NOAH – NMR Supersequences for Small Molecule Analysis and Structure Elucidation

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Introduction

The structure characterization of small molecules by NMR spectroscopy nowadays largely follows well established protocols that are reliant on a core set of 2D correlation experiments that includes COSY, TOCSY, NOESY/ROESY, HSQC, HMQC, and HMBC sequences, or variants of these [1]. Having established themselves as the primary techniques, much focus has now turned to developing experimental methods that allow the faster collection of these data sets, often exploiting the improved sensitivity afforded by modern instrument developments, including cryogenic probes. Improvements in instrument capabilities have led to the introduction of parallel acquisition NMR (PANSY) [2] using multiple receivers allowing to establish molecular structure of small organic molecules from just a single experiment, (PANACEA) [3].

Here we show that as many as five conventional NMR pulse sequences based on 1H direct detection can be combined into a single supersequence. This approach offers significant time savings and increases the efficiency of NMR experiments as compared to conventional data recording since only a single recovery (relaxation) delay (d₁) is employed in the combined pulse sequences.

The Idea

The proposed technique is outlined schematically in Fig. 1a and exploits the concept of polarization sharing (ASAP) [4]. The notion of nested sequences was previously introduced long ago with the COCONOSY experiment which yielded separate 2D COSY and NOESY spectra from the same experiment [5]. In this work we further exploit these techniques by recording multiple two-dimensional data sets per measurement (see Fig. 1), with each designed to optimally utilise coherences from the various reservoirs of proton magnetization within a molecule. We term this concept NOAH (NMR by Ordered Acquisition using 1H-detection). We suggest that hundreds of such combinations are possible [6]. By analogy to the nested phase cycles that are commonly known as supercycles we call such nested pulse sequences NMR supersequences. We illustrate the concept with one of several possible implementations of the NOAH-4 supersequences (Fig. 1b). Having a single recovery delay for all four sequences in this NOAH-4 supersequence greatly reduces the experiment duration and improves the efficiency of precious NMR system usage. Thus all four 2D spectra in this version of the NOAH-4 experiment are recorded starting from a single recovery delay, d₁(Fig. 2).
Pulse Sequence

Fig. 1. a) A schematic representation of the nested (NOAH) supersequences. Only a single recovery delay, d₁, is employed for up to N=5 nested sequences leading to significant time savings; b) The NOAH-4 (MSCN) pulse sequence combining 2D ¹⁵N HMQC, 2D ¹³C HSQC, 2D ¹H-¹H COSY and 2D ¹H-¹H NOESY experiments [6]. The polarity of gradient pulses, g₁, g₄ and all receiver phases are inverted for all even increments. The 180 degree ¹³C pulses are constant adiabaticity WURST pulses.

Fig. 2. A schematic representation of the NOAH-4 supersequence, MSCN (a) and the 2D spectra recorded in a single experiment, (b) ¹H-¹5N HMQC, (c) multiplicity edited ¹H-¹3C HSQC, (d) ¹H-¹3C HMBC, (e) ¹H-¹H COSY and (f) ¹H-¹H NOESY. The sample is 50 mM gramicidin S in DMSO-d₆. The spectra were recorded on an AVANCE III spectrometer equipped with a TCI Cryoprobe.

The second example demonstrates one possibility of the NOAH-5 supersequence, MSBCN that combines ¹H-¹⁵N HMQC (M), multiplicity edited ¹H-¹³C HSQC (S), ¹H-¹³C HMBC (B), COSY (C) and NOESY (N) pulse sequences. The experiment is similar to the NOAH-4 supersequence, MSCN except the HMBC module is incorporated between the HSQC and COSY modules akin to the “afterglow” technique [3]. This NOAH-5 supersequence, produces five 2D spectra in one measurement (Fig. 3).

Fig. 3. Schematic representation of the NOAH-5 MSBCN supersequence (a) and the 2D spectra recorded in a single experiment, (b) ¹H-¹5N HMQC, (c) multiplicity edited ¹H-¹³C HSQC, (d) ¹H-¹³C HMBC, (e) ¹H-¹H COSY and (f) ¹H-¹H NOESY. The sample is 50 mM cyclosporine in benzene-d₆.

Molecular structure from a single NMR supersequence

In the vast majority of cases the structures of small organic molecules can be established from three basic 2D NMR experiments – ¹H-¹5C HSQC, ¹H-¹³C HMBC and ¹H-¹H COSY. These spectra can be obtained significantly faster by combining the required experiments into a single NOAH NMR supersequence. There are several possible variants of such NOAH supersequences. In the NOAH-3 BSC experiment combining ZZ-HMBC [7], HSQC and ASAP-COSY [7] sequences the partially recovered magnetization (Mz) employed for the COSY module is subject to differences in the T₁ relaxation rates of individual protons. In order to reduce the variations in cross-peak intensity across the COSY spectrum, a short spinlock may be applied in analogy with the hetero-nuclear ASAP experiments (Fig. 4a). Accordingly, we call this module ASAP-COSY.
**ASAP-COSY [7]**

The COSY peak intensities become significantly distorted as measured immediately after the ZZ-HMBC and HSQC modules. Following a short spinlock of just 40 ms the relative intensities are largely restored (see SI in ref. [7]). The artifact suppression effect of the short spinlock period is demonstrated in Fig. 4.

**Figure 4**

Fig. 4. a) The ASAP-COSY pulse sequence; phases: \( \Phi_1 = \pi, -\pi \); rec = \( \pi, -\pi \); the adiabatic spinlock, SL was applied using 1 ms long adiabatic WURST-2 pulses with \( Q = 3 \); \( g_0 \) are spoiler gradients, \( g_1 = g_2 \) are coherence selection gradients; b) the conventional 2D COSY spectrum of pamoic acid (d) in DMSO-d6 recorded with the recovery delay, \( d_1 = 0.2 \) s, \( t_2 = 256 \) ms; c) the ASAP-COSY spectrum recorded with identical experimental conditions and SL = 40 ms.

In the first example, the structure of gibberellic acid is solved correctly from the NOAH-3 BSC data (Fig. 5). In the second example (not shown) the structure of 1,2:3,4-O-isopropylidene-D-galacto-pyranose is determined correctly from the spectra recorded using the NOAH-4 BSCN supersequence obtained by appending the NOESY module to the NOAH-3 BSC supersequence [7]. The NOESY module provides additional, stereospecific spatial information about the orientation of the four Me-groups in the molecule that is not available from other data e.g. the chemical shifts or \( ^3J_{HH} \) couplings.
References