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Spin-State-Selective TPPI: A New Method for Suppression of Heteronuclear Coupling Constants in Multidimensional NMR Experiments

T. Schulte-Herbrüggen,* J. Briand,† A. Meissner,* and O. W. Sørensen*

**Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark; and †Department of Physical & Structural Chemistry, UW-2940, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939*

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A novel multidimensional NMR pulse sequence tool, spin-state-selective time-proportional phase incrementation (S^3 TPPI), is introduced. It amounts to application of different TPPIs on the two components of doublets so that their frequencies can be manipulated independently. The chief application is for suppression of large heteronuclear one-bond coupling constants in indirect dimensions of multidimensional experiments without interchanging the two transverse magnetization components of doublets as conventional decoupling does, which is advantageous when they relax at different rates such as by partial compensation of dipolar and CSA relaxation contributions. For experimental confirmation we use a sample of ^{15}N-labeled neural cell adhesion molecule modules 1 and 2, a protein with a molecular weight of about 20 kDa. The new tool is general and can be combined with many multidimensional NMR experiments for proteins.

Key Words: **multidimensional NMR; S^3 TPPI; HSQC; TROSY.**

The fact that transverse relaxation times can vary strongly within multiplet components in high-field spectra of large molecules has triggered the idea that overall sensitivity might improve if heteronuclear decoupling is dispensed with (*1*), although for an IS two-dimensional (2D) multiplet the intensity is distributed over typically two components. The point is that in evolution periods, the magnetization of the narrow resonance of a doublet should not be transferred to the broader resonance where it would decay faster. In an approach dubbed TROSY, Pervushin *et al.* (*1*) select exclusively the 2D component representing correlation between the two transitions of longest T_2 relaxation times. In other words, part of the initial spin order is eliminated.

That poses the question whether it is possible to treat the coherence on the slowly relaxing transition as in TROSY while also maintaining the faster relaxing component in a way so that it adds to the intensity of the genuine TROSY peak. This amounts to finding a way of suppressing heteronuclear couplings without mixing the two components and in fact that is possible when the size of the coupling constants is known.

The basic idea is derived from a recently published new pulse sequence element, TIG–BIRD (*2*), that in a heteronuclear IS two-spin system allows arbitrary and independent manipulations of the two coherences in a doublet and, if required, simultaneous and arbitrary manipulation of other isolated one-spin systems. For example, two doublet components can be given any phases appropriate prior to an evolution period of a multidimensional experiment. Hence one of them can be rendered a phase $\pi J t_1$ and the other a phase $-\pi J t_1$ in order to compensate the respective opposite phases acquired during a period t_1. The net result is that both coherences will appear to have precessed at the chemical shift frequency, i.e., as if conventional decoupling had been applied. We refer to this approach as spin-state-selective time-proportional phase incrementation (S^3 TPPI).

TIG–BIRD in its full generality allowing arbitrary and independent rotations of the multiplet components is much more than is needed for the application in mind: if S^3 TPPI is performed at the beginning of an evolution period the magnetization vectors must only be rotated from the z axis to variable positions in the xy plane (e.g., phases $\pm\pi J t_1$). Another alternative chosen here is to place the S^3 TPPI element at the end of an evolution period, i.e., whatever phase difference is acquired during that period gets compensated by refocusing the two components. To this end a set of four building blocks shown in Fig. 1a can be inserted into multidimensional experiments involving, e.g., ^{1}H–^{15}N correlations of the HSQC type. The parameter t^{m} stands for t mod$(2/J)$, where t is the time of J defocusing to be compensated, i.e., typically the duration of an evolution period. It should be noted that these pulse sequence elements can be understood entirely in terms of a simple vector model.

For the first few t_1 increments where the acquired change in angle between the two magnetization vectors, $\Delta\varphi$, is less than π, reestablishment of the initial angle by the S^3 TPPI element is achieved through effective J evolution also during t_1, provided that the two vectors are interchanged which occurs by the

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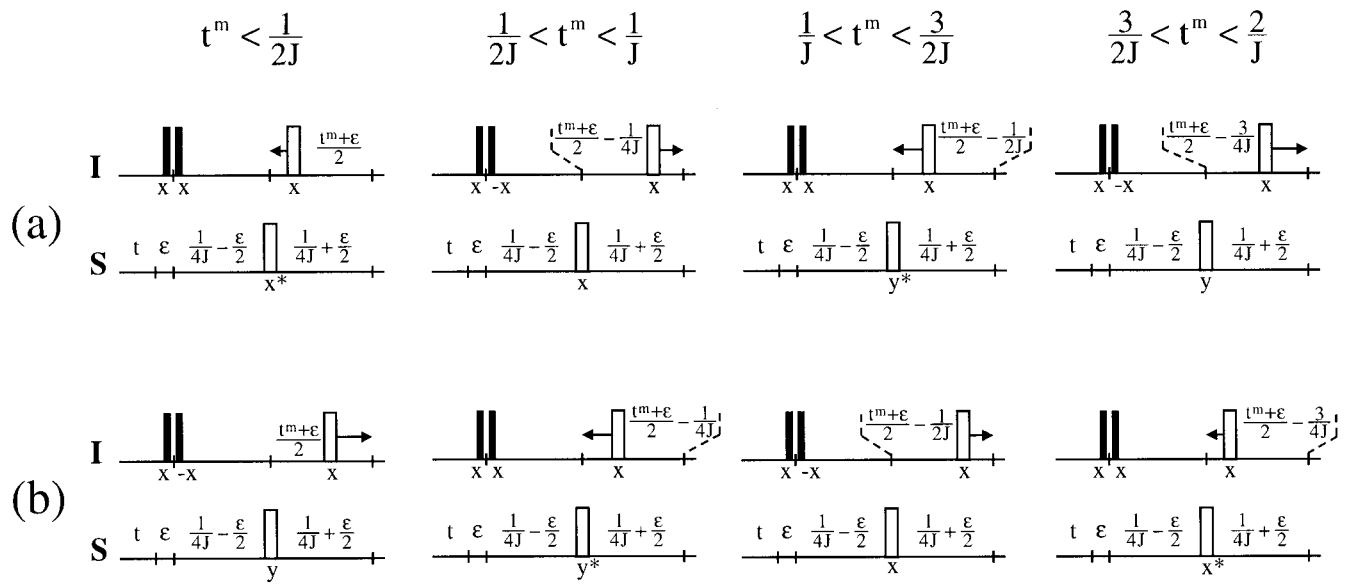

FIG. 1. Set of four pulse sequence building blocks for refocusing of J coupling evolution having occurred during a period t. Apart from chemical shift evolution during t, the elements in (a) invariably leave S-spin magnetization in antiphase with respect to the I spin in an IS two-spin system. t^m is t mod$(2/J)$ and ϵ is the initial t delay. (b) S^3 TPPI version suitable for antiphase to inphase conversion ($2I_zS^{\pm} \rightarrow S^{\pm}$). The elements invariably leaving S-spin magnetization inphase and for inphase to antiphase conversion are identical to those in (a) and (b), respectively, but with a $\pi/2$ phase shift of the pulse phases marked with an asterisk. In practice, it can be advantageous to apply the two adjacent $\pi/2(x)$ pulses as one π pulse and omitting the adjacent $\pi/2(x)-\pi/2(-x)$ pulses.

initial π^I pulse in the first element of Fig. 1a. In the next interval, $\pi \leq \Delta\varphi \leq 2\pi$, $\Delta\varphi$ is compensated through effective J evolution during $t_1 - (2J)^{-1}$ and without interchanging the two magnetizations vectors at the beginning of the S^3 TPPI element (second element in Fig. 1a). The next interval, $2\pi \leq \Delta\varphi \leq 3\pi$, is similar to the first one but a $1/J$ evolution causes a sign change that is compensated by a $\pi/2$ phase shift of the S-spin π pulse (third element in Fig. 1a). The same phase shift is present between the second and fourth elements.

For very large molecules at very high fields the genuine TROSY approach (*1, 3*) is expected to offer the highest sensitivity for ^{1}H–^{15}N correlations because the contribution from the faster relaxing component becomes insignificant and does not compensate losses from application of the additional rotations in the S^3 TPPI element. However, for the currently available magnetic fields and medium-size proteins investigated by NMR, it can be worthwhile to retain the broader peak meaning that our new S^3 TPPI approach should be superior to TROSY. Neglecting pulse imperfections and relaxation losses during additional delays, the S^3 TPPI-based experiment is theoretically also superior to HSQC (*4, 5*) because in indirect dimensions it is more favorable sensitivity-wise to superimpose two components of different transverse relaxation rates than to have both effectively relax at the average rate as $\exp[-t/T_2(\alpha)] + \exp[-t/T_2(\beta)] \geq 2\exp[\{-t/T_2(\alpha) - t/T_2(\beta)\}/2]$.

An experimental comparison was carried out using the three pulse sequences outlined in Fig. 2. Note that S^3 TPPI can only be applied in indirect dimensions so that conventional decoupling is applied during acquisition of the free induction decay of the two-dimensional experiment. That eliminates the TROSY effect in the acquisition dimension and it could only be retained fully in three-dimensional applications. Conventional ^{13}C multipulse decoupling in the proton dimension is well suited to aromatic ^{1}H–^{13}C groups where the protons have small chemical shift anisotropy (*9*) and hence a vanishing TROSY effect.

The experiments were performed on a Bruker DRX 600-MHz spectrometer on a sample of ^{15}N-labeled neural cell adhesion molecule (NCAM) modules 1 and 2, a protein of about 20 kDa molecular weight (*10*). Fig. 3 shows a contour plot of the S^3 TPPI HSQC spectrum where a number of peaks have been framed. Sections through these peaks are also plotted in Fig. 3 along with sections through the corresponding peaks in spectra recorded under identical conditions with the TROSY and HSQC pulse sequences outlined in Figs. 2a and 2b, respectively. Compared to TROSY, sensitivity gains of about 20% or more are observed in the S^3 TPPI HSQC spectrum. The comparison with HSQC (*4, 5*) (displaced dashed lines in the sections in Fig. 3) is somewhat disappointing and overall in the spectrum it is clear that HSQC is the method of choice for this molecule at 600 MHz. We tentatively ascribe this result primarily to poor RF homogeneity of the proton coil—a spectrum recorded after a $5\pi/2$ pulse is accompanied by an intensity loss in excess of 20% in comparison to one recorded after a $\pi/2$ pulse. The loss caused by RF inhomogeneity and possibly transverse relaxation during the slightly longer pulse sequence is too large to be compensated by the more favorable

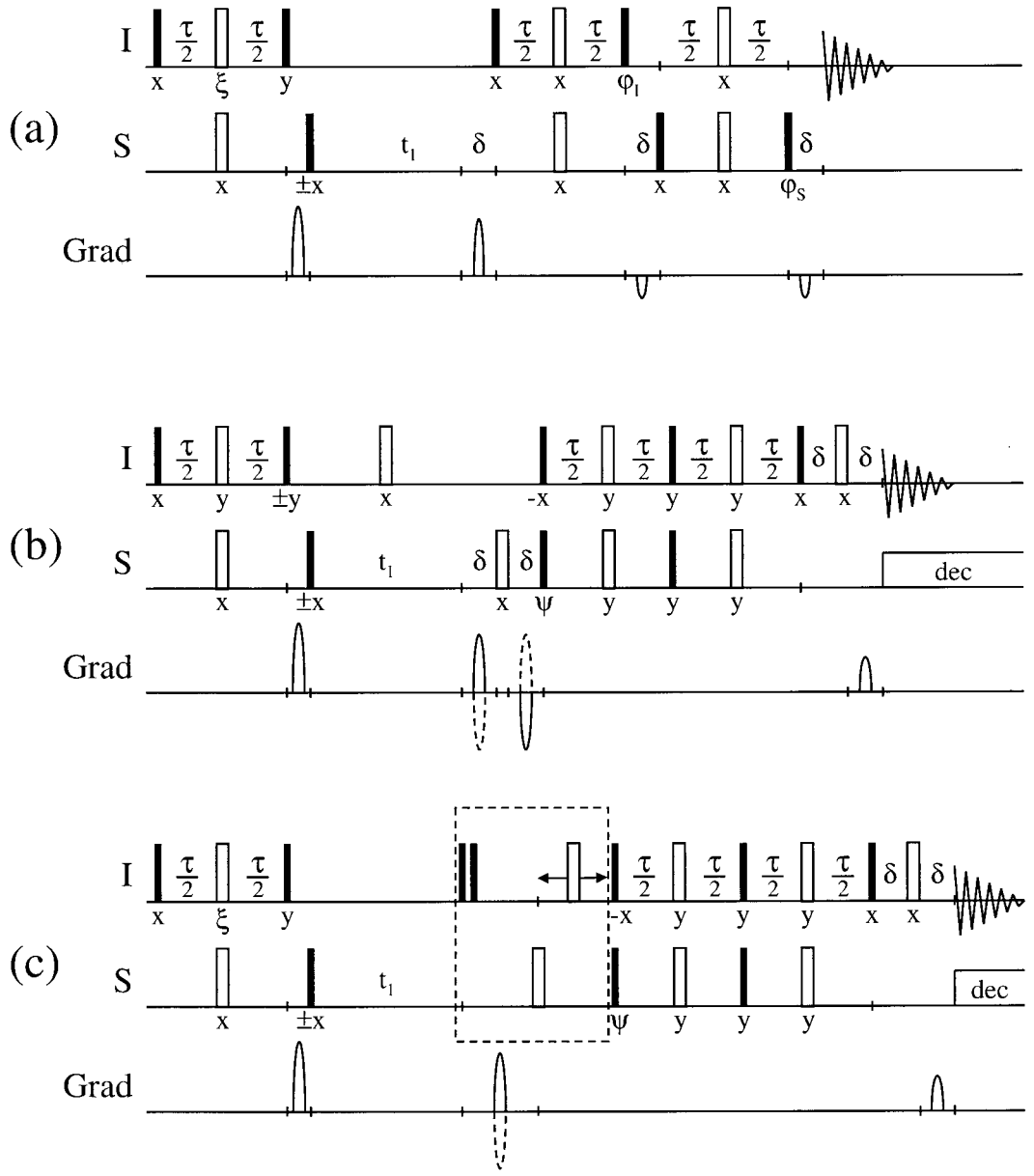

FIG. 2. Pulse sequences for two-dimensional ^{1}H–^{15}N chemical shift correlation: (a) TROSY or $(S^3CT)^2$ HSQC (*1, 3*), (b) HSQC (*4, 5*), and (c) S^3 TPPI HSQC where the dashed box represents the elements in Fig. 1a. Filled and open bars represent $\pi/2$ and π pulses, respectively. The first gradient in all experiments is uncorrelated with the other gradients accompanying echo or antiecho selection while $\tau = (2J_{IS})^{-1}$ and δ is a gradient delay. The phase ξ is x on our Bruker DRX 600 spectrometer while it is y on our Varian Unity Inova 750. Echo experiments: (a) $\varphi_I = \varphi_S = y$ (Bruker DRX 600) or $\varphi_I = \varphi_S = -y$ (Varian Unity Inova 750) combined with gradients of relative amplitudes -7, 3, 2; (b) $\psi = x$ and gradients 5, -5, 1; (c) $\psi = x$ and gradients 10, 1. Antiecho experiments: (a) $\varphi_I = \varphi_S = -y$ (Bruker DRX 600) or $\varphi_I = \varphi_S = y$ (Varian Unity Inova 750) combined with gradients of relative amplitudes -8, 2, 3; (b) $\psi = -x$ and gradients -5, 5, 1; (c) $\psi = -x$ and gradients -10, 1. An indication $\pm$ to a phase means that a two-step phase cycle accompanied by a π phase shift of the receiver reference phase can be employed. The TROSY version in Fig. 2a (*3*) has a $\sqrt{2}$ higher sensitivity than the original one (*6–8*). Pulse programs including the features of water flipback and Watergate for all three experiments are available from the authors upon request.

relaxation characteristics during the t_1 period. A higher static field (i.e., a larger TROSY effect) would be favorable for TROSY and a disadvantage for HSQC and to a smaller extent also for S^3 TPPI HSQC. In other words, we expect that there is a window of molecular size and field strength not dissimilar to typical current values where S^3 TPPI HSQC would offer the highest sensitivity. Any improvement in RF homogeneity will help in this direction. We should also note that S^3 TPPI HSQC is favored relative to HSQC by an increase in t_1^{max}.

In conclusion, we have introduced a novel pulse sequence

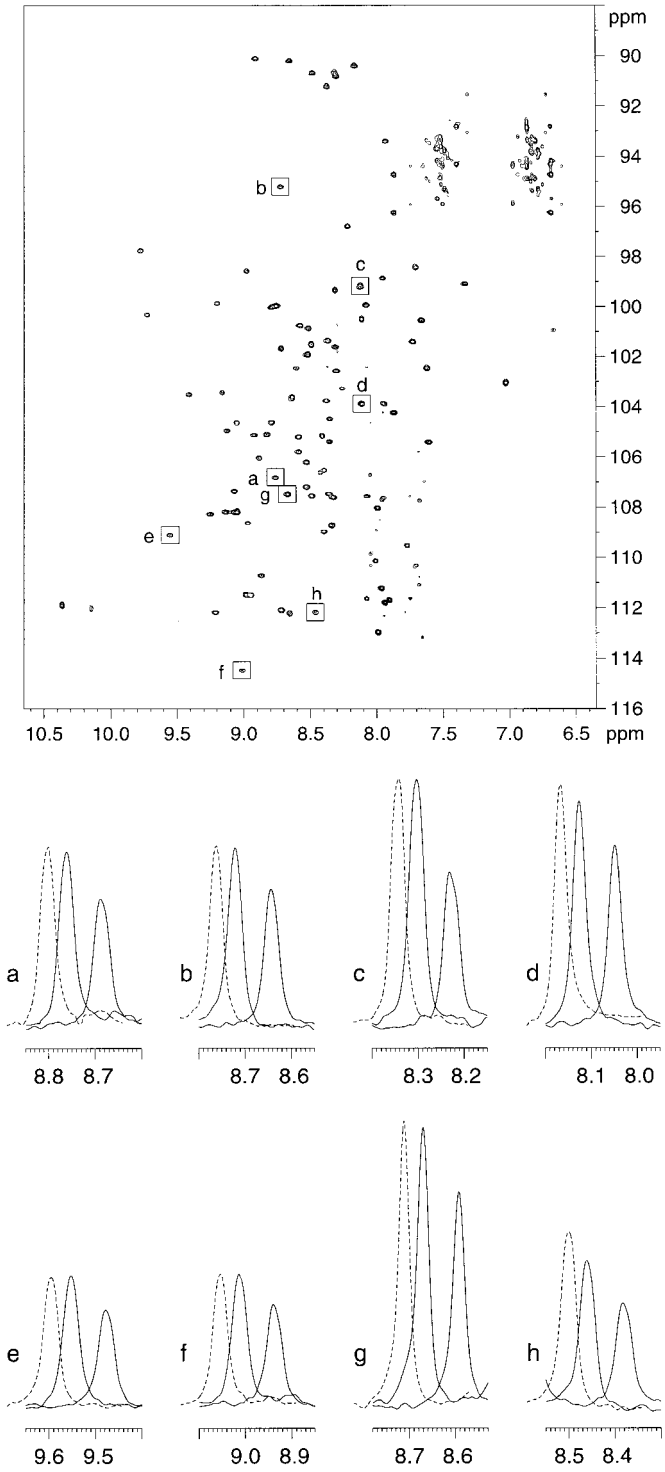

FIG. 3. (Top) 2D ^{15}N–^{1}H correlation spectrum of NCAM recorded with the S^3 TPPI HSQC pulse sequence in Fig. 2c on a Bruker DRX 600-MHz spectrometer. Parameters: $\delta = 1.5$ ms, $\epsilon = 0.163$ ms, and $\tau = 5.43$ ms; 16 scans and 2048 complex data points in F_2; 532 increments in t_1 corresponding to $t_1(\max) = 87$ ms; apodization by a cosine window in t_2 and an 18° shifted cosine window in t_1; zero-filling to $8192(F_2) \times 4096(F_1)$ complex points. 33 t_1 increments are taken per block of the sequences shown in Fig. 1a, so one $2/J$ period comprising all four blocks is completed after 132 increments. (Bottom) For the framed cross peaks, the sensitivity of the S^3 TPPI HSQC experiment (middle line in the sections) is compared to those of TROSY (right-most peak in the sections) and HSQC (displaced dashed lines), all recorded with identical experimental parameters.

element for heteronuclear decoupling in indirect dimensions of multidimensional NMR spectra. The advantage of this approach is that fast and slowly decaying coherences are not mixed, however, both are retained and superimposed. A critical aspect of S^3 TPPI is its sensitivity to variations in J coupling constants; if there is a wide range of J values it will not work properly over the entire range. This point has not been investigated in detail as yet, but the application presented demonstrates that the small variation in $^{1}J_{NH}$ in isotropic solutions of proteins does not seem to pose a problem. Thus S^3 TPPI can be combined with other protein backbone multidimensional NMR techniques involving correlation between ^{15}N and $^{1}H^{N}$.

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