

## X-Relayed $^1\text{H}$ - $^1\text{H}$ Correlated Spectroscopy

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Many biological and synthetic organic molecules contain molecular fragments in which two or more different groups of protons are coupled to a common NMR active heteronucleus, e.g.,  $^{31}\text{P}$ ,  $^{15}\text{N}$ , or  $^{13}\text{C}$ . In such cases, if the protons have no mutual homonuclear coupling, or their coupling is not resolved, it is frequently desirable to establish a through-bond connectivity between the different groups of protons via the spin-spin couplings with the heteronucleus. In principle this can be done by identifying those protons which correlate with a common X resonance in a heteronuclear COSY experiment, or by conventional heteronuclear spin decoupling, but this approach may be severely limited by poor spectral dispersion and sensitivity of the X nucleus. In this paper we introduce X-relayed  $^1\text{H}$ - $^1\text{H}$  correlated spectroscopy (X-relayed  $^1\text{H}$ - $^1\text{H}$  COSY), a new form of heteronuclear relayed coherence transfer spectroscopy (1-3) which can establish such  $\text{H} \rightarrow \text{X} \rightarrow \text{H}$  connectivities directly. While all previously described 2D heteronuclear relayed coherence transfer experiments used detection of the X nucleus in one dimension to establish the heteronuclear scalar coupling networks (1-3), the present experiment avoids the need to interpret, or even record, the NMR spectrum of X.

The experimental scheme is shown in Fig. 1. Transverse proton magnetization is created by the first pulse, and evolves during  $t_1$ . Coherence transfer is brought about by the pair of  $\pi/2$  pulses at the end of  $t_1$ , creating antiphase magnetization of those X nuclei which are coupled to protons. During the period  $\tau$  this coherence evolves exclusively under the influence of the heteronuclear spin-spin couplings, since chemical shifts are refocused by the pair of  $\pi$  pulses at the midpoint of  $\tau$ . Under the action of the  $\pi/2$  pulse at the end of  $\tau$ , coherence is subsequently transferred back to those protons which are coupled to X.

In the 2D spectrum obtained after Fourier transformation in  $t_1$  and  $t_2$ , each connectivity of the type  $\text{H}_k \rightarrow \text{X} \rightarrow \text{H}_l$  is manifested by two cross-peaks in positions defined by the proton chemical shifts  $\delta_{\text{H}_k}$  and  $\delta_{\text{H}_l}$ , i.e.,  $(\omega_1 = \delta_{\text{H}_k}, \omega_2 = \delta_{\text{H}_l})$  and  $(\omega_1 = \delta_{\text{H}_l}, \omega_2 = \delta_{\text{H}_k})$ . The two cross-peaks are the result of coherence transfer along the pathway  $\text{H}_k \rightarrow \text{X} \rightarrow \text{H}_l$  and its reverse,  $\text{H}_l \rightarrow \text{X} \rightarrow \text{H}_k$ . Two "diagonal" peaks also appear, corresponding to the pathways  $\text{H}_k \rightleftharpoons \text{X}$  and  $\text{H}_l \rightleftharpoons \text{X}$ . All other contributions to the spectrum are suppressed by the phase cycle shown in Fig. 1 (which may further be combined with CYCLOPS (4)). The first two steps of the cycle restrict the finally observed proton magnetization to those terms which involved transverse

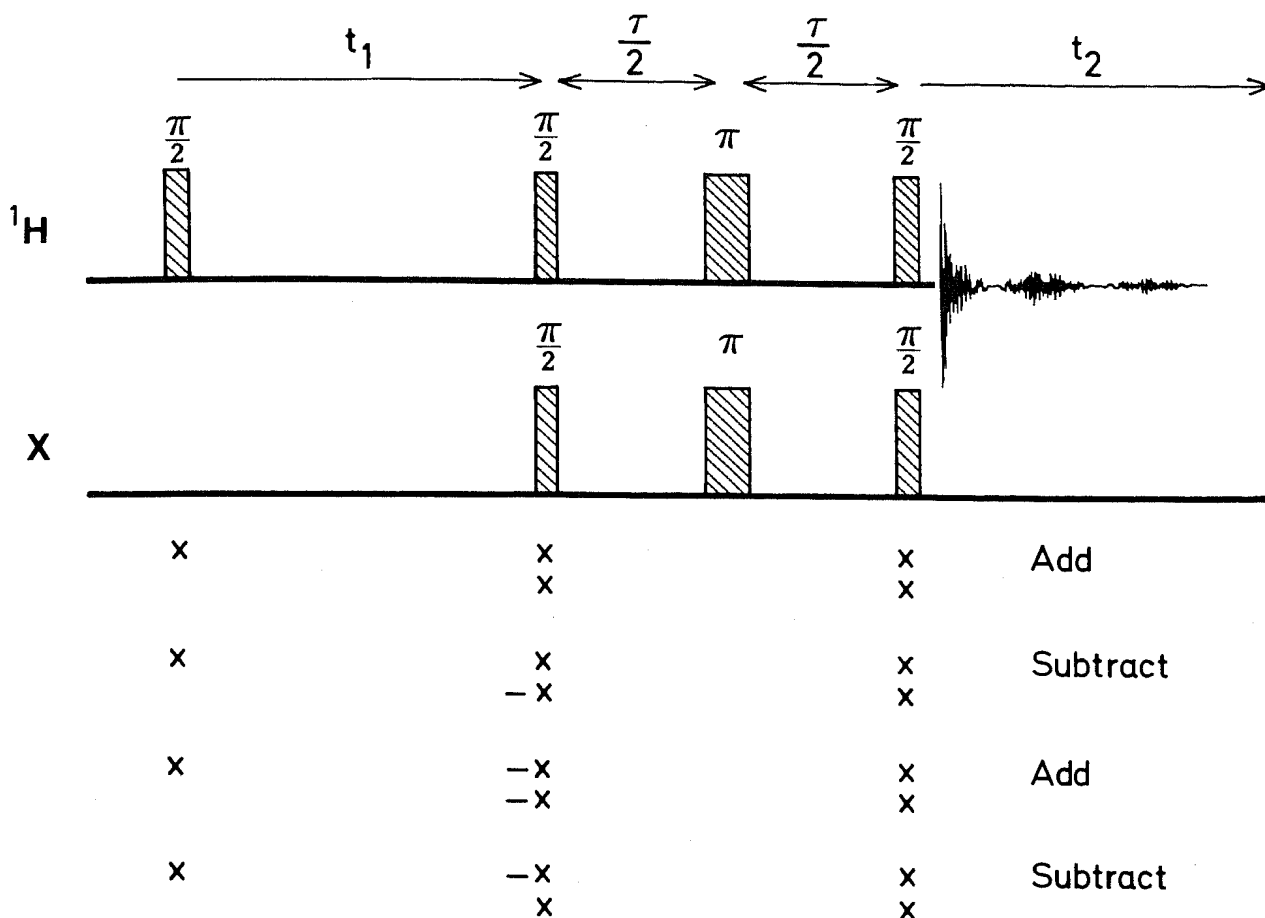
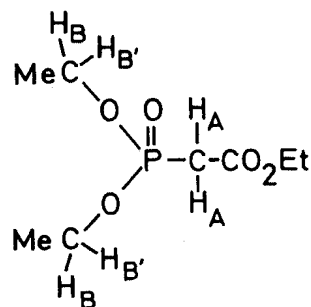


FIG. 1. Pulse sequence and phase cycling for the X-relayed  $^1\text{H}$ - $^1\text{H}$  COSY experiment (see text for explanation).

magnetization of X nuclei during  $\tau$ . The remaining steps discriminate against axial peaks arising from pure longitudinal magnetization of X nuclei during  $t_1$ , as well as against terms originating from heteronuclear zero and double-quantum coherence during the period  $\tau$ .

Figure 2 shows a  $^{31}\text{P}$ -relayed  $^1\text{H}$ - $^1\text{H}$  COSY spectrum obtained from a sample of triethylphosphonoacetate (**1**):



The two magnetically equivalent protons  $\text{H}_\text{A}$  resonate at  $\delta_\text{A}$ , while the four protons  $\text{H}_\text{B}$  and  $\text{H}_\text{B}'$  are virtually degenerate at  $\delta_\text{B}$ ; all six are coupled to phosphorus

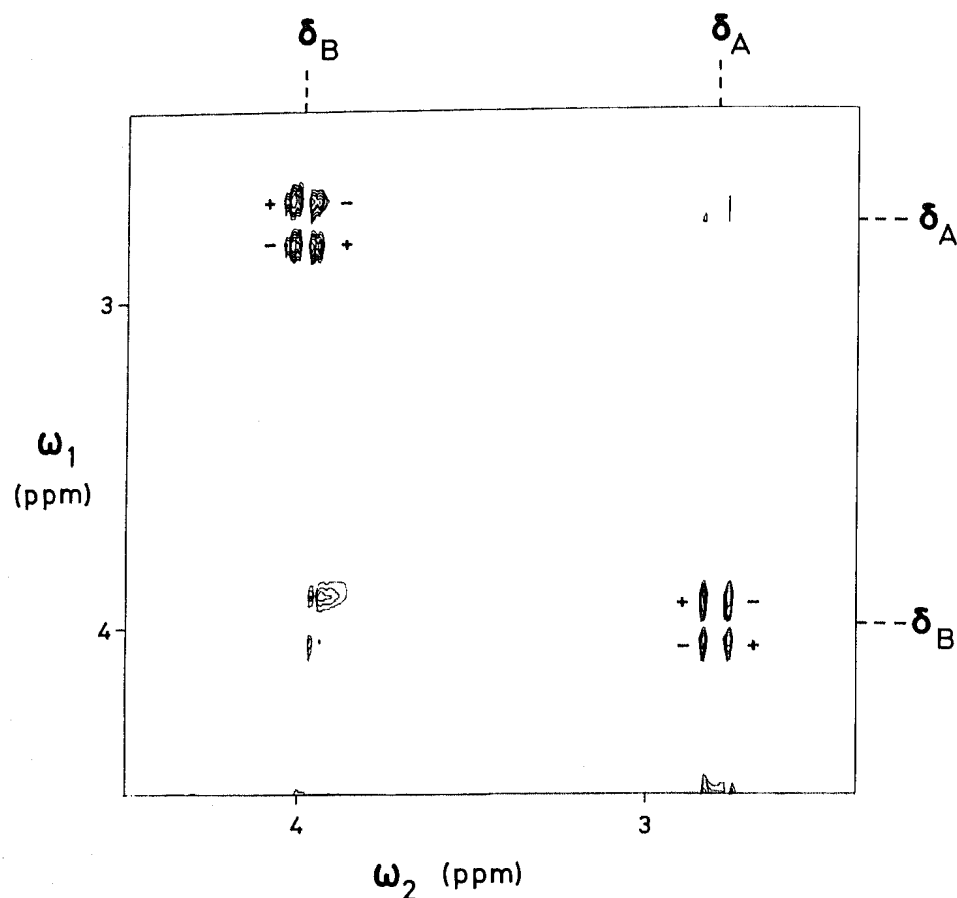


FIG. 2.  $^{31}\text{P}$ -relayed  $^1\text{H}$ - $^1\text{H}$  COSY spectrum obtained from a 10% solution of triethylphosphonoacetate (1) (obtained from Fluka AG) in  $[\text{^2H}_6]$  benzene. Cross-peaks between protons  $\text{H}_A$  and  $\text{H}_B$  indicate their connectivity to a common  $^{31}\text{P}$  atom, through the coupling pathways  $\text{H}_A \rightarrow ^{31}\text{P} \rightarrow \text{H}_B$  and  $\text{H}_B \rightarrow ^{31}\text{P} \rightarrow \text{H}_A$ . Both positive and negative levels are included in the contour plot; chemical shifts are calibrated relative to  $[\text{^2H}_5]$  benzene.

( $J(\text{H}_A, \text{P}) = 21$  Hz,  $J(\text{H}_B, \text{P}) = J(\text{H}_B', \text{P}) = 8$  Hz). In the  $^{31}\text{P}$ -relayed  $^1\text{H}$ - $^1\text{H}$  COSY spectrum obtained with the scheme of Fig. 1 the connectivity of the protons  $\text{H}_A$  and  $\text{H}_B$  to a common  $^{31}\text{P}$  nucleus is clearly indicated by the pair of cross-peaks. Calculations using the operator product formalism of Sørensen *et al.* (5) show that the spectrum can be phased such that both cross-peaks and "diagonal" peaks have a pure absorptive antiphase multiplet structure with respect to the heteronuclear spin-spin couplings in both frequency dimensions. This may be clearly seen in the experimental spectrum. The transfer functions governing the efficiency of the coherence transfer in a spin system  $\text{H}_k\text{-X}_m\text{-H}_l$  during  $\tau$  are, for the cross-peaks,

$$\sin \pi J_{km}\tau \sin \pi J_{lm}\tau \prod_{\substack{i \\ i \neq k,l}} \cos \pi J_{im}\tau e^{-\tau/T_2(X)} \quad [1]$$

and for the diagonal peaks

$$\cos \pi J_{km}\tau \cos \pi J_{lm}\tau \prod_{\substack{i \\ i \neq k,l}} \cos \pi J_{im}\tau e^{-\tau/T_2(X)}, \quad [2]$$

where in each case the product over  $i$  includes all couplings to the X nucleus other than those involved in the relay pathway. The delay  $\tau$  should therefore be set so as to maximize the function [1], taking due note of the dependence on  $T_2$  of the X nuclei,  $T_2(X)$ , when this is short.

When compared to previously described heteronuclear relayed coherence transfer experiments (1-3), the value of the present experiment lies mainly in its relative independence of the spectral properties of the X nucleus. Thus it should be possible to detect simultaneously the presence of many  $H \rightarrow X \rightarrow H$  coupling pathways regardless of the degree of overlap in the NMR spectrum of the X nuclei. Potential ambiguities might arise in the case of strong X-X couplings, which could transmit coherence over longer pathways, such as  $H \rightarrow X \rightarrow X \rightarrow H$ . Such situations could, however, be readily recognized, since the splittings in the proton spectrum due to heteronuclear couplings would cease to be simple doublets.

Among many potential applications for the present experiment an obvious possibility is the use for sequential resonance assignments in oligonucleotides (6), where the otherwise isolated ribose proton spin systems of successive nucleotides may be connected using the couplings to the  $^{31}\text{P}$  atom of the intervening phosphate group. Similarly, this experiment might be employed for connecting the otherwise isolated proton spin systems of successive amino acid residues in polypeptides via  $^{13}\text{C}$  or  $^{15}\text{N}$  spins (7, 8). In complexes of organic ligands with NMR-active metal ions, for example,  $^{113}\text{Cd}$  or  $^{199}\text{Hg}$ , connectivities between nuclear spins located on different ligands may be established via the coupling with the central metal nucleus (9). This opens interesting avenues for studies of metal coordination in macromolecules, such as proteins and nucleic acids (10). More generally we note also that this experiment is one of a general class in which homonuclear 2D proton spectra may be edited according to their interactions with an X nucleus; such experiments may hold considerable potential. Also, although we have discussed only experiments involving proton observation, detection of coherence transfer pathways  $X \rightarrow H \rightarrow X$ , or between different heteronuclei, should be equally feasible.

The experiment was carried out using a Bruker AM 300 spectrometer specially modified to allow proton observation immediately following a sequence of proton (decoupler) and phosphorus (transmitter) pulses. Signals were detected on the decoupler coils of a 10 mm broadband probe, phosphorus pulses being delivered via the X receiver coils. Errors in the phase shifts of the proton pulses led to some residual diagonal intensity for protons not coupled to  $^{31}\text{P}$ , but this problem is not serious since no useful information is contained in even the true diagonal peaks. Residual axial peaks were also present at the lower boundary of the spectrum. The delay  $\tau$  was set to 0.0116 sec, which is close to the optimum value of expression [1] in this case; 64 transients were collected for each of 64  $t_1$  increments during a total experimental time of  $\sim 2.5$  hr. The carrier was placed at the center of the spectrum. Time-proportional phase incrementation (TPPI) of the first pulse (i.e., a  $90^\circ$  phase increment between each  $t_1$  value) was employed to give quadrature detection in  $\omega_1$  (11-13), as was recently also described for the acquisition of absorption mode COSY spectra (14). The spectral width was 1.25 kHz in both dimensions, and 512 points were used in  $\omega_2$ ; during 2D transformation phase-shifted sine-bell window functions and one zero filling were applied in both dimensions.

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