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Highly Selective Excitation in Biomolecular NMR by Frequency-Switched Single-Transition Cross-Polarization

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The last 15 years have witnessed the development of a host of selective excitation methods in NMR, many of which use shaped pulses to select a chosen line or multiplet.[1] To improve selectivity, it is possible to transfer coherences by combining cross-polarization[2] with selective pulses, a method which was initially applied to homonuclear systems,[3] and later extended to heteronuclear systems[4] by transferring coherence back and forth between a selected proton and a chosen nitrogen-15 or other low-γ nucleus. In this Communication we propose a method for highly selective excitation employing two-fold single-transition cross-polarization, which is spin-state selective, so that coherences are transferred between individual transitions rather than between entire multiplets. The method is capable of exciting signals selectively despite severe overlap and benefits from differential line-narrowing.

Conventional cross-polarization methods, which aim at transferring simultaneously all coherences belonging to a multiplet, are limited by transverse relaxation during the transfer and suffer from limited selectivity since the amplitudes of the radio frequency (RF) fields applied during cross-polarization must be larger than the scalar coupling constant. However, it has recently been shown that coherence transfer can also be achieved by single-transition cross-polarization (ST-CP)[5] if the amplitudes of the RF fields are much weaker than the scalar coupling constant.

During ST-CP, both on- and off-resonance transfer processes occur. The on-resonance transfer occurs between the two irradiated transitions. Thus, if the two RF carriers are set at $\omega(I^-S^\alpha)$ and $\omega(I^\alpha S^-)$, the (proton) coherence I_xS^α, represented by a wavy line on the left-hand side of Figure 1, is transferred into a (nitrogen) coherence $I^\alpha S_x$. This on-resonance transfer is represented by a curved solid arrow. Simultaneously, the proton coherence I_xS^β is converted into nitrogen coherence $I^\beta S_x$ by an off-resonance transfer, represented by a curved dashed arrow in Figure 1. On- and off-resonance transfer processes result in coherences with the same phase for appropriate RF pulse lengths and amplitudes. The process is reversed in a second step after switching the RF carriers to $\omega(I^-S^\beta)$ and $\omega(I^\beta S^-)$. The coherences I_xS^α and $I^\alpha S_x$ are thus first interconverted by on-resonance transfer and subsequently by off-resonance transfer. For the coherences I_xS^β and $I^\beta S_x$ the order is reversed. We shall refer to this process as frequency-switched single-transition cross-polarization (FS-ST-CP).

The subspaces in which the two transfer processes occur will remain separated as long as the amplitudes of the RF fields are weak, $\omega_1{}^I = \omega_1{}^S < |2\pi J_{IS}|$. This allows FS-ST-CP to be remarkably selective. If either forward or backward transfer has vanishing efficiency the signals associated with a square pattern disappear. The transfer of coherence is therefore only effective for a single selected square pattern (i.e. for one $^{15}N-^1H$ group) even if other square patterns have one or two corners in common (Figure 2).

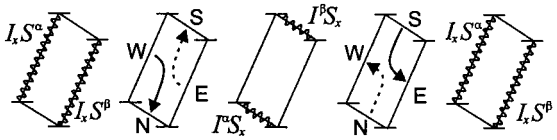

Figure 1. Frequency-switched single-transition cross-polarization (FS-ST-CP). The labels N, W, S, and E (for North, West, South, and East) refer to the positions of the peaks in the $^1H-^{15}N$ heteronuclear single-quantum correlation (HSQC) spectrum (reflecting $\gamma_N<0$). The two proton coherences I_xS^α and I_xS^β (left) are simultaneously converted into two nitrogen coherences $I^\alpha S_x$ and $I^\beta S_x$ (middle) by on- and off-resonance cross-polarization (solid and dashed arrows). The nitrogen coherences are subsequently transferred back to the proton transitions.

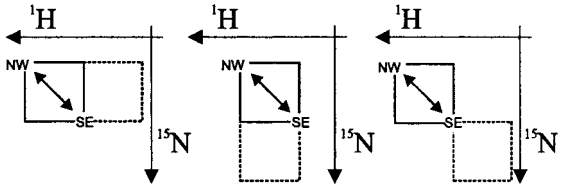

Figure 2. Selection of signals spanning a square pattern due to an $^{15}N-^1H$ spin pair in an undecoupled HSQC spectrum. Even when the SE corner of the selected square is accidentally superimposed with a SW corner of a neighboring square (left), and with a NE corner of another adjoining square (middle), only signals of the selected $^{15}N-^1H$ pair are observed. However, if the SE corner of the chosen square coincides with the NW corner of another square (right), there may be some weak contamination since the SE peak of the second square is at an offset of 2J in both dimensions with respect to the NW corner of the desired square.

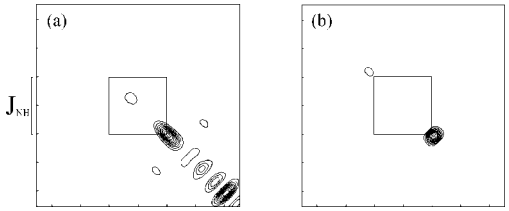

Figure 3. Simulated offset profiles for frequency-switched single-transition cross-polarization representative for a $^{15}N-^1H$ system ($J_{NH} = -90$ Hz) calculated for an experiment with two consecutive coherence transfer steps, as in Figure 4, from ^1H to ^{15}N and back to ^1H. The RF frequencies are positioned (a) on the SE corner of the multiplet for both transfers and (b) on the NW corner for the forward and on the SE corner for the backward transfer with field strengths $\omega_1I/2\pi = \omega_1S/2\pi = 30$ Hz. The lowest contours are 10% of the maximum.

Figure 3 shows a two-dimensional offset profile representative of FS-ST-CP employing an exact integration of the Liouville−von Neumann equation with the complete time-independent Hamiltonian expressed in the doubly rotating frame.[6] The chemical shifts $\omega(I^-)$

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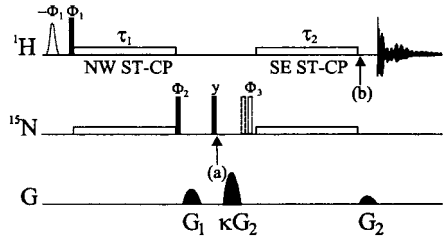

Figure 4. Pulse sequence used to achieve selective excitation of a chosen NH group by FS-ST-CP. To obtain in-phase transfer of both components ($J_{NH} \approx -90$ Hz), the field strengths were typically $\omega_1 I/2\pi = \omega_1 S/2\pi$_= 25 Hz for a transfer time $\tau_1 = \tau_2 = 25$ ms. The phase cycling was $\Phi_1 = y, y, -y, -y$, $\Phi_2 = y, -y, -y, y$, $\Phi_3 = -x$, receiver $\Phi_{rec} = x, -x, x, -x$. All other pulses had an x phase. Pulsed field gradient were 1 ms long. An optional selective pulse on the water resonance can be inserted at the start of the sequence in a water flip-back scheme. For two-dimensional experiments an evolution interval t_1 must be inserted at time point (a). To suppress phase-twisted line shapes in 2D spectra (which come from the quasi-isotropic nature[6] of the transfer process), echo signals were combined with antiechoes, recorded by inverting κ from $-\gamma_I/\gamma_S$ to γ_I/γ_S and Φ_3 from $-x$ to x. A mixing sequence for NOESY or TOCSY may be inserted at time point (b).

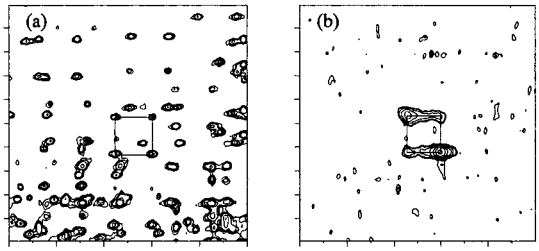

Figure 5. (a) Detail (600 × 600 Hz) from an undecoupled ^{1}H−^{15}N HSQC spectrum of RNase H. (b) The same region employing selective excitation of threonine 34 with the sequence of Figure 4 (the square pattern is centered at 8.1 and 114 ppm.). The number of scans was 128 with 64 t_1 increments. The FS-ST-CP contact times $\tau_1 = 26$ ms and $\tau_2 = 24$ ms were calibrated to yield maximum signal for the SE "TROSY" peak. The lowest contour corresponds to 2% of the maximum intensity.

and $\omega(S^-)$ have been incremented systematically over the full width of the windows in Figure 3 while the scalar-coupling constant was left invariant. The contour plot in Figure 3a corresponds to the efficiency of the excitation scheme described in Figure 4 with a forward transfer from I_xS^β to $I^\beta S_x$ and a backward transfer from $I^\beta S_x$ to I^-S^β without switching the frequencies (two SE transfers). The contour plot in Figure 3b corresponds to the scheme with a frequency switch (FS-ST-CP). Switching the frequencies between the two transfer steps results in a substantial increase in selectivity due to the asymmetry of the offset profiles in the on- and off-resonance subspaces. The complete treatment of the selectivity would require drawing all four observables (the four corners of the square pattern). However, one may remark that the selectivity for the NW peak is almost symmetrical and that the frequency switching leads to very weak efficiency for both off-diagonal responses even for the selected ^{15}N−^{1}H pair (see Figure 5).

Figure 4 shows the pulse sequence used for selective excitation by FS-ST-CP. Efficient water suppression is achieved using two pulsed gradients G_2 and κG_2 that lead to a gradient echo. The efficiency can be improved by about 15% for T34 in *Escherichia coli* RNase H by introducing a selective 90° flip-back pulse on water at the beginning of the sequence. Not only x- and y-, but also z-components of the single-transition coherences are transferred,[6] as can be shown by theory, experiments, and simulations. Since one transfers complex $p = -1$ nitrogen coherence $I^{\alpha,\beta}S^-$ directly into $p = -1$ proton coherence $I^-S^{\alpha,\beta}$, the signals vanish if one chooses gradients with $\kappa = \gamma_I/\gamma_S$ instead of $\kappa = -\gamma_I/\gamma_S$. A nitrogen 180° pulse allows one to obtain an antiecho signal resulting in pure 2D absorption. This pulse can be omitted for 1D spectra.

Figure 5a shows a crowded region of an undecoupled HSQC spectrum of a 0.9 mM sample of ^{15}N-labeled *E. coli* Rnase H in H_2O: D_2O = 9:1 buffered at pH = 5.5 with 100 mM perdeuterated sodium acetate, 1 mM perdeuterated dithiothreitol and 4 mM sodium azide at 303 K and 14 T (600 MHz). The protein was expressed[7] and purified[8] as described elsewhere.

Only signals of the selected ^{15}N−^{1}H pair of threonine 34 appear in Figure 5b. The SE peak is slightly narrower in both dimensions and less attenuated by relaxation during cross-polarization than the NW peak. Indeed, the I_xS^β and $I^\beta S_x$ coherences have longer lifetimes since they benefit from partial cancellation of CSA and dipolar coupling effects.[9] The SE and NW peaks, which have relative heights 3:1, correspond to "TROSY" and "anti-TROSY" peaks, respectively.[10] In very large molecules, only the former will survive. The NE and SW peaks are due to weak "parasitic" transfer.[5]

The overall efficiency of FS-ST-CP, with a two-fold transfer requiring a total of 53 ms, is about 30% for T34 of *E. coli* Rnase H ($M = 17.8$ kDa; $\tau_C = 9.7$ ns at 300 K[7]). This can be determined by comparing the signal amplitudes obtained after a two-fold transfer with those obtained after a four-fold transfer, that is, with the sequence of Figure 4 applied twice in succession. In human ubiquitin ($\tau_C = 4.1$ ns at 300 K[11]), the efficiency is 35% for T14. This is a reasonable price to pay for highly selective excitation in biomolecules.

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