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A general enhancement scheme in heteronuclear multidimensional NMR employing pulsed field gradients

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SUMMARY

General pulse sequence elements that achieve sensitivity-enhanced coherence transfer from a heteronucleus to protons of arbitrary multiplicity are introduced. The building blocks are derived from the sensitivity-enhancement scheme introduced by Cavanagh et al. ((1991) *J. Magn. Reson.*, **91**, 429–436), which was used in conjunction with gradient coherence selection by Kay et al. ((1992) *J. Am. Chem. Soc.*, **114**, 10663–10665), as well as from a multiple-pulse sequence effecting a heteronuclear planar coupling Hamiltonian. The building blocks are incorporated into heteronuclear correlation experiments, in conjunction with coherence selection by the formation of a heteronuclear gradient echo. This allows for efficient water suppression without the need for water presaturation. The methods are demonstrated in HSQC-type experiments on a sample of a decapeptide in H_2O. The novel pulse sequence elements can be incorporated into multidimensional experiments.

Suppression of artifacts is a severe problem in multidimensional NMR of biomolecules. The problem is twofold: firstly, a huge water resonance obscures interesting signals, and secondly, t_1 noise is present in all indirectly detected dimensions of multidimensional NMR spectra. In heteronuclear correlation experiments, the suppression of undesired signals by the application of pulsed field gradients (Hurd and John, 1991; John et al., 1991; Vuister et al., 1991, 1992; Kay, 1993) improves the quality of the spectra tremendously. Best results are achieved when the first gradient is applied during the evolution time of the heteronucleus and a second gradient before the detection period of the protons, which leads to the formation of a heteronuclear gradient echo (Maudsley et al., 1978). However, a factor of $\sqrt{2}$ is lost in signal-to-noise in conventional, amplitude-modulating heteronuclear correlation spectra employing gradient echos for coherence selec-

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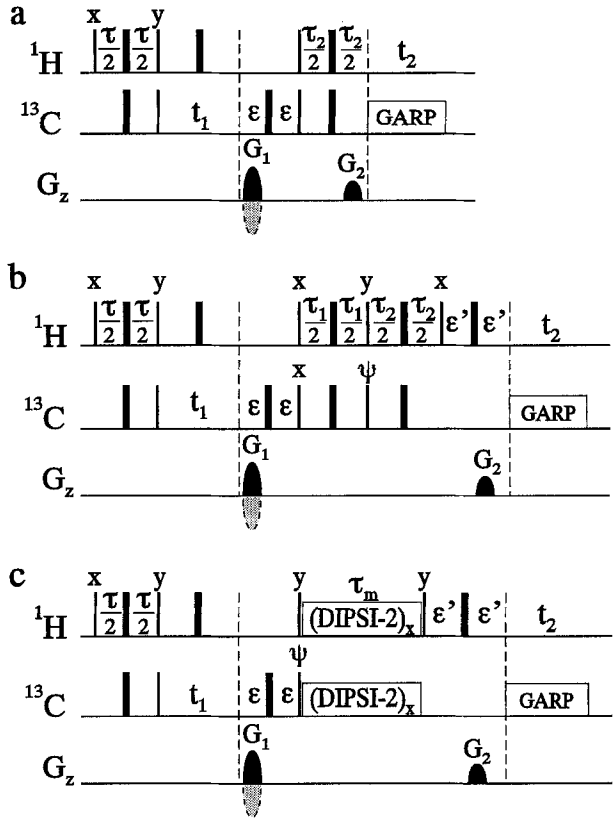

Fig. 1. Pulse sequences for the heteronuclear correlation experiments, using a heteronuclear gradient echo for coherence selection. (a) Amplitude-modulating HSQC experiment. (b) Sensitivity-enhanced HSQC experiment. Depending on τ_1 in the C→H transfer step, the enhancement factors given in Table 1 are observed. (c) HSQC with a heteronuclear TOCSY C→H transfer embedded between 90°(I,S) pulses to achieve the desired planar effective Hamiltonian H_{xy}. The second 90°(S) pulse has been omitted because the relevant components of the density operator consist of in-phase proton magnetization. The gradient acting on carbon is inverted in concert with the phase ψ (y,−y) to select the echo and anti-echo in alternate scans. Which combination of gradients needs to be combined with which phase (ψ = ±y) depends on the particular hardware used. Spectra with pure phases in ω_1 are obtained from the original data set according to the method described by Cavanagh et al. (1991).

tion (Kay et al., 1992; Ross et al., 1993; Schleucher et al., 1993a,b), but this is generally considered a small price to pay for artifact suppression and improved spectral quality. Since amplitude-modulating spectra are state-of-the-art in this respect, we consider them to be reference spectra and assign them unit sensitivity. Yet, it is obviously of interest to increase the sensitivity above this level.

Heteronuclear correlation experiments with increased sensitivity that select the two coherence transfer pathways $S^+ \rightarrow I^-$ or $S^- \rightarrow I^-$ in alternate scans have been introduced for IS spin systems (Cavanagh et al., 1991; Palmer III et al., 1991; Madsen and Sørensen, 1992). Such phase-modulating experiments do not suffer from reduced sensitivity when a heteronuclear gradient echo is applied, as has been shown for HSQC and multidimensional experiments (Kay et al., 1992; Muhandiram et al., 1993; Schleucher et al., 1993a,b).

TABLE 1
THEORETICAL AND **EXPERIMENTAL** ENHANCEMENT FACTORS OBTAINED FROM THE NEW PULSE SEQUENCES[a]

	Sequence Fig. 1b			Sequence Fig. 1c
	$\tau_1 = \tau_2 = 1/2J$	$\tau_1 = 1/4J$; $\tau_2 = 1/2J$	$\tau_1 = 1/6J$; $\tau_2 = 1/2J$	$\tau_m = 0.77/J$
IS	2/**1.95**	1.71/**1.75**	1.5	1.87/**1.71**
I_2S	1/**0.87**	1.41/**1.27**	1.37	1.4/**1.30**
I_3S	1/**0.92**	1.21/**1.08**	1.25	1.14/**1.09**

[a] The experimental values are averages over eight cross peaks for CH, 20 for CH_2 and 3 for CH_3. The enhancement factors are given relative to the sequence in Fig. 1a.

Based on these results we propose general pulse sequence elements (Fig. 1b,c), producing sensitivity enhancement in heteronuclear I_nS moieties. These elements can be implemented in multidimensional NMR experiments detecting protons of arbitrary multiplicity with a sensitivity gain of at least 20%, corresponding to a reduction in experiment time exceeding 30%. Considering that CH_3 protons generally exhibit high sensitivity, so that the experiment should be tuned for CH and CH_2 groups, the effective time saving approaches 50%.

The new elements are demonstrated in HSQC-type experiments. The transfer amplitude f in the C→H transfer step of the sensitivity-enhanced HSQC (Fig. 1b) for the transfer from $2S^{\pm}F_z$ to F^- in an I_nS spin system is given by (F denotes the sum of I spins: $F = \sum_{i=1}^{n} I$):

$$f = \tfrac{1}{2}\sin(\pi J\tau_1) + \sin(\pi J\tau_2)\cos^{n-1}(\pi J\tau_1)$$

The amplitude-modulating HSQC with gradients (Boyd et al., 1992; Davis et al., 1992) corresponds to $\tau_1 = 0$. While τ_2 in the C→H transfer step (Fig. 1b) is kept constant for all multiplicities, the optimum value of τ_1 depends on the number of attached protons ($\tau_1 = (2J)^{-1}$, $(4J)^{-1}$, $(6J)^{-1}$ for n = 1,2,3, respectively). The sensitivity enhancement thus achieved compared to the amplitude-modulating HSQC with gradients is listed in Table 1. For example, choosing $\tau_1 = 1/4J$ yields a sensitivity enhancement of $\sqrt{2}$ for CH_2 groups and of $1 + 1/\sqrt{2}$ for CH groups. For CH groups this can be rationalized by the observation that the effective Hamiltonian is $2\pi JF_yS_y$ during τ_1 and $2\pi JF_xS_x$ during τ_2. Thus, the overall effective Hamiltonian during $\tau_1 + \tau_2$ is the effective planar coupling Hamiltonian $H_{xy} = \pi J(F_xS_x + F_yS_y)$, (Schulte-Herbrüggen et al., 1991) which effects transfer between coherences of specific orders.

An alternative implementation of this effective coupling Hamiltonian for sensitivity enhancement is achieved by heteronuclear TOCSY (Bertrand et al., 1978; Müller and Ernst, 1979; Canet et al., 1990; Brown and Sanctuary, 1991; Ernst et al., 1991; Morris and Gibbs, 1991a,b). The best heteronuclear TOCSY sequences use pulses that are exclusively along one axis, e.g. the x-axis. They create an effective Hamiltonian $H_{zy} = \pi J(F_zS_z + F_yS_y)$ that can be converted to the desired planar form $H_{xy} = \pi J(F_xS_x + F_yS_y)$ by embedding the heteronuclear TOCSY sequence between two $90°(I,S)_y$ pulses (Fig. 1c). Transfer efficiencies under H_{xy} are plotted in Fig. 2 as a function of the mixing time. The optimum, as a compromise for all multiplicities, is around 0.77/J.

The transfer function for antiphase magnetization $2S^-F_z$ to F^- can be derived applying commu-

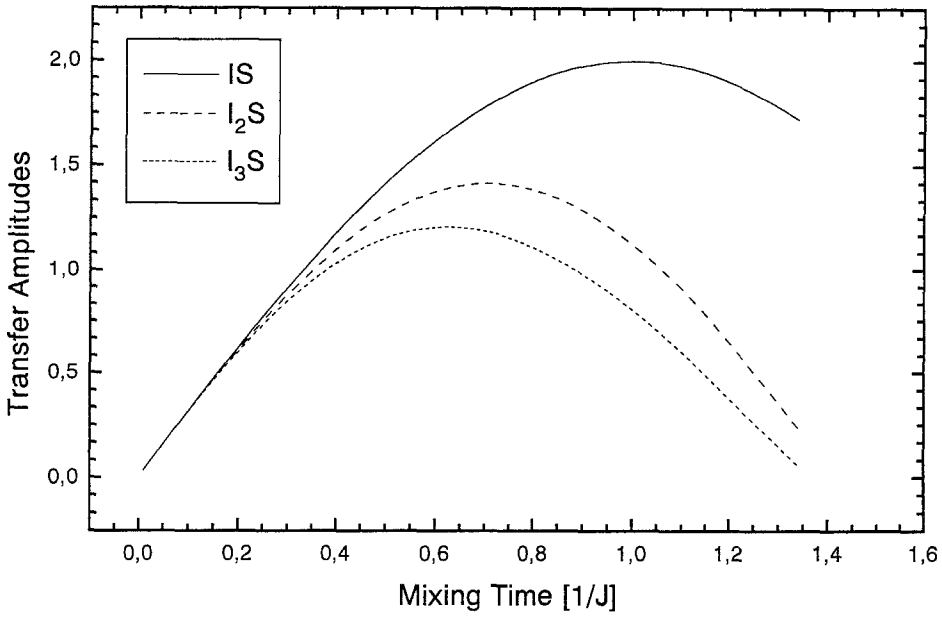

Fig. 2. Transfer efficiency (relative to an amplitude-modulating HSQC with a heteronuclear gradient echo) for the C→H transfer with planar TOCSY, starting from heteronuclear antiphase coherence. The optimal transfer for IS groups is achieved after 1/J, for I_2S groups after $1/\sqrt{2}J$ and for I_3S groups after 0.6249/J.

tation rules to the initial operator and using the Hamiltonian $H_{xy} = \pi J(F_xS_x + F_yS_y)$ ($[A,B]_+$ is the anticommutator: AB + BA). For an IS spin system we find:

$$2S^-F_z \xrightarrow{H_{xy}\tau} 2S^-F_z\cos(\pi J\tau) + iF^-\sin(\pi J\tau)$$

with an optimum at $\tau = (J)^{-1}$ and a maximum of F^- corresponding to an enhancement factor of 2. For an I_2S spin system we obtain:

$$2S^-F_z \xrightarrow{H_{xy}\tau} 2S^-F_z\cos(\pi\sqrt{2}J\tau) + (i/\sqrt{8})[1 + 2S_zF_z,F^-]_+ \sin(\pi\sqrt{2}J\tau)$$

with an optimum at $\tau = (\sqrt{2}J)^{-1}$ and a maximum of $F^-/\sqrt{2}$ corresponding to an enhancement factor of $\sqrt{2}$. For the I_3S spin system the expression is even more complicated. The desired transfer from $2S^-F_z$ to F^- is given by:

$$2S^-F_z \xrightarrow{H_{xy}\tau} iF^-\{(1/6)\sin(\pi J\tau) + (\sqrt{3}/4)\sin(\sqrt{3}\pi J\tau) + (1/12 + 1/(8\cdot\sqrt{3}))\sin[(2-\sqrt{3})\pi J\tau] + (1/12 - 1/(8\cdot\sqrt{3}))\sin[(2+\sqrt{3})\pi J\tau]\}$$

The first maximum, determined numerically, lies at $\tau = 0.6249/J$ with (0.6028) F^- corresponding to an enhancement factor of 1.2056.

Four experiments, each using a heteronuclear gradient echo for coherence selection in ω_1, are compared in Fig. 3, namely an amplitude-modulating HSQC (Davis et al., 1992; Boyd et al.,

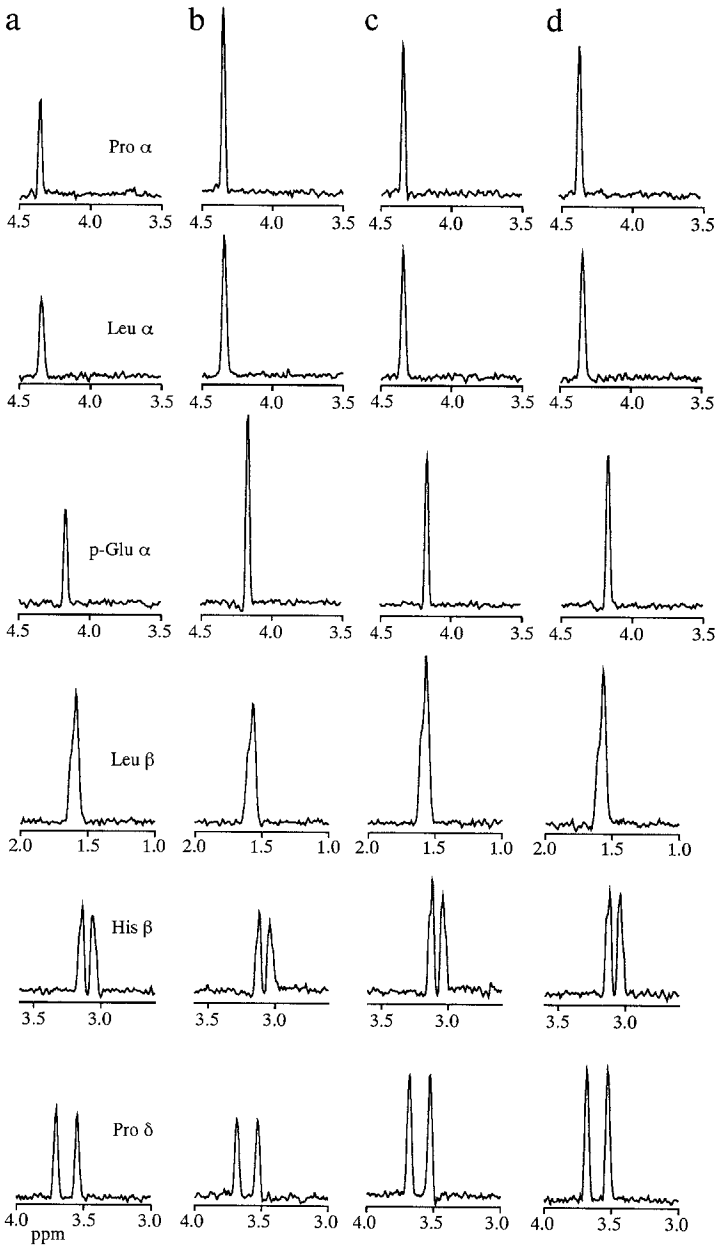

Fig. 3. Traces through Pro-H^{α}, Leu-H^{α}, pyro-Glu-H^{α}, Leu-H_2^{β}, His-H_2^{β}, and Pro-H_2^{δ} of C,H correlation spectra, obtained with the pulse sequences of Fig. 1 on a 20-mM sample of LHRH (p-E-H-W-S-Y-G-L-R-P-G) dissolved in 80% H_2O/20% D_2O and acetic acid. (a) Amplitude-modulating HSQC (Fig. 1a); (b) sensitivity-enhanced HSQC with τ_1 = 3.732 ms (Fig. 1b); (c) sensitivity-enhanced HSQC with τ_1 = 1.866 ms (Fig. 1b); and (d) planar TOCSY sequence with a DIPSI-2 mixing time of 5.5 ms and a $\gamma B_1/2\pi$ = 10 kHz on the carbon and proton channels (Fig. 1c). Spectra were recorded on a Bruker AMX 600 spectrometer, using a triple-resonance probe equipped with a self-shielded gradient coil. Sine-shaped gradients of 2 ms duration were followed by a recovery delay of 300 μs (thus, $\varepsilon = \varepsilon' = 2.3$ ms). The maximum gradient strength used was 0.275 T/m for the first gradient; the second gradient had equal duration and shape and was adjusted for maximum signal intensity ($|G_1/G_2| \approx \gamma_H/\gamma_C$). The carbon and proton carriers were positioned at 40 and 3 ppm, respectively.

1992), a sensitivity-enhanced HSQC (Kay et al., 1992) with $\tau_1 = (2J)^{-1}$, a sensitivity-enhanced HSQC with $\tau_1 = (4J)^{-1}$, and a sensitivity-enhanced HSQC with heteronuclear TOCSY with a mixing time of 0.77/J for the C→H coherence transfer step. The experiments have been performed on a 20-mM solution of the linear decapeptide LHRH in 80% H_2O/20% D_2O. Three traces are shown for CH and CH_2 groups. The expected enhancement factors can be clearly seen in the ω_2 traces through CH and CH_2 groups. In conclusion, we have introduced new general pulse sequence elements that offer increased sensitivity in heteronuclear correlation experiments and should find widespread application in multidimensional NMR employing pulsed field gradients.

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