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Time-Shared X(ω_1)-Half-Filter for Improved Sensitivity in Subspectral Editing

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Experiments with X-half-filter elements allow the separation of the resonances from protons bound and unbound to a spin X into different subspectra. This Communication presents a modified half-filter element where the filter delay is simultaneously used for chemical shift labeling and scalar coupling evolution in a semi-constant time experiment. The filter element is demonstrated with a ^{1}H NOESY spectrum of a 28.5-kDa 2:1 complex between the uniformly ^{13}C-labeled N-terminal domain of *Escherichia coli* arginine repressor and operator DNA.

Key Words: **X(ω_1)-half-filter; semi-constant time; subspectral editing; protein/DNA complex.**

X-half-filter elements allow the separation of the resonances from protons bound to a heteronucleus X from the resonances of all other protons (*1–3*). For example, a NOESY spectrum with a ^{13}C/^{15}N(ω_1)-half-filter recorded of a protein–DNA complex with ^{13}C/^{15}N-labeled protein and unlabeled DNA contains all the intraprotein NOEs in one subspectrum and all intra-DNA NOEs in another subspectrum, facilitating the resonance assignment of the individual macromolecules. Intermolecular NOEs would be found in both subspectra, but could be directed into a separate subspectrum by the use of a double half-filter (*3, 4*).

The main drawback of the half-filter elements is the loss of magnetization by transverse relaxation during the filter delay which is typically $1/J_{HX}$, corresponding to more than 10 ms for ^{15}N-half-filters (*2*). Part of the signal can, however, be recovered by using the half-filter delay simultaneously for frequency labeling in a semi-constant time experiment. This was demonstrated for a filter designed for suppression of the resonances from ^{15}N-bound protons (*5*) and recently proposed for a 3D ^{13}C-edited NOESY experiment with a ^{13}C/^{15}N(ω_1)-half-filter with full editing capabilities (*6*). The present Communication corrects the pulse sequence shown in Ref. (*6*) and experimentally illustrates the performance of the new half-filter element which is in the following referred to as "time-shared X(ω_1)-half-filter."

Figure 1A shows the pulse sequence of a ^{1}H NOESY experiment with a time-shared ^{13}C(ω_1)-half-filter. Two data sets, A and B, are recorded, where the sum spectrum (A + B) contains the intramolecular NOEs between protons not bound to ^{13}C and the difference spectrum (A − B) contains the intramolecular NOEs between ^{13}C-bound protons. For $t_1 = 0$, two FIDs are recorded with the 180°(^{1}H) pulse centered in the delay $2\Delta'$ and the 180°($^{13}C^{aliph}$) pulse either before (data set A)

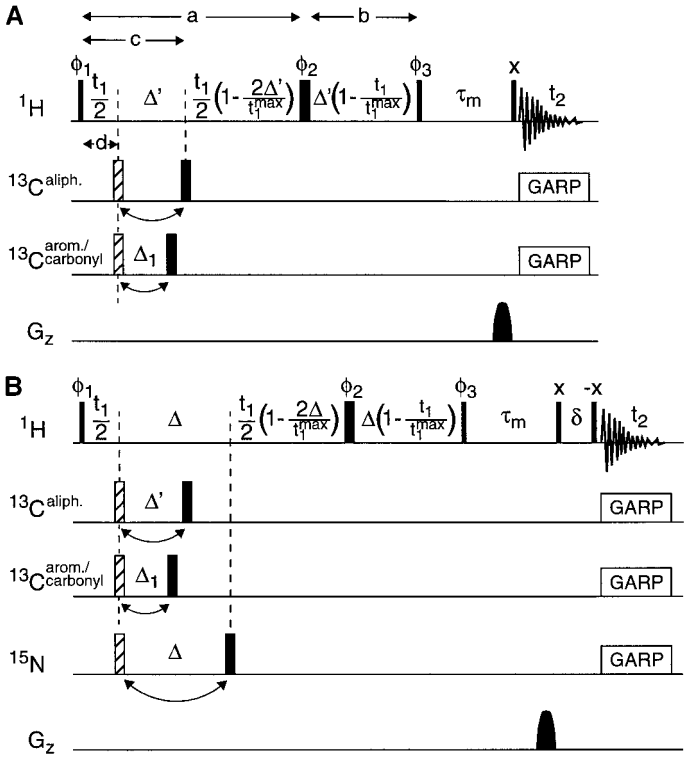

FIG. 1. Pulse sequences with a time-shared ω_1-half-filter. (A) ^{1}H NOESY with a time-shared ^{13}C(ω_1)-half-filter. $\Delta' = 3.6$ ms, $\Delta_1 = 3.1$ ms. Initial values and increments of delays a–d: a = Δ', incr. = $(t_{1max} - \Delta')/N$; b = Δ', incr. = $-\Delta'/N$; c = Δ', incr. = $t_{1max}/(2N)$; $d = 0$, incr. = $t_{1max}/(2N)$, where N = SW*t_{1max} is the number of increments, t_{1max} is the maximum evolution time in seconds, and SW is the sweepwidth in hertz. Narrow and wide bars denote 90° and 180° pulses, respectively. Two data sets are recorded with the 180°(^{13}C) pulses either at the positions indicated by the hatched bars (data set A) or at the positions indicated by filled bars (data set B). Phase cycle: $\phi_1 = 8(x, -x)$ + States–TPPI; $\phi_2 = 4(x), 4(y), 4(-x), 4(-y)$; $\phi_3 = 2(45°, 45°, 225°, 225°)$; receiver = $2(x, -x, -x, x, -x, x, x, -x)$. (B) Same as (A), but with simultaneous ^{15}N/^{13}C editing and a jump-and-return sequence (*11*) for water suppression after the mixing period τ_m. $\Delta = 1/(2J_{HN}) = 5.3$ ms.

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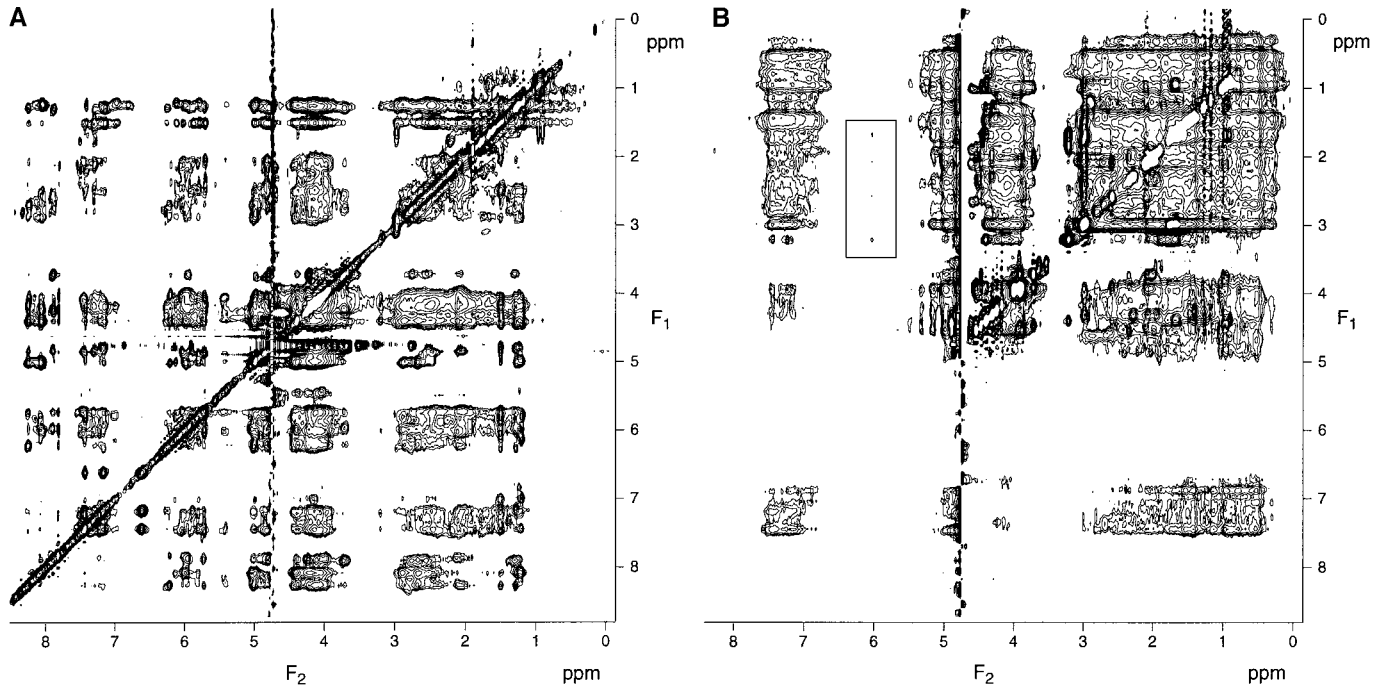

FIG. 2. ^{1}H NOESY spectra with a time-shared ^{13}C(ω_1)-half-filter recorded with a 0.5 mM solution of a 2:1 complex between the N-terminal DNA-binding domain of the *E. coli* arginine repressor and a 16-mer DNA duplex in D_2O at pH 6.9 and 25°C, using the pulse sequence of Fig. 1A. The protein was uniformly ^{13}C-labeled. Experimental parameters: t_{1max} = 14 ms, t_{2max} = 73.1 ms, τ_m = 200 ms, Bruker DMX 600 NMR spectrometer. For simultaneous decoupling of the aliphatic and aromatic ^{1}H resonances, the ^{13}C carrier frequency was shifted to 75 ppm during t_2 and GARP decoupling (*12*) was applied with a field strength of 3.57 kHz. The 180°(^{13}C) pulses were applied as 250-μs band-selective G^3 inversion pulses at 46 and 160 ppm (*13*). (A) Sum spectrum selecting the resonances from protons not bound to ^{13}C in the F_1 dimension. (B) Difference spectrum, selecting ^{13}C-bound protons in the F_1 dimension. The frame identifies intermolecular protein–DNA NOEs.

or after the initial delay Δ' (data set B). With $\Delta' = 1/(2J_{HC})$, the signals from ^{13}C-bound protons assume the same phase as all other proton signals in data set A and the opposite phase in data set B. As $^{1}J_{HC}$ is larger for aromatic than aliphatic CH groups, mismatch between filter delay and $^{1}J_{HC}$ coupling constants can be reduced by applying the 180°(^{13}C) pulses as shaped pulses covering the range of aliphatic and aromatic carbons separately, with $\Delta' = 1/(2J_{HC}^{aliph})$ and $\Delta_1 = 1/(2J_{HC}^{arom})$. The semi-constant time evolution scheme (*7, 8*) of the pulse sequence in Fig. 1A completely refocuses J evolution in data set A, while maintaining effective J evolution periods of $2\Delta'$ and $2\Delta_1$, respectively, in data set B for all t_1 values. For $t_1 = t_{1max}$, there is no delay between the 180°(^{1}H) and the following 90°(^{1}H) pulse, so that the entire filter delay $2\Delta'$ is used for frequency labeling. Compared to the original half-filter element with decoupling (*2, 3*), the time-shared half-filter element shortens the average time of transverse proton magnetization in the 2D experiment by $1/(2J_{HX})$. This can lead to noticeably improved sensitivity in samples with high transverse relaxation rates.

The performance of the time-shared ^{13}C(ω_1)-half-filter was experimentally tested with a 28.5-kDa 2:1 complex between the N-terminal DNA-binding domain of the *Escherichia coli* arginine repressor (*9*) and a palindromic 16-mer DNA duplex. The protein was uniformly labeled with ^{13}C, while the DNA was unlabeled. Data sets A and B were recorded in an FID-interleaved manner using the ^{13}C(ω_1)-half-filtered NOESY pulse sequence of Fig. 1A. The sum of both data sets (Fig. 2A) contains the intra-DNA peaks, while the difference data set (Fig. 2B) contains the intraprotein peaks. Intermolecular NOEs were observed in the difference spectrum between different side chain protons of Arg 2 and a 1′ proton of the DNA (framed in Fig. 2B). Corresponding cross peaks should in principle also be present in the sum spectrum on the other side of the diagonal (*3*) but most of them could not be identified because of overlap with intra-DNA cross peaks. Although most of the lines observed in this complex were very broad presumably due to exchange phenomena, the few well-resolved cross peaks demonstrate a clean separation of the intraprotein from the intra-DNA NOEs with a maximum cross-talk of less than 5% from the protein subspectrum into the DNA subspectrum. Compared to a NOESY spectrum recorded under identical conditions with a conventional ^{13}C(ω_1)-half-filter (*2*) and the same t_{1max} value, the sensitivity of the time-shared version was improved on average 1.3-fold.

In conclusion, a time-shared X-half-filter element minimizes signal loss by relaxation and replaces a conventional X-half-filter element without loss of filter performance. Like the conventional filter element, it can also be used for simultaneous editing of the resonances from ^{15}N- and ^{13}C-bound

protons versus those from ^{14}N- and ^{12}C-bound protons (Fig. 1B) (*6*, *10*). Furthermore, a time-shared $^{15}N(\omega_1)$-half-filter can be derived from the pulse sequence of Fig. 1B by omitting the ^{13}C pulses or by applying only the hatched 180°(^{13}C) pulses in every scan to achieve ^{13}C decoupling in the F_1 dimension.

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