NMR Spectroscopy

International Edition: DOI: 10.1002/anie.201705506
German Edition: DOI: 10.1002/ange.201705506

NOAH: NMR Supersequences for Small Molecule Analysis and Structure Elucidation

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Abstract: *Nested NMR experiments combining up to five conventional NMR pulse sequences into one supersequence are introduced. The core 2D NMR techniques routinely employed in small molecule NMR spectroscopy, such as HSQC, HMQC, HMBC, COSY, NOESY, TOCSY, and similar, can be recorded in a single measurement. In this way the data collection time may be dramatically reduced and sample throughput increased for basic NMR applications, such as structure elucidation and verification in synthetic, medicinal, and natural product chemistry.*

Nowadays, the structure characterization of small molecules by NMR spectroscopy largely follows well-established protocols that are reliant on a core set of 2D correlation experiments that includes correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), nuclear Overhauser effect spectroscopy (NOESY)/ rotating-frame Overhauser effect spectroscopy (ROESY), heteronuclear single quantum correlation (HSQC), heteronuclear multiple quantum correlation (HMQC), and heteronuclear multiple bond correlation (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. These approaches can be broadly classified as utilizing enhanced instrument hardware capabilities, novel pulse sequence design, modified data sampling schemes, or combinations of these. Improvements in instrument capabilities have provided for "ultrafast" spectroscopy,[2] which exploits the spatial encoding of NMR responses to provide a complete correlation data set in a single scan. These have also led to the introduction of parallel acquisition NMR (PANSY)[3] using multiple receivers for the simultaneous detection of multiple nuclei and hence multiple correlation spectra. It has been demonstrated that the molecular structures of small organic molecules can be established from just a single sensitivity demanding experiment (PANACEA)[4] using multiple receivers. The use of interleaved acquisition allows spectra to be recorded from several uncoupled nuclear species in a similar fashion.[5] However, ultrafast and multi-receiver approaches remain uncommon to date, in part because of specific hardware requirements and low sensitivity.

Herein, we show that as many as five conventional NMR pulse sequences based on ^{1}H direct detection can be combined into a single supersequence that requires only a single receiver. 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 (d_1) is employed in the combined pulse sequences.

The proposed technique is outlined schematically in Figure 1a and combines several previously introduced approaches, as discussed in detail in the proceeding text. Specifically tailored pulse sequences have been developed previously to reduce the duration of data acquisition by minimizing the recovery delay between repeated transients, as exemplified in the acceleration by sharing adjacent polarization (ASAP) scheme.[6] This exploits the concept of polarization sharing in which a stored reservoir of magnetization of ^{12}C-bound passive protons is used to replenish the magnetization of the ^{13}C-bound active protons that are used to generate a ^{1}H-^{13}C heteronuclear correlation spectrum. This may be achieved either through the use of NOE transfer[7] or a short (< 50 ms) homonuclear isotropic mixing scheme[6] to pass magnetization from the passive reservoir to the active protons. This short mixing period is used in place of the conventional recovery delay (d_1, typically at least 1 s), in this way reducing the time required to record a heteronuclear correlation spectrum significantly.

Herein, we further exploit the concept of tailored polarization storage by recording multiple 2D data sets nested within a single experiment for greatly accelerated data collection. This utilizes multiple free induction decay (FID) acquisitions per measurement (Figure 1), with each designed to optimally utilize coherences from the various reservoirs of proton magnetization within a molecule. The sequences presented incorporate multiple heteronuclear and homonuclear correlations with each acquisition based on ^{1}H detection to provide optimum sensitivity. We term this concept NOAH (**N**MR by **O**rdered **A**cquisition using ^{1}**H**-detection).

The notion of nested sequences was previously introduced long ago with the combined COSY/NOESY (COCONOSY) experiment,[8] which yielded separate 2D COSY and NOESY spectra from the same experiment. Therein, both 2D experiments share the same recovery delay (d_1) and evolution

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Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.201705506.

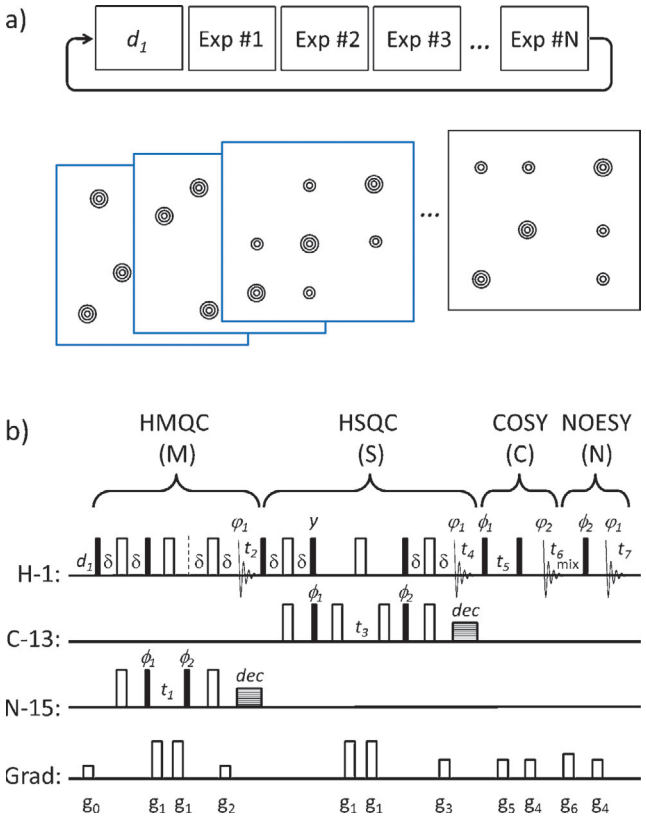

Figure 1. a) A representation of the nested (NOAH) supersequences. Only a single recovery delay (d_1) was employed for up to N = 5 nested sequences leading to significant time savings. b) The NOAH-4 (MSCN) pulse sequence combines 2D ^{15}N HMQC, 2D ^{13}C HSQC (multiplicity editing optional; Supporting Information, Figure S1a), 2D ^{1}H-^{1}H COSY, and 2D ^{1}H-^{1}H NOESY experiments. All pulses were applied with phase *x* unless indicated otherwise (filled = 90°, open = 180° pulses). Key: phase cycles ($\phi_1 = x,-x$, $\phi_2 = x,x,-x,-x$); receiver phases ($\varphi_1 = x,-x,-x,x$, $\varphi_2 = x,-x$); *J*-evolution delays set to $1/4J_{NH}$ and $1/4J_{CH}$ in the HMQC and HSQC modules, respectively (δ delays); NOESY mixing delay (*mix*); common recovery delay (d_1). Gradients [ms, G cm^{-1}]: g_0 = (1,7), g_1 = (1,40), g_2 = (1,8.1), g_3 = (1,20.1), g_4 = g_5 (1,20), g_6 = (1,17). The polarity of gradient pulses g_1 and g_4, and all receiver phases, are inverted for all even increments. The 180° ^{13}C pulses are constant adiabaticity WURST (wideband, uniform rate, smooth truncation) pulses.

period (t_1) with the COSY acquisition occurring within the NOESY mixing time, thus providing the two experiments in the time required for NOESY alone. Similar experiments for recording two spectra simultaneously have been proposed in labeled protein samples in liquids[9] and solids.[10] These should be distinguished from time-shared experiments[11] that collect the same type of spectra from different nuclear species—usually ^{15}N and ^{13}C—and require doubling the number of scans for each additional spectrum to allow for phase encoding.

With the NOAH sequences, we demonstrate the nesting of up to five 2D correlation experiments (NOAH modules); including various combinations of the established methods for small-molecule characterization mentioned earlier. We suggest that hundreds of such combinations are possible (Supporting Information, Table S1). 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 the several possible implementations of the NOAH-4 supersequences (Figure 1b). The pulse sequence starts with the recovery delay (d_1), which is typically the longest pulse sequence event by far. 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. This is achieved by preserving the coherences of interest largely undisturbed for the consecutive modules throughout the supersequence. Following the recovery delay (d_1) the experiment begins with the least sensitive module, ^{1}H-^{15}N HMQC. As for the ASAP HMQC experiment,[6] this module ensures that the bulk magnetization of protons that are not directly bound to ^{15}N is preserved for the subsequent NOAH sequence modules by keeping it along the $+z$ axis. Only 0.37 % of the total proton magnetization is used for this HMQC module. This is followed by the ^{13}C HSQC pulse sequence where the phase cycle and gradients are arranged in such a way as to keep the magnetization of protons that are not directly coupled to ^{13}C undisturbed along the $+z$ axis. A further 1.1 % of the total ^{1}H magnetization is used by this module. The remaining ^{1}H magnetization is then split equally for recording the two ^{1}H-^{1}H correlated experiments COSY and NOESY. These two modules are incorporated into the NOAH-4 supersequence according to the COCONOSY Scheme.[8] At the end of the t_5 (COSY t_1) evolution period a 90° proton read pulse transfers the frequency encoded magnetization to the coupled proton sites. Following this read pulse half of the magnetization is stored along the *z* axis while the other half is refocused by the decoding gradient and observed during the free induction decay (t_6), providing the 2D COSY spectrum. The COSY acquisition period (t_6) is incorporated into the NOESY mixing period. Both the COSY and NOESY experiments in this NOAH-4 pulse sequence share the same t_1 evolution period (t_5). The COSY acquisition period has a delay (*mix*) appended to meet the requirements of the total duration of the NOESY mixing period (t_6 + *mix*). The NOESY module ends with a read pulse and a decoding gradient before the 2D NOESY spectrum is acquired (t_7). Thus, all four 2D spectra in this version of the NOAH-4 experiment are recorded starting from a single d_1 recovery delay.

The NOAH-4 supersequence, MSCN, produces four 2D spectra in one measurement; namely, ^{1}H-^{15}N H**M**QC (M), ^{1}H-^{13}C H**S**QC (S) with optional multiplicity editing, **C**OSY (C), and **N**OESY (N), where highlighted single letter abbreviations are used to identify each module indicated in parentheses (Figure 2). Considering that in most conventional experiments, and in small molecule experiments in particular, the recovery delay (d_1) is typically the most time-consuming part of the experiment by far, the time savings provided by the NOAH supersequences are substantial and increase as more modules are combined into a single supersequence (for a comparison with conventionally recorded data see the Supporting Information, Figures S2 and S3).

The second example demonstrates one possibility of the NOAH-5 supersequence; namely MSBCN, which combines

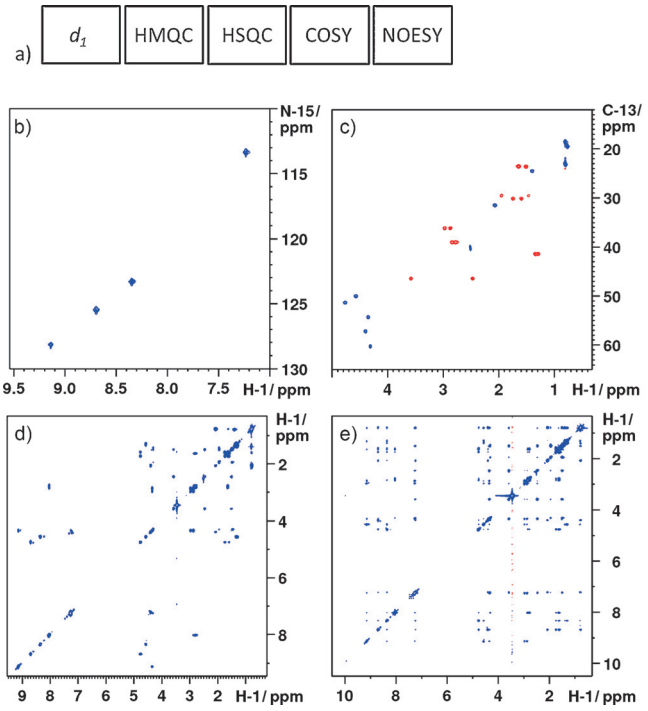

Figure 2. a) A representation of the NOAH-4 supersequence, MSCN. The 2D spectra recorded in a single experiment; b) ^{1}H-^{15}N HMQC, c) multiplicity edited ^{1}H-^{13}C HSQC, d) ^{1}H-^{1}H COSY, e) ^{1}H-^{1}H NOESY. The sample is 50 mM gramicidin S in [D_6]DMSO (dimethyl sulfoxide). The spectra were recorded on an AVANCE III spectrometer equipped with a TCI CryoProbe in 38 min and 26 s with 2 scans per increment and 512 t_1-increments per module resulting in a 2k×2k raw data matrix. Further experimental details and a comparison between NOAH and conventional data are provided in the Supporting Information.

Table 1: Selected examples of useful combinations of NOAH 2D supersequences. Rows in bold indicate those utilizing three radiofrequency (rf) channels (^{1}H/^{13}C/^{15}N); all others require only two (^{1}H/X). Over 250 further supersequence combinations are listed in the Supporting Information.

No.	Exp 1	Exp 2	Exp 3	Exp 4	NOAH code
1	^{13}C HSQC	COSY	–	–	SC
2	^{13}C HSQC	NOESY	–	–	SN
3	^{13}C HSQC	TOCSY	–	–	ST
4	^{13}C HSQC	DQFCOSY	–	–	SD
5	^{13}C HSQC	^{13}C HMBC	–	–	SB
6[a]	^{13}C HMQC	COSY	–	–	MC
7	^{13}C HMQC	NOESY	–	–	MN
8	^{13}C HMQC	TOCSY	–	–	MT
9[b]	^{13}C HMQC	^{13}C HMBC	–	–	MB
10	^{13}C HMQC	NOESY	–	–	MN
11	COSY	NOESY	–	–	CN
12	**^{15}N HMQC**	**^{13}C HSQC**	**COSY**	**–**	**MSC**
13	**^{15}N HMQC**	**^{13}C HSQC**	**NOESY**	**–**	**MSN**
14	**^{15}N HMQC**	**^{13}C HSQC**	**TOCSY**	**–**	**MST**
15	**^{15}N HMQC**	**^{13}C HSQC**	**DQFCOSY**	**–**	**MSD**
16	**^{15}N HMQC**	**^{13}C HSQC**	**^{13}C HMBC**	**–**	**MSB**
17	**^{15}N HMQC**	**COSY**	**NOESY**	**–**	**MCN**
18	^{13}C HMQC	^{13}C HMBC	COSY	–	MBC
19	^{13}C HMQC	^{13}C HMBC	DQFCOSY	–	MBD
20	^{13}C HSQC	COSY	NOESY	–	SCN
21	^{13}C HSQC	^{13}C HMBC	COSY	–	SBC
22	^{13}C HSQC	^{13}C HMBC	DQFCOSY	–	SBD
23	**^{15}N HMQC**	**^{13}C HSQC**	**COSY**	**NOESY**	**MSCN**
24	**^{15}N HMQC**	**^{13}C HSQC**	**^{13}C HMBC**	**COSY**	**MSBC**

[a] Supporting Information, Figure S9; [b] Supporting Information, Figure S10. 2D NMR experiment key: H**S**QC (S), H**M**QC (M), HM**B**C (B), **C**OSY (C), **D**QF-COSY (D), **T**OCSY (T), and **N**OESY (N).

^{1}H-^{15}N H**M**QC (M), multiplicity edited ^{1}H-^{13}C H**S**QC (S), ^{1}H-^{13}C HM**B**C (B), **C**OSY (C), and **N**OESY (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. This NOAH-5 supersequence (Supporting Information, Figure S1b) produces five 2D spectra in one experiment (Figure 3) delivering all the information that is required for small molecule structure elucidation in a single measurement. The technical aspects of this pulse sequence are described in more detail in the Supporting Information.

Further examples of NOAH style supersequences for applications to samples at natural isotopic abundance are listed in Table 1 and in Table S1 (Supporting Information) and illustrative spectra are provided in Figures S2–S11 (Supporting Information). These include (but are not in any way limited to) seven of the most frequently used 2D NMR experiments: H**S**QC (S), H**M**QC (M), HM**B**C (B), **C**OSY (C), double quantum filtered (**D**QF)-COSY (D), **T**OCSY (T), and **N**OESY (N). The efficiency of the NOAH type supersequences can be further improved by combining these experiments with other fast techniques, such as non-uniform sampling,[12] Hadamard spectroscopy,[13] projection spectroscopy,[14] use of multiple receivers,[3] and similar. The utility of the NOAH supersequences has been successfully tested on multiple NMR systems in our laboratory. While not all of the 285 NOAH supersequences listed herein and in the Supporting Information are equally efficient and/or practical, the existence of a large number of possible combinations highlights the need for a systematic classification of this technique, and a more complete description of their design and operation will be provided in future work. While we have only considered 2D experiments herein, preliminary work suggests that similar extensions to 3D and higher dimensional experiments are possible.[9a,10]

Experimental Section

All spectra were recorded on a Bruker AVANCE III NMR spectrometer operating at 700 MHz ^{1}H frequency and equipped with a TCI CryoProbe optimized for ^{1}H detection. Further tests (not shown here) were carried out on a Bruker AVANCE III NMR spectrometer operating at 500 MHz ^{1}H frequency and equipped with a BBFO SMART probe. Three simple additional processing routines (au-programs) were written for automatic data separation (splitx) and the frequency axis adjustment for homonuclear experiments (fixF1) or ^{15}N heteronuclear experiments (fixF1n) following data separation into individual data subsets. These routines, along with the NOAH-4 and NOAH-5 pulse programs, are provided in the Supporting Information. Further sequences are available from the authors upon request and will also be available from the Bruker online User Library.

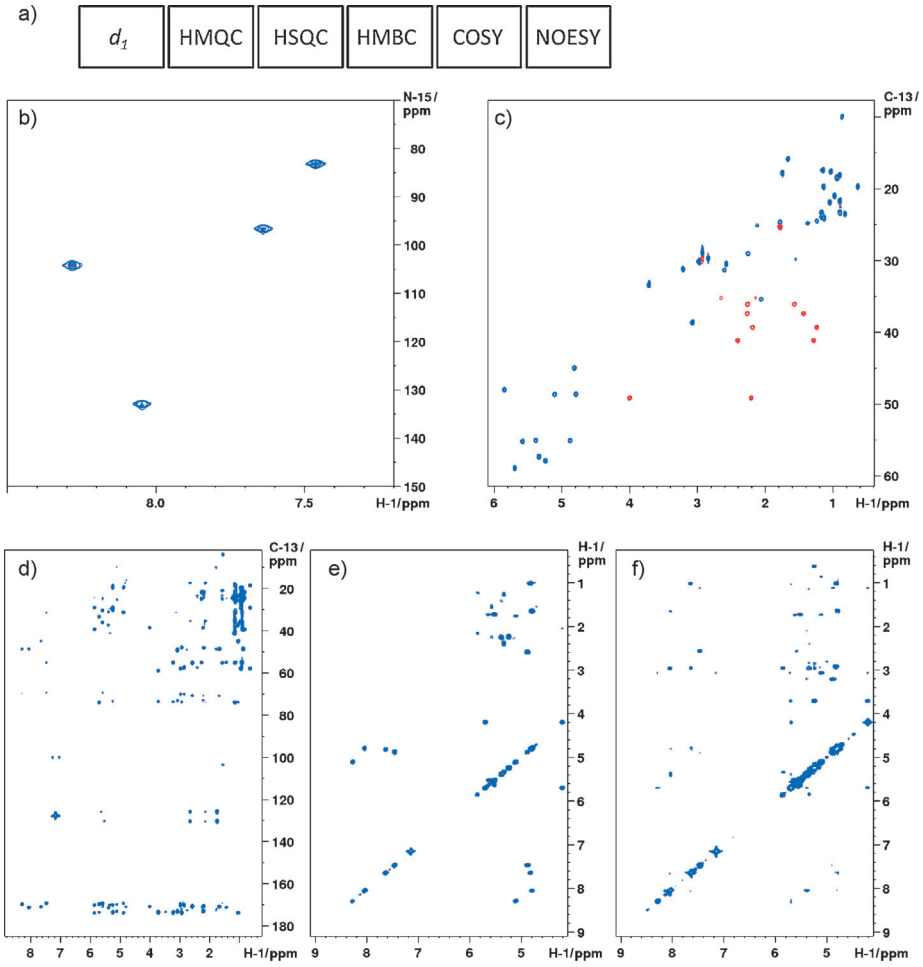

Figure 3. a) A representation of the MSBCN NOAH-5 supersequence. The 2D spectra recorded in a single experiment; b) ^{1}H-^{15}N HMQC, c) multiplicity edited ^{1}H-^{13}C HSQC, d) ^{1}H-^{13}C HMBC, e) ^{1}H-^{1}H COSY, f) ^{1}H-^{1}H NOESY. The sample is 50 mM cyclosporine in [D_6]benzene. The spectra were recorded on an AVANCE III spectrometer equipped with a TCI CryoProbe in 44 min with 2 scans per increment and 512 t_1-increments per module, resulting in a 2k×2560 raw data matrix.

Conflict of interest

The authors declare no conflict of interest.

Keywords: COSY · HSQC · NMR techniques · NOAH · structure elucidation

How to cite: *Angew. Chem. Int. Ed.* **2017**, *56*, 11779–11783
Angew. Chem. **2017**, *129*, 11941–11945

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Manuscript received: May 30, 2017
Revised manuscript received: June 17, 2017
Accepted manuscript online: June 30, 2017
Version of record online: August 18, 2017

