Band-Selective Homonuclear
2D Correlation Experiments

Application Note

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Abstract
This application note demonstrates the utility of the band-selective homonuclear 2D correlation experiments available in the VnmrJ 3 software package. When the information to be obtained originates from a given part of the whole spectral region, then the band-selective homonuclear decoupled approach can significantly reduce the experimental time while simplifying the cross-peak structures of the 2D spectra, and hence increasing signal amplitudes. The capabilities of the method, as well as the simplicity of the experimental setup, are illustrated using several 400 MHz 2D NOESY spectra of a brucine sample.
Introduction

Homonuclear 2D correlation experiments are cornerstones of the structure elucidation strategy used by NMR spectroscopists. More often than not, the time requirement of these experiments is determined by the desired resolution in the indirect frequency domain (F1), where each extra data point requires the acquisition of a pair of 1D spectra. It occurs rather frequently that solving a given structural problem does not require the full correlation map, and that a reduced F1 region may provide all necessary spectral information. In the corresponding pulse sequence (see Figure 1), band-selective excitation along the evolution dimension (bashdNOESY) is typically achieved by the double pulse field gradient spin echo (DPFGSE) excitation sculpting (Figure 1, blue box)\(^1\). This technique allows the operator to either significantly reduce the overall experimental time, or by keeping the time constant to increase the F1 resolution correspondingly. When the DPFGSE block is combined with a nonselective refocusing pulse (see red circle in Figure 1) during the evolution period an extra bonus is earned, namely, splittings originated from scalar coupling partners that are not affected by the region-selective refocusing pulses will be eliminated i.e. band-selective homodecoupling is achieved\(^2\). The result is a simplified multiplet structure in F1 and, consequently, increased signal amplitudes. In situations of overlapping signals, where multiplet-selective 1D experiments are not directly applicable, this approach may provide very powerful alternatives. The examples here are from 2D NOESY measurements (Figure 1), but the method can easily be implemented in other homocorrelated 2D experiments (the corresponding TOCSY and ROESY sequences are available in VnmrJ 3) or in any pulse sequence with \(^1\)H chemical shift evolution.

Experimental

Sample

The sample in this study is brucine, a popular test sample in organic NMR, dissolved in CDCl\(_3\). The 400 MHz proton spectrum and its structural formula are displayed in figure 2.

Instrument and experimental conditions

The experiments were run on an Agilent MR-400 DD2 NMR spectrometer operating at 399.86 MHz and equipped with a 5 mm OneNMR probe and a Performa I gradient amplifier (maximum gradient strength ~20 G/cm). Four different phase sensitive NOESY experiments (A-D) were run on the sample. The relevant parameters are summarized in the following table:

<table>
<thead>
<tr>
<th>Spectrum Width (Hz)</th>
<th>Selectivity of the q3 DPFGSE pulses (Hz)</th>
<th>Number of Increments</th>
<th>Acquisition Time (s)</th>
<th>Experiment Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 4006</td>
<td>not applicable</td>
<td>480</td>
<td>0.256</td>
<td>440</td>
</tr>
<tr>
<td>B 408</td>
<td>350</td>
<td>48</td>
<td>0.256</td>
<td>46</td>
</tr>
<tr>
<td>C 92.5</td>
<td>80</td>
<td>12</td>
<td>0.256</td>
<td>13</td>
</tr>
<tr>
<td>D 92.5</td>
<td>80</td>
<td>24</td>
<td>1.02</td>
<td>30</td>
</tr>
</tbody>
</table>
Figure 1. Pulse sequence display and acquisition panel of the band-selective 2D NOESY pulse sequence. The blue square depicts the DPFGSE block used to narrow the F1 excitation band, the orange one depicts the optional zero-quantum suppression element, and the 180 deg. nonselective decoupling pulse is labeled with a red circle.

Figure 2. The 400 MHz $^1$H spectrum of brucine in CDCl$_3$ with the chemical structure of brucine inset.
All other acquisition parameters were kept constant: relaxation delay = 2.5 s, mixing time = 0.6 s, number of repetitions = 8 for each increment, and zero-quantum suppression\(^3\) was used in each experiment. No linear prediction was applied in t1 prior to Fourier transformation. Matching cosine square window functions were applied both in F2 and F1. The conventional NOESY spectrum (spectrum A) is shown in Figure 3.

Figure 3. Conventional (full) 2D NOESY correlation map of brucine at 400 MHz. The total experimental time was 7 h 20 min.
Experimental setup

The bashdNOESY experiment requires a unique set of frequency-selective shaped pulses to be calculated every time the experiment is used. While this might sound like a complicated task, in practice it is a simple operation because the necessary tools are provided in the corresponding panels. The frequency bands for the selective pulses are chosen interactively from the 1D PROTON spectrum and the shaped pulse (including the one for zero-quantum suppression) is then created automatically by clicking the Select button.

1. Collect a PROTON spectrum as a study in the Study Queue.
2. Load the PROTON spectrum into the current workspace, select Continue Study, and then select (HH)bsNOESY from the Experiment Selector (Figure 4).
3. In the Study Queue double click on bashdNOESY, select the requested region from the proton spectrum by placing the cursors on each side of the desired frequency range (in this case from 3.5 to 4.35 ppm) and click the Select button. This step will set the F1 spectral window (sw1) as well.
4. Customize the relevant acquisition parameters: relaxation and mixing time, number of transients and increments. Click the BB homodec during t1 radio button to achieve band-selective homodecoupling in F1.
5. The experiment is now ready to acquire bashdNOESY data. Use the green Save to save the customized parameters then the Submit button in the Study Queue to initiate data collection (Figure 4).

Figure 4. Setting up the region-selective bashdNOESY pulse sequence. After collecting a PROTON spectrum, bashdNOESY is added to the Study Queue.
Results and Discussion

**Band-selective experiment**

Figure 5. shows the band-selective bashdNOESY 2D spectrum (spectrum B) together with the matching expansion of the whole 2D NOESY correlation map (spectrum A). The information content of the two depicted regions, as well as the digital resolution in F1 (see Table 1 in the experimental section), is practically identical but there are two remarkable differences:

1. Spectrum B was run in one tenth of the time (46 min) required for spectrum A (440 min).
2. The band-selective spectrum was run with the homodecoupling option, therefore splittings originating from coupling partners outside the refocused region are eliminated. The result is a more compact multiplet structure in the F1 dimension and correspondingly higher signal amplitudes.

Figure 5. Band-selective 2D bashdNOESY spectrum (B, run in 46 min) together with the matching expansion of the whole 2D NOESY correlation map (A, run in 440 min). Though the two spectra have identical data density in F1, spectrum B shows narrower cross-peaks in F1 due to homonuclear decoupling.

Figure 6. Setting up the multiplet-selective bashdNOESY experiment. After collecting a PROTON spectrum, bashdNOESY is added to the Study Queue. The two cursors select a very narrow spectral region covering only the three overlapping protons (H11α, H14 and H18α) between 2.96 and 3.15 ppm. When customization is complete, the experiment is ready to be submitted.
Multiplet-selective experiment in case of signal overlap

The F1 frequency region involved in the DPFGSE step can be as narrow as the width of a proton multiplet. This, of course, would not justify running a 2D experiment – there is an appropriate selective NOESY1D sequence available for this purpose. The situation, however, is different in the case of unresolved multiplets like protons H11a, H14, and H18a in the brucine spectrum (3.04-3.1 ppm). Direct selective excitation here is not applicable, therefore using the stepNOESY pulse sequence or chemical shift-selective filters might provide alternatives. The former includes a TOCSY step and requires a stand-alone coupling partner, while the latter is based on the knowledge or best guess of the chemical shift difference of the overlapping signals, and is really complicated if more than two protons are involved.

If the overlapping protons are not coupled to each other, then the bashdNOESY sequence is the most convenient solution. The experimental setup is shown in figure 6 and the 2D spectra in figure 7. Since the F1 spectral window is very narrow, only a few increments (12 in spectrum C and 24 in spectrum D) are necessary to achieve full signal separation in reasonably short times. Due to the complete F1 decoupling, the NOE correlations of the three overlapping protons are completely uncoupled and the relevant traces from spectrum D (see Figure 8) show no cross-contamination.

Figure 7. Multiplet-selective 2D bashdNOESY spectra (C and D) together with the matching expansion of the whole 2D NOESY correlation map (A). The high-resolution spectrum (D) clearly shows singlet structures and hence complete signal separation in the F1 dimension. (The vertical insets are from the high resolution 1H spectrum where the multiplet structure is retained.)

Figure 8. High resolution proton spectrum of brucine together with two NOESY traces from spectrum D (see Figure 7.) Note that due to broadband homodecoupling in F1, the NOE correlations from protons H11a and H14 are fully separated though the same protons are unresolved in the high resolution 1H spectrum.
Conclusions

The band-selective bashdNOESY pulse sequence and its close relatives, bashdROESY and bashdTOCSY, in VNMRJ3 provide very powerful tools for structure elucidation. When only partial information is required they offer significant time saving and simplified F1 multiplet structure against their nonselective 2D equivalent. They are particularly handy for the spectroscopists when correlations of overlapping signals are to be identified.

References


