Relaxation Artifacts and Their Suppression in Multidimensional E.COSY-type NMR Experiments for Measurement of J Coupling Constants in ^{13}C- or ^{15}N-Labeled Proteins

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NMR structure elucidation of biomolecules in solution probes internuclear distances by cross-relaxation and dihedral angles via coupling constants. For extracting *J* couplings, a widely used type of technique is based on the E.COSY principle,[1] where small *J* values are readily measured when associated with large well-resolved ones. In ^{13}C- or ^{15}N-labeled proteins the large one-bond couplings $^1J_{CH}$ and $^1J_{NH}$ lend themselves for this purpose with particular ease.[2]

A prerequisite for accurate measurement of *J* coupling constants by E.COSY-type techniques is that passive spins remain unperturbed during the mixing process as the simple cross-peak patterns otherwise would be corrupted. There are two sources of perturbation of typically heteronuclear passive spins during mixing in multidimensional NMR experiments applied to ^{13}C- or ^{15}N-labeled proteins. The first is pulse imperfections; i.e., even though the ideal overall rotations are zero or π, some spins will experience other rotations. The second is relaxation of passive spins, inducing transitions between their α and β states.[3,4] Figure 1a illustrates the problem schematically: in addition to the desired full line contour peaks, passive spin flips give rise to the components depicted by dashed lines. These peaks are the main ones in the corresponding complementary E.COSY-type spectrum[1] that is characterized by passive spins having changed their spin states during mixing. Whenever the line widths dominate the *J* coupling constants of interest, such as in proteins, desired and undesired peaks overlap and show up as single peaks with maxima shifted in the direction of the undesired ones. Hence, with the *J* of interest being taken as the horizontal displacement between the resulting peaks, too small a value is measured.[4]

Another effect to be taken into account in determining *J* coupling constants is known as scalar relaxation of the second kind.[5−7] This effect is important, however not relevant, for the methods covered in this communication.

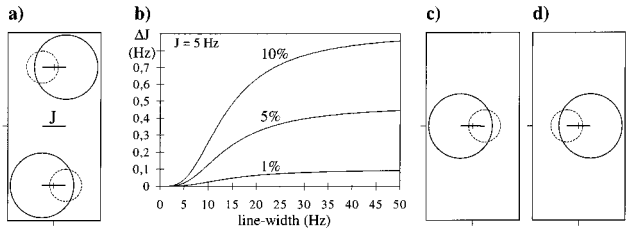

Figure 1. (a) Schematic E.COSY-type 2D contour spectrum with cross-talk to the multiplet components of the complementary spectrum indicated by the dashed contours. (b) Curves illustrating reduction in measured *J* coupling constant in a doublet of 5 Hz line separation and with Lorentzian line shape as a function of line width for 1%, 5%, and 10% levels of cross-talk. (c,d) Same as (a) but with S^3 editing and decoupling in the vertical dimension.

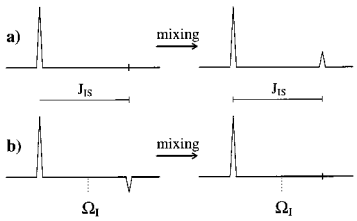

Figure 2. Schematic spectra illustrating relaxation cross-talk. (a) Relaxation of a passive spin during the mixing process gives rise to cross-talk. (b) Preparation of the spin system by an S^3 method with a level of cross-talk of opposite sense so as to compensate for cross-talk by cross-relaxation.

Although 10% error signals earlier have not been considered a problem, recent work with proteins indicates that considerable systematic errors can be introduced already at this level. For various levels of cross-talk the effect is quantified as a function of line width in Figure 1b given two Lorentzian components split by $J = 5$ Hz. For narrow lines the effect is negligible, but it grows significantly with increasing line width, the relevant parameter being the degree of overlap between the desired and undesired peaks. Clearly, a systematic error approaching 1 Hz for a 5 Hz coupling constant hampers accurate structure determination of proteins by NMR.

This communication describes a simple recipe to compensate for these systematic errors. Since the cross-talk signals correspond to the signals of the complementary E.COSY spectrum, an appropriate linear combination of normal and complementary spectra would suppress them. The most sensitive approach for this is to combine any of the recently introduced S^3 (spin state selective) methods[8] with whatever E.COSY-type multidimensional method of interest. The S^3 methods edit the two components in Figure 1a into two subspectra and allow for decoupling of $^1J_{XH}$ in the vertical dimension, leading to the two spectra in Figure 1c,d instead of the one in Figure 1a.

Plain insertion of an S^3 element into the pertinent E.COSY-type pulse sequence would result in the situation illustrated in Figure 2a, where a clean edited spectrum is observed for zero mixing time (in the absence of pulse imperfections) whereas the cross-talk signal emerges for finite mixing times. Hence, the spin system may be prepared in a state with "negative" cross-talk to compensate what happens during mixing as shown in Figure 2b. The result is a clean final spectrum that without any additional measures allows for accurate measurement of *J* coupling constants. Preparation of the spin system in a state with "negative"

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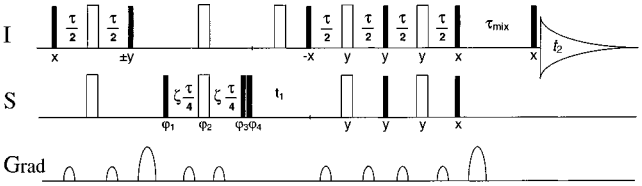

Figure 3. Heteronuclear correlated S^3E-J_{IS}−NOESY pulse sequence employed for recording the spectra presented in Figure 4. Two data sets with the phase vectors (φ_1, φ_2, φ_3, φ_4) are recorded, i.e., A: {(π/4, 0, 0, 0) − (π/4, 0, π/2, π/2)} and B: {(π/4, 0, 0, π) − (5π/4, 0, π/2, 3π/2)}. Combining as A + B and A − B yields the edited subspectra with a relative phase shift of π/2 in t_1. Details about the experiment are given in an earlier publication.[8] ζ denotes the parameter shortening the original delay $\tau = (4J)^{-1}$ for compensation of cross-talk.

cross-talk is for S^3E achieved by choosing a delay different from $(4J)^{-1}$. For example the S^3E E.COSY-type pulse sequence in Figure 3 requires a slightly shorter delay, i.e., $\zeta < 1$. The intensity expressions for the two resonances are in both subspectra proportional to $\sin(\pi J\zeta\tau) \pm \cos(\pi J\zeta\tau)$ and may be rationalized by a simple vector model.[8b]

Simultaneous total elimination of cross-talk for all cross-peaks in a pertinent protein spectrum depends on the size of the J coupling constant relevant for the S^3 element and the rate constant for build up of the undesired cross talk signals. Both can vary between individual subgroups in a protein and thus impede broadband suppression of cross-talk. Should this happen, there is always the option of suppressing cross-talk on an individual cross-peak basis by forming appropriate linear combinations of the two subspectra generated.

In practice, the variation in $^1J_{NH}$ in the backbone of proteins is so limited that all NH sites with approximately the same mobility can be cleaned up from undesired cross-talk simultaneously. The same holds true for $^1J_{CH}$ in $C^\alpha H$ groups of the backbone although two different linear combinations may be required for α helix and β sheet, respectively.[9]

Because of other sources of error, it is not meaningful to insist on cross-talk suppression below a level of about 2−3%. Thus, the proposed method is general for these important applications, and whenever the suppression is incomplete and postprocessing correction is required, it is an indicator of local motion or variation in proton density—a feature that could prove useful in other contexts.

The scheme for compensating relaxation artifacts has been tested on the protein [^{15}N]RAP 17−97 (N-terminal domain of α_2-macroglobulin receptor associated protein)[10] using the pulse sequence in Figure 3[8b] for measurement of heteronuclear J coupling constants involving ^{15}N of the backbone. Figure 4 illustrates the results with 1D sections of the Gln 33 residue. On the far left in Figure 4a is shown the S^3E-edited $^1J_{NH}$ backbone amide correlation peak in an uncompensated experiment with cross-talk clearly visible. This cross-talk is in no way obvious in the cross-peak sections in the rest of Figure 4a, thus leading to systematically smaller apparent coupling constants. In contrast, the cross-talk is almost perfectly suppressed by selecting a shorter delay in the S^3E element (corresponding to an initial negative cross-talk of 10.3% for this residue), and as expected, all cross-peaks in Figure 4b show larger effective coupling constants as compared to the uncompensated ones. This level of cross-talk suppression was achieved throughout the backbone of the protein but not for all side chain nitrogens. A consistency check was performed in Figure 4c where 10.3% cross-talk is generated artificially by linear combinations of the compensated spectra in Figure 4b; in fact the same values of the J coupling constants are measured as in the uncompensated spectra in Figure 4a.

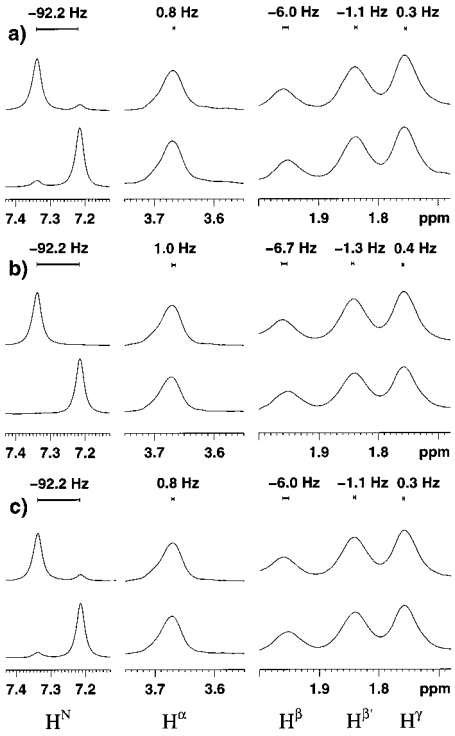

Figure 4. 1D sections from spectra of the [^{15}N]RAP 17−97 protein recorded at 298 K on a Varian Unity Inova 750 MHz spectrometer using the pulse sequence in Figure 3. The sections are taken at the chemical shift frequency of ^{15}N in Gln33. Parameters: NOESY mixing time 60 ms; water presaturation 1.5 s plus during NOESY mixing; τ = 5.262 ms; States-TPPI mode; t_1(max) = 42.6 ms; 128 scans. A data matrix of 256 × 8192 points covering 3000 × 10000 Hz was zero-filled to 512 × 8192 and apodized with cosine square and 7 Hz exponential line broadening in t_1 and t_2, respectively, prior to Fourier transformation. The coupling constants were estimated from the 1D sections using Bruker XWIN NMR 1.3 software. (a) Sections from an uncompensated experiment recorded with τ = 5.262 ms. (b) An experiment compensating cross-relaxation and pulse imperfections cross-talk using a delay $\zeta\tau$ = 4.843 ms determined in a 1D setup experiment. (c) Sections with artificially generated cross-talk formed by 1:0.103 linear combinations of the cross-talk free sections in (b).

In practice, prior to the multidimensional E.COSY-type experiment the size of the artifacts can be estimated from direct-correlation peaks (far left in Figure 4) and possibly even in simple one-dimensional (1D) setup experiments and the S^3 element adjusted to suppress them in the actual experiment. A quantitative theory shows that a rough estimate of the rate constant for these passive spin flips in the slow-tumbling limit of large molecules is $-(2T_1(S))^{-1}$, where $T_1(S)$ is the conventional T_1 of the passive spin S, i.e., $\Gamma_{I_zS^\alpha,I_zS^\beta} \approx -(2T_1(S))^{-1} = -(1/2)\Gamma_{S_z,S_z}$.

In conclusion, we have introduced a general approach for eliminating systematic errors caused by cross-relaxation of passive spins in E.COSY-type multidimensional experiments using $^1J_{XH}$ coupling constants for E.COSY-type multiplet spreading. It should find widespread use in protein NMR as more accurate J coupling constants allow for more accurate structures.

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