

# Calculation of Coherence-Transfer Behavior under Planar versus Isotropic Mixing Hamiltonians and Application to Heteronuclear $J$ Cross-Polarization Experiments in Solution-State NMR Spectroscopy

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The heteronuclear  $J$  cross-polarization technique has been demonstrated by a number of groups to be an effective means for achieving coherence transfer in solution-state NMR spectroscopy (1). While the majority of modern experiments rely on the approach of "spin-order transfer by RF pulses" (1), such as the INEPT (2) or DEPT (3) techniques, for effecting the desired transfer of spin order among nuclear spins of different species, the general approach of "spin-order transfer under an average Hamiltonian" (1) has some advantages in a number of specific situations (1, 4–14). Of relevance for the present paper is the work of Kellogg and co-workers (9–11), which demonstrated very well the advantages of the heteronuclear cross-polarization technique in transferring spin order between  $^{31}\text{P}$  and  $^1\text{H}$  nuclei in nucleic acids. As Kellogg and co-workers, as well as others (12), have pointed out, the cross-polarization techniques can have a sensitivity advantage over INEPT methods when both heteronuclear and homonuclear coherence-transfer steps are desirable, since both processes occur simultaneously under the influence of the cross-polarization Hamiltonian.

The purpose of the present Note is to address a specific aspect of the theoretical calculations presented by Kellogg and Schweitzer (11) for the coherence-transfer behavior in the heteronuclear ( $^1\text{H}$ ,  $^{31}\text{P}$ ) spin system present in dinucleotide fragments. These calculations employed a computer program (ZTOCSY) which was written specifically to simulate coherence-transfer behavior in homonuclear spin systems under the influence of an isotropic mixing Hamiltonian (17). Kellogg and Schweitzer indicated that the program was used in their study without modification, and that the presence of heteronuclear spins was taken into account by scaling down the heteronuclear coupling constants by 50%, to reflect the reduced rate of coherence transfer between two spins of different nuclear species compared to that between two spins of the same species. However, as we shall demonstrate below, merely scaling the heteronuclear coupling constant is

only appropriate for a two-spin system, while calculations involving three or more spins require the use of the proper Hamiltonian; use of the ZTOCSY program with its isotropic mixing Hamiltonian is invalid in the application described by Kellogg and Schweitzer. None of the qualitative conclusions in the Kellogg and Schweitzer study are affected by the computational error, but the quantitative results are modified somewhat. Some aspects of the distinct nature of coherence transfer under isotropic mixing and  $J$  cross polarization have been explicitly described by Chandrakumar and co-workers (15, 16), and implicitly by a number of groups.

In the discussions below, we will use the following, somewhat loose, nomenclature. The acronym TOCSY (*total correlation spectroscopy*) will be used, as it was originally defined (18), to refer to coherence transfer in a homonuclear spin system under the influence of an isotropic mixing Hamiltonian (*vide infra*). The acronym CP (*cross polarization*) will refer to coherence transfer between spins of different nuclear species under the influence of a planar coupling Hamiltonian (19) of the general form  $\mathbf{H} = \pi J(I_y S_y + I_z S_z)$ . In the original applications of CP experiments, on-resonance CW RF fields were applied to the two nuclear species, with the relative amplitudes fulfilling the Hartmann–Hahn matching condition (4, 20, 21). However, in current applications in solution-state studies, phase-modulated RF schemes are normally employed to increase the spectral bandwidth over which efficient, heteronuclear coherence transfer can be achieved (1). The CP sequences typically employed, such as DIPSI-2 (22) applied to each nuclear species, are generally quite effective at promoting homonuclear coherence transfer as well; thus, we shall use the term CP-TOCSY to refer to the case where heteronuclear coherence transfer via an effective, planar Hamiltonian and homonuclear transfer via an isotropic mixing Hamiltonian occur simultaneously. We avoid the use of the term hetero-TOCSY, as this should perhaps be reserved for the situation in which an "isotropic"

Hamiltonian has effectively been created for the heteronuclear coherence-transfer process (23).

For the purposes of this Note, we will consider the coherence-transfer behavior in a linear AMX spin system (i.e.,  $J_{AX} = 0$ ). For a homonuclear spin system, the idealized isotropic mixing (TOCSY) nuclear spin Hamiltonian is

$$\mathbf{H}_{\text{im}} = 2\pi J_{\text{AM}}\mathbf{I}_A \cdot \mathbf{I}_M + 2\pi J_{\text{MX}}\mathbf{I}_M \cdot \mathbf{I}_X, \quad [1]$$

where  $J_{\text{AM}}$  and  $J_{\text{MX}}$  are the scalar coupling constants and  $\mathbf{I}_A$ ,  $\mathbf{I}_M$ , and  $\mathbf{I}_X$  are the spin-angular-momentum vector operators for spins A, M, and X, respectively. In a heteronuclear spin system, with spin A belonging to one nuclear species and spins M and X to a second species, the idealized CP-TOCSY mixing Hamiltonian, in the doubly rotating reference frame (4), is

$$\mathbf{H}_{\text{cp}} = \pi J_{\text{AM}}(I_{A_y}I_{M_y} + I_{A_z}I_{M_z}) + 2\pi J_{\text{MX}}\mathbf{I}_M \cdot \mathbf{I}_X, \quad [2]$$

where  $I_{\alpha y}$  and  $I_{\alpha z}$  ( $\alpha = A, M$ ) are Cartesian components of the corresponding vector operators. The evolution of a spin system under the influence of a time-independent Hamiltonian can be conveniently followed via a density-operator formalism (24), with the density operator at an arbitrary time  $t$  being given by

$$\rho(t) = \exp\{-i\mathbf{H}t\}\rho(0)\exp\{i\mathbf{H}t\}, \quad [3]$$

where  $\rho(0)$  is the density operator at  $t = 0$ . Any spin operator, such as  $I_{\alpha x}$  ( $\alpha = A, M, X$ ), can be evaluated at any time as

$$\langle I_{\alpha x}(t) \rangle = \text{Tr}[I_{\alpha x}\rho(t)], \quad [4]$$

where  $\langle \rangle$  denotes the expectation value of, in this case,  $I_{\alpha x}$ , and Tr stands for the trace of the operator product enclosed in brackets.

In the special case of a heteronuclear two-spin system, with the spins designated A and M, the isotropic mixing Hamiltonian would simplify to

$$\begin{aligned} \mathbf{H}_{\text{im}} &= 2\pi J_{\text{AM}}\mathbf{I}_A \cdot \mathbf{I}_M \\ &= 2\pi J_{\text{AM}}(I_{A_x}I_{M_x} + I_{A_y}I_{M_y} + I_{A_z}I_{M_z}). \end{aligned} \quad [5]$$

Since the term  $I_{A_x}I_{M_x}$  commutes with the other two terms in Eq. [5], the evolution of the density operator, Eq. [3], under the isotropic Hamiltonian can be factored to give

$$\begin{aligned} \rho(t) &= \exp\{-i2\pi J_{\text{AM}}(I_{A_y}I_{M_y} + I_{A_z}I_{M_z})\} \\ &\quad \times \exp\{-i2\pi J_{\text{AM}}I_{A_x}I_{M_x}\}\rho(0) \\ &\quad \times \exp\{i2\pi J_{\text{AM}}I_{A_x}I_{M_x}\} \\ &\quad \times \exp\{i2\pi J_{\text{AM}}(I_{A_y}I_{M_y} + I_{A_z}I_{M_z})\}. \end{aligned} \quad [6]$$

If the initial density operator is proportional to the  $x$  components of the angular-momentum operators (i.e.,  $I_{A_x}$  or  $I_{M_x}$ ), which would normally be true when invoking a CP Hamiltonian of the form of Eq. [2], then Eq. [6] simplifies to

$$\begin{aligned} \rho(t) &= \exp\{-i2\pi J_{\text{AM}}(I_{A_y}I_{M_y} + I_{A_z}I_{M_z})\}\rho(0) \\ &\quad \times \exp\{i2\pi J_{\text{AM}}(I_{A_y}I_{M_y} + I_{A_z}I_{M_z})\}. \end{aligned} \quad [7]$$

Equation [7] is identical to the result one would obtain for a CP experiment on a two-spin system (i.e., using Eq. [2] with  $J_{\text{MX}} = 0$ ), except for the additional factor of two in front of the scalar coupling constant. Thus, the coherence transfer in a heteronuclear two-spin system for a CP experiment could be calculated using an identical procedure as for a homonuclear two-spin system under isotropic mixing, with the trivial modification that the heteronuclear scalar coupling constant employed in the calculation should be half of its actual value. This is, in fact, the manner in which Kellogg and Schweitzer (11) employed the ZTOCSY program to simulate heteronuclear coherence transfer in their study. As demonstrated above, this procedure is valid for a two-spin system.

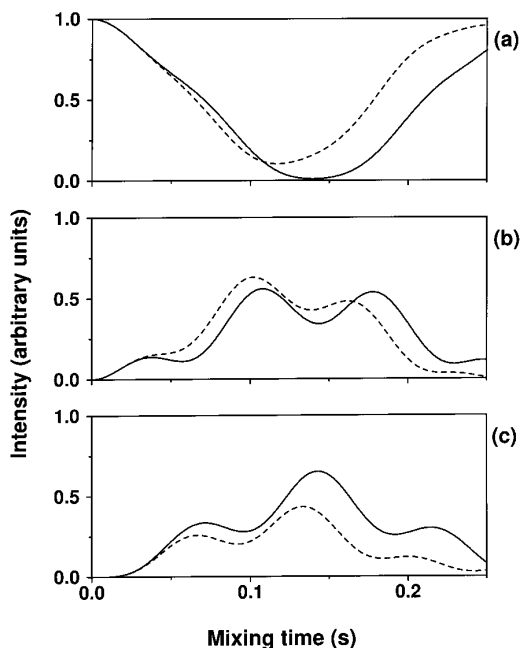
However, the simple expedient of halving the heteronuclear coupling constants is not valid in the general case, as one can see by considering the linear AMX spin system. In this case, and starting with the isotropic mixing Hamiltonian of Eq. [1], one cannot factor Eq. [3] as was done for the two-spin case to produce Eq. [7], since the term  $I_{A_x}I_{M_x}$  does not commute with all of the other terms in Eq. [1]. Thus, the evolution of a three-spin (or more) system, as expressed by Eq. [3], will differ in a nontrivial way for the TOCSY experiment (using a generalized version of Eq. [1]) versus the CP-TOCSY experiment (using the generalized version of Eq. [2]).

The difference in coherence-transfer behavior between homonuclear TOCSY and heteronuclear  $J$  cross-polarization experiments for a three spin AX<sub>2</sub> system can be seen directly by inspecting the analytical expressions describing these processes. Under isotropic mixing, the transfer of magnetization in a homonuclear system from spin A to the X spins is given by (15, 25)

$$M_X \propto \frac{4}{9}[1 - \cos(3\pi J\tau)]M_A, \quad [8]$$

where all multispin terms have been ignored,  $\tau$  is the mixing time,  $J$  is the homonuclear coupling constant, and  $M_A$  and  $M_X$  are the A and X spin magnetizations, respectively. The analogous expression for the transfer of magnetization in a heteronuclear spin system under the influence of the cross-polarization Hamiltonian is (8, 21)

$$M_X \propto \frac{1}{2}[1 - \cos(\pi\sqrt{2}J\tau)]M_A. \quad [9]$$



**FIG. 1.** Theoretical simulations of the time dependence of coherence transfer in a linear, heteronuclear three-spin system  $S-I_1-I_2$ , where  $S$  and  $I$  denote distinct nuclear species. The heteronuclear coupling constant ( $S-I_1$ ) is 10.14 Hz, while the homonuclear  $I_1-I_2$  coupling constant is  $-11.8$  Hz. Magnetization is assumed to start on the heteronucleus  $S$ , and the transfer of magnetization aligned along the doubly rotating-frame  $x$  axis is calculated: (a)  $S$  spin magnetization; (b)  $I_1$  spin magnetization; and (c)  $I_2$  spin magnetization. The solid lines represent the data calculated with the heteronuclear Hamiltonian, Eq. [2], while the dashed lines represent the data calculated with the homonuclear Hamiltonian, Eq. [1], but with the heteronuclear scalar coupling constant reduced by half to 5.07 Hz.

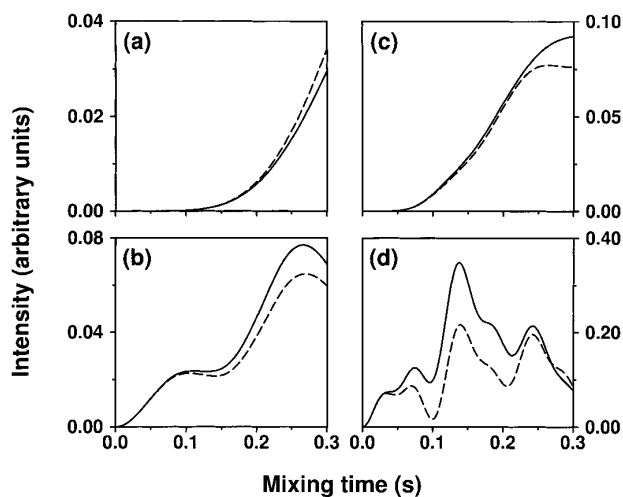
An inspection of Eqs. [8] and [9] clearly indicates the distinct nature of the coherence-transfer behavior under isotropic mixing versus cross polarization. Substituting  $J/2$  for  $J$  in the isotropic mixing result, Eq. [8], does not provide the correct description of the coherence-transfer process in the cross-polarization experiment.

In order to demonstrate the difference in coherence-transfer behavior for linear, heteronuclear  $A(MX)$ , and homonuclear  $AMX$  spin systems, numerical simulations were performed and some characteristic results are shown in Fig. 1. The heteronuclear coupling constant  $J_{AM}$  is 10.14 Hz, while the homonuclear coupling constant  $J_{MX}$  is  $-11.8$  Hz; in all cases,  $J_{AX} = 0$  Hz. Magnetization was assumed to start on spin  $A$ , and the transfer of this magnetization to spins  $M$  and  $X$  is calculated as a function of the length of the mixing time. The dashed curves in Fig. 1 were calculated using the ZTOCSY program (17), with  $J_{AM}$  being entered as half its actual value (i.e., 5.07 Hz was used in the ZTOCSY calculation), while the solid curves were calculated using the correct Hamiltonian for the CP-TOCSY experiment, Eq. [2]. For the latter calculations, standard numerical procedures were

employed to evaluate Eqs. [3] and [4] [see Refs. (8, 25), for example]. While the qualitative behavior of the coherence-transfer functions shown in Fig. 1 is similar for the isotropic and planar Hamiltonian calculations, there obviously are significant quantitative differences.

To demonstrate the magnitude of the effects to be expected for a more complicated spin system, such as the dinucleotide fragment considered in the Kellogg and Schweitzer study, coherence-transfer behavior was calculated for the nine-spin system described by Kellogg and Schweitzer (11). Unfortunately, the coupling constants were not explicitly listed in their paper, and we were unable to identify a set of values from the referenced literature to allow us to reproduce the simulated data in Fig. 7 of Ref. (11), so we derived our own set of coupling constants. The 1', 5', and 5'' protons from the 5' residue were omitted, as were the 1', 2', and 2'' protons from the 3' residue. Calculations were carried out with backbone coupling constants set as appropriate for B-form DNA (Figs. 2a and 2b) and A-form DNA (Fig. 2c and 2d). For determining the  ${}^3J_{P-5'}$ ,  ${}^3J_{P-5''}$ ,  ${}^3J_{P-3'}$ ,  ${}^3J_{4'-5'}$ , and  ${}^3J_{4'-5''}$  scalar coupling constants, knowledge of the backbone  $\beta$ ,  $\gamma$ , and  $\epsilon$  torsion angles is required (26). The torsion angles for the B DNA were obtained from Table 1 in Ref. (27):  $\beta = 180^\circ$ ,  $\gamma = 57^\circ$ , and  $\epsilon = 173^\circ$ . The torsion angles for the A DNA were extracted from Table 11-2 of Ref. (28):  $\beta = 172^\circ$ ,  $\gamma = 41^\circ$ , and  $\epsilon = -146^\circ$ . The phosphorus-proton coupling constants were calculated using a Karplus equation with parameters given by Lankhorst *et al.* (29):  ${}^3J_{HCO-P} = 15.3 \cos^2\phi - 6.1 \cos\phi + 1.6$ , where  $\phi$  is the relevant proton-phosphorus torsion angle derived from the backbone angles  $\beta$  and  $\epsilon$  (26). The  ${}^3J_{4'-5'}$  and  ${}^3J_{4'-5''}$  values were estimated from the  $\gamma$  torsion angle using the graph in Fig. 16 of Ref. (30). For the deoxyribose ring, a C2'-endo conformation (pseudorotation angle of  $18^\circ$ ) (28) was assumed for the B DNA, and a C3'-endo conformation (pseudorotation angle of  $162^\circ$ ) for the A DNA, with a maximum pucker amplitude  $\Phi_m$  of  $36^\circ$  in both cases; the  ${}^3J_{2'-3'}$ ,  ${}^3J_{2''-3'}$ , and  ${}^3J_{3'-4'}$  coupling constants were obtained from Table III of Ref. (31). The geminal proton-proton coupling constants were obtained from Ref. (32). The values of all the coupling constants are provided in the legend to Fig. 2.

In calculating the coherence-transfer functions shown in Fig. 2, the magnetization was assumed to start on the  ${}^{31}P$  nucleus between the nucleotides, and the time dependence of coherence transfer to the 3' proton of the 3' residue is shown in Fig. 2a for B DNA and Fig. 2c for A DNA, while Figs. 2b and 2d show the transfer to the 3' proton of the 5' residue for B and A forms of DNA, respectively. The solid lines in Fig. 2 show the simulated coherence-transfer behavior calculated using the planar mixing Hamiltonian, Eq. [2] in generalized form, while the simulations shown as dashed lines were calculated with the ZTOCSY program (i.e., isotropic Hamiltonian) and with the heteronuclear scalar cou-



**FIG. 2.** Simulated coherence-transfer functions for a dinucleotide fragment, showing the transfer of magnetization to the 3' residue (a, c) and to the 3' proton of the 5' residue (b, d) from the  $^{31}\text{P}$  nucleus linking the nucleotides, as a function of the mixing time. A total of nine spins were considered [the 1', 5', and 5'' protons of the 5' residue and the 1', 2', and 2'' protons of the 3' residue were omitted to match the configuration used in Ref. (11)]. The full, planar Hamiltonian was used for calculating the data shown by solid lines, while the data shown as dashed lines were calculated using an isotropic mixing Hamiltonian, with the heteronuclear coupling constants halved. A C2'-endo deoxyribose ring pucker and backbone torsion angles appropriate for B-form DNA were used for (a, b), while the data of (c, d) are relevant for A-form DNA with a C3'-endo ring pucker. The scalar coupling constants, determined as described in the text, were (a, b)  ${}^3J_{\text{P-5}'} = 2.4$  Hz,  ${}^3J_{\text{P-5}''} = 2.4$  Hz,  ${}^3J_{\text{P-3}'}$  = 1.6 Hz,  ${}^2J_{\text{5}'-5}'' = -11.8$  Hz,  ${}^3J_{\text{4}'-5}' = 2.6$  Hz,  ${}^3J_{\text{4}'-5}'' = 1.1$  Hz,  ${}^3J_{\text{2}'-3}' = 5.3$  Hz,  ${}^3J_{\text{2}''-3}'' = 0.8$  Hz,  ${}^3J_{\text{3}'-4}' = 0.8$  Hz, and  ${}^2J_{\text{2}'-2}'' = -14.1$  Hz; and (c, d)  ${}^3J_{\text{P-5}'} = 3.6$  Hz,  ${}^3J_{\text{P-5}''} = 1.5$  Hz,  ${}^3J_{\text{P-3}'}$  = 8.5 Hz,  ${}^2J_{\text{5}'-5}'' = -11.8$  Hz,  ${}^3J_{\text{4}'-5}' = 1.5$  Hz,  ${}^3J_{\text{4}'-5}'' = 2.9$  Hz,  ${}^3J_{\text{2}'-3}' = 6.8$  Hz,  ${}^3J_{\text{2}''-3}'' = 9.7$  Hz,  ${}^3J_{\text{3}'-4}' = 8.6$  Hz, and  ${}^2J_{\text{2}'-2}'' = -14.1$  Hz. All other coupling constants were set to zero.

pling constants halved. The simulated data shown in Fig. 2 demonstrate that the differences to be expected between the isotropic and planar Hamiltonian calculations can be rather small in some cases and quite significant in others, depending on the exact values of the scalar coupling constants. The magnitude of the differences will also depend on the coupling topology. The small differences observed in Figs. 2a–2c result from the small  ${}^1\text{H}-{}^{31}\text{P}$  couplings relative to other couplings in the network, while the differences in Fig. 2d are more substantial as a result of the larger  ${}^3J_{\text{P-3}'}$  coupling constant.

In summary, we have indicated that an isotropic mixing Hamiltonian, as employed in the ZTOCSY simulation program (17), should not be used to calculate coherence-transfer behavior for heteronuclear cross-polarization experiments, except in the specific case of a two-spin system. Instead, the Hamiltonian which properly describes the homonuclear and heteronuclear interactions should be used. We emphasize, however, that none of the qualitative con-

clusions or experimental results contained in the Kellogg and Schweitzer study (11) are affected; only the quantitative aspects of the simulated data shown in Fig. 7 of their paper need to be reconsidered.

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