4: $T_p = 100$ µs, $\Delta\omega^0 = 150$ kHz, $\gamma B_1/2\pi^{max} = 11960$ Hz, $\zeta = 10$, tan $\kappa = 20$) provides again an increase of the diagonal and the antidiagonal bandwidth for the transfer. (F) FLOPSY-16 with $\gamma B_1/2\pi = 10$ kHz.

used for the simulations. The transfer time was always set to 1/2J. Experimentally the C,C and H,H J-transfer based adiabatic TOCSY sequences were tested on a 10 mg $^{13}C$,$^{15}N$ labeled sample of Ubiquitin in a $H_2O/D_2O$ 90/10 solution at pH 5.0 and 303 K (commercially available from VLI Research, Inc., Malvern, PA) in a 5 mm microcell Shigemi tube (320 µl). All experiments were performed on Bruker DRX 800 MHz and DRX 600 MHz spectrometers (Bruker Analytik GmbH, Rheinstetten, Germany) equipped with TXI HCN z-gradient probes. Spectra were processed using XWINNMR2.6 from Bruker. One z-filtered HCCH-TOCSY experiment was also carried out on a $^{13}C$,$^{15}N$ labeled sample (1 mM) of the fumarate sensor histidine kinase *Dcu*S (17.4 kDa). The protein sample was at pH 6.5, 50 mM sodium phosphate buffer, 200 mM NaCl, 0.8 mM CHAPS, 50 mM Glycin, 50 pM Pefabloc SC (Fluka AG, Buchs, Switzerland), 0.01% $NaN_3$ and $H_2O/D_2O$ 90/10. Also this sample was measured at 303 K in a 5 mm microcell Shigemi tube.

## Results and discussion

Figure 3 compares the offset dependent transfer efficiencies of two standard TOCSY variants, DIPSI-2 (A) and DIPSI-3 (B), with the adiabatic TOCSY variant published by Kupce et al. (D: ca-WURST-8, *P5M4* (Kupce et al., 1998)) and with our *P9M16*, tanh/tan based TOCSY experiments (C). The offset dependence of the latter is similar to the DIPSI-3 sequence. DIPSI-2 is slightly more broadband along the antidiagonal but less broadband along the diagonal. The sequence shown in D, the original starting point of our work, is very broadband along the diagonal but deviates strongly from the desired square shaped offset dependence. Going to shorter pulses the broadband nature of the transfer improves dramatically both along the diagonal as well as along the antidiagonal. Using a BIR-4 pulse (Figure 3E) the bandwidth along the diagonal increases by a factor of two to three as compared to the DIPSI (A, B) or FLOPSY (F) sequence. The bandwidth along the antidiagonal increases about 50% when compared to the DIPSI-2 sequence.

To evaluate different TOCSY sequences we have used a novel z-filter HCCH-TOCSY experiment (Figure 2) that can accommodate different TOCSY schemes. TOCSY spectra of ubiquitin using the tanh/tan *P9M16* or the DIPSI-3 sequence are shown in Figure 4. Due to the fact that for a given average power the mixing schemes have discrete non identical durations we could not exactly match the mixing times for tanh/tan *P9M16* and DISPI-3. To allow a fair comparison of the different mixing sequences we have taken 1D traces out of the 800 MHz 2D spectra (all shown in Figure 4C). Mixing times for DIPSI-3 were multiples of approx. 5.4 ms and multiples of approx. 7.2 ms for the tanh/tan *P9M16* sequence. This allows us to compare the intensities of the different mixing sequences. The adiabatic tanh/tan *P9M16*

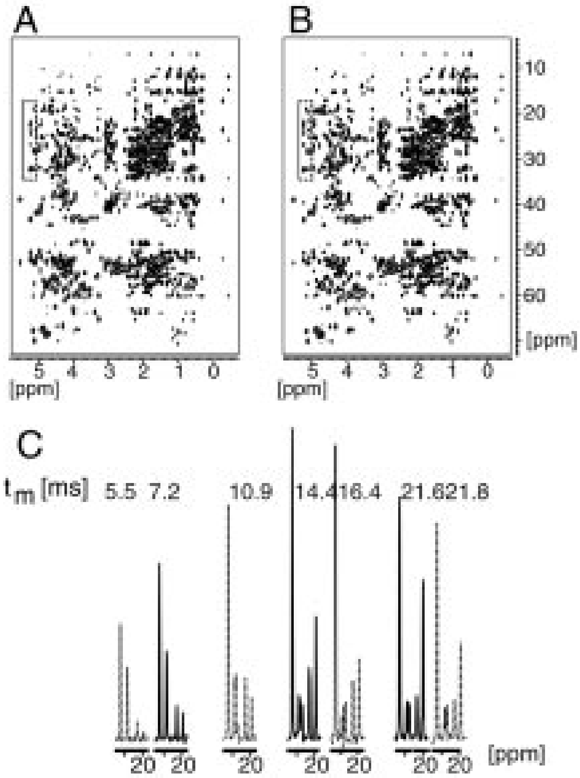


*Figure 4.* For ubiquitin 2D spectra of the HCCH-TOCSY sequence (Figure 2) are shown ($T_m \sim 12$ ms, 800 MHz spectrometer). (A) A DISPI-3 sequence was used for the C,C TOCSY (10 kHz). (B) A *P9M16*, tanh/tan (tanh/tan: $T_p = 50$ µs, $\Delta\omega^0 = 150$ kHz, $\gamma B_1/2\pi^{avr} = 9750$ Hz, $\gamma B_1/2\pi^{max} = 10504$ Hz, $\zeta = 10$, tan $\kappa = 20$, Q = 2) sequence was used. Due to different loop times of the mixing sequences (given by the different length of the supercycles) a direct comparison of the intensities of the spectra is difficult. Therefore slices out of the marked boxes are compared in (C). A build up curve against the mixing time is plotted (black lines are *P9M16*, tanh/tan and dashed lines are DIPSI-3). For comparable mixing times (last two traces at $t_m = 21.6$ ms and $t_m = 21.8$ ms) a gain in sensitivity is observed (10–30%).

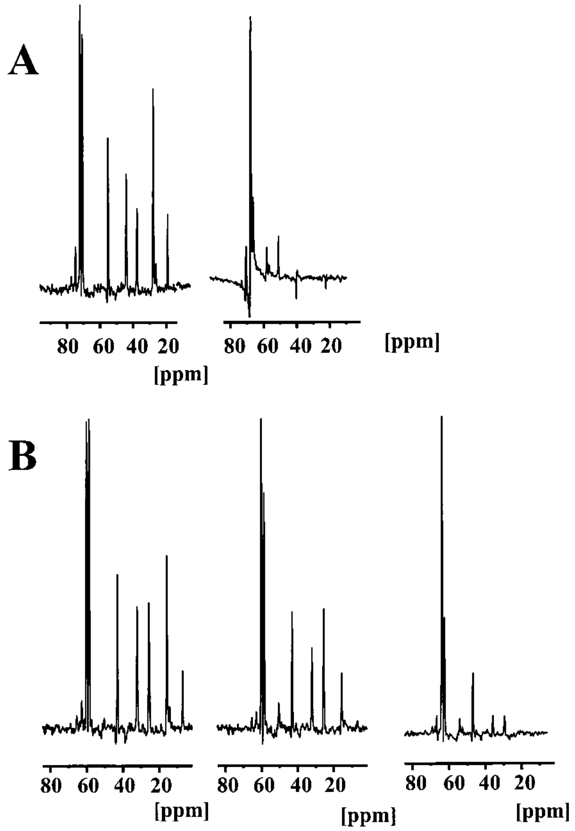


*Figure 5.* Traces out of 2D z-filter HCCH-TOCSY spectra of ubiquitin recorded with: (A) DIPSI-3 with a basic RF field strength of $\gamma B_1/2\pi = 10$ kHz (left) and one decreased by 2 dB (right); $T_m \sim 12$ ms. (B) BIR-4, *P9M16* (BIR-4: $T_p = 100$ µs, $\Delta\omega^0 = 150$ kHz, $\gamma B_1/2\pi^{avr} = 9750$ Hz, $\gamma B_1/2\pi^{max} = 10504$ Hz, $\zeta = 10$, tan $\kappa = 20$, Q = 2) $T_m \sim 14$ ms. The actual RF field strength used was the nominal one ($\gamma B_1/2\pi^{max} = 10504$ Hz, left), the nominal one decreased by 2 dB (middle) and the nominal one decreased by 4 dB (right). The BIR-4 *P9M16* sequence is much more robust towards pulse miscalibration and $B_1$ inhomogeneities.

TOCSY is about 10–30% more sensitive than a DIPSI-3 sequence. Comparing the last two traces in Figure 4, which are taken at 21.6 ms and 21.8 ms, respectively, this enhancement effect can be seen most easily. Even though this mixing time is not ideal, since magnetization has been lost due to relaxation, the adiabatic tanh/tan *P9M16* TOCSY (full black line) has a higher intensity than the DIPSI-3 sequence (dashed line).

The different mixing schemes proposed in this article are quite long and can therefore only accommodate a limited discrete set of mixing times. However, different pulses like tanh/tan and BIR-4 with different length and similar performance are available to provide more flexibility. For H,H J-transfer experiments we propose a *P5P9M16* ((P5: 0°, 150°, 60°, 150°, 0°) expanded first by P9 (P9: 0°, 15°, 180°, 165°, 270°, 165°, 180°, 15°, 0°) and then by MLEV16) supercycle with either tanh/tan or BIR-4 pulses. These supercycles provide excellent performance when compared with FLOPSY-16, which is known to be close to ideal considering the transfer efficiency along the antidiagonal (Glaser and Quant, 1996). *P5P9M16* is even broader along the antidiagonal than FLOPSY-16 (Figure 6).

All adiabatic TOCSY sequences have in common that they are insensitive against pulse miscalibration and $B_1$ field inhomogeneities. In Figure 5 spectra of a DIPSI-3 sequence with $\gamma B_1/2\pi = 10$ kHz are compared with those of a BIR-4 *P9M16* sequence (details are given in the caption of Figure 5). Reduction of

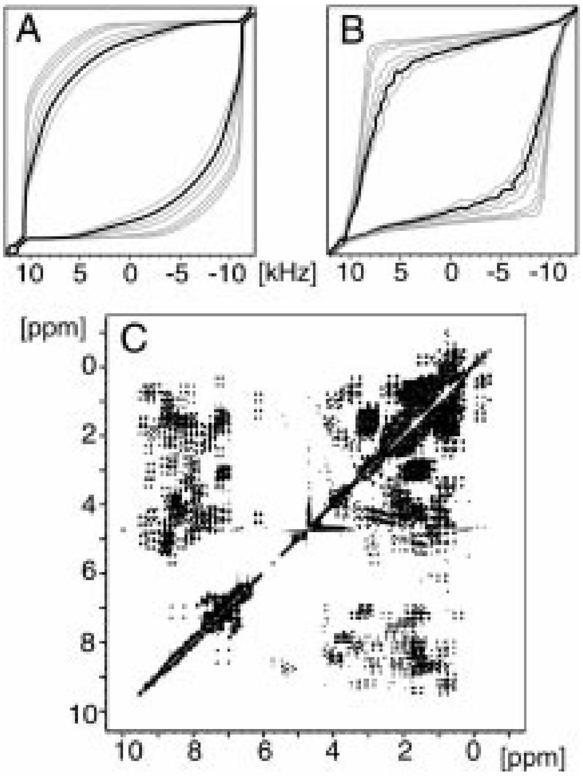


*Figure 6.* Simulation of the transfer efficiencies: (A) FLOPSY-16 with $\gamma B_1/2\pi = 10$ kHz, and (B) tanh/tan, *P5P9M16* (tanh/tan: $T_p = 50$ µs, $\Delta\omega^0 = 150$ kHz, $\gamma B_1/2\pi^{avr} = 9750$ Hz, $\gamma B_1/2\pi^{max} = 10504$ Hz, $\zeta = 10$, tan $\kappa = 20$, Q = 2). (C) H,H TOCSY spectrum using (B) recorded on Ubiquitin.

the RF field strength in steps of two dB influences the transfer efficiency of DIPSI-3 much more than that of the BIR-4 *P9M16* sequence. Even a RF field strength miscalibrated by +4 dB, which is equivalent to using a 50° pulse instead of a 90° pulse, still yields transfer with the BIR-4 *P9M16* sequence. Adiabatic pulses are even more robust against an increase of RF power compared to the just discussed decrease since additional RF field strength will yield a higher Q factor and thus an even better adiabatic behavior. This is not the case for TOCSY sequences based on square pulses. Sequences like DIPSI and FLOPSY tolerate only small RF field strength miscalibrations in either direction. Especially FLOPSY-16 has shown to be very prone to miscalibration. The robustness of adiabatic TOCSY sequences therefore makes them highly attractive for automated biomolecular NMR spectroscopy where robustness of the sequence against miscalibration is beneficial.

## Conclusions

We have presented novel and more efficient building blocks for adiabatic TOCSY J-transfer. Using tanh/tan or BIR-4 adiabatic pulses expanded by supercycles like *P9M16* for C,C transfer or P5P9M16 for H,H transfer, we have achieved a gain in sensitivity of about 10–30% for the tanh/tan *P9M16* C,C sequence when compared to a traditional DIPSI-3 C,C experiment. More important, these TOCSY sequences, especially when combined with BIR-4 pulses have shown to tolerate significant pulse miscalibration and $B_1$ inhomogeneity. These adiabatic TOCSY sequences have been tested using a novel z-filter version of a HCCH-TOCSY on ubiquitin and the histidine kinase fumarat sensor protein *Dcu*S (17.4 kDa) yielding equally good spectra and therefore promise to be applicable to a wide range of systems of different sizes.

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