

Adiabatic Mixing in the Liquid State

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Adiabatic spin inversion has been used in the liquid state very efficiently for decoupling purposes. Here we show that it can also be adapted for spin mixing experiments, such as the TOCSY and clean TOCSY experiment, and is superior to previously employed mixing sequences. The main advantage of adiabatic mixing sequences over the conventional mixing schemes used in liquid state experiments is an extremely low sensitivity to RF field inhomogeneity and miscalibration of the B_1 field strength. The method is evaluated experimentally by comparing results obtained with different mixing schemes in the basic 2D TOCSY experiment. In addition to higher reliability, adiabatic mixing provides a sensitivity improvement of ca. 20% as compared to conventional mixing schemes. This is explained by higher signal losses due to RF inhomogeneity in the experiments employing traditional mixing schemes. More significant sensitivity improvements can be expected in situations where RF homogeneity is traditionally poor, for example, in large volume probes and magnetic resonance imaging experiments. © 1998 Academic Press

Clear advantages of adiabatic decoupling (1–5) over traditional (composite pulse) decoupling methods (6, 7) suggest that similar benefits could be expected from adiabatic methods in experiments involving spin mixing as a coherence transfer step (8, 9). Consistently, adiabatic cross-polarization techniques have recently become very popular in solid state NMR (10–12). Here we demonstrate that adiabatic inversion pulses can be used to construct efficient isotropic mixing schemes for coherence transfer in homonuclear spin systems in liquids (13).

The main advantages of using adiabatic pulses as main building blocks for constructing spin decoupling waveforms are extremely efficient use of RF power, high tolerance to RF field inhomogeneity and miscalibration, and excellent off-resonance performance in band-selective homodecoupling (4, 14, 15) applications. Similar advantages have been demonstrated in adiabatic cross-polarization experiments in solids (12). A principal difference between the two applications, decoupling and cross polarization, is that in

the decoupling experiments adiabatic pulses are used to invert spins adiabatically, whereas in the cross-polarization experiments, the Hartmann–Hahn condition (16) is approached adiabatically (10, 11). Although the latter technique can be used in liquids (17, 18), its main disadvantage is that considerably longer contact (mixing) times are needed, which makes it unattractive for applications to molecules with fast relaxing spin systems, such as biopolymers. To cope with this, we show below that adiabatic inversion pulses can be optimized for use in spin mixing experiments, such as in adiabatic TOCSY and clean TOCSY experiments. Since the principle of matching the Hartmann–Hahn condition used here is different from that published previously (10, 17, 18), we call this experiment adiabatic mixing of the second kind.

Both, the wide band spin mixing and decoupling experiments are based on repetitive application of composite inversion pulses to the given spin system, and therefore both experiments may appear very similar at first glance. Nevertheless, the theoretical requirements for obtaining a good performance are quite different (19).

The effective Hamiltonian, H_{mix} , in an offset range where the isotropic mixing is efficient can be written as (8, 9)

$$H_{\text{mix}} = -\omega_{\text{I,eff}}\mathbf{I} - \omega_{\text{S,eff}}\mathbf{S} + 2\pi J_{\text{eff}}\mathbf{I} \cdot \mathbf{S}, \quad [1]$$

where the effective scalar coupling, J_{eff} , determines the efficiency of the polarization transfer. The effective coupling can be related to the unperturbed J coupling by introducing a scaling factor λ_{mix} ,

$$J_{\text{eff}} = \lambda_{\text{mix}}J. \quad [2]$$

A similar scaling factor λ_{dec} is introduced in the theory of wideband decoupling (6, 7). Note, however, that good decoupling requires the λ_{dec} be as small as possible ($\lambda_{\text{dec}} \ll 1$), whereas in spin mixing experiments considerable efforts are

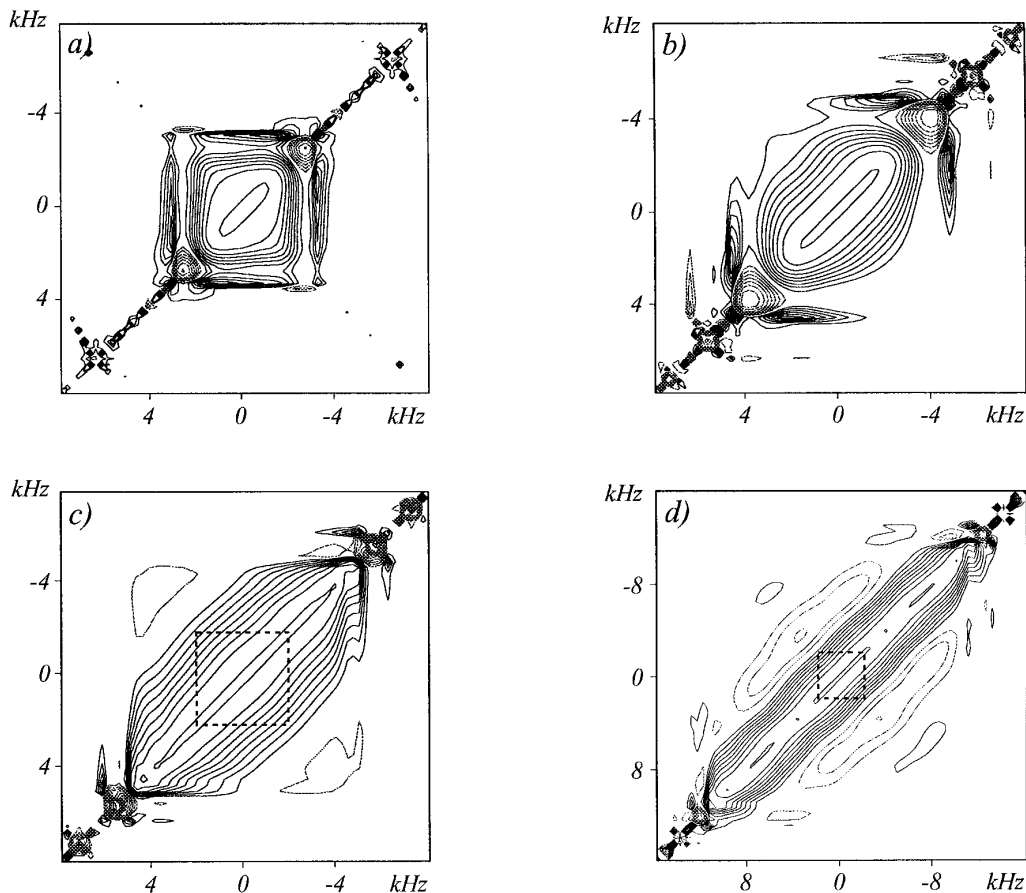


FIG. 1. The coherence transfer efficiency calculated for different mixing waveforms assuming $J = 10$ Hz. (a) A planar WURST-8 pulse with MLEV-16 phase cycle, $T_p = 0.1$ ms, $B_1(\text{max}) = 6.95$ kHz, $B_1(\text{RMS}) = 5.57$ kHz. (b) The same as in (a) but with a 20-step supercycle. (c) Frequency-modulated WURST-8 pulse, $T_p = 0.25$ ms, sweep width of 16.0 kHz, $B_1(\text{max}) = 5.60$ kHz, corresponding to $Q_0 = 2.0$ and $B_1(\text{RMS}) = 4.52$ kHz. (d) The same as in (c) except sweep width of 32 kHz was used, $B_1(\text{max}) = 9.70$ kHz, corresponding to $Q_0 = 3.0$, $B_1(\text{RMS}) = 7.84$ kHz.

made in order to maximize the scaling factor ($\lambda_{\text{mix}} \leq 1$). Stated otherwise, the quality of broadband decoupling mainly depends on the long-term properties of the RF field, whereas for broadband spin mixing experiments the short-term properties are the primary concern. As pointed out by Fujiwara *et al.* (20), there is a direct relationship between the intensity of sidebands in a decoupling experiment and coherence transfer efficiency in a corresponding mixing experiment. Therefore, we can expect that relatively short adiabatic pulses will be most efficient in mixing experiments.

In homonuclear spin mixing experiments employing a monochromatic (CW) RF field, the coherence transfer function a_{IS} can be expressed analytically (21) as

$$a_{IS} = \frac{1}{1+b} \sin^2(\Omega_{IS}t), \quad [3]$$

where

$$b = \frac{\Delta_{\text{HH}}}{|J_{\text{eff}}|} \quad [4]$$

$$\Omega_{IS} = \frac{1}{2} \sqrt{\Delta_{\text{HH}}^2 + J_{\text{eff}}^2} \quad [5]$$

$$\Delta_{\text{HH}} = \omega_{eI} - \omega_{eS} \quad [6]$$

$$J_{\text{eff}} = \frac{1}{2} J [1 + \cos(\theta_I - \theta_S)] \quad [7]$$

$$\omega_e = \sqrt{\omega_1^2 + \Delta\omega^2} \quad [8]$$

$$\tan \theta = \frac{\Delta\omega}{\omega_1}. \quad [9]$$

$\Delta\omega$ is the offset from the carrier frequency and ω_I is the RF

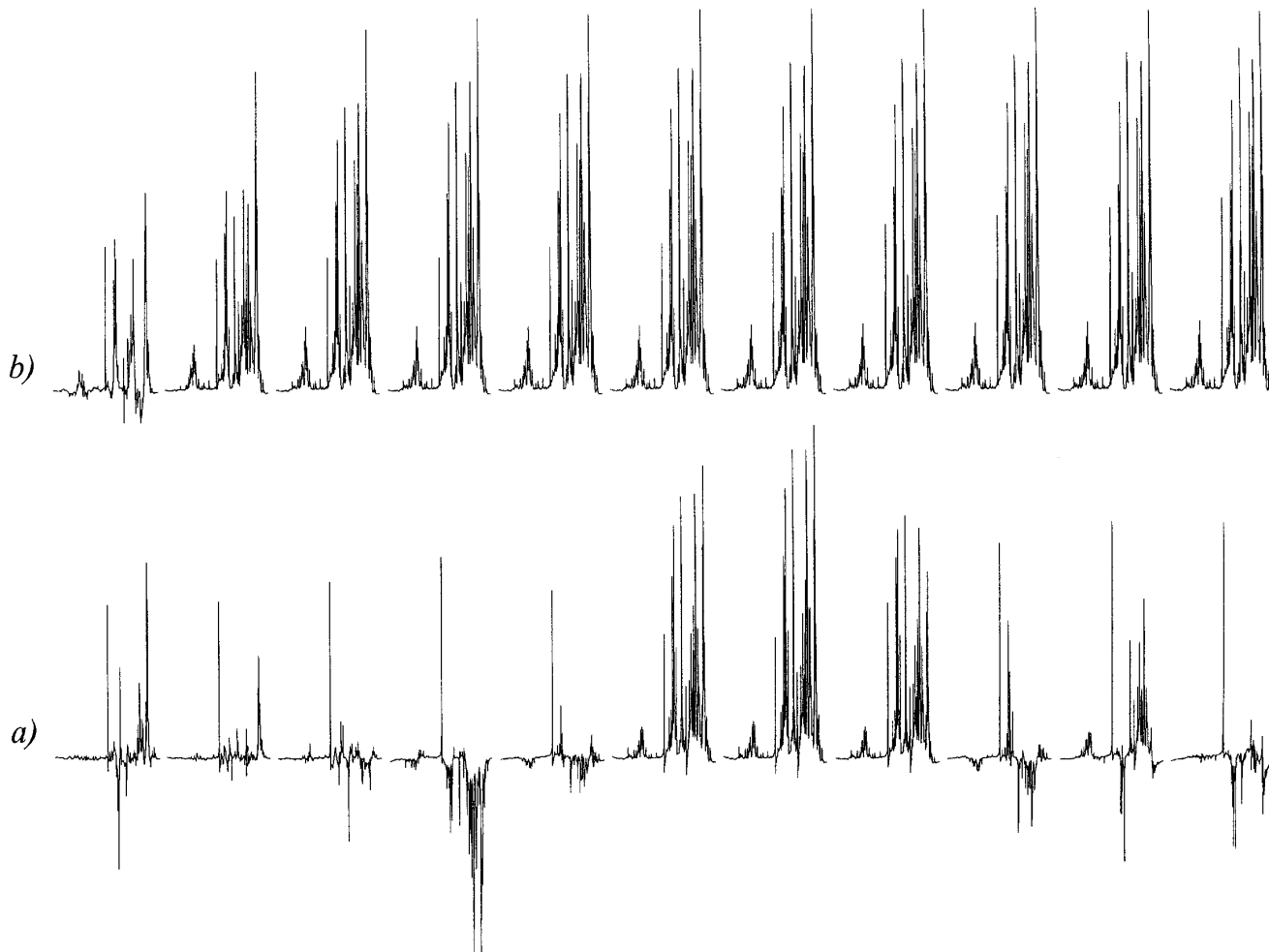


FIG. 2. Tolerance of 1D TOCSY spectra to miscalibration of the RF field strength. (a) DIPSI-2 composite pulse was used for spin mixing. $T_p = 7.66$ ms, with optimum B_1 field strength of 3.75 kHz; the RF power level was incremented in 1-dB steps from 2.16 to 6.84 kHz. (b) WURST-8 mixing waveform, $T_p = 0.25$ ms, sweep width of 16.0 kHz, was used with a 20-step supercycle (20); the RF power level was increased in 1-dB steps from 3.96 kHz (3.2 kHz RMS) to 12.52 kHz (10.12 kHz RMS). The spectra were recorded with the 14-kDa protein villin 14 T (25), 5 mM in D_2O , using a mixing time of 60 ms on a Varian UnityPlus spectrometer operating at a frequency of 400 MHz.

field strength; all frequencies are in rad/s. The transfer is most efficient if the Hartman–Hahn mismatch $\Delta_{HH} = 0$.

In the presence of a frequency-modulated adiabatic pulse, the effective coupling and the Hartmann–Hahn mismatch become time dependent and the efficiency of the coherence transfer is most conveniently analyzed numerically. On the other hand, we can predict the efficiency of the adiabatic mixing by decomposing the adiabatic pulse into N steps such that for each step of length τ_n there is a corresponding fixed frequency $\Delta\omega_n$. This essentially can be represented as an interleaved DANTE sequence consisting of N independent monochromatic mixing fields which match the Hartmann–Hahn condition for all spin pairs symmetric with respect to the instant frequency, $\Delta\omega_n$. Obviously, for the given total length of the adiabatic pulse T_p , the time τ_n spent at each particular frequency $\Delta\omega_n$ will be less for larger N . Therefore, as the

modulation depth of the adiabatic pulse increases, it becomes less efficient for spin mixing.

In order to achieve efficient spin inversion in a short time scale we must use optimized frequency sweep pulses (4, 22). Low RF peak amplitudes, which are usually preferred in experiments employing relatively long irradiation schemes, can conveniently be generated using the so called WURST- n modulation scheme (2) employing the optimum frequency sweep (22), which can be defined in terms of RF amplitude ω_1 ,

$$\omega_1(t) = \omega_1(\max)[1 - |\sin(\beta t)|^n], \quad [10]$$

and frequency offset $\Delta\omega$ from the carrier

$$\Delta\omega(t) = \lambda_{\text{mod}} \int [1 - |\sin(\beta t)|^n]^2 dt, \quad [11]$$

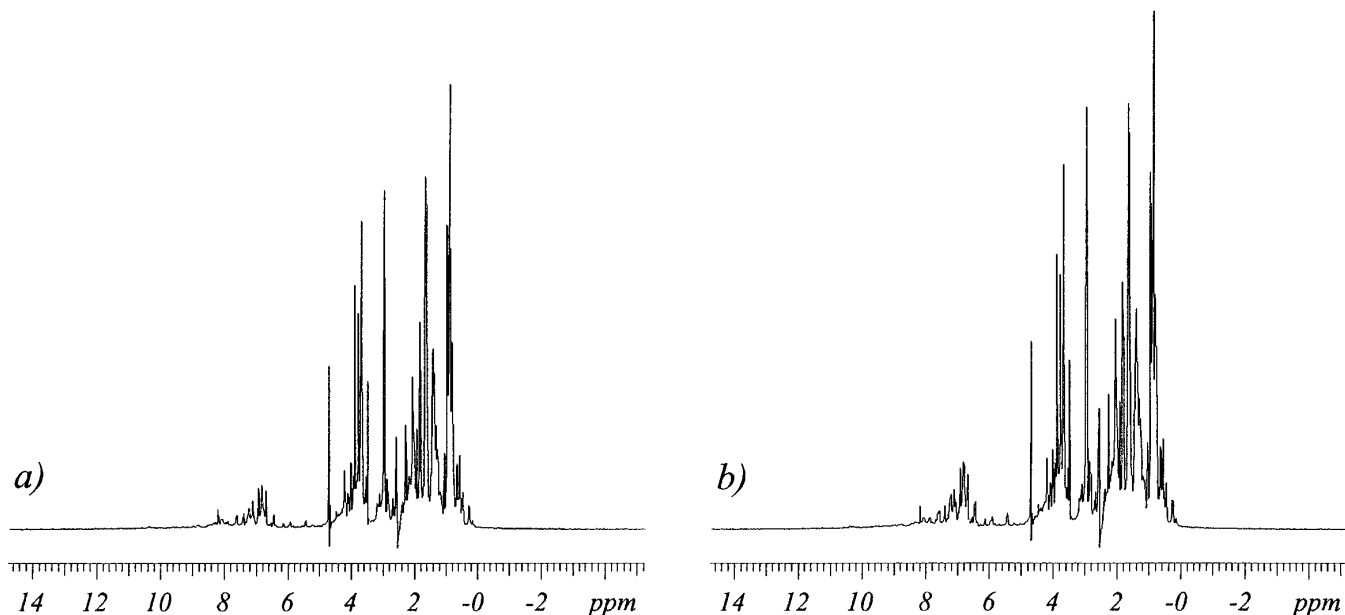


FIG. 3. Comparison of the first increments from the 2D spectra shown in Fig. 4 acquired with (a) DIPSI-2 mixing sequence and (b) WURST-8 mixing.

where β is a time scaling factor and λ_{mod} is a frequency scaling factor (modulation depth). The RF field strength for adiabatic pulses ($\lambda_{\text{mod}} > 0$) is determined by the adiabaticity factor, Q ,

$$\omega_1(\text{max}) = \sqrt{Q\lambda_{\text{mod}}\beta}. \quad [12]$$

The overall coherence transfer efficiency of adiabatic mixing for various frequency modulation schemes is displayed in Fig. 1. If the modulation depth λ_{mod} is set to zero (no frequency modulation), the overall performance of the mixing scheme using the MLEV-16 phase cycle (6) is shown in Fig. 1a. The 20-step supercycle (20, 23), which thus far has been used only in decoupling experiments, does not improve the overall efficiency of the coherence transfer (see Fig. 1b). However, it does make the mixing sequence less sensitive to the RF field mis-setting (23).

Introduction of a relatively mild frequency modulation ($\lambda_{\text{mod}} = 4$) improves the overall performance of the mixing experiment by making the effective bandwidth somewhat wider (see Fig. 1c). At the same time the tolerance to the RF field miscalibration increases considerably. Making the modulation deeper by increasing the pulse length or sweep width, or both, leads to further improvement of the inversion properties of the adiabatic pulse. Unfortunately, this does not increase the effective coherence transfer bandwidth. Although the mixing bandwidth readily extends along the diagonal of the 2D map (see Fig. 1d), the coherence transfer efficiency along the anti-diagonal is essentially conserved and does not extend beyond the bandwidth of the planar waveform (Fig. 1a). Therefore, the main advantage of using the frequency-modulated adiabatic

pulses for spin mixing is a high tolerance to RF inhomogeneity and miscalibration. The increase in the effective bandwidth is not nearly as efficient as it is in the case of adiabatic decoupling.

The benefits from the increased B_1 tolerance of the adiabatic mixing are demonstrated in Fig. 2, where the spectra of the first increment of the standard ^1H - ^1H correlated 2D TOCSY experiment are compared at different levels of mis-setting of the RF field amplitude. The traditional mixing sequences (8, 9) such as DIPSI-2 (Fig. 2a), DIPSI-3, FLOPSY-8, or MGS- n (not shown here) are highly sensitive to RF field strength. Mis-setting the RF amplitude of the mixing waveform by only 1 dB can substantially degrade the overall sensitivity of such experiments. In contrast, the adiabatic mixing is extremely insensitive to RF field strength once the necessary intensity threshold is reached (Fig. 2b).

The overall performance of the conventional (DIPSI-2) and adiabatic (WURST-8) mixing sequences is shown in Figs. 3a and 3b, respectively. The corresponding ^1H - ^1H correlated 2D TOCSY experiments (24) employing the two mixing techniques were recorded for the 14.4-kDa protein villin 14T. Although the overall performance is comparable, for the reasons discussed above, the adiabatic experiment (Fig. 3b) provides a slightly better sensitivity (15–20%). This can be attributed to signal loss in the conventional mixing experiments due to RF inhomogeneity, whereas the experiments based on adiabatic mixing are affected to a much lower extent. Overall, the adiabatic TOCSY is much more reliable in practical biomolecular NMR, where the actual strength of the RF field is strongly sample dependent.

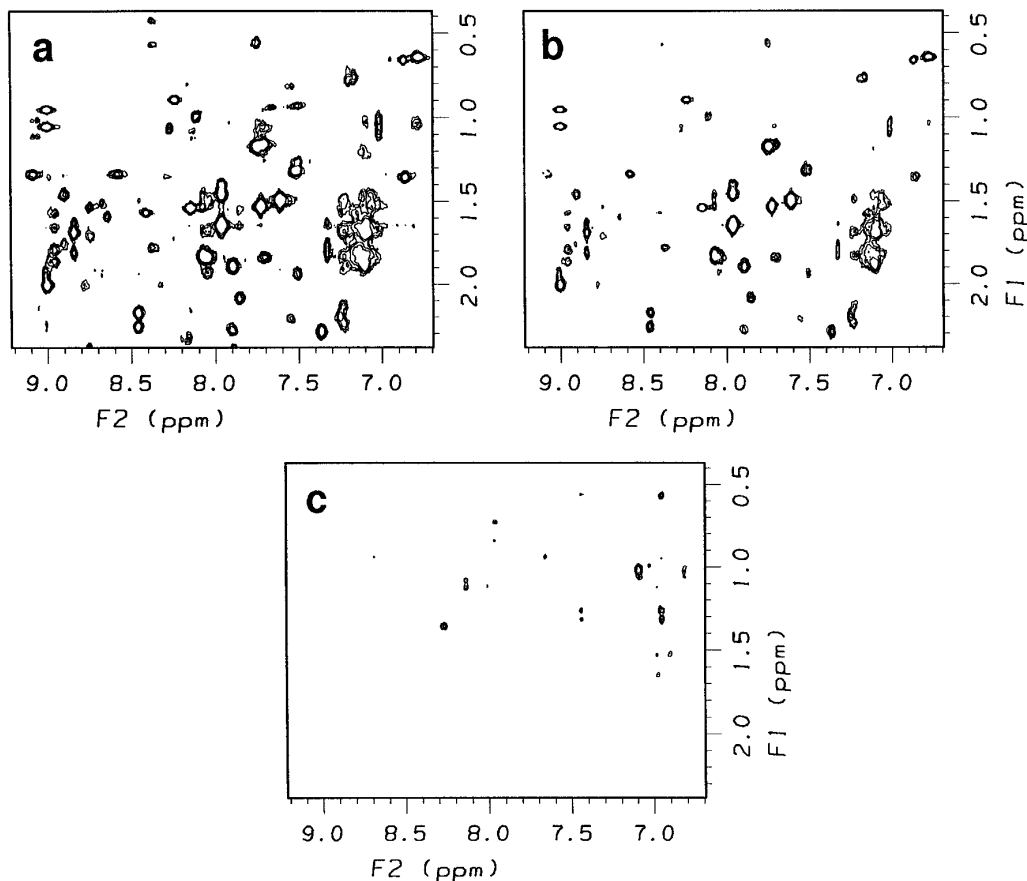


FIG. 4. Comparison of a selected region of 2D ^1H - ^1H correlated TOCSY spectra of a 5 mM lysozyme sample (standard Wilmad reference sample) in 90% $\text{H}_2\text{O}/10\%$ D_2O , 25°C. The spectra were acquired at 600 MHz, using a 60 ms mixing time and a RMS RF field strength of 10.5 kHz for the adiabatic mixing. (a) Contour plot of the relaxation-compensated (“clean”) TOCSY spectrum, acquired with the WURST-2 mixing sequence. (b) Positive and (c) negative contour levels for the uncompensated TOCSY spectrum, acquired with the WURST-8 mixing sequence. Both mixing sequences used 150- μs -long pulses and 35-kHz-wide frequency sweeps superimposed with a 20-step supercycle (20, 23). The two spectra were acquired, processed, and plotted with identical parameters.

An inherent problem in ^1H - ^1H TOCSY experiments is the deleterious effects of nuclear spin cross relaxation during the mixing period (26–28). A significant loss in sensitivity can result from the destructive interference of coherent and incoherent magnetization transfer processes, since the nuclear spin cross relaxation during the mixing time is normally dominated by transverse cross relaxation. Such cross-relaxation effects can also generate cross peaks from two-step transfers, which have the same sign as most TOCSY peaks, thus leading to ambiguity in the origin of the observed cross peaks. Griesinger *et al.* (26) and Bearden *et al.* (27) introduced a general methodology for minimizing the effects of cross relaxation in TOCSY spectra which relies on the fact that longitudinal and transverse cross-relaxation rates have opposite signs in large molecules. By designing a mixing sequence such that the spin trajectories balance the longitudinal and transverse cross-relaxation rates, a so-called “clean” TOCSY spectrum can be obtained with optimum sensitivity for the desired cross peaks

representing coherence transfer through scalar couplings, and minimum contributions from cross-relaxation pathways. Since the original clean TOCSY experiments were reported, a number of variations have been developed to try to improve the efficiency of the mixing sequences (28). The most common approaches involve the incorporation of time delays at appropriate points in the mixing sequence, as in the original clean TOCSY experiments (26, 27), or the use of crafted pulses designed to balance the effects of longitudinal and transverse cross relaxation (28). Relaxation compensation can easily be built into adiabatic mixing sequences by simply adjusting the power index, n (see Eqs. [10] and [11]). Both simulations and experimental observations indicate that optimum performance is obtained by using the WURST-2 pulse (4). Similar results can be expected with optimum sweep Gaussian and WURST-1 pulses (22). Note that increasing the RF peak power (decreasing the index n) does not affect the RMS power requirements for adiabatic mixing sequences. This is a remarkable advantage

with respect to constant amplitude windowless mixing sequences, where introduction of extra delays increases the actual (RMS) power consumption for the given effective mixing bandwidth.

The effectiveness of the relaxation-compensation properties of the WURST-2 sequence is demonstrated in Fig. 4. TOCSY experiments using the WURST-2 (Fig. 4a) and WURST-8 (Figs. 4b and 4c) mixing sequences were performed on a sample of the protein lysozyme (standard Wilmad reference sample) in 90% H₂O/10% D₂O, with a mixing time of 60 ms, using an INOVA 600-MHz spectrometer; in both cases, the RMS-RF field strength was 10.5 kHz. A selected spectral region is shown, containing the high field to amide/aromatic cross peaks. Positive contour levels of the WURST-2 clean TOCSY spectrum are plotted in Fig. 4a; no peaks with negative sign were observed. The corresponding positive contour levels of the uncompensated WURST-8 TOCSY spectrum are plotted in Fig. 4b, and the negative levels are plotted separately in Fig. 4c for ease of viewing. All acquisition, processing, and plotting parameters are identical for the WURST-8 and WURST-2 data sets. Inspection of the data shown in Fig. 4 clearly demonstrates the improved sensitivity which results from the minimization of cross-relaxation contributions in the spectra. Note that the sensitivity gain we observe here (WURST-2 vs WURST-8) comes on top of the sensitivity gain demonstrated in Fig. 3 (WURST-8 vs DIPSI-2).

Here we have demonstrated that the performance of homonuclear ¹H-¹H TOCSY experiments can be improved with adiabatic mixing techniques. Adiabatic mixing is highly insensitive to RF field inhomogeneity and mis-calibration. As a result, a significant sensitivity improvement can be obtained with respect to the conventional experiments. Cross relaxation artefacts can be eliminated by adjusting the power index, *n*, to obtain an adiabatic clean TOCSY. A more substantial sensitivity gain can be expected under experimental conditions where the RF homogeneity is poor, such as usually encountered in large volume probes and in magnetic resonance imaging. Although the effective bandwidth cannot be increased to the extent demonstrated for adiabatic decoupling, a moderate increase is still achievable. Overall, adiabatic mixing experiments prove to be more sensitive and much more reliable in practical situations encountered in biomolecular NMR. We expect that this approach can readily be implemented in homonuclear ¹³C-¹³C TOCSY experiments, such as the HCCH-TOCSY, or in heteronuclear cross-polarization experiments in liquids.

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