

Spin-state-selective coherence transfer via intermediate states of two-spin coherence in IS spin systems: Application to E.COSY-type measurement of J coupling constants

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Summary

It is demonstrated that a new pulse sequence element, Spin-State-Selective Coherence Transfer (S³CT), via an intermediate state of heteronuclear IS zero- or double-quantum coherence can transfer the two single-quantum coherences on one of the spins exclusively to any one of the two single-quantum coherences on the other spin. This fact is used for editing into two subspectra that are most suitable for extraction of homo- or heteronuclear J coupling constants when S³CT is combined with homonuclear coherence transfer during a mixing period. Experimental confirmation is obtained using a ¹⁵N-labeled 58-residue protein, the C-terminal Kunitz domain from human type VI collagen. The J coupling constants determined include ³J_{H^N-H^α and ³J_{N-H^β related to the φ and χ¹ angles, respectively.}}

Among the techniques for measurement of J coupling constants, those employing the so-called E.COSY approach (Griesinger et al., 1985,1986,1987) have become very popular because J couplings otherwise buried in the line width or in a complicated multiplet structure can be made clearly visible in two-dimensional (2D) spectra or 2D sections of higher dimensional spectra. Especially when a heteronuclear spin such as ¹⁵N or ¹³C is involved, the large heteronuclear one-bond coupling constants to protons can be exploited to spread out cross peak multiplets in order to make accessible structurally important J coupling constants (Montelione et al., 1989; Sørensen, 1990; Griesinger et al., 1994).

Recently, a further possibility for simplification of E.COSY-type cross peak multiplet structures was introduced (Meissner et al., 1997a,b), namely by imposing a restriction on the preparation sequence in such a way that editing into two separate subspectra corresponding to the spin of the attached nucleus being in the α or β state can be done, i.e. Spin-State-Selective Excitation (S³E). Since it is these two parts of the 2D multiplet that need to be compared in order to extract the interesting coupling constants, decoupling of the heteronucleus during the

evolution time is feasible. This feature greatly eases the measurement of these coupling constants, as the sections to compare are at the same position in the pertinent dimension of the edited subspectra, i.e. most suitable for automatic extraction by computer.

This communication describes an alternative to S³E that achieves the same end of editing into two subspectra according to the attached spin being in the α or β state; however, in an entirely different way. S³E, shown in Fig. 1a, is part of the preparation sequence, whereas the new pulse sequence element shown in Fig. 1b, S³CT (Spin-State-Selective Coherence Transfer), achieves the editing by a restricted coherence transfer at an appropriate place in a multidimensional pulse sequence. S³CT and earlier work (Fäcke and Berger, 1996; Ross et al., 1996) easily generalized to produce the same result can all be reduced to one basic idea that is well illustrated by a simple rotation represented in an energy level diagram of a two-spin system.

Along the lines of our systematic strategy for derivation of pulse sequences (Sørensen, 1989; Zhang et al., 1994; Nielsen et al., 1996; Meissner et al., 1997a) we start from the idea represented in the energy level diagram and

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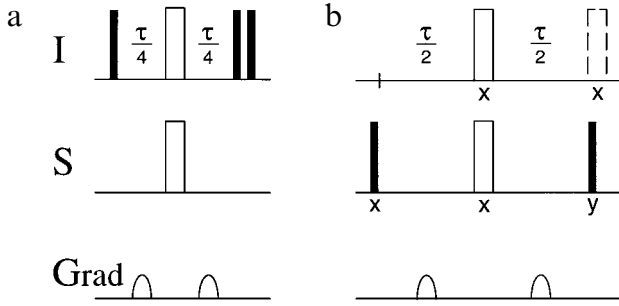


Fig. 1. (a) S^3E and (b) S^3CT pulse sequence elements for editing of spectra according to the spin state of the S spin. Filled and open bars represent $\pi/2$ and π pulses, respectively. The phases of the S^3E pulses are given in Meissner et al. (1997a,b), while the phases of the S^3CT pulses are indicated below the pulses. The dashed π pulse on the I spin is optional (see text). The delay τ is $(2J)^{-1}$.

derive the sequence from there. In Fig. 2 a selective π rotation on the S spin is indicated; it is the transition corresponding to the I spin being in the α spin state, but one could of course just as well have chosen the other one. Clearly this π pulse transfers (a) double- (2Q) and (b) zero-quantum (ZQ) coherence into single-quantum coherence (IQC) on the I spin. In operator terms we can write explicitly:

$$I^-S^- = |\beta\beta\rangle\langle\alpha\alpha| \xrightarrow{\pi I^{\alpha}S_y} |\beta\beta\rangle\langle\alpha\beta| = I^-S^{\beta} \quad (1a)$$

$$I^-S^+ = |\beta\alpha\rangle\langle\alpha\beta| \xrightarrow{\pi I^{\alpha}S_y} -|\beta\alpha\rangle\langle\alpha\alpha| = -I^-S^{\alpha} \quad (1b)$$

$$I^+S^- = |\alpha\beta\rangle\langle\beta\alpha| \xrightarrow{\pi I^{\alpha}S_y} -|\alpha\alpha\rangle\langle\beta\alpha| = -I^+S^{\alpha} \quad (1c)$$

$$I^+S^+ = |\alpha\alpha\rangle\langle\beta\beta| \xrightarrow{\pi I^{\alpha}S_y} |\alpha\beta\rangle\langle\beta\beta| = I^+S^{\beta} \quad (1d)$$

where the transformations in Eqs. 1a and b correspond to Figs. 2a and b, respectively.

It follows from Eq. 1 that each of the four possible two-spin coherences in a two-spin system, which can be separated by appropriate phase cycles, gets transferred to a different IQC on the I spin. Hence there is the basis for S^3CT editing.

It is of course possible to have the heteronuclear 2Q and ZQ coherences evolve during an evolution period of a 2D experiment, but it would be inconvenient to extract the interesting J coupling constants because peaks to compare would occur at different ω_1 frequencies with the 2Q and ZQ spectra being the β and α subspectra, respectively. It is more convenient to have either the I- or S-spin chemical shifts in the ω_1 dimension and then only excite the two-spin coherences as an intermediate state prior to the transformation in Eq. 1 or Fig. 2.

It has already been demonstrated that $\pi I^{\alpha}S_y$ can be realized by a selective pulse (Fäcke and Berger, 1996), but for general applications it is more attractive to have a non-selective pulse sequence (Ross et al., 1996). The conversion of $\pi I^{\alpha}S_y$ into a non-selective sequence is straightforward and analogous to earlier examples (Sørensen,

1989; Zhang et al., 1994; Nielsen et al., 1996; Meissner et al., 1997a):

$$\begin{aligned} e^{i\pi I^{\alpha}S_y} &= e^{i(\pi/2)(2I_zS_y+S_y)} \\ &= e^{i(\pi/2)S_x} e^{i(\pi/2)2I_zS_z} e^{-i(\pi/2)S_x} e^{i(\pi/2)S_y} \\ &= e^{i(\pi/2)S_x} e^{i(\pi/2)2I_zS_z} e^{i\pi(I_x+S_x)} e^{i\pi I_x} e^{i(\pi/2)S_y} e^{-i(\pi/2)S_z} \\ &= e^{i(\pi/2)S_x} e^{iH_0/4J} e^{i\pi(I_x+S_x)} e^{iH_0/4J} e^{i(\pi/2)S_y} e^{i\pi I_x} e^{-i(\pi/2)S_z} \end{aligned} \quad (2)$$

where standard operator and propagator transformation formulas (e.g. Sørensen, 1989) have been used and H_0 represents the unperturbed Hamiltonian. For the applications in this paper the final z rotation can be omitted, because it would commute with the relevant part of the density operator at that point. Also the πI_x pulse at the end can be omitted, as it only interchanges the coherence order of the I spin ($I^+ \leftrightarrow I^-$), i.e. permutes the coherences on the right-hand side of Eq. 1. The pulse sequence derived from Eq. 2 is shown in Fig. 1b.

One can immediately envisage eight different 2D pulse sequences employing S^3CT , since there are three binary options: measure homo- or heteronuclear long-range coupling constants, let I- or S-spin chemical shifts evolve during the evolution period, and finally should 1H isotropic TOCSY or NOESY (or relayed COSY) mixing be employed.

Figure 3 shows the eight sequences which theoretically (i.e. neglecting relaxation and pulse imperfections) exhibit the same sensitivity as their S^3E -based counterparts (Meissner et al., 1997a,b). All start with the transformation of I_z magnetization to longitudinal two-spin order $2I_zS_z$. Undesired magnetization from the solvent and other

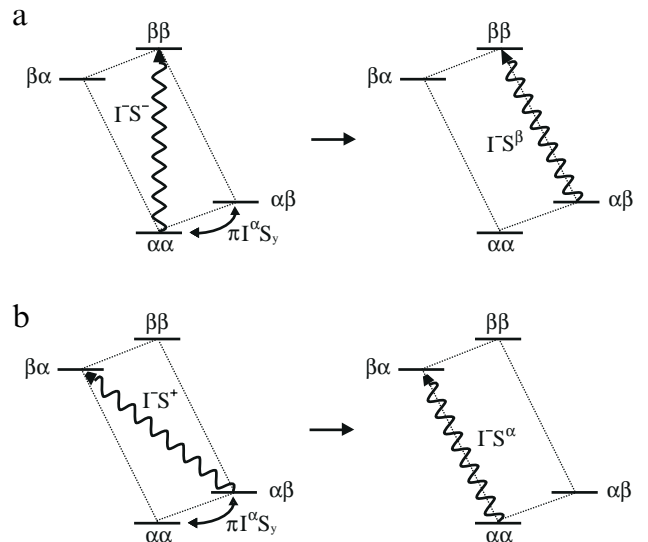


Fig. 2. Two-spin-1/2 energy level diagram showing the effects on the 2QC and ZQC of the rotation indicated by the curved double arrows. (a) Conversion of 2QC (I^-S^-) to one of the two I-spin IQCs (I^-S^{β}) by a selective single-quantum S-spin π pulse. (b) Conversion of ZQC (I^-S^+) to the other I-spin IQC (I^-S^{α}) by the same selective single-quantum π pulse.

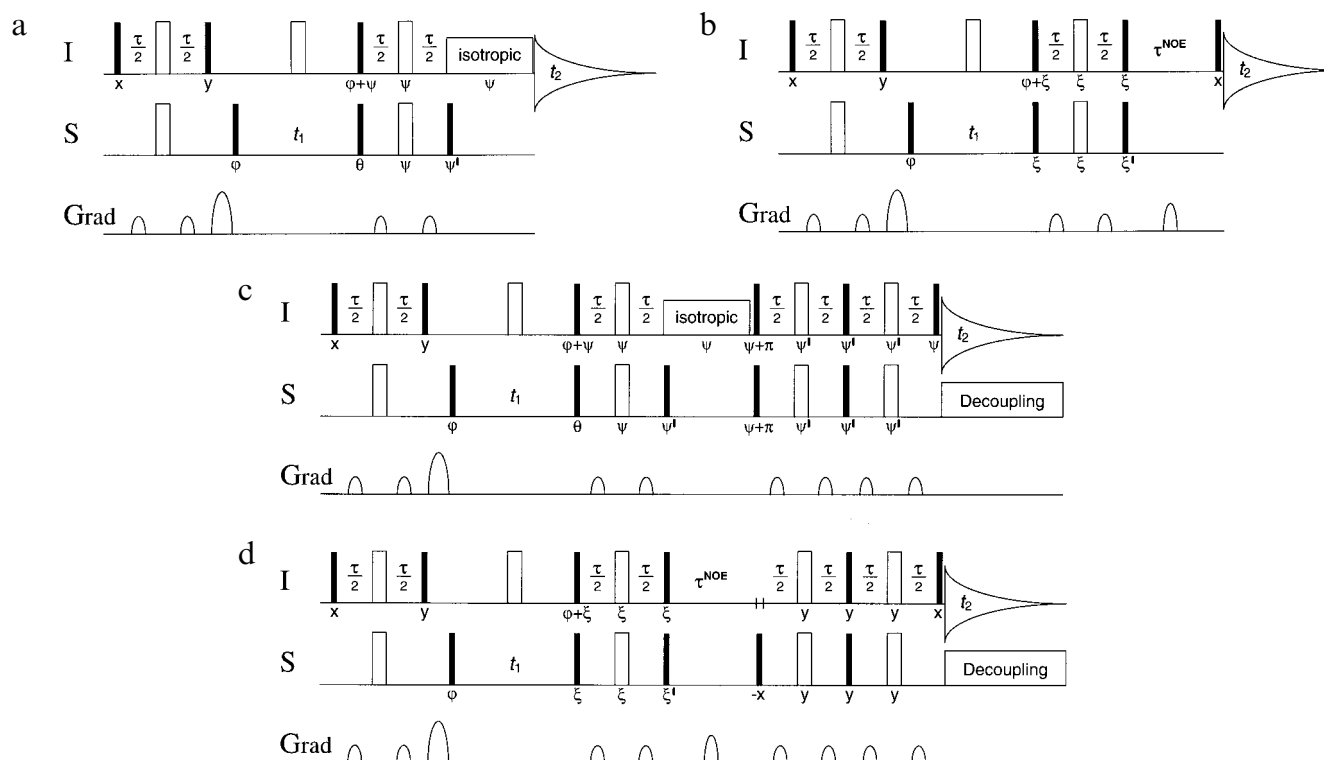


Fig. 3. S^3CT E.COSY-type pulse sequences for determination of homo- or heteronuclear J coupling constants. Filled and open bars represent $\pi/2$ and π pulses, respectively. The phases are indicated below the pulses: $\psi' = \psi + \pi/2$ and $\xi' = \xi + \pi/2$. Sequences a–d have S-spin chemical shift evolution in t_1 , while sequences e–h have I-spin chemical shift evolution in t_1 . Heteronuclear coupling constants can be measured with sequences a, b, e, and f while homonuclear coupling constants can be measured with sequences c, d, g, and h. The frame designated *isotropic* corresponds to an isotropic TOCSY mixing sequence, e.g. DIPSI-2 (Shaka et al., 1988). For NOESY we employ the phase cycling of Derome and Williamson (1990). In all eight sequences, the phase ϕ is to be cycled to create two data sets which contain both 2QC and ZQC: ($\phi = x, -x$ with receiver x, x) and ($\phi = y, -y$ with receiver x, x). Addition and subtraction will then yield the edited ZQ and 2Q subspectra, respectively. For the S^3CT TOCSY spectra, pure absorption is obtained by combining the echo and antiecho spectra (Cavanagh and Rance, 1990; Kay et al., 1992; Sattler et al., 1996). Thus, the phase ψ is cycled in four steps for selection of echo or antiecho. In the heteronuclear S^3CT TOCSY sequences a and c, ψ is cycled ($x, -x$ with receiver x, x) and ($y, -y$ with receiver x, x). Addition or subtraction of these two data sets will yield the antiecho or echo spectra, respectively. Combined with the cycle of ϕ , four subspectra are obtained: A ($\phi = x, -x, x, -x$; $\psi = x, x, -x, -x$ with receiver x, x, x, x), B ($\phi = y, -y, y, -y$; $\psi = x, x, -x, -x$ with receiver x, x, x, x), C ($\phi = x, -x, x, -x$; $\psi = y, y, -y, -y$ with receiver x, x, x, x), and D ($\phi = y, -y, y, -y$; $\psi = y, y, -y, -y$ with receiver x, x, x, x), which all must be recorded for $\theta = \psi$ and for $\theta = \psi + \pi$. The combinations $\{(A-B) - (C-D)\}_{\theta=\psi}$ and $\{(A+B) + (C+D)\}_{\theta=\psi+\pi}$ yield the echo and the antiecho parts, respectively, for one half of the multiplets. For the other half, echo and antiecho parts are obtained as $\{(A-B) - (C-D)\}_{\theta=\psi+\pi}$ and $\{(A+B) + (C+D)\}_{\theta=\psi}$, respectively. In the homonuclear correlated S^3CT TOCSY sequences e and g, echo and antiecho selection are obtained for $\theta = \psi + \pi$ and $\theta = \psi$ respectively, and with appropriate echo or antiecho phase cycles for ψ . The two edited subspectra are then obtained as the 2Q and ZQ parts, respectively. For all S^3CT NOESY sequences, ξ is cycled in two steps to select $\Delta p = \pm 1$ (i.e. $x, -x$ with receiver $x, -x$) and the States-TPPI mode (Marion et al., 1989) is employed.

protons not bound to an S spin will at this point exist as transverse magnetization and can therefore be attenuated by a z-gradient pulse. The $\pi/2$ pulse of phase ϕ prior to the t_1 evolution period creates antiphase S magnetization (sequences a–d) or antiphase I magnetization (sequences e–h). Then a $\pi/2$ pulse with a phase containing ϕ immediately after the t_1 evolution period creates two-spin coherence, i.e. 2QC and ZQC. Discrimination between 2QC and ZQC is achieved by the phase cycle for ϕ . When 2QC has been selected, the S^3CT element will transfer these to I^-S^B and I^+S^B . During the mixing period, transfer of I-spin magnetization to a third spin T of the same isotope occurs. In the case of isotropic TOCSY mixing we therefore have T^-S^B and T^+S^B after the mixing period, of which only the former is detected with the normal scheme of quadra-

ture detection (Bain, 1984; Bodenhausen et al., 1984). If ZQC were selected instead of 2QC we would end up with T^-S^A and T^+S^A .

The IS ZQ/2Q π rotation (Cavanagh et al., 1991; Schulte-Herbrüggen et al., 1991; Kay et al., 1992; Willker and Leibfritz, 1992) at the end of sequences c, d, g, and h in Fig. 3 transfers I_1S^B (I_1S^A) to I^B1_S (I^A1_S) and, accordingly, T^-S^B (T^-S^A) to T^I^B (T^I^A). Applying a ZQ/2Q π rotation therefore allows the homonuclear $^nJ_{TI}$ coupling constant to be measured (Willker and Leibfritz, 1992).

Figure 4 shows part of the amide region of a 1H spectrum of the ^{15}N -labeled 58-residue C-terminal Kunitz domain from human type VI collagen ($\alpha 3(VI)$) (Sørensen et al., 1996, 1997) and the corresponding parts of 1D 2Q- S^3CT and ZQ- S^3CT subspectra recorded with the hetero-

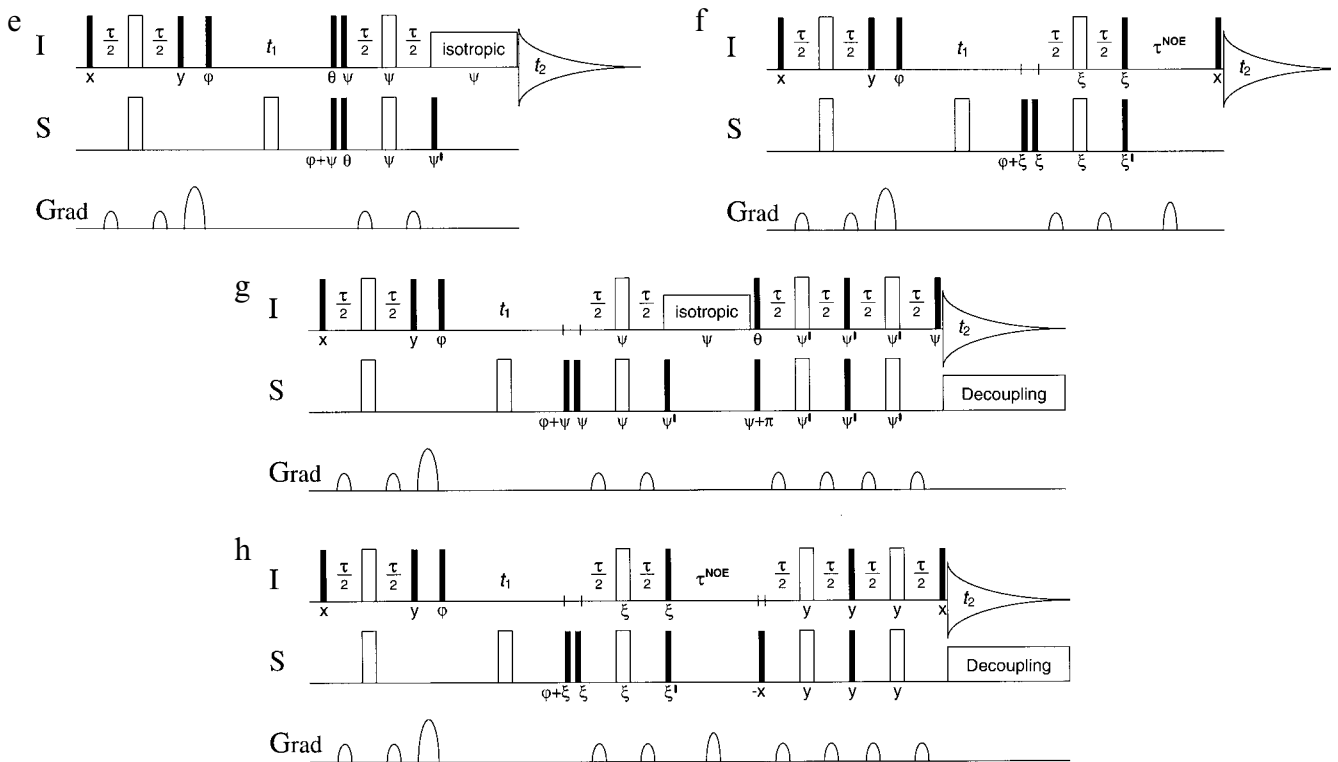


Fig. 3. (continued).

nuclear S^3CT J_{XH} NOESY sequence (Fig. 3b) employing $t_1 = \tau^{NOE} = 0$. It is immediately apparent that the desired editing according to the spin state of the nitrogen has been achieved with the S^3CT element.

Figure 5 shows excerpts from S^3CT spectra of ^{15}N - $\alpha 3(VI)$ recorded with the sequences in Figs. 3a and g and ^{15}N as the S spin. The homo- or heteronuclear coupling constants are easily extracted from the relative peak displacement in the two subspectra. The three-bond H^N-H^α and $N-H^\beta$ J coupling constants, which provide information about the ϕ and χ^1 angles, respectively, can be obtained from spectra employing either TOCSY or NOESY transfer. In contrast, the $H_{i-1}^\alpha-N_i$ J coupling constant, which is related to the ψ angle, requires experiments employing NOESY transfer.

The S^3CT pulse sequences with heteronuclear chemical shift evolution during t_1 are shorter than the corresponding S^3E pulse sequences, as no ZQ/2Q π rotation is needed prior to homonuclear mixing in S^3CT . The S^3E sequences are, on the other hand, shorter than their S^3CT counterparts if 1H chemical shift evolution is desirable in t_1 . The two experiments also differ in their sensitivity to J mismatch. Figure 6 shows the intensities of the desired and undesired doublet components as a function of $\Delta J/J_0$ ($\tau = (2J_0)^{-1}$) in subspectra of both S^3CT and S^3E . It is evident that cross talk is far less dependent on proper tuning of τ in S^3CT . However, it is also clear that the improved suppression of cross talk is obtained at the expense of the intensity of the desired doublet component.

Another factor that contributes to cross talk in S^3CT (and equally in S^3E) is relaxation of the S spin during the mixing time.

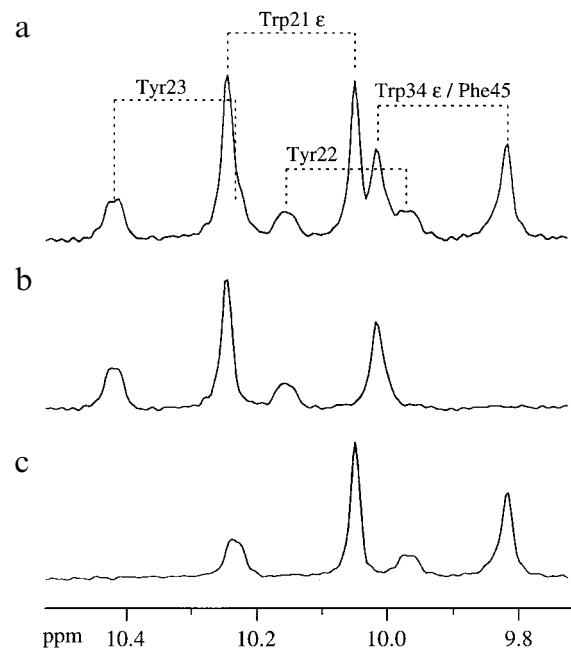


Fig. 4. (a) Part of the amide region of the 1H spectrum of the ^{15}N -labeled C-terminal Kunitz domain from human type VI collagen ($\alpha 3(VI)$). (b) and (c) 1D edited S^3CT subspectra recorded with the heteronuclear S^3CT J_{XH} NOESY sequence (Fig. 3b) employing $t_1 = \tau^{NOE} = 0$.

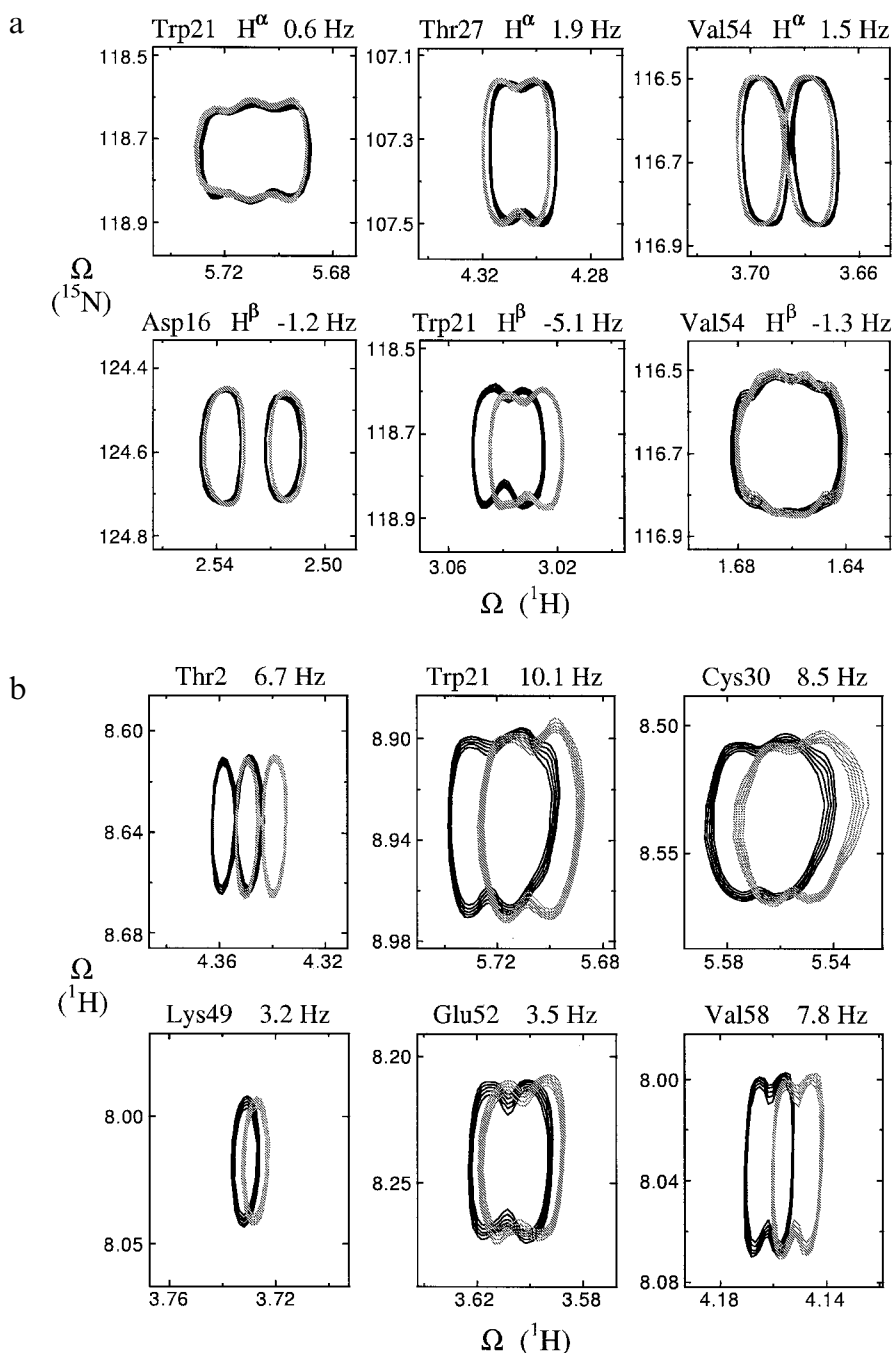


Fig. 5. Excerpts from hetero- and homonuclear correlated S^3CT TOCSY spectra of ^{15}N - $\alpha 3(\text{VI})$ (90%/10% $\text{H}_2\text{O}/\text{D}_2\text{O}$) recorded with (a) the sequence in Fig. 3a and (b) the sequence in Fig. 3g, respectively, on a Varian Unity Inova 750 MHz spectrometer. The edited subspectra (black and gray) have been overlaid using the software package Pronto (Kjær et al., 1994). The J coupling constants are heteronuclear two- and three-bond ^{15}N - ^1H coupling constants in (a) and homonuclear H^{N} - H^{α} coupling constants in (b). Parameters are (a) TOCSY mixing time 40 ms; solvent presaturation 1.5 s; $t_1(\text{max})=51.2$ ms; 64 scans and 128 increments; $\tau=5.56$ ms; Gaussian ($l_b=-7.5$ Hz, $g_b=0.2$ Hz) and sine-bell (shifted $\pi/3$) window functions in t_2 and t_1 , respectively. Spectral widths of 2500 Hz and 10000 Hz were covered by a data matrix of 256×8192 points zero-filled to 512×8192 points prior to Fourier transformation. (b) TOCSY mixing time 40 ms, solvent presaturation 1.5 s; $t_1(\text{max})=25.6$ ms; 32 scans and 256 increments; $\tau=5.56$ ms; WALTZ-16 decoupling during acquisition; Gaussian ($l_b=-7.5$ Hz, $g_b=0.2$ Hz) and sine-bell (shifted $\pi/3$) window functions in t_2 and t_1 , respectively. Spectral widths of 10000 Hz in both dimensions were covered by a data matrix of 512×8192 points zero-filled to 1024×8192 points prior to Fourier transformation. The coupling constants were estimated from 1D sections with a precision of about 0.2 Hz.

In conclusion, we have described a new pulse sequence element, Spin-State-Selective Coherence Transfer (S^3CT), and compared it to Spin-State-Selective Excitation (S^3E).

It has been shown how application of the S^3CT element at an appropriate place in a pulse sequence enables editing into two subspectra according to the spin state of the

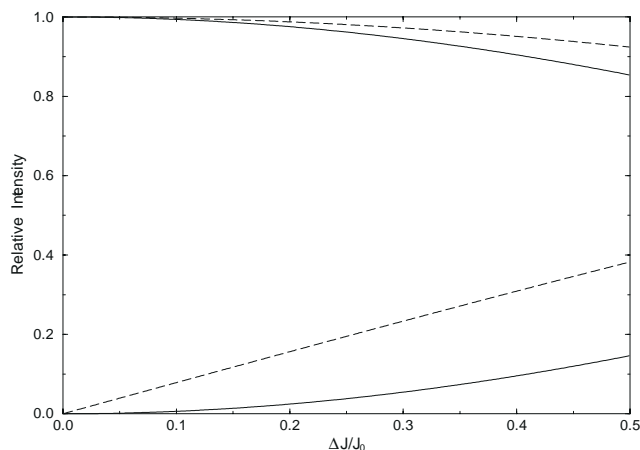


Fig. 6. Intensity curves for desired (upper curves) and undesired (lower curves) doublet components in edited S³CT (solid lines) and S³E (dashed lines) spectra as a function of $\Delta J/J_0$, where $J = J_0 + \Delta J$ is the coupling constant of the actual doublet and J_0 is the one used in setting the τ delay. The curves represent $0.5|(1 \pm \sin(\pi J/2J_0))|$ (S³CT) and $|\cos(\pi J/4J_0) \pm \sin(\pi J/4J_0)|/\sqrt{2}$ (S³E).

attached spin, thereby facilitating easy E.COSY-type measurement of both homo- and heteronuclear coupling constants. The S³CT element has the advantage of being very tolerant to J mismatch and is therefore especially suited for systems with a wide range of one-bond coupling constants.

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