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Integration of spin-state-selective excitation into 2D NMR correlation experiments with heteronuclear ZQ/2Q π rotations for $^1J_{XH}$-resolved E.COSY-type measurement of heteronuclear coupling constants in proteins

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Summary

Spin-State-Selective Excitation (S^3E), which for example selectively excites amide proton resonances corresponding to exclusively either the α or the β spin state of the covalently bound ^{15}N atom is employed for E.COSY-type extraction of heteronuclear J coupling constants. Instead of having one spectrum with two peaks (corresponding to the α or β spin state of ^{15}N), S^3E generates two spectra, each with only one peak for each ^{15}N nucleus. These two spectra are generated from the same data set, so that there is no reduction in sensitivity compared to conventional $^1J_{NH}$-resolved methods. Another interesting feature in comparison with conventional methods is that $^1J_{NH}$ can be suppressed during the evolution period, meaning that no heteronuclear multiplet structure is visible in the ω_1 frequency dimension. The S^3E pulse sequence element is combined with NOESY for measurement of $^3J_{N\text{-}H\beta}$ and $J_{N\text{-}H\alpha}$ coupling constants in either a hetero- or a homonuclear correlated version. Experimental confirmation is obtained using the protein RAP 17–97 (N-terminal domain of α_2-macroglobulin Receptor Associated Protein).

E.COSY-type (Griesinger et al., 1985,1986,1987) multidimensional NMR techniques combined with ^{13}C and ^{15}N isotopic labeling have made it possible to measure many valuable long-range coupling constants in proteins (Montelione et al., 1989; Griesinger et al., 1994). The key feature of these techniques is that a mixing sequence is very restrictive in terms of the coherence transfers allowed; typically only correlations between connected transitions occur.

An additional possibility for simplification of cross peak multiplets is by putting restrictions on the preparation sequence, as illustrated for a two-dimensional (2D) experiment in Fig. 1a, so that only part of the resonances are excited. Such a pulse sequence element, S^3E (Spin-State-Selective Excitation), is outlined in Fig. 1b and explained in more detail below. In another publication, Meissner et al. (1997) combined S^3E with 2D correlation sequences like NOESY or TOCSY for measurement of *homonuclear* coupling constants in an E.COSY-type way. Two pulse sequences were presented that led to 2D spectra with ^1H-^1H homonuclear and $^{13}C/^{15}N$-^1H heteronuclear correlations, respectively.

In this communication we incorporate the S^3E technology into techniques for measurement of *heteronuclear* coupling constants from homo- or heteronuclear correlated 2D spectra. It extends earlier work by Montelione et al. (1989), who showed that E.COSY-type patterns suitable for measurement of heteronuclear long-range coupling constants between ^{15}N and ^1H nuclei occur automatically in standard NOESY, TOCSY or relayed COSY spectra of a ^{15}N-enriched protein sample. The new techniques are demonstrated on a ^{15}N-enriched sample of the protein RAP 17–97 (N-terminal domain of α_2-macroglobulin Receptor Associated Protein; Nielsen, 1996; P.R. Nielsen, L. Ellgaard, M. Etzerodt, H.C. Thøgersen and F.M. Poulsen, personal communication, 1997).

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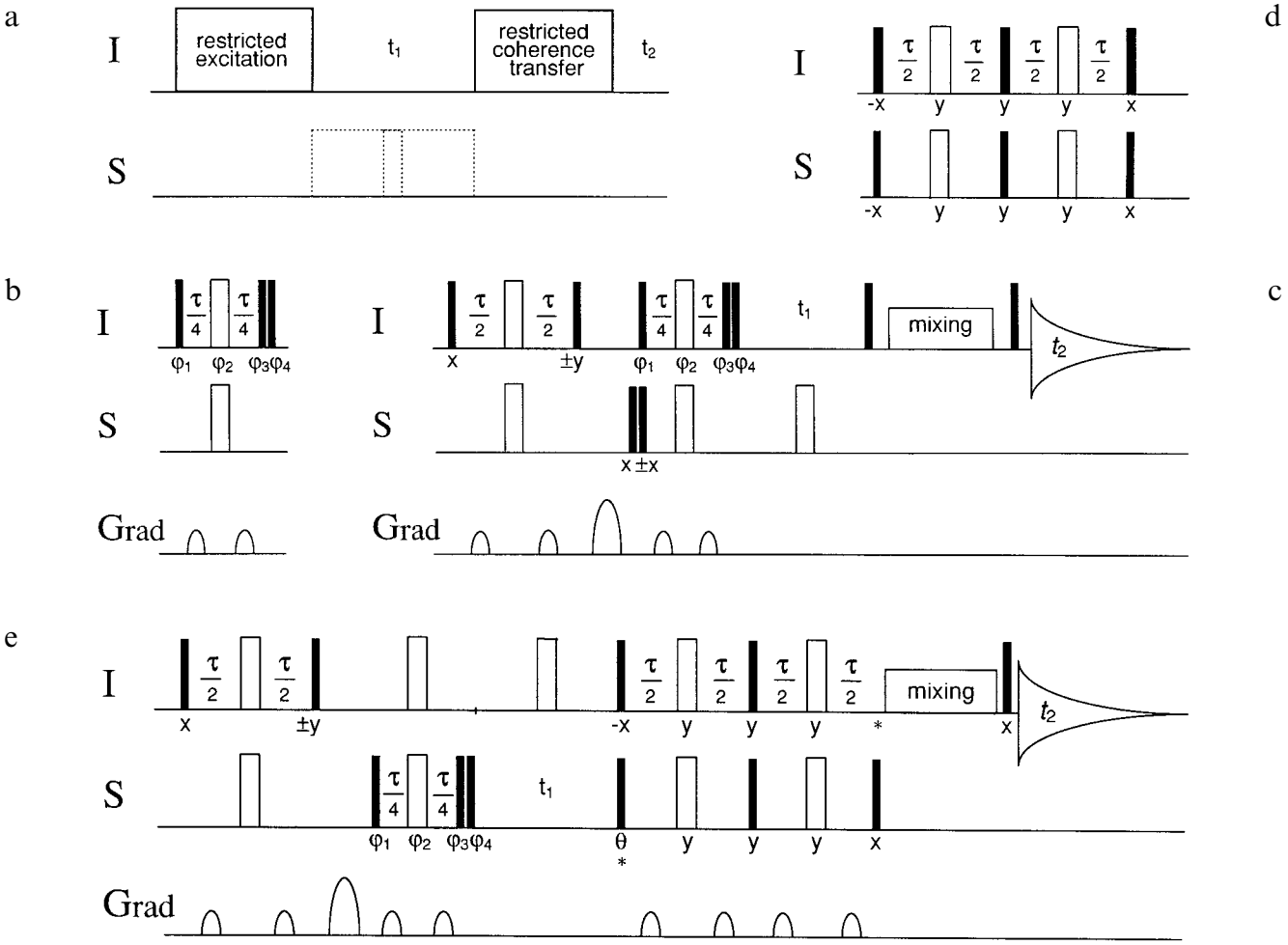

Fig. 1. (a) Schematic 2D pulse sequence combining restrictions in the preparation and mixing sequences. (b) S^3E pulse sequence element for editing of S^α and S^β resonances in I-spin spectra. With phase vectors (φ_1, φ_2, φ_3, φ_4) two data sets are combined initially, i.e., A: $\{(\pi/4,0,0,0)-(\pi/4,0,\pi/2,\pi/2)\}$, B: $\{(\pi/4,0,0,\pi)-(5\pi/4,0,\pi/2,3\pi/2)\}$ and then A + B and A − B yield the edited subspectra with a $\pi/2$ relative phase displacement. (c) Homonuclear S^3E J_{IS} NOESY/TOCSY pulse sequence. The first four pulses and the gradients ensure efficient suppression of magnetization of protons not attached to S spins prior to S^3E. (d) Heteronuclear ZQ or 2Q π rotation element. (e) Heteronuclear S^3E J_{IS} NOESY/TOCSY pulse sequence. Filled and open bars indicate $\pi/2$ and π pulses, respectively, and phases are indicated below the pulses. The delay τ is $(2\,{}^1J_{IS})^{-1}$. Phases ±x and ±y indicate independent two-step phase cycles with opposite receiver phase, while $\theta = x$ and $\theta = -x$ is used for echo and antiecho, respectively. In the NOESY variant in (e) the S pulse marked with * is omitted and an I-spin $(\pi/2)_x$ pulse is added at the position of the * on that channel.

In ^{13}C- and ^{15}N-labeled proteins, E.COSY-type cross peak multiplets normally consist of two clusters of resonances separated by a 2D displacement vector $\mathbf{J} = ({}^1J; {}^nJ)$ where the long-range coupling constant to be measured can be homo- or heteronuclear. The measurement is done by comparing ω_2 1D sections separated by ^1J in ω_1, but even when all chemical shifts are known in advance it is still to be determined exactly which traces to compare and also additional overlap may have been introduced relative to the corresponding spectrum without ^1J visible in the multiplets. Inclusion of S^3E into the preparation sequence circumvents these two problems, because the two clusters of resonances are edited into two subspectra with one cluster in each spectrum and because ^1J can be suppressed by decoupling during the evolution period, so that the two clusters both appear centered at the chemical shift frequency associated with the evolution period. Schematic spectra illustrating these points are shown in Fig. 2. Note that, ideally, these spectra with or without S^3E all exhibit the same signal-to-noise ratio, as the edited S^3E spectra are constructed from the same data set.

It is an interesting feature of S^3E that it can be understood entirely in terms of a vector model. Immediately before the second $\pi/2$ pulse, the two I-spin magnetization vectors are aligned along the x- and y-axis, respectively, of the rotating frame, as illustrated in Fig. 3. At this point the S^3E phase cycles amount to application of effectively a zero degree or a π_x or π_y pulse. Since a π pulse inverts the phase of only one of the two magnetization vectors, appropriate linear combinations will edit the S^α and S^β subspectra. Likewise, S^3E could also be employed to edit the I^α and I^β S-spin subspectra.

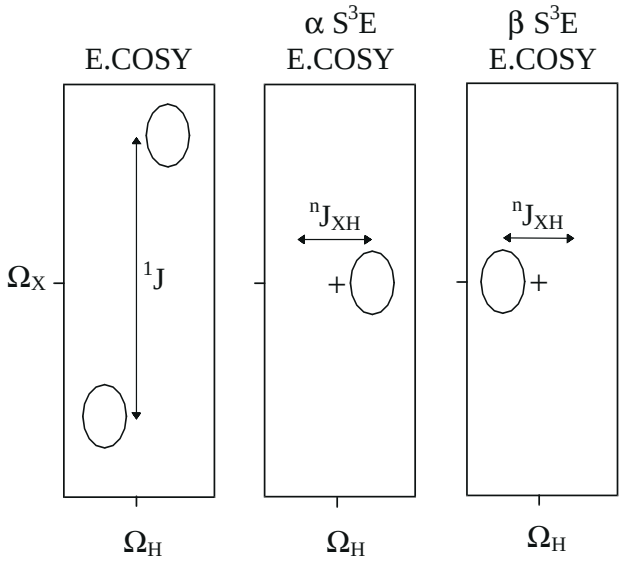

Fig. 2. Schematic S^3E E.COSY-type spectra illustrating the points discussed in the text. X can be a proton or a heteronucleus, while ^1J is always heteronuclear for the application described here.

In a protein enriched with ^{15}N, where S^3E editing is of the ^1H doublets with respect to ^{15}N being in the α or the β spin state, the edited spectra resulting from application of the homonuclear correlated pulse sequence in Fig. 1c will show H^N-H^β and H^N-H^α cross peaks with displacement vectors $\mathbf{J}^N = (^1J(N\text{-}H^N); {}^3J(N\text{-}H^\beta))$ and $\mathbf{J}^N = (^1J(N\text{-}H^N); {}^nJ(N\text{-}H^\alpha))$ (n = 2, 3), respectively. As indicated above, $^1J(N\text{-}H^N)$ can be suppressed by the π^S pulse in t_1 so that only the ω_2 coordinate appears in the cross peaks. Excerpts from a spectrum of ^{15}N-RAP 17–97 recorded with this pulse sequence are shown in Fig. 4.

There are a number of reasons why it would be desirable to have a heteronuclear 2D correlation spectrum from which to extract the heteronuclear long-range coupling constants: the usually larger chemical shift dispersion compared to protons, the absence of diagonal peaks, and the possibility to employ heteronuclear coherence transfer echoes in combination with pulsed field gradients for artifact suppression. It is straightforward to modify the pulse sequence in Fig. 1c to start with an INEPT transfer from protons to the heteronuclei for evolution during t_1 and then transfer back to protons afterwards. However, in order to measure $^nJ_{IS}$ coupling constants, it is necessary that the individual coherences having evolved during t_1 be associated with one and only one spin state of S and that this spin state not be perturbed by the mixing process. The solution to this problem is the pulse sequence element outlined in Fig. 1d, which was first applied to a problem of the type in question by Willker and Leibfritz (1992). It represents a heteronuclear zero- or double-quantum (ZQ/2Q) π rotation; whether it is ZQ or 2Q depends on the exact phase settings of the π/2 pulses and on the relative sign of the two gyromagnetic ratios. For the purpose of the discussion we consider this rotation as $\pi(I_xS_x + I_yS_y)$, which is a ZQ (2Q) π rotation for IS spin systems of like (opposite) gyromagnetic ratios, i.e.

$$I^\alpha S^\beta \xleftrightarrow{\pi(I_xS_x + I_yS_y)} I^\beta S^\alpha \qquad (1)$$

In pulsed field gradient NMR, the effect of this element is better known in the form

$$I^{\alpha/\beta} S^- \xleftrightarrow{\pi(I_xS_x + I_yS_y)} \mp i I^- S^{\alpha/\beta} \qquad (2)$$

called planar mixing (Schulte-Herbrüggen et al., 1991) or sensitivity enhancement (Cavanagh et al., 1991; Kay et al., 1992; Madsen et al., 1992,1993; Schleucher et al., 1994). Usually the corresponding echo transformation is implemented by insertion of a π pulse or an equivalent π phase shift of a π/2 pulse (Cavanagh et al., 1991; Schulte-

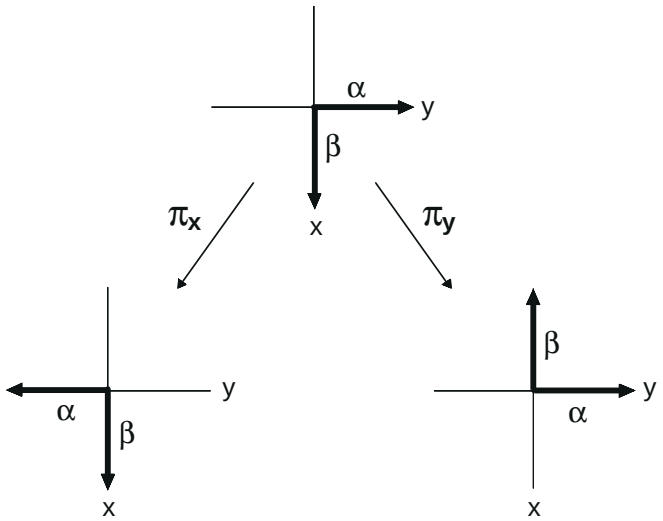

Fig. 3. Vector model illustration of the editing step in S^3E. The two vectors in the I- or S-spin rotating frame represent the coupled spin being in the α or β state, respectively. The editing is based on the fact that a π pulse on the active spin inverts only one of the two magnetization vectors.

TABLE 1
OVERVIEW OF THE RELEVANT PLACEMENTS OF HETERONUCLEAR ZQ/2Q π ROTATIONS AROUND HOMONUCLEAR MIXING ELEMENTS AND THE IMPLICATIONS IN TERMS OF TYPE OF CORRELATION (HOMO- OR HETERONUCLEAR) AND LONG-RANGE COUPLING CONSTANTS THAT CAN BE MEASURED[a]

ZQ/2Q π rotation	None	Before mixing	After mixing	Before and after mixing
I-I correlation	$^{n}J_{IS}$		$^{n}J_{II}$	
S-I correlation		$^{n}J_{IS}$		$^{n}J_{II}$

[a] Apart from S^{3}E the experiment without ZQ/2Q π rotations is due to Montelione et al. (1989), while the others are due to Willker and Leibfritz (1992).

Herbrüggen et al., 1991; Kay et al., 1992; Schleucher et al., 1994; Sattler et al., 1996), e.g.:

$$I^{\alpha/\beta}S^{+} \xleftarrow{\pi S_y} \xleftarrow{\pi(I_xS_x + I_yS_y)} \pm i\, I^{-}S^{\alpha/\beta} \qquad (3)$$

From Eqs. 2 and 3 it follows that the stipulation that the individual coherences having evolved during t_1 be associated with one and only one spin state of S is fulfilled for the ZQ/2Q π rotation after t_1 and the succeeding homonuclear mixing does not perturb the S spin states. Hence the displacement vectors associated with e.g. the H^N-H^β cross peaks in spectra recorded with the pulse sequence in Fig. 1e are also $\mathbf{J}^N = (^1J(N\text{-}H^N);\ ^3J(N\text{-}H^\beta))$, where $^1J(N\text{-}H^N)$ can be suppressed by the π^I pulse in t_1.

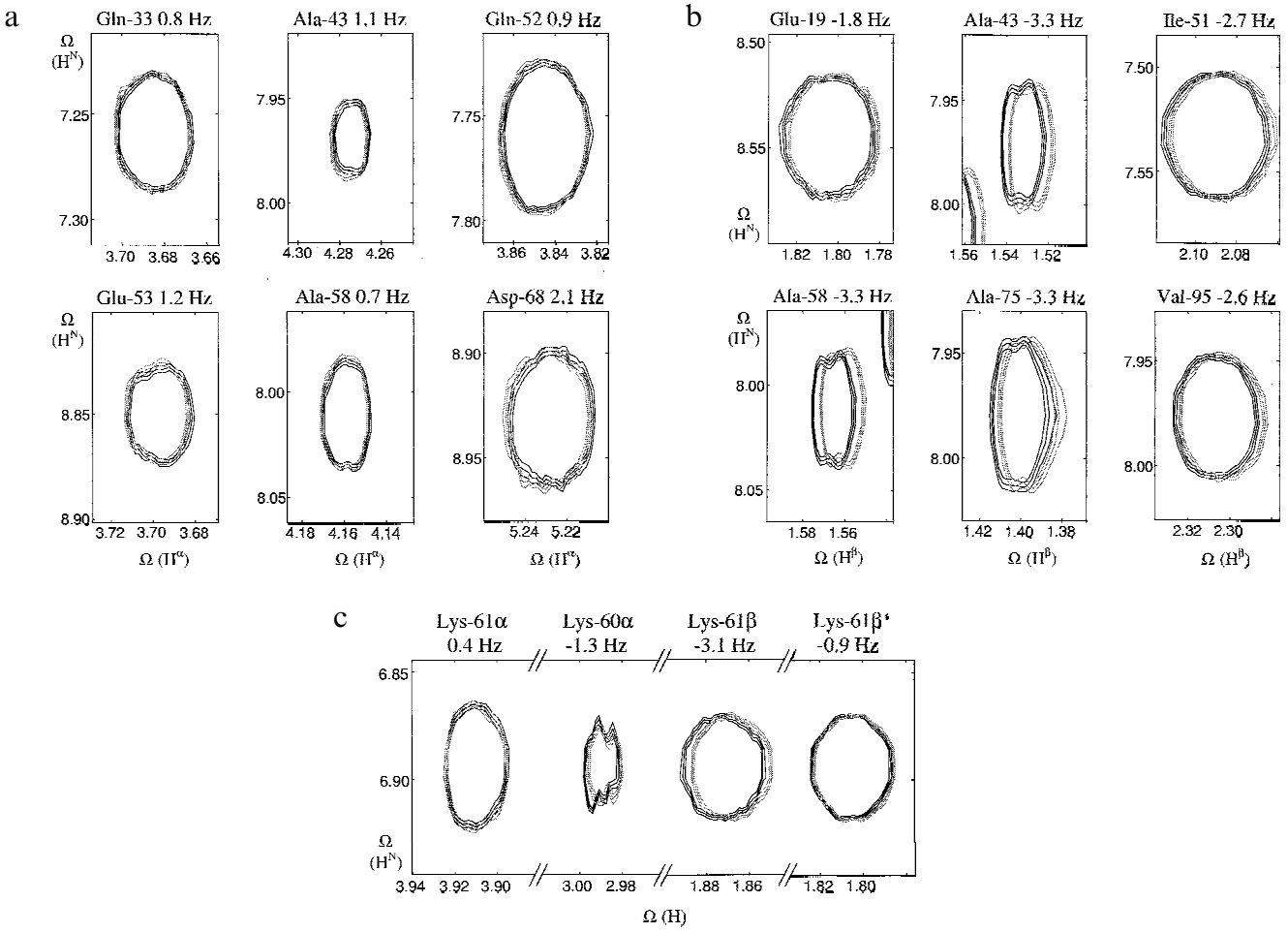

Fig. 4. Representative cross peaks from edited homonuclear S^{3}E-J_{NH}-NOESY spectra of ^{15}N-RAP 17–97, recorded with the sequence in Fig. 1c on a Varian Unity Inova 750 MHz spectrometer. The two edited subspectra (full and dotted lines, respectively) have been overlaid using the software package PRONTO (Kjær et al., 1994). Parameters: NOESY mixing time 60 ms; water presaturation 1.5 s plus during NOESY mixing; $t_1(\max) = 25.6$ ms; 64 scans and 256 increments; $\tau = 5.26$ ms; States-TPPI mode and cosine window functions. The coupling constants were estimated from 1D sections with a precision of about ±0.2 Hz. A much higher precision can be obtained by taking the entire 2D peak shapes into account. The cross peaks in (a) and (b) represent $^2J_{N\text{-}H^\alpha}$ and $^3J_{N\text{-}H^\beta}$, respectively, and those in (c) all protons with two- or three-bond couplings to the backbone ^{15}N of Lys^{61} ($^2J_{N\text{-}H^\alpha}$, $^3J_{N\text{-}H^\alpha(i-1)}$ and $^3J_{N\text{-}H^{\beta/\beta'}}$).

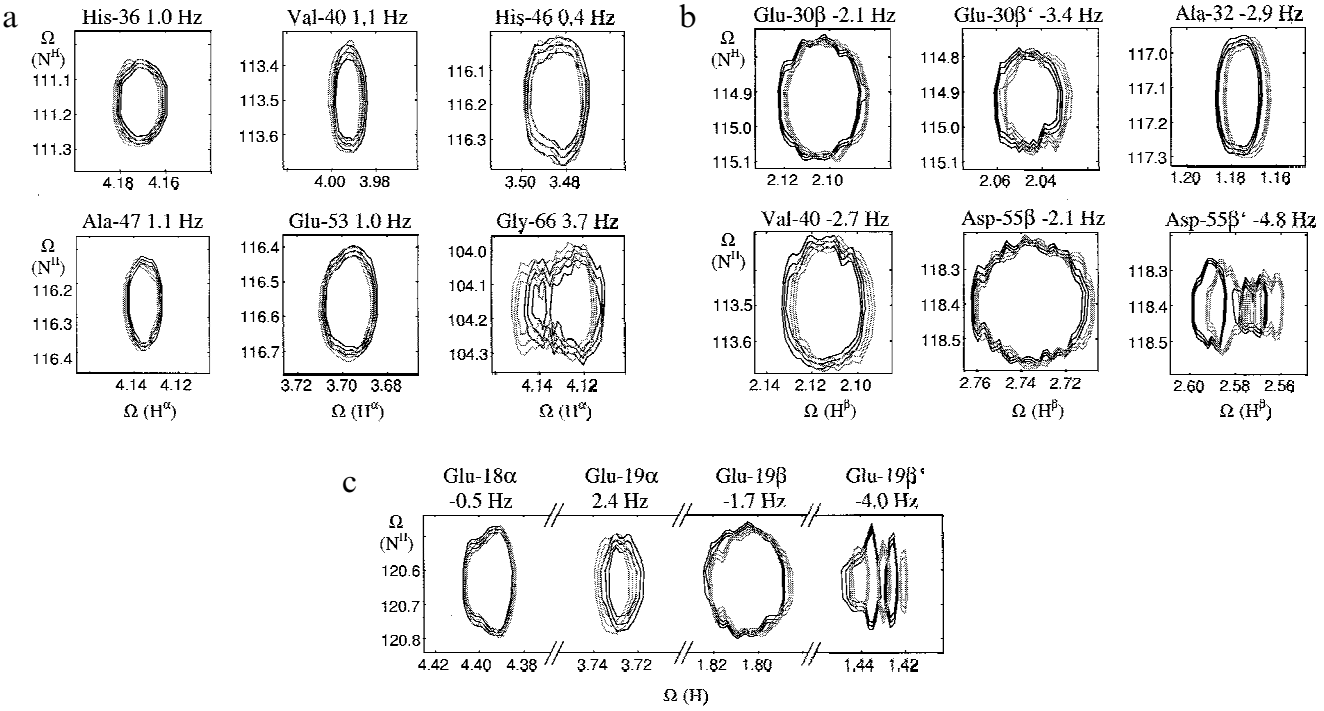

Fig. 5. Representative cross peaks from heteronuclear S^3E-J_{NH}-NOESY spectra of ^{15}N-RAP 17–97, recorded with the sequence in Fig. 1e on a Varian Unity Inova 750 MHz spectrometer. The same parameters as in Fig. 4 were employed, except for 128 scans and 128 increments; t_1(max) = 42.6 ms; cosine square and cosine window functions in t_1 and t_2, respectively. The cross peaks in (a) and (b) represent $^2J_{N\text{-}H^\alpha}$ and $^3J_{N\text{-}H^\beta}$, respectively, and those in (c) all protons with two- or three-bond couplings to the backbone ^{15}N of Glu^{19} ($^3J_{N\text{-}H^\alpha(i-1)}$, $^2J_{N\text{-}H^\alpha}$ and $^3J_{N\text{-}H^{\beta/\beta'}}$).

Excerpts from a spectrum of ^{15}N-RAP 17–97 recorded with this pulse sequence are shown in Fig. 5.

A ZQ/2Q pulse sequence element can be inserted before or after the homonuclear mixing sequence, which amounts to four options and there is the further option of having I or S spin chemical shifts evolve during t_1, i.e. a total of eight possibilities. Four of these make sense for the applications described here and by Meissner et al. (1997), and they are outlined in Table 1 with the information they yield.

In conclusion, we have introduced new pulse sequences combining S^3E for editing of the two doublet lines of IS two-spin systems with E.COSY-type 2D experiments for accurate measurement of heteronuclear coupling constants. The critical part of the pulse sequences with respect to sensitivity is the TOCSY or NOESY transfer among proton spins; the sensitivity of the new experiments is comparable to those of HSQC-TOCSY or HSQC-NOESY. The main advantage over earlier approaches is represented by the fact that the S^3E element allows heteronuclear decoupling during t_1, which results in two subspectra most suitable for extraction of interesting coupling constants.

Acknowledgements

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