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A general method for diagonal peak suppression in homonuclear correlated NMR spectra by spatially and frequency selective pulses ☆

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ABSTRACT

Homonuclear two- and multidimensional NMR spectra are standard experiments for the structure determination of small to medium-sized molecules. In the large majority of homonuclear correlated spectra the diagonal contains the most intense peaks. Cross-peaks near the diagonal could overlap with huge tails of diagonal peaks and can therefore be easily overlooked. Here we present a general method for the suppression of peaks along the diagonal in homonuclear correlated spectra. It is based on a spatially selective excitation followed by the suppression of magnetization which has not changed the frequency during the mixing process. In addition to the auto correlation removal, these experiments are also less affected by magnetic field inhomogeneities due to the slice selective excitation, which on the other side leads to a reduced intensity compared to regular homonuclear correlated spectra.

1. Introduction

In a typical 2D homonuclear correlated spectrum the diagonal contains the most intense peaks, although all the relevant information is contained in the cross peaks. These intense signals can obscure nearby cross peaks. Furthermore, the diagonal is often responsible for the so called t_1-noise, artifacts along the indirect dimension. Intense diagonal peaks also limit the dynamic range of the spectrometer, leading to a lower sensitivity of low intensity signals. The stronger the diagonal peaks in relation to the cross peaks are, the bigger are the problems they cause. In particular, NOESY-type spectra, where the intensity ratio of diagonal versus cross peaks is quite extreme, often suffer from strong diagonal peak artifacts which can easily obscure nearby cross peaks.

Several different strategies for diagonal peak suppression have been reported in the literature. The first approach is based on suppressing diagonal peaks by recording two spectra, a regular 2D spectrum and one containing only the diagonal [1,2]. The latter is obtained by setting the mixing time to zero. Subtraction of the diagonal-only spectrum from the regular one provides a diagonal-free spectrum. However, this approach only works if there is no significant relaxation during the mixing time and does not alleviate the t_1-noise or dynamic range problem since one still has to record datasets with a diagonal. In addition, by using this technique, the acquisition of two different comparable spectra requires a high accuracy of the parameter settings. Otherwise subtraction artifacts will lead to insufficient suppression of the diagonal [2–4]. The second method destroys the magnetization of the excited nucleus by a defocus, mixing, refocus sequence [5]. The mixing period is implemented between two 90° pulses. The magnetization of the excited nucleus, which has not been transferred during the mixing period, undergoes a 180° rotation. A last 90° pulse transfers this magnetization into the z-direction leading to no visible signal of the diagonal in the spectrum. This method leads to an unusual appearance of the 2D spectra, showing cross peaks on diagonals with a slope $\Delta\omega_1/\Delta\omega_2 = 2$. Another method, which has been used to suppress diagonal peaks in a NOESY spectrum uses a combination of two jump-and-return sequences before and after the mixing and a pulsed field gradient to suppress magnetization that evolved with the same frequencies before and after mixing [6]. By this approach the signal intensities in the 2D spectrum are modulated by a sheared sinusoidal profile with zero intensity on the diagonal as a result of the jump-and-return sequences. For multidimensional (3D and 4D) ^{15}N-edited NOESY-type spectra the suppression of diagonal peaks has also been described by selecting only magnetization transfer pathways where the spin-state has been changed. This approach, which allows the observation of cross peaks underneath the diagonal, only works on TROSY-type spectra on proteins and for ^{15}N-bound protons [7–13]. Especially for 3- and 4D NOESY type spectra diagonal peak suppression is very convenient as it makes the use of sparse data sampling techniques much easier due to a significant reduction of the spectral dynamic range [10,11].

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2. Theory and methods

Here we present a completely different, generally applicable, approach for diagonal peak suppression in homonuclear two- and multidimensional spectra, which is based on transforming a homonuclear system into a spatially-separated heteronuclear system by using frequency-selective pulses during a weak field gradient [14–20]. To obtain a diagonal peak suppressed homonuclear 2D spectrum we use the pulse sequences shown in Fig. 1. A selective 90° pulse during a weak gradient excites different signals in different slices of the NMR sample tube. After the mixing period (shown for TOCSY and NOESY type spectra) the excited signals that did not change their frequency significantly during mixing (i.e. the diagonal peak signals but also any underlying or very close-by cross peaks) can be suppressed by using any signal/solvent suppression scheme, when applied during the same weak gradient field. For this purpose we used an excitation sculpting scheme (a combination of a hard and a selective 180° pulse sandwiched by two strong gradients) [21]. To increase the efficiency of the diagonal suppression this element was repeated with different purging gradient strength.

The method of spatially dependent selective spin excitation in solution NMR has been used previously, for example for homonuclear broadband decoupling [14–18,20]. Because of the weak field gradient, the resonance frequencies of the NMR signals are shifted, depending on the position in the sample. The range of frequency shifts of these signals is given by

$$\Delta\omega = sG\gamma \tag{1}$$

where G is the strength of the gradient, γ is the gyromagnetic ratio and s is the sample length to be measured, in our case about 1 cm. Therefore, if we want to use a selective pulse to excite a range of 10 ppm of a proton spectrum on a 500 MHz spectrometer we need at least a gradient strength of 1.2 G/cm. The spatial dependence of the resonance frequencies is shown in Fig. 2.

For a better understanding we illustrate the presented method by a hypothetical molecule. The molecule has three protons with

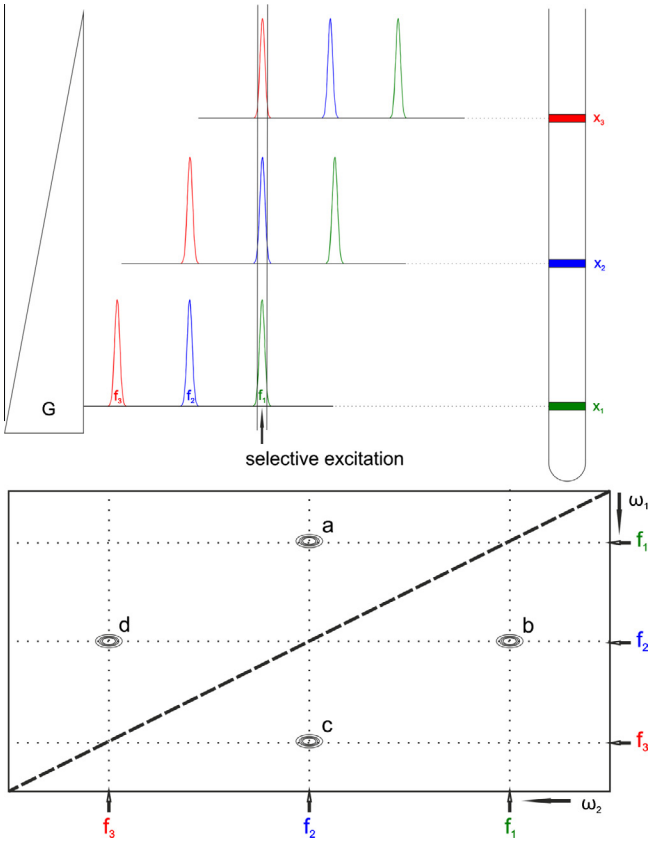

Fig. 2. Top panel: The principle of the selective excitation during a weak field gradient: Small, continuous, variations of the magnetic field lead to a shifting of the spectrum. A selective pulse applied in the middle leads to the excitation of all resonances, but each signal is irradiated in a different slice of the sample tube. Bottom panel: A schematic example of a diagonal suppressed homonuclear 2D spectrum, where proton 2 correlates with proton 1 and 3, but 1 not directly with 3. Auto-correlation (diagonal) peaks are suppressed by using an excitation sculpting block on the signals that were originally, selectively excited in the same slice.

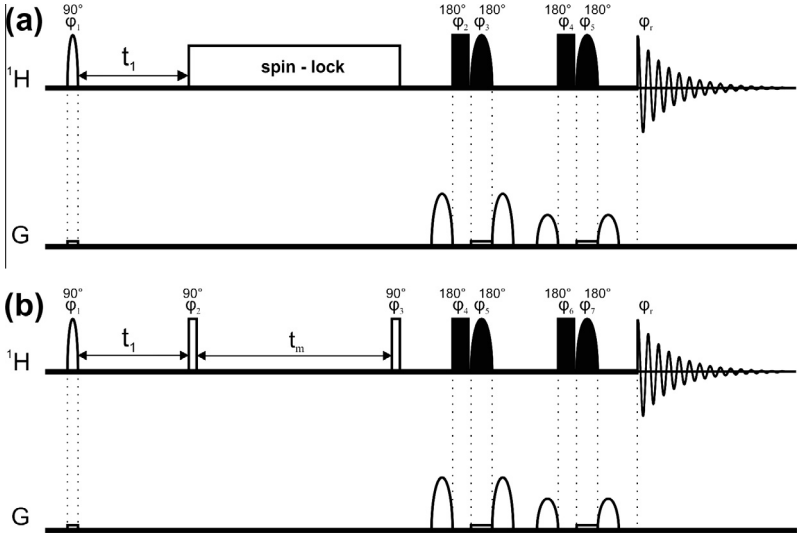

Fig. 1. The pulse sequence of a diagonal suppressed TOCSY experiment is shown in (a). The slice-selective excitation is achieved by a 40 ms EBURP-2 pulse, applied during a weak magnetic field gradient (~1 G/cm). After the evolution and TOCSY mixing, for which we used a 12.5 kHz DIPSI-2 sequence at 500 MHz, the originally excited magnetization, which would produce the diagonal peaks is suppressed by two consecutive excitation sculpting blocks. The latter contain 4 ms rectangular shaped selective 180° pulses during a field gradient which has the same strength of the one used during initial excitation. The following phase cycling was used for (a): $\varphi_1 = x, -x$; $\varphi_2 = x, x, y, y$; $\varphi_3 = -x, -x, -y, -y$; $\varphi_4 = x, x, x, x, y, y, y, y$; $\varphi_5 = -x, -x, -x, -x, -y, -y, -y, -y$; $\varphi_r = x, -x, -x, x, -x, x, x, -x$. The pulse sequence of a diagonal suppressed NOESY experiment is shown in (b). The selective 90° and 180° pulses are the same as in a. The phase cycle for (b) is: $\varphi_1 = x, x, -x, -x$; $\varphi_2 = x$; $\varphi_3 = x, x, x, x, -x, -x, -x, -x, y, y, y, y, -y, -y, -y, -y$; $\varphi_4 = \varphi_6 = x, x, x, x, y, y, y, y$; $\varphi_5 = -x, -x, -x, -x, -y, -y, -y, -y$; $\varphi_7 = x, y$; $\varphi_r = x, -x, -x, x, x, -x, -x, x, y, -y, -y, y, y, -y, -y, y$. 90° and 180° pulses are indicated by white and black bars, respectively.