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Homonuclear Hartmann–Hahn transfer with reduced relaxation losses by use of the MOCCA-XY16 multiple pulse sequence

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Abstract

Homonuclear Hartmann–Hahn transfer is one of the most important building blocks in modern high-resolution NMR. It constitutes a very efficient transfer element for the assignment of proteins, nucleic acids, and oligosaccharides. Nevertheless, in macromolecules exceeding ~10 kDa TOCSY-experiments can show decreasing sensitivity due to fast transverse relaxation processes that are active during the mixing periods. In this article we propose the MOCCA-XY16 multiple pulse sequence, originally developed for efficient TOCSY transfer through residual dipolar couplings, as a homonuclear Hartmann–Hahn sequence with improved relaxation properties. A theoretical analysis of the coherence transfer via scalar couplings and its relaxation behavior as well as experimental transfer curves for MOCCA-XY16 relative to the well-characterized DIPSI-2 multiple pulse sequence are given.

Keywords: Hartmann–Hahn transfer; Isotropic mixing; Relaxation; TOCSY; Invariant trajectory

1. Introduction

Since its discovery, homonuclear Hartmann–Hahn (TOCSY) transfer through scalar couplings [1–3] has found useful applications in all fields of NMR-spectroscopy. In biological NMR, multidimensional TOCSY-based experiments have become the most commonly used tools to achieve side chain assignment in proteins [4,5], the assignment of ribose and deoxyribose resonances in nucleic acids [6–8] and the assignment of sugars in general [9]. Many complete assignments of proteins and nucleic acids up to a molecular weight of ~15 kDa can be found in the literature and have been obtained with the help of such experiments. Even though homonuclear isotropic mixing affects very effective transfer through a chain of coupled spins, rather long mixing times of 50–70 ms in ^{1}H-TOCSY experiments and 15–20 ms in ^{13}C-TOCSY experiments are often necessary for assignment. In cases where magnetization transfer through small couplings is desired, as for example the transfer H1′ to H2′ in RNA, even longer mixing times are necessary. With computer optimized multiple pulse sequences used today, proton and carbon magnetization are transverse for a large fraction of the TOCSY-period and can show significant losses due to fast T_2-relaxation rates. As a result, multidimensional TOCSY-based experiments become very insensitive for large molecular weight macromolecules. Improvements have been achieved in this type of experiments by reducing the time in which magnetization is transverse with the introduction of semi-constant time experiments [10,11] or by the use of fractional ^{2}H labeling to reduce the dipolar relaxation contribution [12,13]. The use of tailored Hartmann–Hahn multiple pulse sequences (TACSY) also improves coherence transfer significantly for a given task like the transfer from H_α to H^N [14,15].

In this article, we propose the use of a broadband TOCSY multiple pulse sequence with improved relaxation properties for coherence transfer in ^{1}H spin systems. The investigation was triggered by empirical observations made during studies of ^{1}H–^{19}F residual

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dipolar coupling constants [17,18], where magnetization was transferred using a dipolar TOCSY: It was observed that while a TOCSY mixing period of approximately 100 ms using the DIPSI-2 multiple pulse sequence [16], with the magnetization spin-locked along the x-axis, left no detectable magnetization for the ^{1}H–^{19}F-E.COSY-DTOCSY applied to the 2′F-labeled 21mer R1inv [17], a mixing period of 268 ms (!) using the MOCCA-XY16 sequence [19–22] still showed reasonable sensitivity. In this study we systematically characterize the offset dependence with respect to isotropic mixing and the relaxation properties of the MOCCA-XY16 sequence. Efficient transfer is demonstrated on the model peptide PA_4 and a ^{15}N,^{13}C-labeled 36-mer RNA and applicability as a homonuclear TOCSY sequence for medium-sized proteins is shown.

2. Theory

The basic building block of a MOCCA (MOdified phase Cycled CArr-Purcell-type) [19] multiple pulse sequence is shown in Fig. 1: Two delays of length $\Delta/2$ surround a 180° pulse of duration d. This basic motif is expanded in at least a minimum cycle of two building blocks, but for practical purposes more sophisticated supercycles like MLEV-16 [23], M4P9 [24,25] or XY16 [21,22] are applied. The transfer efficiency for a given spin system with residual dipolar couplings depends on the coupling topology of the spin system and the orientation of the transferred magnetization [19,26–28]. To date, the most efficient transfer via dipolar couplings for two coupled spins has been found to be achieved by the transfer $I_z \rightarrow I_z$ through the MOCCA-XY16 multiple pulse sequence [19]. As shown later in this article, the same combination of polarization transfer and isotropic mixing sequence is also well-suited for transfer via scalar couplings and provides for significant improvement in the relaxation behavior of the correlated spins.

The effective Hamiltonian of isotropic mixing sequences in a two spin system is approximately given by

$$\mathscr{H}_{iso} = 2\pi\left\{J_x^{\text{eff}} I_{1x} I_{2x} + J_y^{\text{eff}} I_{1y} I_{2y} + J_z^{\text{eff}} I_{1z} I_{2z}\right\} \tag{1}$$

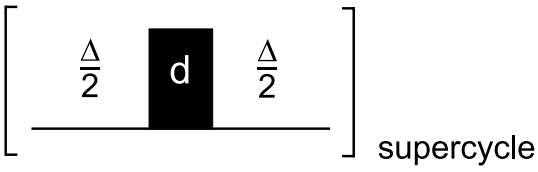

Fig. 1. The basic building block of the Modified Carr–Purcell-type dipolar mixing sequences (MOCCA) consists of a 180° pulse surrounded by two delays of equal length. The minimum supercycle consists of two such building blocks but more effective schemes like M4P9, MLEV-16, and XY16 are usually used. The multiple pulse sequence is virtually identical with the RFDR-sequence used in solid state experiments [48,49].

with the effective coupling constants J_x^{eff}, J_y^{eff}, $J_z^{\text{eff}} \leqslant J$. Most efficient transfer is given on resonance where automatically $J_x^{\text{eff}} = J_y^{\text{eff}} = J_z^{\text{eff}} = J$ is fulfilled [3]. For the MOCCA-XY16 multiple pulse sequence this efficient transfer is still achieved to a good approximation as long as the condition for zero order average Hamiltonian theory

$$\tau_{\text{cyc}} = 2(\Delta + d) \ll \frac{1}{\nu_{\text{off}}^{\max}} \tag{2}$$

with the minimum cycle time τ_{cyc} and the maximum offset $\nu_{\text{off}}^{\max}$ is fulfilled. As a consequence the bandwidth for the homonuclear Hartmann–Hahn transfer of a MOCCA multiple pulse sequence is limited by the delay Δ and the duration d (see also [19]).

For a characterization of the isotropic mixing transfer properties of the MOCCA-XY16 multiple pulse sequence offset profiles were simulated using the program SIMONE [29]. In the simulations the polarization transfer via a 10 Hz J-coupling in a two spin system at the time $1/(2J) = 50$ ms was calculated. Relaxation was neglected but B_1-field inhomogeneity was included as a Gaussian distribution of the rf-amplitude with a full width at half height of 10%. The resulting offset profiles for the isotropic mixing transfer are shown in Fig. 2 for the well-characterized DIPSI-2 sequence and the MOCCA-XY16 sequence with $d = 40\,\mu$s and three different values (20, 88, and 176 μs) for the delay Δ.

The simulations are chosen to show the effect of different delays on the offset profile of isotropic transfer. While the MOCCA-XY16 sequence with $\Delta = 20\,\mu$s shows a square-shaped transfer profile, increased delays lead to a more rounded offset profile with the range of efficient isotropic mixing distributed closer to the diagonal. The DIPSI-2 offset profile for comparison was calculated with an rf-amplitude of 7 kHz which is compatible to the MOCCA-XY16 sequence with $d = 40\,\mu$s and $\Delta = 88\,\mu$s with the square root of the average power of 6990 Hz. Both sequences show satisfying isotropic mixing bandwidth measured at the anti-diagonal with an approximately 20% larger bandwidth for the DIPSI-2 sequence. The MOCCA-XY16 sequences with $\Delta = 20\,\mu$s and $\Delta = 176\,\mu$s instead result in the square root of the average power of 10210 and 5380 Hz, respectively, and cannot directly be compared to the DIPSI-2 sequence.

The auto relaxation behavior of an isotropic mixing multiple pulse sequence can be qualitatively described by the invariant trajectory approach [3,30,31]. This method uses the motion of a normalized magnetization vector of a single spin $\mathbf{n}(t) = \{n_x(t), n_y(t), n_z(t)\}$ under the action of a basis sequence after which the magnetization vector returns to its initial orientation ($\mathbf{n}(\tau_b) = \mathbf{n}(0)$). The effective autorelaxation rate ρ_{eff} of the invariant trajectory of an uncoupled spin is then given by

$$\rho_{\text{eff}} = w_t \rho_t + w_l \rho_l \tag{3}$$

with the transverse autorelaxation rate ρ_t and the longitudinal relaxation rate ρ_l and the transverse and longitudinal weights

$$w_t = \frac{1}{\tau_b} \int_0^{\tau_b} \left\{ n_x(t)^2 + n_y(t)^2 \right\} dt, \tag{4}$$

and

$$w_l = \frac{1}{\tau_b} \int_0^{\tau_b} \left\{ n_z(t)^2 \right\} dt. \tag{5}$$

In the case of molecules with long correlation times τ_c, the transverse relaxation rate ρ_t is significantly larger than the longitudinal rate ρ_l and therefore is the determining factor of the overall relaxation behavior. Fig. 3 shows the corresponding offset dependencies of the transverse weight w_t for the pulse sequences introduced in Fig. 2. While the DIPSI-2 sequence (Fig. 3D) has an on resonance transverse weight of about 0.5, the corresponding minima for the MOCCA sequences (Figs. 3A–C) are 0.33, 0.19, and 0.1, respectively. In general, a longer delay Δ in the MOCCA-XY16 sequence leads to a smaller transverse weight w_t and hence to a lower overall relaxation rate. With increased offset also w_t increases for all four sequences. In the case of the MOCCA-XY16 sequences this can be attributed to the offset dependence of the inversion properties of the 180° pulses applied during the sequence. Taking the offset dependence of the polarization transfer into account the average transverse weight w_t over the transfer bandwidth is slightly increased relative to the on resonant value.

The invariant trajectory approach used here is based on a single uncoupled spin. However, as was discussed previously [30,31], the approach is a good approximation also for coupled spin systems to which homonuclear Hartmann–Hahn pulse sequences are applied. For a qualitative understanding of the relaxation processes in coupled spin systems we have a closer look at the operators present during the Hartmann–Hahn period. As was shown analytically for a number of spin systems [1,32–36], polarization transfer under an ideal isotropic mixing Hamiltonian creates only polarization and zero quantum operator terms. This is a result of the fact that the isotropic mixing Hamiltonian commutes with F_z, the z-component of the total angular momentum operator, which implies that the multiple-quantum order of the

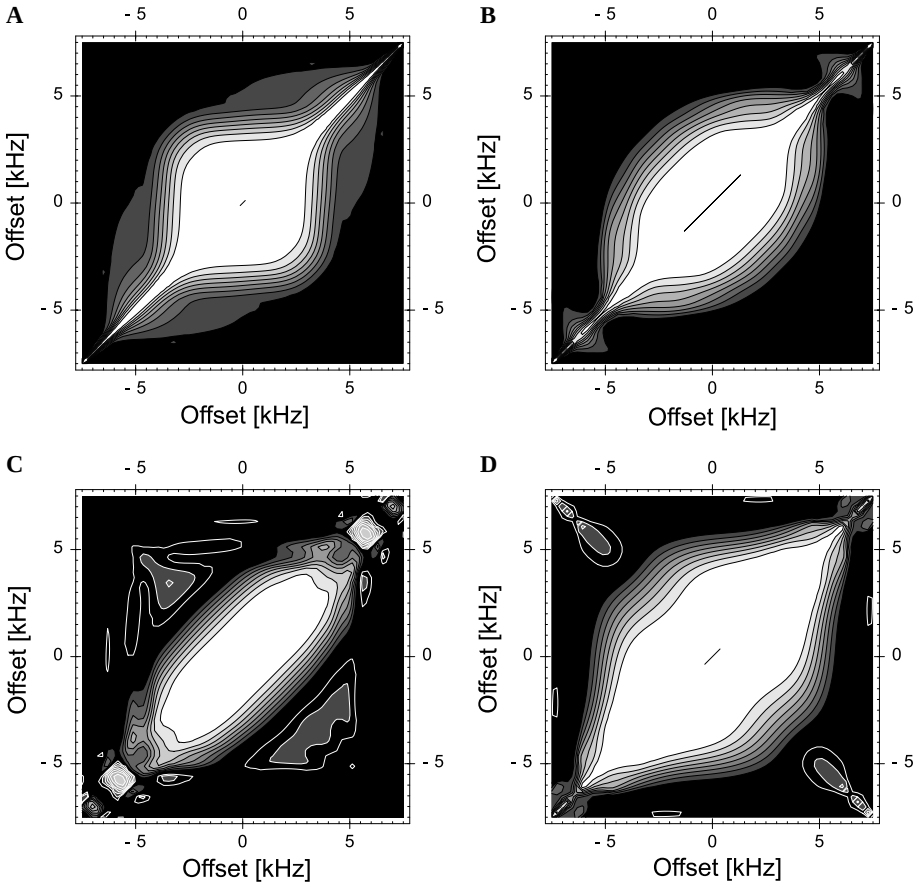

Fig. 2. Offset dependence of polarization transfer for the MOCCA-XY16 sequence with $d = 40\,\mu s$ and $\Delta = 20\,\mu s$ (A), $\Delta = 88\,\mu s$ (B), and $\Delta = 176\,\mu s$ (C), and the DIPSI-2 sequence [16] with an rf-amplitude of 7 kHz (D). The offset profiles were calculated with the simulation software SIMONE [29] assuming a Gaussian B_1-field distribution with a width of 10% at half height.

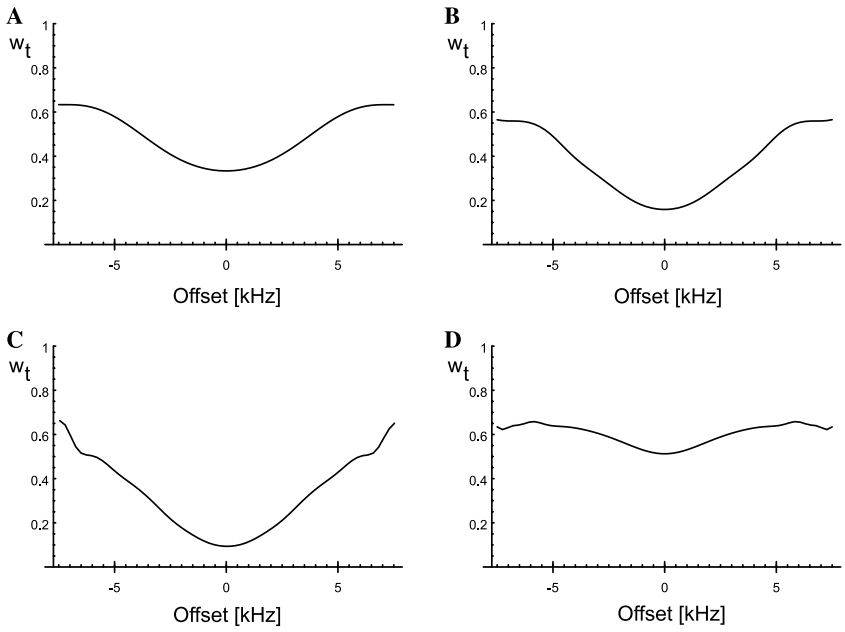

Fig. 3. Offset dependence of the transverse weight w_t as given by Eq. 4 of the MOCCA-XY16 sequence with $d = 40\,\mu s$ and $\Delta = 20\,\mu s$ (A), $\Delta = 88\,\mu s$ (B), and $\Delta = 176\,\mu s$ (C), and the DIPSI-2 sequence (D) calculated using the invariant trajectory approach. The invariant trajectories were simulated using the simulation software SimOne with an flipangle increment of 15° for the integration. The reduced weight of transverse magnetization can directly be correlated to the improved relaxation behavior of the MOCCA-XY16 sequences since T_2-relaxation represents the major relaxation pathway. For (D) the normalized magnetization vector for the invariant trajectory was assumed to start perpendicular to the irradiation axis. As for all phase alternating sequences a magnetization vector starting parallel with the irradiation would lead to an effective spin lock and $w_t \sim 1.0$ [31].

density operator is preserved during the mixing process [37,3]. In contrast to the ideal average Hamiltonian, the application of a real Hartmann–Hahn mixing multiple pulse sequence results in all kinds of intermediate operators. However, the main effect of the pulse sequence is a rotation of the polarization and ZQ-terms of the ideal isotropic mixing Hamiltonian into inphase and antiphase coherences. Considering an isolated two-spin system with dipole–dipole interaction as the most important relaxation pathway, polarization and ZQ-operators relax very slowly in the spin diffusion limit and correspond to the longitudinal relaxation rate of an uncoupled spin. Inphase and antiphase coherences, instead, both relax significantly faster and correspond to the transverse relaxation rate for a single spin in the invariant trajectory. Rotation due to the pulse sequence is well described via the invariant trajectory approach using a single magnetization vector, thus, the method serves as a good model for the auto relaxation rates. However, since the MOCCA-XY16 multiple pulse sequence is modified such that the longitudinal weight is increased it should be noted that this may result in NOE contribution during TOCSY transfer.

3. Experimental

The first experimental verification of the homonuclear Hartmann–Hahn transfer via scalar couplings using the MOCCA-XY16 multiple pulse sequence was given in Fig. 4 of [38] and was also observed in high-resolution magic angle spinning experiments using the virtually identical RFDR pulse sequence [39]. Nevertheless, for a more accurate characterization of the isotropic mixing properties coherence transfer curves were recorded on the cyclic pentapeptide D-Pro–Ala–Ala–Ala–Ala which we will refer to as PA_4 in the following. The peptide was dissolved in DMSO-d_6 and at 600 MHz and 300 K the sample did not show any transfer due to nuclear Overhauser enhancement (NOE) (Fig. 4). For the acquisition of the transfer curves the amide protons were selectively excited by the use of the DPFGSE technique [40] using a 2 ms G3 pulse for inversion [41]. Four series of one dimensional experiments were recorded with increasing mixing times for the transfer periods using NOE, DIPSI-2, and MOCCA-XY16 with $\Delta/d = 2.2$ and $\Delta/d = 4.4$.

The resulting FIDs were exponentially apodized with a linebroadening of 50 Hz and the transfer curves were extracted from the intensities of the corresponding resonances. The well-characterized DIPSI-2 multiple pulse sequence was used as a reference for the MOCCA sequences. It was applied with a rf-amplitude of 6 kHz while the rf-amplitude of the MOCCA sequences was 12.5 kHz for the 180° pulses. However, taking the delays of the MOCCA-XY16 sequences into account the average introduced rf-power corresponds to 6990 Hz for $\Delta = 88\,\mu s$ and 5380 Hz for $\Delta = 176\,\mu s$.

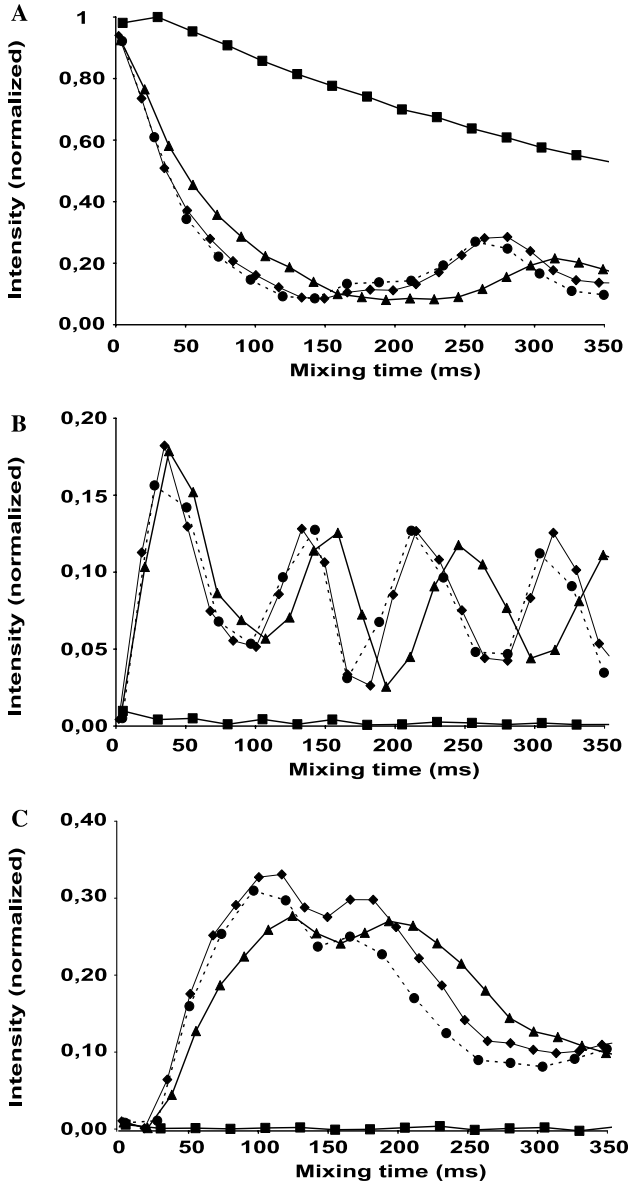

Fig. 4. Experimental polarization transfer curves for Ala 5 of the cyclic pentapeptide PA_4. Starting from the amide proton transfer to H^N (A), H^α (B), and H^β (C) is shown for the MOCCA-XY16 sequence with $d = 40\,\mu s$, and $\Delta = 88\,\mu s$ (solid line, diamonds), $\Delta = 176\,\mu s$ (solid line, triangles) and the DIPSI-2 sequence (dashed line, circles). Since no NOE could be measured for the sample (solid line, squares) the transfer curves display pure TOCSY/ROE properties of the sequences.

The MOCCA-XY16 sequence with $\Delta/d = 2.2$ shows slightly better transfer properties compared to the DIPSI-2 sequence which might be explained by the stronger ROE present during transfer of the latter sequence. The MOCCA-XY16 sequence with $\Delta/d = 4.4$ instead displays a transfer with reduced oscillation frequencies, corresponding to reduced effective coupling constants that can be attributed to the smaller bandwidth of this sequence.

The results for the pentapeptide PA_4 encouraged us to acquire coherence transfer curves on a ^{15}N, ^{13}C-labeled 36mer RNA with the base sequence 5′-rGGU GAC UCC AGA GGU CGA GAG ACC GGA GAU AUC ACC-3′ dissolved in D_2O containing 5 mM phosphate buffer pH 6.7, 80 mM NaCl and 0.1 mM EDTA [42]. For the acquisition of the transfer curves the residual water was presaturated and the H5 to ribose region in the spectrum selectively excited. Again, series of 1D's as pseudo two-dimensional experiments were recorded with incremented mixing times in the indirect dimension. Fig. 5 shows the transfer curves for NOE, DIPSI-2, and MOCCA-XY16 with $\Delta/d = 4.4$ mixing for the H5 to H6 transfer of a separated pyrimidine resonance. Drastic differences in the polarization transfer curves of the DIPSI-2 versus the MOCCA-XY16 multiple pulse sequence are visible: The maximum transfer of the DIPSI-2 sequence is only 41% of the maximum transfer of the MOCCA sequence. In contrast to the small pentapeptide PA_4 a strong negative NOE is present in the 36mer RNA during the MOCCA sequence that significantly influences the transfer. Nevertheless, at a mixing time of ~33 ms even the difference of MOCCA and NOE transfer curves, which can be attributed to Hartmann–Hahn transfer and strongly overestimates the NOE contribution, shows a ~40% increase compared to the maximum of the DIPSI-2 transfer curve. Also the amount of magnetization remaining for detection after 80 ms and even 300 ms (data not shown) mixing time is an indicator for the far better relaxation properties of the MOCCA-XY16 sequence compared to conventional TOCSY-sequences like DIPSI-2.

In a number of TOCSY experiments it is usually the aim to get the assignment of a defined spin system and connectivities due to NOE are undesired. A "clean" MOCCA sequence is possible in principle, but unfor-

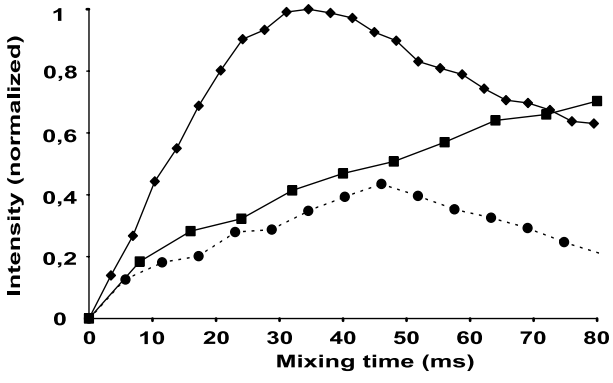

Fig. 5. Experimental polarization transfer curves for the H5–H6 transfer of a resolved signal of a ^{15}N,^{13}C-labeled 36mer RNA. The transfer of the MOCCA-XY16 sequence with $d = 40\,\mu s$ and $\Delta = 176\,\mu s$ (diamonds), the DIPSI-2 sequence (circles), and NOESY (squares) are shown. At the maximum transfer of the MOCCA-XY16 sequence the transferred polarization is larger than the sum of NOE and DIPSI-2, indicating the improved relaxation behavior.

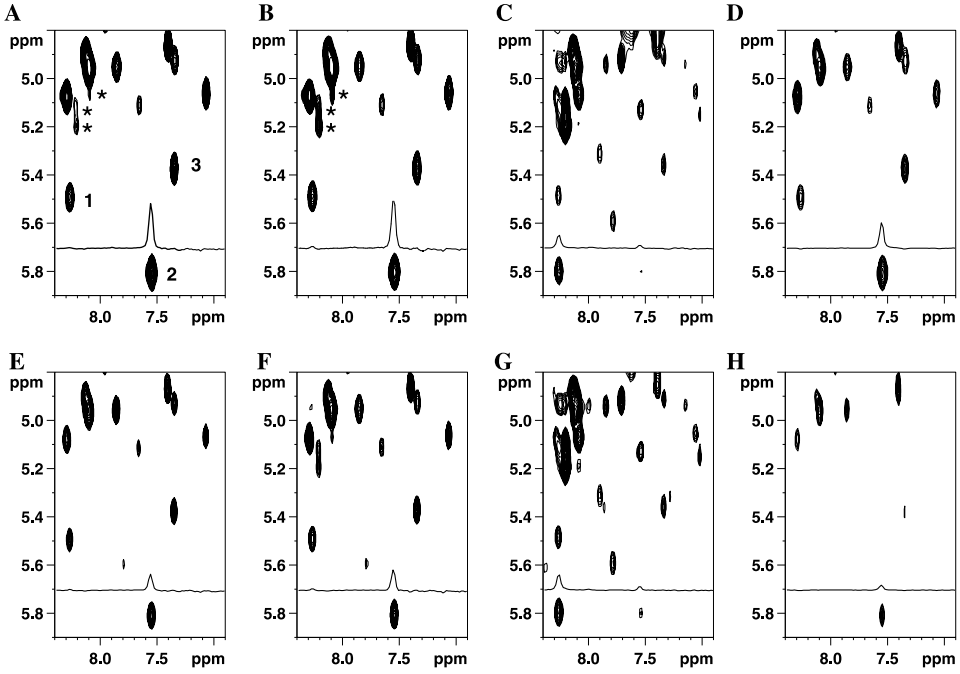

Fig. 6. Two-dimensional experiments with various mixing elements were recorded on hen-egg white lysozyme as a model system for a medium-sized protein. The MOCCA-XY16 sequence with $d = 40\,\mu s$ and $\Delta = 88\,\mu s$ (A,E) and $\Delta = 176\,\mu s$ (B,F), a NOE transfer step (C,G), and polarization transfer with the DIPSI-2 sequence (D,H) were used for the experiments with approximate mixing times of 70 ms (A–D) and 120 ms (E–H). For a better estimate of the relative signal intensities slices at 5.8 ppm in the indirect dimension are shown with a cross peak solely due to NOE at 8.25 ppm and a cross peak mainly due to isotropic mixing at 7.55 ppm in the acquired dimension. Significant signals due to NOE are marked with a star. As expected, the intensities of these peaks increase with the delay Δ used in the MOCCA sequence. Compared to the DIPSI-2 sequence the improved relaxation behavior of the MOCCA-XY16 sequences is clearly evident.

Table 1
Relative cross peak integrals for signals marked in Fig. 6 A at several mixing times

Mixing time	30 ms			70 ms			120 ms		
	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
MOCCA $\Delta/d = 2.2$	1.00	0.95	1.00	0.85	0.90	0.86	0.70	0.82	0.79
MOCCA $\Delta/d = 4.4$	0.87	0.91	0.98	1.00	1.00	1.00	1.00	1.00	1.00
NOESY	0.12	0.02	0.09	0.32	0.05	0.22	0.81	0.14	0.57
DIPSI-2	0.87	1.00	0.90	0.47	0.59	0.47	0.24	0.36	0.28

tunately this would imply a given ratio of transverse versus longitudinal weight of 1:2 in the spin diffusion limit [30] which is fulfilled for $\Delta/d = 0.5$ (see Fig. 3A). Such a sequence, of course, is not optimal in terms of reduced relaxation. We therefore compared the MOCCA-XY16 sequence with $\Delta/d = 2.2$ and $\Delta/d = 4.4$ with the DIPSI-2 sequence and a NOESY: Each of the four two-dimensional experiments was applied to unlabeled hen egg-white lysozyme for approximate mixing times of 30, 70 (Figs. 6A–D), and 120 ms (Figs. 6E–H). Hen egg-white lysozyme as a medium-sized protein already displays significant negative NOE (Figs. 6C and G) and some of the strong NOE signals give rise to cross peaks also in the MOCCA-XY16 experiments, especially with long delays Δ ((Figs. 6A and B). However, the NOE contribution is generally small. On the other hand, especially for long mixing times the MOCCA-XY16 sequence shows its relaxation advantage over the DIPSI-2 multiple pulse sequence (slices in Fig. 6, Table 1).

4. Discussion

Loss of magnetization during TOCSY mixing periods is mainly determined by the T_2-relaxation rate of transverse magnetization. A characterization of multiple pulse sequences with respect to the overall relaxation rate can be given by the ratio of transverse coherence versus longitudinal polarization present during the sequence. This ratio is especially low for the MOCCA-XY16 multiple pulse sequence since only during the time d of the 180° pulse is ~50% of the coherence oriented in the transverse plane. In principle, the relaxation properties can be steadily improved by an increase of the

delay Δ, but at the same time the bandwidth of the isotropic mixing sequence is reduced and transfer due to NOE increases. For a given bandwidth an optimal Δ/d-ratio can be specified in the sense of minimum rf-power necessary and the maximum rf-amplitude available. In our case this ratio was close to 2.2 as was also found for the transfer via dipolar couplings [19], but in many cases different ratios might yield better results. For practical applications where the optimum compromise between bandwidth and relaxation rates is desired, it is probably best to start with the maximum rf-amplitude available for the 180° pulses and a Δ/d-ratio of 2.2 and then steadily increase the delay Δ for an improved relaxation rate so that the experiment still shows the necessary bandwidth. In experiments on the pentapeptide PA_4, in the absence of NOE, the favorable isotropic mixing properties of MOCCA-XY16 could be demonstrated. Reduced effective relaxation rate could also be shown in polarization transfer curves measured on a $^{15}N,^{13}C$-labeled 36mer RNA. For large molecules, NOE is a problem for relaxation enhanced MOCCA sequences used for assignment purposes: For relaxation optimized MOCCA sequences the delays with the magnetization stored along z are maximized and therefore also the NOE contribution. While ROE contributions in conventional TOCSY-sequences can be compensated by clean versions like the DIPSI-2rc [43] or clean-CITY [44] in which the ROE is substracted by delays with NOE of opposite sign, a clean MOCCA sequence in the spin diffusion limit would require a ratio $\Delta/d = 0.5$ which would imply very limited improvement of relaxation behavior compared to conventional clean-TOCSY sequences. However, as shown in one of the original publications [45], the spin diffusion limit does not apply to most biomolecules and the range of optimal compensation of ROE versus NOE is significantly shifted towards $\Delta/d > 0.5$. NOE-artifacts for at least medium-sized proteins are acceptably small (Fig. 6), also since most atoms with strong NOE connectivities are scalar coupled, anyway. Of course, a proton-based assignment of very large molecules with very efficient spin diffusion based on MOCCA-XY16 TOCSY experiments might not be possible. However, in applications where the isotropic mixing period is introduced to efficiently distribute magnetization to the surrounding spins (as for example in E.COSY-type experiments [46,47]) the NOE contribution is of minor importance and the MOCCA-XY16 sequence is not limited by molecular size.

5. Conclusion

We showed that Modified Carr–Purcell-type dipolar mixing (MOCCA) sequences optimized for efficient transfer via dipolar couplings are also well-suited for isotropic mixing through scalar couplings. Polarization transfer with the MOCCA-XY16 multiple pulse sequence results in a significantly reduced effective relaxation rate during the TOCSY-period, but at the same time transfer via NOE is introduced especially for large delays Δ and molecules with long correlation times. A significant improvement of ^{13}C–^{13}C-TOCSY periods applied to labeled molecules with short T_2 relaxation using the MOCCA-XY16 sequence as the isotropic mixing sequence can be expected. Further investigations concerning this important application are in progress.

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