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NMR experiments for the sign determination of homonuclear scalar and residual dipolar couplings

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Abstract

A modified version of the JHH-TOCSY experiment, 'signed COSY', is presented that allows the determination of the sign of residual dipolar ^{1}H-^{1}H coupling constants with respect to the sign of one-bond ^{1}H-X coupling constants in linear three-spin systems X-^{1}H-^{1}H, where X = ^{13}C or ^{15}N. In contrast to the original JHH-TOCSY experiments, the signs of J_{HH} couplings may be determined for CH_2-CH_2 moieties and for uniformly ^{13}C/^{15}N-labelled samples. In addition, sensitivity is enhanced, diagonal peaks are suppressed and cross peaks are observed only between directly coupled protons, as in a COSY spectrum.

Residual dipolar couplings between ^{1}H spins are readily measured in dilute liquid crystalline phases and contribute long-range conformational restraints in structure determinations (Tjandra and Bax, 1997). Since the sign of dipolar couplings varies for different orientations of the coupling partners, the sign must be determined to extract the full information content. This can be difficult for ^{1}H-^{1}H couplings, if the protons are not also scalar coupled (Tian et al., 1999a,b) or do not have a common coupling partner (Cai et al., 1999; Permi et al., 1999). The JHH-TOCSY experiment (Willker and Leibfritz, 1992, 1994; Xu et al., 1999) produces an E.COSY-type spectrum that reveals the relative signs of J_{HX} and J_{HH} couplings in linear X-^{1}H-^{1}H spin systems, as well as the magnitude of the J_{HH} coupling. Unfortunately, the JHH-TOCSY experiment works neither for uniformly ^{13}C/^{15}N-labelled proteins nor for CH_2-CH_2 moieties, severely limiting its applicability. Here we present a modified pulse sequence which does not measure the magnitude of J_{HH}, but which does allow the determination of the relative signs of J_{HH} and J_{HX} couplings in uniformly labelled proteins and in CH_2-CH_2 groups. In addition, the new experiment minimizes signal overlap by generating only COSY cross peaks and is therefore referred to in the following as 'signed COSY'.

The JHH-TOCSY experiment has been described in detail earlier (Willker and Leibfritz, 1992). Signed COSY experiments differ from JHH experiments by the use of heteronuclear decoupling during the evolution time t_1 and cycling of the phase $\phi 7$ of the second last 90°(^{1}H) pulse (Figure 1). Therefore, splittings by $^1J_{HX}$ couplings are avoided and only antiphase magnetization is detected during the acquisition time t_2 which can refocus into observable magnetization only for non-vanishing J_{HH} couplings.

We use Cartesian product operators for a brief description of the relevant coherence transfer pathway in the experiment of Figure 1a, assuming a linear three-spin system ^{13}C-^{1}H-^{1}H′, where ^{13}C and ^{1}H are coupled by a one-bond scalar coupling $^1J_{HC}$, and ^{1}H and ^{1}H′ are coupled by a much smaller, scalar or dipolar coupling, and there is no coupling between ^{13}C and ^{1}H′. Starting from equilibrium magnetization of the ^{13}C-bound proton, H_z, antiphase magnetiza-

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Supplementary material: A signed COSY spectrum of gramicidin S at natural isotopic abundance can be obtained from the authors on request.

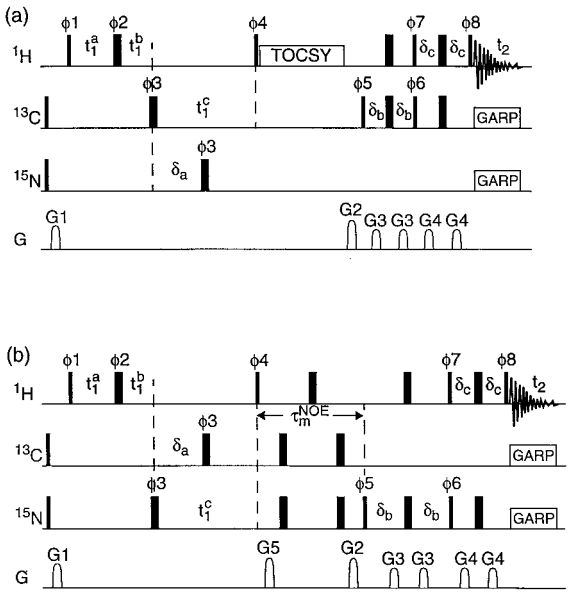


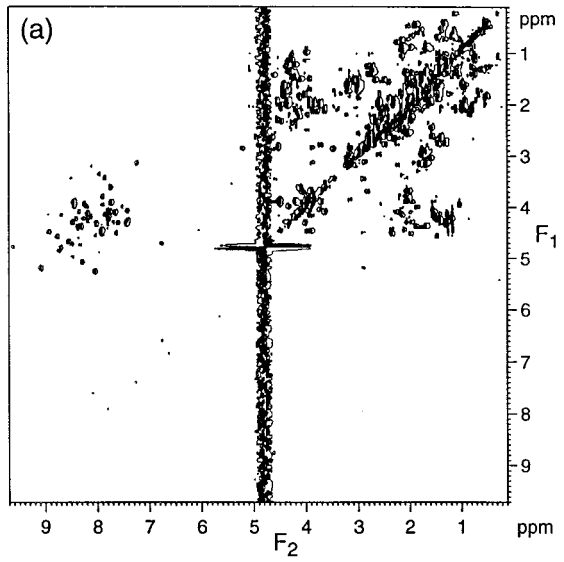
Figure 1. Pulse sequences of signed COSY experiments. Narrow and wide filled squares denote non-selective 90° and 180° pulses, respectively. Phases are x unless indicated differently. Phase cycle: ϕ_1 = x; ϕ_2 = 64(y),64(−y); ϕ_3 = 32(x),32(−x); ϕ_4 = 16(y),16(−y); ϕ_5 = x,−x; ϕ_6 = 8(y), 8(−y); ϕ_7 = 4(y),4(−y); ϕ_8 = 2(x),2(−x); receiver = 2(x,−x,−x,x),4(−x,x,x,−x),2(x,−x,−x,x). The States-TPPI scheme is applied to ϕ_1 and ϕ_2. Pulsed field gradients (strength in G cm^{-1}, duration in ms, axis): G1 (17.5,1.0,z), G2 (12.5,1.0,x+y+z), G3 (5.0,0.5,x), G4 (5.0,0.5,z), G5 (−7.5,1.0,x+y+z). The experiments are semi-constant time in the t_1 dimension, i.e. $t_1^{a,b,c} = (\delta_a, 0, \delta_a)$ for $t_1 = 0$ and $t_1^{a,b,c} = (0, t_{1max}/2 - \delta_a, t_{1max}/2+\delta_a)$ for $t_1 = t_{1max}$, with corresponding time increments (Grzesiek and Bax, 1993; Logan et al., 1993). (a) Signed COSY with TOCSY transfer. Sign comparison to $^1J_{HC}$. Delays: $\delta_a = 1/(4^1J_{HC})$, $\delta_b = \delta_c = 1/(8^1J_{HC})$. (b) Signed COSY with NOE transfer. Sign comparison to $^1J_{HN}$. Delays: $\delta_a = \delta_b = 1/(4^1J_{HN})$, $\delta_c = 1/(8^1J_{HN})$.

tion $2H_xC_z$ is generated and frequency-labeled during the evolution time t_1. The subsequent 90°(^{1}H) pulse converts this magnetization into longitudinal two-spin order $2H_zC_z$. Part or all of it is transferred into $2H'_zC_z$ by the following TOCSY mixing scheme and converted into $2H'_zC_y$ by the following 90°(^{13}C) pulse. This term evolves into $4H'_zH_zC_x$ during the delay $2\delta_b$ and is converted into $4H'_xH_xC_z$ by the subsequent 90°(^{1}H,^{13}C) pulses. After refocusing to $2H'_xH_y$ during the delay $2\delta_c$, the final 90°(^{1}H) pulse generates the term $2H'_xH_z$. The resulting cross peak between H in F_1 and H′ in F_2 is purely absorptive, with in-phase and antiphase lineshapes in the F_1 and F_2 dimensions, respectively.

In the absence of relaxation and additional couplings, the delays $2\delta_a$, $2\delta_b$ and $2\delta_c$ should be of equal length and set to $1/(2^1J_{HC})$ for maximum sensitivity. In this situation, the H-H′ cross peak intensity depends on $\sin^3(\pi^1J_{HC}2\delta)$. As the magnetization evolves during t_2 with $\sin(\pi J_{HH'}t_2)$, the sign of $J_{HH'}$ is reflected in the sign of the cross peak, assuming that the TOCSY mixing preserves the sign of the magnetization. If H′ is also bound to a ^{13}C spin, $2\delta_c$ must be set to $1/(4^1J_{HC})$ to avoid complete defocusing under $^1J(^1H',^{13}C)$. In addition, $2\delta_b$ must be set to $1/(4^1J_{HC})$, if H and H′ are bound to the same ^{13}C spin. If either $2\delta_b$ or $2\delta_c = 1/(2^1J_{HC})$, all diagonal peaks are suppressed. Diagonal peaks from protons not bound to ^{13}C are suppressed independent of the delay settings. Furthermore, zero-quantum coherences present after the TOCSY mixing period are suppressed by the subsequent part of the pulse sequence.

If the two ^{1}H spins are close in space, more effective magnetization transfer may be achieved by NOE rather than TOCSY mixing. Figure 1b shows a pulse sequence which uses NOE mixing to relate the sign of a ^{1}H-^{1}H coupling to the sign of a $^1J_{HN}$ coupling. 180° pulses are inserted in the NOE mixing interval τ_m^{NOE} to suppress dipole–CSA cross-correlation effects (Levitt and Di Bari, 1994). As backbone amide groups contain only a single proton, the delay $2\delta_b$ can be set to $1/(2^1J_{HN})$ for improved sensitivity.

For experimental verification, signed COSY spectra were recorded with a ^{13}C/^{15}N-labeled sample of the 8.5 kDa N-terminal DNA-binding domain of the *E. coli* arginine repressor (ArgR-N) (Sunnerhagen et al., 1997) in an isotropic phase. The spectrum of Figure 2 was recorded with the pulse sequence of Figure 1a. Many of the cross peaks observed in a conventional ^{1}H-^{1}H COSY spectrum were also observed in the signed COSY spectrum. Cross peaks with aromatic resonances are missing because of off-resonance effects (^{13}C carrier frequency was at 44 ppm). The spectral region shown in Figure 2b illustrates how the signs of the geminal and vicinal ^{1}H-^{1}H couplings are reflected in the signs of the cross peaks. A further control experiment performed with gramicidin S in DMSO at natural isotopic abundance verified that all COSY cross peaks are observable in a signed COSY and have the expected sign, including the CH_2-CH_2 moieties in proline (see Supplementary material). A signed COSY experiment recorded with ArgR-N using NOE instead of TOCSY mixing ($\tau_m^{NOE} = 100$ ms) was found to yield improved sensitivity only for cross peaks between protons closer than 2.3 Å (data not shown).



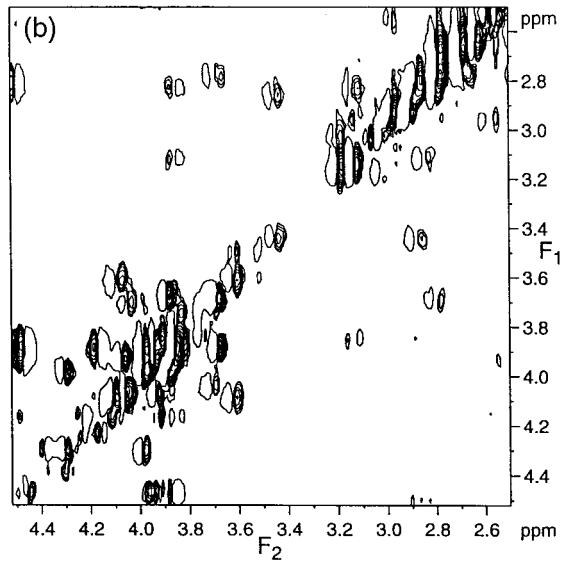


Figure 2. Overview (a) and selected spectral region (b) of a signed COSY spectrum recorded with an isotropic 0.9 mM solution of $^{13}C/^{15}N$-labeled ArgR-N in 90% H_2O/10% D_2O at 27 °C and pH 5.9. Only the lowest contour level was drawn for negative peak intensities. The spectrum was recorded with the pulse sequence of Figure 1a. Experimental parameters: $t_{1max} = 30$ ms, $t_{2max} = 119$ ms, 40 ms clean CITY mixing (Briand and Ernst, 1991), 500 MHz ^1H frequency, Bruker DRX-500 NMR spectrometer, water suppression by selective pre-irradiation, total recording time 15 h.

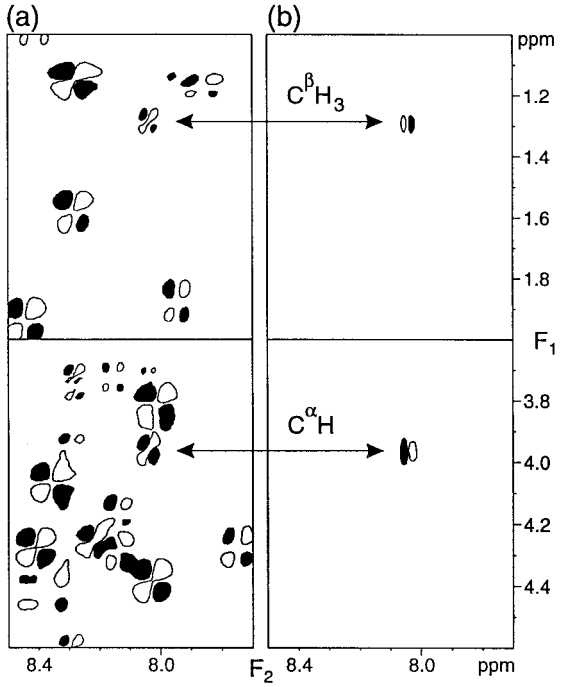


Figure 3. Comparison between conventional and signed COSY spectra recorded with a 15 mM solution of BPTI in a lyotropic phase composed of 5% $C_{12}E_6$/*n*-hexanol, in equimolar ratio (Jonströmer and Strey, 1995), in 90% H_2O/10% D_2O at pH 4.8 and 22 °C. Spectral regions containing H^β-H^N (upper panel) and H^α-H^N (lower panel) cross peaks are shown. Negative contour levels are filled. (a) DQF-COSY spectrum recorded during 6 h, using $t_{1max} = 55$ ms and $t_{2max} = 163$ ms. (b) Signed COSY spectrum recorded with the pulse sequence of Figure 1a using the same parameters as for the spectrum of Figure 2 but with a total recording time of 60 h.

As liquid crystalline solutions with ArgR-N were unstable, the signed COSY experiment of Figure 1b was tested in liquid crystalline solution with a sample of basic pancreatic trypsin inhibitor (BPTI) at natural isotopic abundance. Because of the low ^{13}C abundance, only the most intense cross peaks present in a conventional COSY spectrum (Figure 3a) were also observable in the signed COSY spectrum (Figure 3b). The $C^\beta H_3$-H^N and $C^\alpha H$-H^N cross peaks observed for Ala 58 reveal different signs of the respective ^1H-^1H couplings which become apparent only in the signed COSY spectrum.

The signed COSY experiments of Figure 2 rely on sign conservation of the magnetization transferred during the TOCSY mixing period. This holds for scalar couplings and short mixing times (Rance, 1988), if transverse cross relaxation effects are compensated for (Griesinger et al., 1988; Briand and Ernst, 1991; Cavanagh and Rance, 1992). For dipolar couplings,

a different Hamiltonian applies and TOCSY spectra can yield negative cross peaks (Tolman and Prestegard, 1994; Hansen et al., 1998). In our hands, the clean CITY mixing scheme (Briand and Ernst, 1991) yielded exclusively positive cross peaks for proteins in dilute liquid crystalline phase.

In conclusion, signed COSY experiments offer a powerful tool for the determination of the signs of scalar and dipolar coupling constants by relating them to the known sign of a one-bond coupling constant. The experiments are straightforward to set up and spectral analysis is easy. They can readily be expanded into three-dimensional experiments by converting the delay $2\delta_b$ into an incrementable evolution time. As the experiments may also be performed with samples at natural isotopic abundance, they are applicable to a large variety of compounds.

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