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Joint Composite-Rotation Adiabatic-Sweep Isotope Filtration

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Abstract

Joint composite-rotation adiabatic-sweep isotope filter are derived by combining the composite-rotation [A. C. Stuart, K. A. Borzilleri, J. M. Withka, and A. G. Palmer, *J. Am. Chem. Soc.* 121, 5346–5347 (1999)] and adiabatic-sweep [C. Zwaehlen, P. Legault, S. J. F. Vincent, J. Greenblatt, R. Konrat, and L. E. Kay, *J. Am. Chem. Soc.* 119, 6711–6721 (1997); Ě. Kupče and R. Freeman, *J. Magn. Reson.* 127, 36–48 (1997)] approaches. The joint isotope filters have improved broadband filtration performance, even for extreme values of the one-bond ¹H-¹³C scalar coupling constants in proteins and RNA molecules. An average Hamiltonian analysis is used to describe evolution of the heteronuclear scalar coupling interaction during the adiabatic sweeps within the isotope filter sequences. The new isotope filter elements permit improved selective detection of NMR resonance signals originating from ¹H spins attached to an unlabeled natural abundance component of a complex in which other components are labeled with ¹³C and ¹⁵N isotopes.

Keywords

composite pulse; J-filter; NOESY; structure determination; vts SAM domain

Introduction

Isotope-filtered and isotope-edited techniques are essential to the investigation of biological complexes by NMR spectroscopy (Otting and Wüthrich, 1990). In these studies, one or a subset of the components of a complex are enriched in one or more NMR-active heteronuclei, for example ¹⁵N or ¹³C, and the other components are natural abundance or depleted in NMR-active isotopes. An isotope-filter selectively suppresses and an isotope-editor selectively detects resonance signals from ¹H spins directly attached to the NMR-active heteronuclei. These techniques rely on the differential evolution of ¹H spins subject to a large or small (near-zero) heteronuclear scalar coupling Hamiltonian. An alternative, more specific, nomenclature refers to an isotope-filter as a low-pass *J*-filter and an isotope-editor as a high-pass *J*-filter. Isotope filters are incorporated into NMR pulse sequences, such as COSY or TOCSY experiments, to permit resonance assignments of an unlabeled component of a complex and are incorporated into NOESY experiments to selectively detect NOE cross peaks between the ¹H spins within the unlabeled species or between the unlabeled and labeled components (Breeze, 2000, Peterson, et al., 2004).

The efficiency of isotope filters is reduced by variations in the one-bond scalar coupling constant between the ¹H spin and the heteronucleus. This difficulty is particularly severe

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for $^1\text{H}_n\text{-}^{13}\text{C}$ ($n = 1, 2, 3$) moieties because J_{CH} depends on the nature of the chemical group, varying from approximately ~120 Hz for CH_3 groups to ~220 Hz for the $^1\text{H}^{\epsilon 1}\text{-}^{13}\text{C}^{\epsilon 1}$ spin pair in histidine and the $^1\text{H}8\text{-}^{13}\text{C}8$ spin pair in purine nucleotides. Two general approaches have emerged for improving the robustness of low-pass J -filters despite variation in J_{CH} . The one approach relies on the isomorphism between pulse rotations and scalar coupling evolution (Levitt, 1986). Composite pulses compensated for B_1 inhomogeneity are converted to sequence elements compensated for variation in J_{CH} (Stuart, et al., 1999). The other approach uses the empirical relationship between the heteronuclear scalar coupling constant and isotropic ^{13}C chemical shifts. Designed adiabatic broadband inversion pulses are used to effectively scale the coupling constants to a uniform value during an INEPT or other polarization transfer period (Kupče and Freeman, 1997, Zwanen, et al., 1997).

In the present paper, the two approaches are combined to generate joint composite-rotation adiabatic-sweep isotope filter sequences, or low pass J -filters. The theoretical performance of the proposed sequences is evaluated using an average Hamiltonian description of the evolution of the heteronuclear scalar coupling interaction during an adiabatic sweep. The proposed sequences are validated using a ^{13}C -labeled Ala, Tyr, and His amino acid mixture, representing the range of J_{CH} encountered in proteins, and by recording a $^1\text{H}\text{-}^1\text{H}$ F1-filtered, F2-edited NOESY spectrum of the $[\text{U-}^{13}\text{C}, \text{U-}^{15}\text{N}]$ vts SAM domain complexed with its unlabeled cognate RNA ligand (Edwards, et al., 2006).

Theory

Evolution of a spin system under the heteronuclear scalar coupling Hamiltonian during an adiabatic sweep has been treated theoretically by Kay and coworkers (Zwanen, et al., 1997, Zwanen, et al., 1998). The adiabatic sweep is divided into a series of equal time intervals short enough that the amplitude and resonance offset of the rf field can be treated as constant during each interval. The evolution of the density matrix during the i th interval is calculated using as initial conditions the state of the density operator after the $(i - 1)$ th interval. Thus, evolution through the entire adiabatic sweep is calculated iteratively for the N intervals Δt_i for $i = 1, \dots, N$, beginning with the initial conditions at the start of the adiabatic sweep. To simplify these calculations, Kay and coworkers developed compact expressions for efficiently calculating the evolution of a reduced set of basis operators during each interval.

In most applications, and in particular for isotope filtration, the state of the density operator does not need to be known at any arbitrary point within an adiabatic sweep; rather the net evolution of the density operator at the end of the sweep is of primary concern. In these circumstances, an average Hamiltonian theoretical treatment of evolution under the heteronuclear scalar coupling Hamiltonian is sufficiently accurate and yields simple intuitive results. Average Hamiltonian theory has been applied to adiabatic pulses for isolated spin systems, i.e. in the absence of scalar couplings, by Mitschang and Rinneberg (Mitschang and Rinneberg, 2003) and to homonuclear scalar coupled systems by Bennett and coworkers (Bennett, et al., 2003).

The experimental situation of interest is illustrated in Figure 1. The initial state of the density operator for an I - S spin system ($I = ^1\text{H}$ and $S = ^{13}\text{C}$) is described by an expansion in terms of in-phase (I_x and I_y) and antiphase transverse I spin product operators ($2I_xS_z$ and $2I_yS_z$). An adiabatic sweep of duration τ_p is applied to the S spin and a refocusing 180° is optionally applied to the I spin at time τ_{inv} . The sequence element in Fig. 1a is formally equivalent to the sequence $-\tau_p\text{-}[180^\circ(I)]$ in which the Hamiltonian in the rotating frames of reference of the I and S spins during the period τ_p is given by

$$\mathcal{H}(t) = \Omega(t)S_z + \omega_I(t)S_x + a(t)2\pi J_{IS}I_zS_z \quad [1]$$

and the bracketed $180^\circ(I)$ pulse is included only if a refocusing pulse is applied. The refocusing pulse inverts the scalar coupling interaction at time τ_{inv} ; the effect of this pulse is represented by the function $a(t)$ with values

$$a(t) = \begin{cases} 1 & \text{when } 0 \leq t \leq \tau_{inv} \\ -1 & \text{when } \tau_{inv} < t \leq \tau_p \\ 1 & \text{when no refocussing pulse is used} \end{cases} \quad [2]$$

Without loss of generality, the I spin is assumed to be on-resonance in the rotating frame. The rotating frame of the S spin is defined relative to the instantaneous radiofrequency field of the adiabatic sweep, $\omega_{rf}(t)$; therefore, the frequency offset of the S spin is given by the time-dependent function $\Omega(t) = \omega_S - \omega_{rf}(t)$ and ω_S is the resonance frequency of the S spin. The amplitude of the adiabatic sweep, assumed to be applied with x -phase is given by $\omega_1(t)$. The amplitude and the tilt angle of the effective field in the rotating frame are given by $\omega_e(t) = [\Omega(t)^2 + \omega_1(t)^2]^{1/2}$ and $\tan[\theta(t)] = \omega_1(t)/\Omega(t)$, respectively. The time-variation of $\omega_e(t)$ and $\theta(t)$ are assumed to satisfy the adiabatic condition, in which the adiabaticity factor $Q = \omega_e/|d\theta(t)/dt| \gg 1$; typically, the adiabaticity factor at resonance, $\Omega(t) = 0$, is chosen to satisfy $Q_0 > 5$.

Evolution under this Hamiltonian is analyzed most conveniently in a tilted frame of reference whose z' -axis is oriented along the instantaneous direction of $\omega_e(t)$. Transformation to the tilted frame is defined by $\widehat{\mathcal{H}}(t) = U_1 \mathcal{H}(t) U_1^{-1}$ with $U_1 = \exp[i\theta(t)S_y]$:

$$\widehat{\mathcal{H}}(t) = \omega_e(t)S'_z + a(t)2\pi J_{IS} \{I_z S'_z \cos[\theta(t)] - I_z S'_x \sin[\theta(t)]\} \quad [3]$$

The propagator for evolution under this Hamiltonian can be written as (Haeberlen and Waugh, 1968)

$$U(t) = U_0(t)U_j(t) \quad [4]$$

in which

$$U_0(t) = T \exp[-iS'_z \int_0^t \omega_e(t') dt'] \quad [5]$$

$$U_j(t) = T \exp[-i \int_0^t \widetilde{\mathcal{H}}(t') dt'] = \exp[-i \widetilde{\mathcal{H}} t] \quad [6]$$

T is the Dyson time-ordering operator (Evans and Powles, 1967), and $\widetilde{\mathcal{H}}(t)$ is the effective Hamiltonian in an interaction frame rotating around the z' -axis with frequency $\omega_e(t)$. In this frame, illustrated in Fig. 1c, the Hamiltonian is given by:

$$\begin{aligned} \widetilde{\mathcal{H}}(t) &= a(t)2\pi J_{IS} I_z S'_z \cos[\theta(t)] - a(t) \sin[\theta(t)] 2\pi J_{IS} I_z U_0(t) S'_x U_0^{-1}(t) \\ &= a(t)2\pi J_{IS} I_z S'_z \cos[\theta(t)] - a(t) \sin[\theta(t)] 2\pi J_{IS} I_z \{S'_x \cos[\omega_e(t)t] - S'_y \sin[\omega_e(t)t]\} \end{aligned} \quad [7]$$

The average Hamiltonian is

$$\overline{\mathcal{H}} = \overline{\mathcal{H}}^{(0)} + \overline{\mathcal{H}}^{(1)} + \dots \quad [8]$$

in which

$$\overline{\mathcal{H}}^{(0)} = \frac{1}{t} \int_0^t \tilde{\mathcal{H}}(t_1) dt_1 \quad [9]$$

$$\overline{\mathcal{H}}^{(1)} = \frac{-i}{2t} \int_0^t dt_2 \int_0^{t_2} [\tilde{\mathcal{H}}(t_2), \tilde{\mathcal{H}}(t_1)] dt_1 \quad [10]$$

In the applications envisioned, the transverse components of the interaction frame Hamiltonian given in Eq. [7], are negligible owing to rapid evolution of the sinusoidal terms that depend on $\omega_e(t)$. Thus, the zero-order average Hamiltonian is given by

$$\overline{\mathcal{H}}^{(0)} = \frac{1}{\tau_p} \int_0^{\tau_p} a(t') \cos[\theta(t')] dt' 2\pi J_{IS} I_z S'_z = 2\pi \bar{J}_{IS} I_z S'_z \quad [11]$$

in which the reduced scalar coupling constant is defined by

$$\bar{J}_{IS} = \frac{J_{IS}}{\tau_p} \int_0^{\tau_p} a(t') \cos[\theta(t')] dt' \quad [12]$$

In the absence of a refocusing pulse:

$$\bar{J}_{IS} = \frac{J_{IS}}{\tau_p} \int_0^{\tau_p} \cos[\theta(t')] dt' = J_{IS} \langle \cos\theta \rangle \quad [13]$$

in which angle brackets indicate the average value over the adiabatic sweep. The density operator in the laboratory frame at the end of the adiabatic sweep is given by:

$$\begin{aligned} \sigma(\tau_p) &= \exp[-i\theta(\tau_p)S_y] \mathbf{U}_0(\tau_p) \exp[-i\overline{\mathcal{H}}^{(0)} \tau_p] \sigma(0) \\ &\times \exp[i\overline{\mathcal{H}}^{(0)} \tau_p] \mathbf{U}_0^{-1}(\tau_p) \exp[i\theta(\tau_p)S_y] \\ &= \exp[-i\pi S_y] \exp[-i\overline{\mathcal{H}}^{(0)} \tau_p] \sigma(0) \exp[i\overline{\mathcal{H}}^{(0)} \tau_p] \exp[i\pi S_y] \end{aligned} \quad [14]$$

The second equality is obtained because the propagator $\mathbf{U}_0(\tau_p)$ commutes with $\overline{\mathcal{H}}^{(0)}$ and the initial density operator and because the adiabatic sweep achieves a total rotation $\theta(\tau_p) = \pi$. Thus, evolution of the density operator during τ_p is obtained by the simple product operator rules for evolution under a reduced scalar coupling Hamiltonian $\overline{\mathcal{H}}^{(0)}$ followed by an ideal πS_y rotation; for example:

$$\begin{aligned} I_y &\xrightarrow{\tau_p} I_y \cos(\pi \bar{J}_{IS} \tau_p) + 2I_x S_z \sin(\pi \bar{J}_{IS} \tau_p) \\ 2I_x S_z &\xrightarrow{\tau_p} -2I_x S_z \cos(\pi \bar{J}_{IS} \tau_p) + I_y \sin(\pi \bar{J}_{IS} \tau_p) \end{aligned} \quad [15]$$

If a $180^\circ(I)$ refocusing pulse is applied during the adiabatic sweep, then these results are modified by applying the $180^\circ(I)$ pulse to the operators present after τ_p .

For a chirp pulse, a linear frequency sweep with a constant value of ω_1 , Eq. [13] can be integrated to give

$$\begin{aligned} \langle \cos\theta \rangle &= \frac{1}{2\Delta F} \left\{ \left[(\Delta F + 2\delta)^2 + 4\omega_1^2 \right]^{1/2} - \left[(\Delta F - 2\delta)^2 + 4\omega_1^2 \right]^{1/2} \right\} \\ &\approx \frac{2\delta}{\Delta F} \end{aligned} \quad [16]$$

in which ΔF is the sweep bandwidth and δ is the frequency offset between the S spin and the center of the frequency sweep. The second line is obtained assuming that $\Delta F^2 \gg 2\omega_1^2 + 4\delta^2$. When this approximation is valid, evolution of the scalar coupling during an adiabatic sweep (in the absence of a refocusing 180° pulse applied to the I spins), can be represented as the sequence element $\tau' - 180^\circ(S) - (\tau_p - \tau')$, in which

$$\tau' = \frac{\tau_p}{2} \left(1 + \frac{2\delta}{\Delta F} \right) \quad [17]$$

In Eq. [17], τ' is equal to the time at which the adiabatic frequency sweep is resonant with the S spin (Zwahlen, et al., 1997). These results are approximately correct for the WURST family of adiabatic pulses (Kupče and Freeman, 1995) as well, provided that δ is restricted to the central constant-amplitude region of the pulse.

The accuracy of the average Hamiltonian result of Eq. [12] is illustrated in Figure 2 for the pulse sequence element of Fig. 1a using a series of WURST-20 adiabatic pulses (Kupče and Freeman, 1995) with increasing field strengths and shorter durations, typical of values used for these pulses in biological NMR spectroscopy. As shown in Fig. 2d, the accuracy of the average Hamiltonian result is weakly dependent on the adiabaticity of the sweep, provided that adiabaticity is maintained ($Q_0 \geq 5$). However, accuracy depends strongly on $\omega_{1\max}\tau_p$, in which $\omega_{1\max}$ is the peak value of $\omega_1(t)$, for two reasons. First, the accuracy of the approximation $\overline{\mathcal{H}} \approx \overline{\mathcal{H}}^{(0)}$, that is, keeping only the zero-order Hamiltonian in Eq. [8], requires that $\omega_{1\max} \gg \pi J_{IS}$. Second, the cosine and sine terms that depend on $\omega_e(t)t$ in Eq. [7] are averaged to zero only if $\omega_{1\max}\tau_p \gg 1$. Thus, the accuracy of the above results is reduced for either weak rf fields or short pulse lengths.

Rotations under on-resonance radiofrequency pulses in the operator space $\{I_x, I_y, I_z\}$ are isomorphous to rotations under the scalar coupling Hamiltonian in the operator space $\{2I_zS_z, 2I_xS_z, I_y\}$ (Levitt, 1982). Thus, a composite 90° pulse that efficiently rotates I_z magnetization into the transverse plane independently of variation in the nominal rotational angle can be converted into a pulse-interrupted-free-precession period that transforms I_y magnetization efficiently into two-spin operators independently of variation in J_{IS} . The composite 90° pulse sandwich $\beta_0(2\beta)_{2\pi/3}$, in which the bracketed term is the nominal rotation angle and the subscript is the phase of the pulse, has previously been used by Stuart and coworkers to develop a highly efficient composite-rotation isotope filter (Stuart, et al., 1999). Using the aforementioned isomorphism, the composite filter propagator is given by:

$$\begin{aligned} \mathbf{U} &= \exp[-i(2\beta)2I_{2\pi/3}S_z] \exp[-i\beta 2I_zS_z] \\ &= \exp[-i(2\beta)2\{I_z\cos(2\pi/3) + I_x\sin(2\pi/3)\}S_z] \exp[-i\beta 2I_zS_z] \\ &= \exp[-i(2\pi/3)I_y] \exp[-i(2\beta)2I_zS_z] \exp[i(2\pi/3)I_y] \exp[-i\beta 2I_zS_z] \end{aligned} \quad [18]$$

The leftmost I_y rotation is not needed to implement an isotope filter and can be eliminated to simplify the propagator to

$$\mathbf{U} = \exp[-i4\pi J_{IS}\tau I_zS_z] \exp[i(2\pi/3)I_y] \exp[-i2\pi J_{IS}\tau I_zS_z] \quad [19]$$

in which $\beta = \pi J\tau$ and the filter is tuned to for a nominal scalar coupling constant J_0 by setting the evolution delay $\tau = 1/(2J_0)$. This propagator is realized by the ideal filter pulse sequence:

$$\begin{array}{lcl} I : & \frac{\tau}{2} - & 180_x^\circ \quad -\frac{\tau}{2} - 120_y^\circ - \tau - & 180_x^\circ \quad -\tau - \\ S : & & 180_x^\circ & 180_x^\circ \end{array} \quad [20]$$

A product operator analysis of the sequence shows that the evolution of I -spin coherence between points a and b of Fig. 3a is given by $-I_y \rightarrow -\varepsilon I_y$ in which:

$$\varepsilon = \cos^3[\pi J_{IS} \tau] \quad [21]$$

For a filter, the two-spin product operators created in this sequence, $2I_x S_z$ and $2I_y S_z$, are purged by a 90° pulse applied to the S spins. In other applications, optimal transfer to $2I_x S_z$ longitudinal two-spin order can be obtained by appending a final 30° rotation to the sequence. For ^1H spins attached to ^{12}C nuclei, $J_{IS} = 0$ and $\varepsilon = 1$ under all conditions (ignoring relaxation).

As emphasized by Kay and coworkers (Zwahlen, et al., 1997) and by Kupče and Freeman (Kupče and Freeman, 1997), one-bond C-H scalar coupling constants and isotropic chemical shifts are linearly related for ^1H spins attached to ^{13}C nuclei in amino acid residues and RNA nucleotides:

$$J_{IS} \approx 120 + 0.365\delta_C \text{ (amino acids)} \quad [22]$$

$$J_{IS} \approx 101 + 0.710\delta_C \text{ (RNA)} \quad [23]$$

in which J_{IS} is measured in Hz and δ_C is the chemical shift measured in ppm (relative to DSS). As shown by Eq. [12], the reduced scalar coupling constant during an adiabatic sweep also depends on the S -spin resonance offset. Accordingly, the pulse element shown in Fig. 1a can be substituted for the central pair of 180° pulses in an INEPT element. The properties of the adiabatic sweep are chosen to obtain broadband polarization transfer from initial $-I_y$ magnetization to $2I_x S_z$ for the desired range of J_{IS} . This strategy has been elegantly incorporated into double-tuned isotope filters by Kay and coworkers (Zwahlen, et al., 1997).

Two implementations of a joint composite-rotation adiabatic-sweep filter element are shown in Figure 3. The two filter sequences have essentially the same theoretical performance. In the sequence of Fig. 3a, the two adiabatic pulses have the same frequency bandwidth and are swept in the same sense; therefore, the values of $\bar{J}_{IS}$ calculated using Eq. [12] are identical for the two adiabatic sweeps. A product operator analysis of the sequence shows that the evolution of I -spin coherence between points a and b of Fig. 3a is given by $-I_y \rightarrow -\varepsilon I_y$ in which:

$$\varepsilon = \cos^3[\tfrac{1}{2}\pi J_{IS} \{2\tau_a - \tau_p(1 - \bar{J}_{IS}/J_{IS})\}] \quad [24]$$

In the sequence of Fig. 3b, the second adiabatic pulse, of length τ_p , has the same sweep bandwidth, but is swept in the opposite sense as the first adiabatic sweep. Therefore $\langle \cos[\theta(t)] \rangle_{\text{sweep2}} = -\langle \cos[\theta(t)] \rangle_{\text{sweep1}} = \langle \cos\theta \rangle$ using Eq. [13]. A product operator analysis of the sequence shows that the evolution of I -spin coherence between points a and b of Fig. 3b is given by $-I_y \rightarrow -\varepsilon I_y$ in which:

$$\varepsilon = \cos^3[\tfrac{1}{2}\pi J_{IS} \{2\tau_a - \tau_p(1 - \langle \cos\theta \rangle)\}] \quad [25]$$

The filters are implemented by numerically optimizing the adiabatic sweeps in order to minimize ε in Eqs. [24] and [25] over the desired range of J_{IS} (while maintaining adiabaticity of the sweep as a constraint). For ^1H spins attached to ^{12}C nuclei, $J_{IS} = 0$ and $\varepsilon = 1$ under all conditions (ignoring relaxation). Figure 4 compares the theoretical performance of the joint composite-rotation adiabatic-sweep isotope filter for scalar coupling constants in proteins with other approaches that have been described in the literature. The corresponding theoretical performance for scalar coupling constants in RNA is shown in Figure 5. The improvements obtained with the new sequences, particularly at the low and high extremes of the scalar

coupling constants, are evident. The sequence of Fig. 3a is derived from the basic composite rotation $\beta_0(2\beta)_{2\pi/3}$ in a more straightforward fashion; however, as discussed below, the sequence of Fig. 3b has only 180° pulses on the I -spin when transverse coherences are present and allows convenient water suppression with the excitation sculpting (Hwang and Shaka, 1995) approach. In addition, resonance offset effects are smaller for a 60° pulse compared with a 120° pulse.

The above theoretical analyses do not include the effects of relaxation. The joint composite-rotation adiabatic-sweep isotope filters are longer than the corresponding composite-rotation sequences of Stuart and coworkers (Stuart, et al., 1999), for example, the sequence of Fig. 3b is longer by $3\tau_p/2$, leading to reduced sensitivity, owing to relaxation losses. Thus, the lengths of the adiabatic sweeps also should be minimized while satisfying Eqs. [24] and [25]. Transverse relaxation for ^1H spins attached to ^{13}C nuclei is more efficient than for ^1H spins attached to ^{12}C nuclei, because the ^1H - ^{13}C dipolar interaction constitutes an additional relaxation pathway. Consequently, the apparent filter efficiency, measured as the ratio of peak intensities for ^1H spins attached to ^{12}C nuclei and for ^1H spins attached to ^{13}C nuclei is increased in larger proteins with larger transverse relaxation rate constants.

Methods

The new isotope filter elements can be used as building blocks in a number of different pulse sequences as shown in Figures 6 and 7. Figure 6a shows an F1/F2-edited, F3-filtered HMQC-NOESY experiments based on a semi-constant-time HMQC pulse sequence. The excitation sculpting water suppression technique (Hwang and Shaka, 1995) has been built into the filter element, without increase in any delays, for water suppression. Figure 6b shows an F1-filtered, F2/F3-edited NOESY-HMQC pulse sequence. The excitation sculpting technique has been built into the HMQC pulse sequence for water suppression. Both of these sequences use phase cycling for ^{13}C editing and subtraction artifacts may pose a significant problem if large excesses of unlabeled ligands (or buffer components) are present in the sample; however, the HMQC-NOESY sequence in particular has the advantage for use with cryogenic probes of not requiring decoupling during acquisition. Figure 7 shows a F1-filtered, F2/F3-edited NOESY-HSQC pulse sequence that uses the gradient-selected sensitivity-enhanced pulse sequence element (Kay, et al., 1992, Palmer, et al., 1991, Schleucher, et al., 1994) for improved ^{13}C editing.

The accuracy of the average Hamiltonian treatment of adiabatic sweeps and the performance of the proposed composite-rotation adiabatic-sweep isotope filter elements were validated using a sample containing [99%- ^{13}C]-labeled Ala, His, and Tyr amino acids (~ 1 mM in 100% D_2O) (Cambridge Isotopes). The reduced scalar coupling constant for the Ala $^1\text{H}^\alpha$ resonance was measured as a function of resonance offset using the pulse sequence in the inset to Figure 8b. The performance of the proposed filter was measured using the sequence in Figure 3b by recording a single-transient free-induction decay immediately following the filter sequence (i.e., no additional isotope-editing was performed after the filter). Weak presaturation was used prior to the first pulse for water suppression. A ^1H - ^1H F1-filtered, F2-edited NOESY spectrum was recorded using a 0.5 mM sample of a 1:1 complex between [U- ^{13}C , U- ^{15}N] VTS SAM domain and natural abundance TCE 13mer RNA (10% D_2O /90% H_2O , pH = 6.25) described elsewhere (Edwards, et al., 2006). The spectrum was recorded using the pulse sequence of Figure 7 by setting $t_2 = 0$. The spectrum was recorded in ~ 18 hours using $(t_1 \times t_2)$ spectral widths of $(6250 \text{ Hz} \times 12500 \text{ Hz})$ as a (175×1024) complex matrix. A total of 128 scans were recorded per complex t_1 point. The NOE mixing time was 250 ms. All experiments were recorded using a Bruker DRX600 NMR spectrometer equipped with a cryogenic triple resonance probe with a z -axis gradient.

Results

Figure 8 illustrates the application of average Hamiltonian theory for treating scalar coupling interactions during adiabatic sweeps. Figure 8a shows the instantaneous values of $\cos[\theta(t)]$ during an adiabatic sweep for three different values of the resonance offset. The value of $\cos[\theta(t)]$ is null at the moment during the adiabatic sweep that the rf field is resonant with the nuclear spin. The value of $\langle\cos\theta\rangle$ is given by the integral of the curves and is equal to zero only for a spin resonant with the rf field at the center of the sweep. Figure 8b shows the reduced scalar coupling constant measured for the Ala $^1\text{H}^\alpha$ resonance as a function of resonance offset from the value of the rf field as the center of the sweep for the spin-echo pulse sequence shown in the inset. Measured values of the reduced coupling constant are shown as solid circles. The line is not a fit to the data; rather, the line is calculated using average Hamiltonian theory and independently determined parameters used for the adiabatic sweep in the pulse sequence element.

Figure 9 shows the degree of suppression achieved by the joint composite-rotation adiabatic-sweep filter in a single scan using the pulse sequence of Fig. 3b (nearly identical results are obtained using the pulse sequence of Fig. 3a). The residual filter breakthrough peaks, even for the His $^1\text{H}^\epsilon$ resonances (8.28 and 8.66 ppm) and Ala $^1\text{H}^\beta$ resonances (1.26 and 1.50 ppm) are < 0.01 (smaller than the centerband resonance peaks for ^1H spins attached to ^{12}C nuclei, resulting from the $\sim 1\%$ of carbon sites that are not isotopically enriched in the 99% labeled amino acids). Improvements compared with the individual composite-rotation isotope filter and the adiabatic-sweep isotope filter are particularly evident for the His $^1\text{H}^\epsilon$ resonances.

Figure 10 displays the region of the ^1H - ^1H F1-filtered, F2-edited NOESY spectrum corresponding to the F1 RNA sugar region and F2 protein methyl region. A number of RNA-protein crosspeaks are visible (along with a ridge of water-to-protein NOE crosspeaks). The residual diagonal resonances, resulting from filter breakthrough, are inverted and approximately are of the same intensity as the stronger NOE crosspeaks.

Discussion and Conclusion

Two distinct approaches for improving the performance of isotope filters, one based on composite-rotations (Stuart, et al., 1999) and the other based on designed adiabatic sweeps (Kupče and Freeman, 1997, Zwaalen, et al., 1997), have been reported previously. Combination of the two approaches yields superior filtration efficiency, compared with methods individually, particularly for ^1H spins with coupling constants that are near the extrema of the range of values or that do not satisfy empirical relationships between isotropic chemical shifts and scalar coupling constants. The latter property makes the new filter sequences particularly useful for molecular complexes that are weakly aligned in anisotropic media (Tjandra and Bax, 1997). In such samples, the residual heteronuclear dipole coupling adds to the heteronuclear scalar coupling constant and renders the approximate relationships between scalar coupling constants and isotropic shifts in Eqs. [22] and [23] less accurate. The joint composite-rotation adiabatic-sweep isotope filter can be incorporated as a building block in a variety of NMR pulse sequences to obtain NMR spectra that are isotope-filtered in one or more frequency domains.

Theoretical optimization and analysis of the isotope filter pulse sequences are simplified by using average Hamiltonian theory to describe evolution of I -spin coherence under the heteronuclear scalar coupling Hamiltonian while an adiabatic sweep is applied to the S spin in an I_nS spin system. The compact results that emerge from average Hamiltonian theory in this context are likely to be applicable to other experimental NMR methods that utilize adiabatic sweeps in heteronuclear scalar-coupled spin systems.

The joint composite-rotation adiabatic-sweep isotope filter is extremely efficient in suppressing coherence from ^1H spins directly attached to ^{13}C nuclei. In all existing isotope filters, increased suppression efficiency inevitably is obtained at cost of increased filter length. Recently optimal control theory has been applied to the design of solution NMR pulse sequences for coherence transfer with optimal relaxation properties (Khaneja, et al., 2004); similar methods may prove fruitful for further developments in J -filtration.

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References

- Bennett AE, Gross JD, Wagner G. *J Magn Reson* 2003;165:59–79. [PubMed: 14568517]
- Breeze AL. *Prog NMR Spectrosc* 2000;36:323–372.
- Edwards TA, Butterwick JA, Zeng L, Gupta YK, Wang X, Wharton RP, Palmer AG, Aggarwal AK. *J Mol Biol* 2006;356:1065–1072. [PubMed: 16405996]
- Evans WAB, Powles JG. *Proc Phys Soc* 1967;92:1046–1054.
- Grzesiek S, Bax A. *J Biomol NMR* 1993;3:185–204. [PubMed: 8477186]
- Haeberlen U, Waugh JS. *Phys Rev* 1968:453–467.
- Hwang TL, Shaka AJ. *J Magn Reson, Ser A* 1995;112:275–279.
- Kay LE, Keifer P, Saarinen T. *J Am Chem Soc* 1992;114:10663–10665.
- Khaneja N, Li JS, Kehlet C, B BL, Glaser SJ. *Proc Natl Acad Sci USA* 2004;101:14742–14747. [PubMed: 15466716]
- Kupče Ě, Freeman R. *J Magn Reson, Ser A* 1995;115:273–276.
- Kupče Ě, Freeman R. *J Magn Reson* 1997;127:36–48.
- Levitt MH. *J Magn Reson* 1982;48:234–264.
- Levitt MH. *Prog NMR Spectrosc* 1986;18:61–122.
- Logan TM, Olejniczak ET, Xu RX, Fesik SW. *J Biomol NMR* 1993;3:225–231. [PubMed: 8477187]
- Marion D, Ikura M, Tschudin R, Bax A. *J Magn Reson* 1989;85:393–399.
- McCoy M, Mueller L. *J Magn Reson, Ser A* 1993;101:122–130.
- Mitschang L, Rinneberg H. *J Chem Phys* 2003;118:5496–5505.
- Otting G, Wüthrich K. *Q Rev Biophys* 1990;23:39–96. [PubMed: 2160666]
- Palmer AG, Cavanagh J, Wright PE, Rance M. *J Magn Reson* 1991;93:151–170.
- Peterson RD, Theimer CA, Wu H, Feigon J. *J Biomol NMR* 2004;28:59–67. [PubMed: 14739639]
- Schleucher J, Schwendinger M, Sattler M, Schmidt P, Schedletzky O, Glaser SJ, Sørensen OW, Griesinger C. *J Biomol NMR* 1994;4:301–306. [PubMed: 8019138]
- Shaka AJ, Barker PB, Freeman R. *J Magn Reson* 1985;64:547–552.
- Stuart AC, Borzilleri KA, Withka JM, Palmer AG. *J Am Chem Soc* 1999;121:5346–5347.
- Tjandra N, Bax A. *Science* 1997;278:1111–1114. [PubMed: 9353189]
- Zwahlen C, Legault P, Vincent SJF, Greenblatt J, Konrat R, Kay LE. *J Am Chem Soc* 1997;119:6711–6721.
- Zwahlen C, Vincent SJF, Kay LE. *J Magn Reson* 1998;130:169–175. [PubMed: 9500889]

Figure 1.

Frequency-modulated interaction frame. (a) An adiabatic sweep is applied to the S spin while an 180° pulse is applied to the I spins at time t_{inv} . (b) The functional form of $a(t)$ is illustrated. (c) The tilted reference frame is shown schematically and the geometrical meanings of the main parameters are illustrated.

Figure 2.

Accuracy of average Hamiltonian theory. Results from average Hamiltonian theory calculated using Eq. [12] for the sequence element of Fig. 1a with $\tau_{inv} = \tau_p/2$ are compared to numerical calculations using the Liouville equation. The sweep used the WURST-20 shape (Kupče and Freeman, 1995) with a sweep bandwidth $\Delta F/2\pi = 60$ kHz (swept from -30 kHz to $+30$ kHz) centered at a ^{13}C frequency of 0 ppm (for a 600 MHz NMR spectrometer). In (a) $\tau_p = 2.359$ ms, $\omega_{1max}/2\pi = 5$ kHz, (b) $\tau_p = 1.0$ ms, $\omega_{1max}/2\pi = 7$ kHz, and (c) $\tau_p = 0.5$ ms, $\omega_{1max}/2\pi = 9.8$ kHz. The adiabaticity factors on-resonance at the center of the sweeps are $Q_0 =$ (a) 6.65 , (b) 5.13 , and (c) 5.03 . The solid line shows results for average Hamiltonian theory and the dashed line shows the exact numerical calculations. (d) The relative error $(\bar{J}_{IS}^{AHT} - \bar{J}_{IS}^0)/\bar{J}_{IS}^0$, in which $\bar{J}_{IS}^{AHT}$ is calculated using using Eq. [12] and $\bar{J}_{IS}^0$ is calculated using the Liouville equation, is plotted for a ^{13}C spin 150 ppm off-resonance from the center of the adiabatic sweep, using a nominal value of $J_{IS} = 175$ Hz and $\Delta F/2\pi = 60$ kHz (swept from -30 kHz to $+30$ kHz). Shown are (dashed thick line) the percent error obtained when Q_0 is fixed at a value of 7.18 and (dashed thin line) $\omega_{1max}\tau_p/2\pi$ is varied and (solid thick line) the percent error obtained when $\omega_{1max}\tau_p/2\pi$ is fixed at a value of 15.5 and (solid thin line) Q_0 is varied.

Figure 3.

Joint composite-rotation adiabatic-sweep isotope filters using (a) 120° *I*-spin rotation and (b) 60° *S*-spin rotation. Both filters are derived from the composite-rotation $\beta_0(2\beta)_2\pi/3$. Narrow and wide black bars represent 90° and 180° pulses, respectively; 180° pulses on the *I* spins can be applied as composite pulses. The narrow open bar represents an (a) 120° or (b) 60° pulse. Wide open bars applied at the ^{13}C frequency represent the two adiabatic sweeps. The direction of the frequency sweep is from low-to-high frequency (methyl-to-aromatic; $m \rightarrow a$) or high-to-low frequency (aromatic-to-methyl; $a \rightarrow m$) as indicated. The center of the frequency sweep is positioned at the center of the desired frequency range (~ 65 ppm for proteins and ~ 110 ppm for RNA). In (a), τ_{inv} is set so that the *I*-spin 180° pulse occurs when the instantaneous frequency is ~ 10 ppm upfield of the *S*-spin resonances (~ 0 ppm for proteins, ~ 50 ppm for RNA). All pulses have x -phase unless otherwise stated. Phase cycles are $\phi_1 = x, -x$; and receiver phase = x . Gradients $G_1 - G_4$ satisfy the relationships (a) $G_2\tau_2 = G_3\tau_3$ and (b) $G_1\tau_1 - G_2\tau_2 + G_3\tau_3 + G_4\tau_4 = 0$, in which G_k and τ_k are the gradient strength and duration of the k th gradient pulse. In (a) the value of τ_a is determined numerically; in (b), $\tau_a = 1/(2J_0) + \tau_p/2$. The delay τ_c is long enough to encompass the 90° pulse with phase ϕ_1 and the gradient G_4 .

Figure 4.

Net I -spin coherence, ε , for isotope filters applied to proteins. (a) Second-order isotope filter with $\varepsilon = \cos^2[\pi J_{IS}/(2J_0)]$ and $J_0 = 144$ Hz. The filter is realized by setting $\tau_d = \tau_f = 1/(2J_0)$ and eliminating the pulse labeled “hg” in Fig. 3b of Kay and coworkers (Zwahlen, et al., 1997). (b) Isotope filter derived from a $\beta_0(2\beta)_{2\pi/3}$ composite rotation with $\varepsilon = \cos^3[\pi J_{IS}/(2J_0)]$ and $J_0 = 144$ Hz (Stuart, et al., 1999). (c) Adiabatic sweep filter taken from Fig. 3c of Kay and coworkers (Zwahlen, et al., 1997). Calculations used $\tau_a = 2.1$ ms, and the WURST-20 adiabatic sweep (Kupče and Freeman, 1995) centered at 0 ppm with $\tau_p = 1.944$ ms, $\Delta F/2\pi = 60$ kHz and $\omega_{1max}/2\pi = 5$ kHz. (d) Joint composite-rotation adiabatic-sweep isotope filter of Fig. 3b with ε given by Eq. [25]. Calculations used $\tau_a = 4.48$ ms and the WURST-20 adiabatic sweep (Kupče and Freeman, 1995) centered at 65 ppm with $\tau_p = 2.0$ ms, $\Delta F/2\pi = 30$ kHz and $\omega_{1max}/2\pi = 5$ kHz. Contour lines are drawn at $\varepsilon = \{0.005, 0.01, 0.02, 0.03, 0.04, 0.06, 0.08, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40\}$. Heavier lines are drawn for $\varepsilon = \{0.01, 0.04\}$.

Figure 5.

Net I -spin coherence, ε , for isotope filtration in RNA. Joint composite-rotation adiabatic-sweep isotope filter of Fig. 3b with ε given by Eq. [25]. Calculations used $\tau_a = 3.69$ ms and the WURST-20 adiabatic sweep (Kupče and Freeman, 1995) centered at 110 ppm with $\tau_p = 1.8$ ms, $\Delta F/2\pi = 20$ kHz and $\omega_{1max}/2\pi = 4.5$ kHz. Contour lines are drawn at $\varepsilon = \{0.005, 0.01, 0.02, 0.03, 0.04, 0.06, 0.08, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40\}$. Heavier lines are drawn for $\varepsilon = \{0.01, 0.04\}$.

Figure 6.

(a) F1/F2-edited, F3-filtered HMQC-NOESY and (b) F1-filtered, F2/F3-edited NOESY-HMQC pulse sequences. Narrow and wide black bars represent 90° and 180° pulses, respectively. The narrow open bar represents a 60° pulse. Wide open bars applied at the ^{13}C frequency represent the two adiabatic sweeps; open bars applied at the ^1H frequency represent water selective rectangular 180° pulses. All pulses have x-phase unless otherwise stated. Phase cycles are $\phi_1 = x, -x$; $\phi_2 = x, x, -x, -x$; $\phi_3 = 8(x), 8(-x)$; $\phi_4 = 4(x), 4(-x)$; $\phi_5 = x$; and receiver phase = $x, -x, -x, x, -x, x, x, -x$. Gradients $G_1 - G_4$ satisfy the relationships $G_1\tau_1 - G_2\tau_2 + G_3\tau_3 + G_4\tau_4 = 0$, in which G_k and τ_k are the gradient strength and duration of the k th gradient pulse. In (a), these gradients also achieve excitation sculpting water suppression (Hwang and Shaka, 1995); in (b), water suppression uses gradients G_6, G_7 , and G_8 , satisfying $G_6\tau_6 - G_7\tau_7 + G_8\tau_8 = 0$. Other gradients are for artifact suppression. Quadrature detection in t_1 and t_2 is achieved by the TPPI-States method (Marion, et al., 1989). (a) Semi-constant-time frequency labeling (Grzesiek and Bax, 1993, Logan, et al., 1993) is used for t_1 by variation of t_{1a} , t_{1b} , and t_{1c} . The initial values of these delays are $t_{1a}(0) = 2\tau$ and $t_{1b}(0) = t_{1c}(0) = 0$. The value of t_{1a} is decremented by Δt_{1a} and t_{1b} , and t_{1c} are incremented by Δt_{1b} and Δt_{1c} , respectively. These values are chosen to satisfy the requirement that the minimum value of t_{1a} must be greater than the duration of G_4 , $1/\text{SW}_1 = \Delta t_{1a} + \Delta t_{1b} + \Delta t_{1c}$, and $\Delta t_{1a} - \Delta t_{1b} +$

$\Delta t_{1c} = 0$, in which SW1 is the spectral width in the t_1 dimension. Decoupling of ^{13}C O spins is accomplished using SEDUCE-1 (McCoy and Mueller, 1993). Decoupling during acquisition uses GARP (Shaka, et al., 1985) (or an adiabatic decoupling procedure). Parameters for the filter element are described in the caption to Fig. 3b. Other delays are $\Delta = 5.4$ ms for isotope filtration of ^{15}N -attached ^1H spins, $2\tau = 3.47$ ms, and ξ is longer than the duration of G8.

Figure 7.

F1-filtered, F2/F3-edited NOESY-HSQC isotope filtered pulse sequence. Narrow and wide black bars represent 90° and 180° pulses, respectively. The narrow open bar is a 60° pulse. Wide open bars are the two adiabatic sweeps. All pulses have x-phase unless otherwise stated. Phase cycles are $\phi_1 = x, -x$; $\phi_2 = x, x, -x, -x$; $\phi_3 = 8(x), 8(-x)$; $\phi_4 = 4(x), 4(-x)$; $\phi_5 = x$; and receiver phase = $x, -x, -x, x, -x, x, x, -x$. Gradients G1 – G4 satisfy the relationships $G_1\tau_1 - G_2\tau_2 + G_3\tau_3 + G_4\tau_4 = 0$, in which G_k and τ_k are the gradient strength and duration of the k th gradient pulse. Gradients G7 and G10 are used for gradient coherence selection; other gradients are for artifact suppression. Quadrature detection in t_1 is achieved by the TPPI-States method (Marion, et al., 1989); echo-antiecho quadrature detection in t_2 is achieved by inverting ϕ_5 and the sign of gradient G7 (Kay, et al., 1992); axial peaks are shifted by inverting ϕ_1 , and the receiver phase. Decoupling during t_2 and t_3 use the SEDUCE-1 (McCoy and Mueller, 1993) and GARP (Shaka, et al., 1985) (or an adiabatic decoupling procedure), respectively. Parameters for the filter element are described in the caption to Fig. 3b. Other delays are $\Delta = 5.4$ ms for isotope filtration of ^{15}N -attached ^1H spins, $\tau = 3.47$ ms, ζ is longer than the duration of G7, and ξ is longer than the duration of G10.

Figure 8.

J-scaling during adiabatic sweep. (a) Plots of $\cos[\theta(t)]$ versus t during an adiabatic sweep. The sweep used the WURST-20 shape (Kupče and Freeman, 1995) with $\tau_p = 2.359$ ms, a sweep bandwidth $\Delta F/2\pi = 60$ kHz (swept from -30 kHz to $+30$ kHz), and $\omega_{1max}/2\pi = 5$ kHz. The solid line shows results for a spin on-resonance with the center of the sweep ($\delta = 0$); the dotted (dashed) line shows results for a spin with resonance offset $\delta/2\pi = -9$ kHz ($+9$ kHz). Values of $\langle \cos\theta \rangle = (3.2 \times 10^{-5}) \Omega$ are linear over the range $\delta/2\pi = -20$ kHz to $+20$ kHz as obtained using Eq. [13]. (b) Experimental validation of the average Hamiltonian expression for the reduced scalar coupling constant. The scalar coupling constant for the Ala $^1\text{H}^\alpha$ resonance was measured as a function of offset from the center of the adiabatic sweep on a 600 MHz NMR spectrometer using the pulse sequence shown in the inset. The adiabatic sweep used the same parameters as in (a). The points give the experimental result and the line gives the calculated (not fitted) values obtained from Eq. [12].

Figure 9.

Isotope filtration efficiency. A sample containing [U- ^{13}C] Ala, [U- ^{13}C] Tyr, and [U- ^{13}C] His amino acids was used to test the isotope filtration performance in a single (one-scan) transient. (a) One-pulse ^1H NMR spectrum; (b) isotope filtered ^1H NMR spectrum using the filter of Fig. 3b; (c) isotope-filtered ^1H NMR spectrum using the filter taken from Fig. 3c of Kay and coworkers (Zwahlen, et al., 1997); and (d) isotope-filtered ^1H NMR spectrum using in the filter described by Stuart and coworkers (Stuart, et al., 1999). In (b) $\tau_a = 4.608$ ms, and the WURST-20 adiabatic sweep (Kupče and Freeman, 1995) was centered at 68 ppm with $\tau_p = 2.115$ ms, $\Delta F/2\pi = 32.5$ kHz and $\omega_{1\max}/2\pi = 5$ kHz. Gradients had the shape of the center lobe of a sine function and a length of 500 μs . Gradient amplitudes were $G1 = 11$ G/cm, $G2 = 26$

G/cm, $G3 = -15$ G/cm, and $G4 = 30$ G/cm. In (c) $\tau_a = 2.1$ ms, and the WURST-20 adiabatic sweeps (Kupče and Freeman, 1995) were centered at 0 ppm with $\tau_p = 1.944$ ms, $\Delta F/2\pi = 60$ kHz and $\omega_{1max}/2\pi = 5$ kHz. The spectrum in (a) is scaled by a factor 0.2. The insets in (b) and (c) shown the region from 8 to 9 ppm expanded vertically by a factor of 3 to better show the His $^1\text{H}^{\varepsilon 1}$ resonances at 8.28 and 8.66 ppm. Resonance peaks for ^1H spins attached to ^{12}C nuclei, resulting from the $\sim 1\%$ of carbon sites that are not isotopically enriched, are labeled with “*” in (b). Buffer contaminants are labeled with “o” in (a).

Figure 10. NOESY-HSQC spectra for VTS SAM domain/TCE RNA complex. The spectrum was acquired using the pulse sequence of Fig. 7 by setting $t_2 = 0$. Filter parameters were identical as described for Fig. 9b. Other experimental details are given in the text.