A Two-Dimensional NMR Experiment for the Correlation of Spin-Locked and Free-Precession Frequencies

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A numerous and growing set of experiments involve continuous application of radio frequency (rf) fields to a homonuclear ensemble of spins: included are the TOCSY or HOHAHA experiments;[1,2] the CAMELSPIN or ROESY experiment;[3] nutation spectroscopy;[4] $T_{1\rho}$ experiments;[5] the TROESY experiment;[6] the ZFHF-ZQ experiment;[7] etc. In every such experiment, there are several processes which occur during the application of the rf: relaxation between eigenstates, including coherent magnetization transfer; zero-quantum, single-quantum, and multiple-quantum evolution, including coherent magnetization transfer; and eventual establishment of a nonequilibrium steady state. In designing and performing these experiments, it has been the aim to find conditions or experimental protocols in which one of the processes is isolated for observation while the others are in some way suppressed or compensated in order to observe only the effects of the target process.

This effort has not been completely successful. The most troublesome pair[8,9] to separate has been transverse cross-relaxation (targeted in CAMELSPIN and ROESY) and homonuclear Hartmann–Hahn exchange (HH) (targeted in TOCSY or HOHAHA). For macromolecules with $\omega\tau_c \gg 1$ and $2\sigma_1 = -\sigma_2$, near-complete suppression of cross-relaxation in TOCSY experiments has been achieved by pulse sequences designed to distribute the spins between orientations perpendicular and parallel to the static magnetic field such that the longitudinal and transverse cross-relaxation exactly cancels.[8–11] The computer-optimized pulse sequence, clean-CITY,[11] achieves essentially complete suppression of the cross-relaxation between pairs of nuclei giving signals at close to the carrier frequency and 70% suppression for signals grouped as far away as $\gamma B_1/2$. The target HH process is detected with efficiencies of 70–100% for signals appearing within $\gamma B_1/5$ of the carrier. In general, the use of heteronuclear decoupling sequences and derivatives is not 100% efficient in detecting the HH process, as shown by Waugh.[12]

The converse problem, suppression of HH exchange while observing cross-relaxation, has also given difficulty. The suggestion[13] that the use of a series of small flip-angle pulses for spin-locking would suppress the HH exchange is apparently incorrect.[14] HH exchange can be partly suppressed by sweeping the rf during spin-locking.[15] The TROESY experiment[6] suppresses HH exchange but suffers from the disadvantage when applied to macromolecules that the cross-relaxation rate measured is one-fourth of the transverse relaxation rate. The search for methods to isolate HH exchange and transverse cross-relaxation is currently very active.[16,17]

Since all of the above-listed processes will normally occur and resist clean and simultaneous suppression, an appealing alternative is to find a method to observe them separately in a single experiment. The prototype experiment suggested here is the simplest possible 2-D experiment, for which we propose the acronym LOUSY (Lock On Unprepared Spins). The pulse sequence [RD – spin lock(t_1) – FID(t_2)] consists of a relaxation delay (RD), application of rf during time t_1, and acquisition during t_2. A 2-D spectrum is obtained by double Fourier transformation with respect to t_1 and t_2.

The events occurring during this experiment unfold as follows: during RD, the spin system recovers to its thermal equilibrium state, $|\rho_0\rangle$ (using superoperator notation[18]). Evolution during t_1 is best followed by transforming the density vector $|\rho\rangle$ into the basis of eigenstates of the Hamiltonian including rf, $|{}^{r}\rho\rangle$. Then

$$(|{}^{r}\rho(t)\rangle - |{}^{r}\rho_{ss}\rangle) = e^{-(iL-R)t}\,(|{}^{r}\rho_0\rangle - |{}^{r}\rho_{ss}\rangle) \qquad (1)$$

where L and R are the Liouvillian and relaxation superoperators in the rf basis. The steady-state density vector $|{}^{r}\rho_{ss}\rangle$ is obtained as described previously.[19]

$$-(iL - R)|{}^{r}\rho_{ss}\rangle = R|{}^{r}\rho_0\rangle \qquad (2)$$

During the period t_1, the elements of $|{}^{r}\rho\rangle$ will evolve as follows: (1) Diagonal elements of $|{}^{r}\rho(t)\rangle$ will relax toward $|{}^{r}\rho_{ss}\rangle$ (which is itself nearly diagonal). Both zero-frequency decay and the signals arising from $|\rho_{ss}\rangle$ will occur in the 2-D spectrum at $\omega_1 = 0$ and along ω_2 at the free-precession frequencies. It is possible to separate the steady-state contributions from the decay by subtracting the FID for the steady state from each FID obtained while stepping t_1. (2) Zero-quantum coherences of $|{}^{r}\rho(t)\rangle$ are responsible for isotropic mixing or Hartmann–Hahn exchange. The evolution frequencies for two coupled spins is closely given by[19]

$$\omega_{HH} = \left[(2\pi J)^2\cos^4\left(\frac{\theta_I + \theta_S}{2}\right) + (\omega_{eI} - \omega_{eS})^2\right]^{1/2} \qquad (3)$$

where $\theta_I = \tan^{-1}(\omega_I/\omega_{rf})$, $\omega_{eI} = (\omega_I^2 + \omega_{rf}^2),^{1/2}$, etc. Cross peaks arising from zero-quantum coherences will be observed at $\omega_1 = \omega_{HH}$ and $\omega_2 = \omega_I \pm J/2$ and $\omega_S \pm J/2$. The intensities of these peaks will depend on the HH mixing coefficient, $(J\omega_{rf})/(\omega_I^2 - \omega_S^2)$. (3) Single-quantum coherences will oscillate with frequencies centered around ω_{eI}, ω_{eS}, etc. If the applied rf is inhomogeneous, they will decay rather quickly, with time constant $T_{2\rho}^*$ (analogous to T_2^* in laboratory frame experiments). With some care, however, the correlation of rotating and laboratory frame frequencies for each spin can be observed, leading to a family of "diagonal" peaks falling around the hyperbola $\omega_1 = (\omega_{rf}^2 + \omega_2^2)^{1/2}$. In addition, COSY-type multiplets may be observed around the cross peaks at $\omega_1 = (\omega_{rf}^2 + \omega_I^2)^{1/2}$, $\omega_2 = \omega_S$, and the reverse. (4) Double and higher quantum coherences will decay even more quickly because of rf field inhomogeneity but may in some cases be observed.

When the rf is turned off at the end of time t_1, the state of the system will be given by $|{}^{r}\rho(t_1)\rangle$, which can then be transformed back to give $|\rho(t_1)\rangle$.

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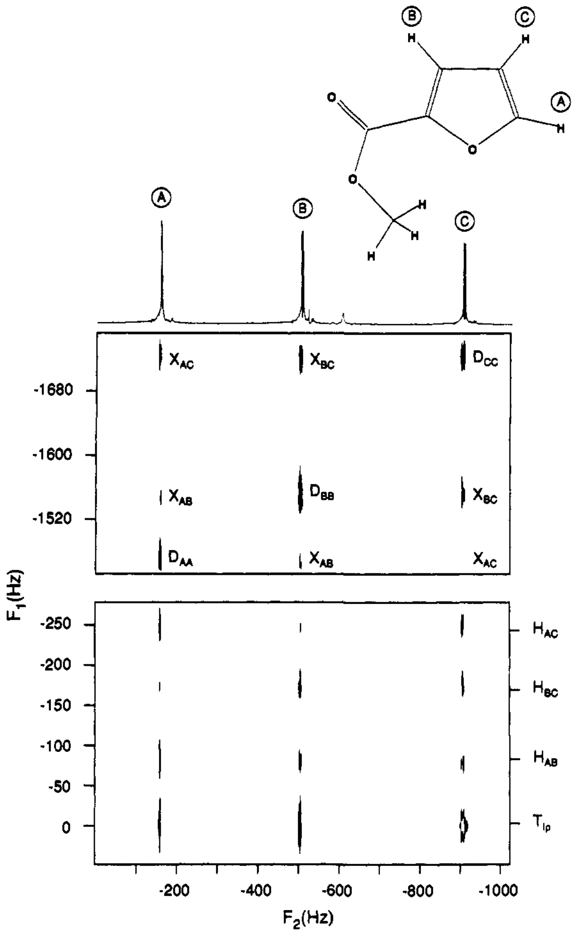

Figure 1. LOUSY spectrum of methyl 2-furoate. Peaks are labeled $T_{1\rho}$, H, D, and X, identifying rotating-frame relaxation, Hartmann–Hahn, "diagonal", and "cross" peaks, respectively, while the subscripts indicate the active nuclei.

Figure 1 illustrates the application of LOUSY to a three-spin system, methyl furoate. An rf field strength of 1470.6 Hz (0.345 G) was used. Spectral widths in the first and second frequency axes were 4096 and 2048 Hz. 2048 points per FID were acquired during t_2, for 398 increments in t_1. A steady-state FID was subtracted from all FIDs. Only the N-type spectrum is shown. The data were zero-filled to 4096 × 4096. A sine-squared window shifted by 60° was used for the FIDs. Along the ω_1 direction, an unshifted sine-squared window was followed by a Kaiser window.

The following correlation peaks are observed: (1) Peaks at $\omega_1 = 0$ Hz and $\omega_2 = 158$, 505, and 905 Hz correspond to the decay of the diagonal elements of $|^r\rho(t_1)\rangle$. Since $|^r\rho_{ss}\rangle$ was subtracted, these are designated as $T_{1\rho}$ peaks. (2) Peaks clustered at $\omega_1 = 76$, 172, and 248 Hz, and $\omega_2 = 158$, 505, and 905 Hz are the HH or TOCSY peaks. If the Fourier transformation is carried out retaining the phases, then two peaks are observed in each case corresponding to zero-quantum transitions with the passive spin parallel or antiparallel to its effective field. The HH peaks are labeled as HH_{AB}, HH_{AC}, HH_{BC} along the ω_1 direction in Figure 1. (3) Peaks at (158, 1479), (505, 1555), and (905, 1723) are "diagonal" peaks and connect directly the frequencies of free precession with the frequencies of precession about the effective fields in the presence of the rf. In Figure 1 these are labeled D_{AA}, D_{BB}, D_{CC}. (4) Peaks at (158, 1723), (505, 1479), and (505, 1723) are COSY-type cross peaks and are labeled X_{AC}, X_{AB}, X_{BC}.

Fourier transformation with respect to the time of rf excitation has been performed previously in several contexts. Aue *et al.*[20] have applied it in mapping the rf field strength. It has been used extensively by Samoson and Lippmaa[4] in the investigation of nutation of odd half-integer spins in solids and by Waugh[21] for measuring the frequency of the ^{13}C–^{1}H HH exchange in solids. We believe that this experiment for measuring homonuclear HH exchange in liquids is novel and has interesting potential uses. For example, the 3-D spectrum obtained from $90_x^\circ - t_1 -$ spin lock(t_2) – FID(t_3), after subtraction of the steady-state FID, will have peaks at $(\omega_1, 0, \omega_S)$ which are the ROESY or CAMELSPIN spectra of cross-relaxation effects, free of HH effects, while the planes with $\omega_2 = \omega_{HH}$ will provide maps similar to those from TOCSY for coupled networks of spins without cross-relaxation effects.

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