An α/β-HSQC-α/β Experiment for Spin-State Selective Editing of IS Cross Peaks

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A generalized version of the TROSY experiment allows the spin-state selective editing of the four multiplet components of 15N-1H cross peaks of amide groups in proteins into four different subspectra, with no penalty in sensitivity. An improvement by 2 in sensitivity results, if only two of the four multiplet components are selected. Use of the experiment for the measurement of 1JHN coupling constants in sensitivity results, if only two of the four multiplet components are selected. Use of the experiment for the measurement of 1JHN coupling constants in sensitivity results, if only two of the four multiplet components are selected. Use of the experiment for the measurement of 1JHN coupling constants in sensitivity results, if only two of the four multiplet components are selected. Use of the experiment for the measurement of 1JHN coupling constants in sensitivity results, if only two of the four multiplet components are selected.
The phase cycling (Fig. 1) causes the loss of about 1/4/J_{15N} of the signal intensity present in the FIDs as the average relaxation, a sensitivity advantage by \sqrt{2} over the original TROSY experiment, where half of the signal intensity present in the FIDs is removed by phase cycling (6). Furthermore, a second subspectrum containing another component of each cross peak multiplet is obtained from the same data which can be used to measure the J_{15N} coupling constant. If effects from relaxation and long-range couplings are neglected, the sensitivity of the modified experiment is half that of an HSQC spectrum recorded with decoupling in both dimensions and sensitivity enhancement (15, 16).

For experimental verification, spectra were recorded with a 15N/2H labeled domain of _Staphylococcus aureus_ gyrase B, which has a molecular weight of 45 kDa for the unlabeled protein and a rotational correlation time of 23 ns (13). Figure 2A shows an overview of the 15N–1H correlations observed in the \(\beta\)-HSQC-\(\beta\) subspectrum. This multiplet component is narrowed in both dimensions by cross correlation between dipole–dipole and CSA relaxation (6). Figures 2B to E show a comparison of cross-sections through the \(\beta\)-HSQC-\(\beta\) (Fig. 2B) and \(\beta\)-HSQC-\(\alpha\) (Fig. 2C) subspectra, recorded with the pulse sequence of Fig. 1, with the corresponding cross-sections through a spectrum recorded with the same pulse sequence, but using the original TROSY phase cycle (6) (Fig. 2D) and through a conventional FHSQC spectrum recorded with heteronuclear decoupling in both frequency dimensions (13) (Fig. 2E). As expected, the new editing scheme yields the \(\beta\)-HSQC-\(\beta\) subspectrum with the same signal-to-noise ratio as the original TROSY experiment, but in half the time (Fig. 2B and C). The cross peak intensities in the \(\beta\)-HSQC-\(\beta\) subspectrum are, however, on average 15% lower than those observed in a conventional, decoupled FHSQC spectrum recorded without sensitivity enhancement (Fig. 2B and E). Figure 3 shows the four multiplet components of a representative 15N–1H cross peak from the four different subspectra recorded with the scheme of Fig. 1 and Table 2. On average, the peak intensities do not vary more than twofold between the different subspectra. Neglecting relaxation, a sensitivity advantage by \sqrt{2} would be predicted for the FHSQC compared to the \(\beta\)-HSQC-\(\beta\) spectrum. In addition, transverse relaxation during the additional delay 2\(\tau\) (Fig. 1) causes the loss of about 1/\sqrt{2} of the magnetization in the \(\beta\)-HSQC-\(\beta\) spectrum, as the average relaxation time of NH terms in the gyrase domain is about 21 ms (13). While the cross correlation effects at 600 MHz 1H frequency were thus not sufficient to raise the signal heights in the

**TABLE 1**

<table>
<thead>
<tr>
<th>Term no.</th>
<th>(t_1)</th>
<th>90°(H)</th>
<th>2(\tau)</th>
<th>90°(H), 90°(N)</th>
<th>2(\tau)</th>
<th>90°(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>H(_N)(_C)cos((\Omega_t)_cos((\pi_J_t)))</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
<td>H(_x)</td>
<td>H(_N)_x</td>
</tr>
<tr>
<td>II</td>
<td>H(_N)_x(_C)sin((\Omega_t)_cos((\pi_J_t)))</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
</tr>
<tr>
<td>III</td>
<td>N(_C)_x(_C)sin((\pi_J_t))</td>
<td>N(_x)</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
<td>H(_x)</td>
<td>H(_N)_x</td>
</tr>
<tr>
<td>IV</td>
<td>N(_C)_x(_C)cos((\pi_J_t))</td>
<td>N(_x)</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
<td>H(_N)_x</td>
</tr>
</tbody>
</table>

* For \(\tau = 1/(4J)\).
* Trigonometric factors are shown only in this column.
* Depends on \(\tau\) with \(\sin(\pi/2\tau)\).
* Independent of \(\tau\).
* Depends on \(\tau\) with \(\sin^2(\pi/2\tau)\).
TABLE 2

Linear Combinations Yielding the Four Different Subspectra

<table>
<thead>
<tr>
<th>Subspectrum</th>
<th>$^{15}$N–$^1$H cross peak multiplet components$^a$</th>
<th>Linear combinations$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-HSQC−$\beta$</td>
<td>x x</td>
<td>$a = A + B$, $b = A - B$, $a + b_{90}$</td>
</tr>
<tr>
<td></td>
<td>x *</td>
<td></td>
</tr>
<tr>
<td>$\alpha$-HSQC−$\alpha$</td>
<td>* x</td>
<td>$a = A + B$, $b = A - B$, $a - b_{90}$</td>
</tr>
<tr>
<td></td>
<td>x x</td>
<td>$c = C + D$, $d = C - D$, $c + d_{90}$</td>
</tr>
<tr>
<td>$\alpha$-HSQC−$\beta$</td>
<td>x *</td>
<td>$c = C + D$, $d = C - D$, $c - d_{90}$</td>
</tr>
<tr>
<td></td>
<td>x x</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Only the filled multiplet component is observed; crosses identify the location of absent multiplet components. The top right component of the cross peak multiplet is assumed to be high-field (most shielded) in both frequency dimensions (18).

$^b$The data sets A–D are recorded with $\phi_{5,5} = y, x$ (A); $-y, -x$ (B); $-y, x$ (C); $y, -x$ (D) (Fig. 1). The subscript ‘90°’ denotes a 90° phase correction of the respective data set in both dimensions which can be achieved in the time domain, e.g., by exchanging the real and imaginary part of each complex data point and taking the complex conjugate.

$\beta$-HSQC−$\beta$ spectrum above those observed in the decoupled FHSQC spectrum, a $\beta$-HSQC−$\beta$ spectrum recorded at 800 MHz under otherwise identical conditions showed on average 1.1 ± 0.3 times higher signals than the corresponding decoupled FHSQC spectrum, and 3.4 ± 1.0 higher signals than the $\alpha$-HSQC−$\alpha$ spectrum.

For measurements of $^1J_{\text{HC}}$ couplings, the narrowest multiplet components are those which are low-field in the $^{13}$C dimension (5). Four data sets must be recorded, however, to obtain two subspectra at the same $F_1$ chemical shift with the scheme of Fig. 1 (Table 2). Such a protocol still yields spectra with twofold better sensitivity than the use of a double $a/\beta$-half-filter (5), although the sensitivity gap could be reduced by the use of a double $a/\beta$-half-filter with shorter filter delays. The $a/\beta$-HSQC−$a/\beta$ experiment of Fig. 1 would be expected to generate more crosstalk between the different subspectra, when the delay $\tau$ does not perfectly match $1/(4J)$, since the magnitude of the different terms depends differently on $\tau$ (Table 1). Crosstalk would further be expected from different relaxation rates of the terms shown in Table 1, although crosstalk was experimentally found to be unimportant in our $^{15}$N−$^1$H experiments. The most important limitation for measurement of one-bond coupling constants by the $a/\beta$-HSQC−$a/\beta$ experiment may be small phase instabilities arising from the fact that the last 90°($^1$H) pulse cannot be phase cycled without compromising the sensitivity enhancement scheme. Finally, the pulse sequence of Fig. 1 reduces the information content as it suppresses the $^{15}$N−$^1$H cross peaks from NH$_2$ and NH$_3$ groups. On the other hand, this effect is beneficial for the measurement of $^1J_{\text{HN}}$ couplings from cross peaks of backbone amide groups.
which overlap with those from side chain NH$_2$ groups in a conventional HSQC spectrum.

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