The CBC NMR Laboratory Application Notes

Non-uniform Sampling (NUS) Method in 2D NMR Data Acquisition and Processing

Non-uniform sampling is a new NMR data acquisition and processing technique[1] that allows a rapid data collection for multidimensional NMR experiments. The NUS method can save up to 75% of data acquisition time without compromising spectral quality in terms of resolution and/or sensitivity of 2D NMR spectrum. For a low concentration sample, the sensitivity could be enhanced by a factor of 2 with a NUS method (assuming a 25% sparsely data sampling) comparing with that required by a traditional linear sampling method. Traditionally, a 2D NMR dataset is collected as an array of 1D spectra as a function of evolution time. The evolution time is linearly varied successively as shown in Figure 1 A), while in a NUS method, the 1D spectra are collected as a function of randomly selected evolution times as shown in Figure 1 B). Since only a fraction of linear incremental 1D spectra is collected along evolution dimension, the total experimental acquisition time is significantly reduced.

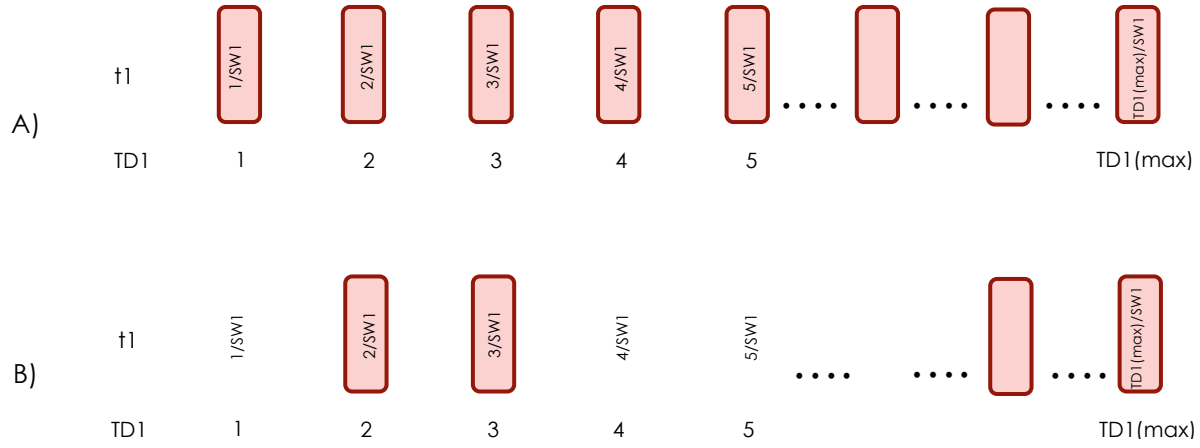

FIGURE 1: A) TRADITIONAL 2D DATA COLLECTION: T1 IS THE EVOLUTION TIME, TD1 IS NUMBER OF EVALUTION TIME INCREMENTS; TD1(MAX) IS THE MAXIMUM NUMBER OF TIME INCREMENTS. SW1 IS THE SPECTRAL WIDTH ALONG THE EVOLUTION DIMENSION. B) NUS DATA COLLECTION: A SUBSET IS COLLECTED RANDOMLY, OFTEN INCLUDING THE LAST POINT (TD1(MAX)/SW1) OF A TRADITIONAL LINEAR DATA COLLECTION.

The total acquisition time of a NUS method depends on data sampling density (i.e. the fraction of normally time-incremental spectra acquired). Sampling density of 25 % is often sufficient to preserve resolution of a normal 2D spectrum. The sensitivity of the NUS spectrum depends on number of scans of each increment, which is often kept the same as the normal 2D spectrum.

As an example, the ^{1}H-^{13}C edited HSQC spectra of strychnine (6 mg in 500 µL $CDCl_3$) acquired on AVIII 400 MHz NMR spectrometer are given in Figure 2. Figure 2 A) shows the aliphatic region of a traditional 2D HSQC spectrum obtained (ns = 4, td1 = 128, recycle time = 1.5 seconds). The blue and red cross peaks represent methyl and methylene groups, respectively. The total acquisition time of this HSQC experiment is 28 minutes. Figure 2 B) shows the same region of 2D NUS HSQC acquired at a sampling density of 25% with a total data acquisition time of 7 minutes. As a comparison, Figure 2 c) shows a 2D

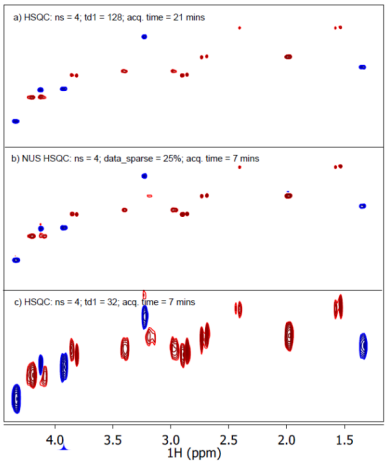

FIGURE 2: EDITED HSQC SPECTRA OF STRYCHNINE A) LINEARLY SAMPLING OF 128 INCREMENTS B) NUS SAMPLING C) LINEARLY SAMPLING WITH 32 INCREMENTS

HSQC spectrum of strychnine acquired using a traditional linear sampling but with only 32 increments in the evolution dimension (td1 = 32). Although it also took only 7 minutes to acquire this spectrum, but the spectral resolution along ^{13}C dimension is not acceptable.

Three NUS 2D spectra (COSY; HSQC; HMBC) (known to the UD NMR users as "2DCOMB") for the strychnine sample acquired on AVIII 600 NMR spectrometer are given in Figure 3. Number of scans used was 2, 2, and 4 for 2D COSY, HSQC, and HMBC accordingly. Since td1(max) is set to 256, only 64 randomly selected 1D spectra were acquired with a sampling density of 25% for each spectrum. The total experimental time for the NUS 2DCOMB is only 18 minutes, significantly reduced from 72 minutes that would otherwise be required for this sample if a normal 2DCOMB is used.

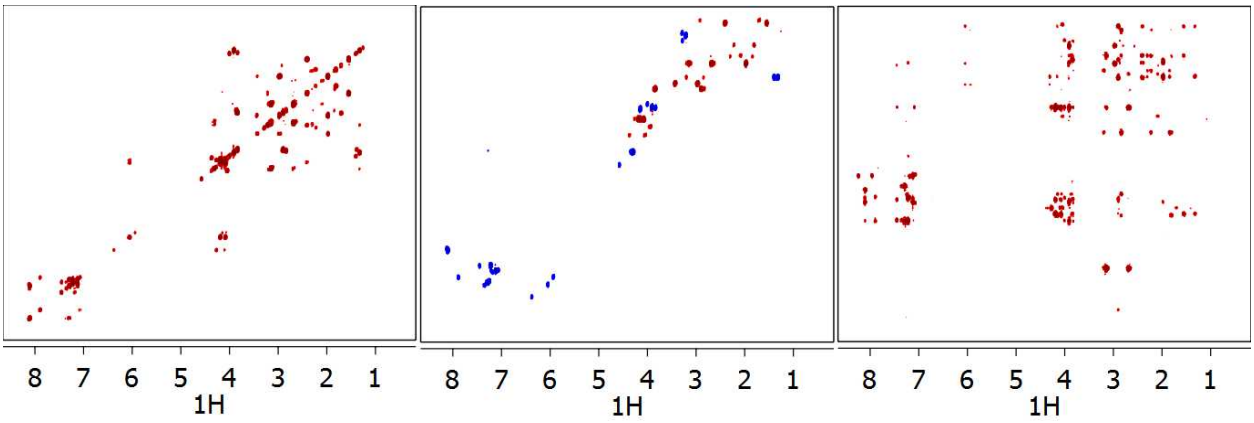

FIGURE 3. 2D NUS COSY, HSQC, AND HMBC OF STRYCHNINE ACQUIRED ON AVIII 600 MHZ NMR SPECTROMETER. NUMBER OF SCANS IS 2, 2, AND 4 FOR COSY, HSQC, AND HMBC, ACCORDINGLY. THE RECYLE DELAY IS 1.5 SECONDS FOR ALL 2D EXPERIMENTS AND THE TD1(MAX) IS SET TO 256.

Implementation of NUS 2D Experiments

The proper implementation of NUS experiments would lead to significant saving in experimental time particularly for the 2D experiments. The impact of reduction in experimental time is twofold: most of 2D NMR experiments on the AVIII 600 MHz NMR automation have been designated as the night-queue experiments to assure a quick turnaround during daytime. With the implementation of NUS method, most of these 2D experiments can now be reassigned as the day-queue experiments such that one is now able to acquire 1D and 2D NMR data for a sample in the same day on the AVIII 600 NMR spectrometer. Secondly, the cost of obtaining a 2D NMR spectrum can now be saved up to 75 %, which could ultimately promote a wide application of multidimensional NMR spectroscopy in the structure elucidation among our facility users.

Operational Instruction:

NUS Data Acquisition:

A Bruker NMR spectrometer running Topspin 3.1 or higher can be used for the NUS data acquisitions in an automation mode. Under this criterion, AVIII 400 and AVIII 600 NMR spectrometers can be used for the NUS data acquisition without any modification of hardware and software. It is unfortunate that the AV400/CPQNP and AV600/CPTXI NMR spectrometers in our laboratory are not suitable for data acquisition of NUS 2D experiments.

Operation Instruction on AVIII 400 MHz NMR Spectrometer:

Set up a 2D experiment as usual, before staring data acquisition, type *expt* to calculate experiment time under linear sampling condition. In **AcquPars** tab, find a line called "**FnTYPE**", click on "down-triangle" symbol next to the line and then select an option of "*non-uniform sampling*". Type *expt* again to calculate the

experimental time, it should ¼ of that required by a linear sampling method. Now start data acquisition by type *zg*. After 2D data set is collected, transfer the data to your own computer, "drag and drop" your dataset onto the MNova data panel, NUS process starts automatically. Usually the MNova NMR software package (9.1 or higher) produces an excellent phase-sensitive or magnitude 2D spectrum without a user intervention. Occasionally, additional adjustment in data apodization parameters is needed to improve the spectral quality. The way of adjustment of apodization parameters is exactly the same as that applied to a normal 2D dataset.

Operation Instruction on AVIII 600 MHz NMR Spectrometer:

The new 2D NUS experiments available on the AVIII 600 automation are summarized in Table I. All experiments listed in the table are now designated as day-queue experiment. When these experiments are performed, the **iconnmr** may show an error message because TopSpin software cannot be used to process the NUS datasets unless a special Bruker license is in place. Just transfer the NUS data to your own computer. "Drag and drop" your NUS dataset onto MNova software data panel, NUS data processing starts automatically.

Table I: 2D NUS Experiments Available for Automation on AVIII600 NMR Spectrometer

Name	NUS Sampling Density (%)	Acquisition Time (NUS) (minutes)*	Type	Exp. Time for a Traditional Sampling (minutes)
COSY_NUS_25	25	10	Day queue	40
HSQCED_NUS_25	25	10	Day queue	40
HMBC_NUS_25	25	15	Day queue	60
2DCOMB_NUS_25 (COSY,HSQC,HMBC)	25	35	Day queue	140

Note: *number of scans are set to 4, 4, and 8 for COSY, HSQC, and HMBC accordingly, one may reduce number of scans to save the acquisition time if the sample concentration is reasonable.

NUS Data Processing

The NUS methods have been reported for a number of years. However, implementation of 2D NUS experiments to an open-access NMR laboratory has been facing a few obstacles: the NUS 2D data acquired on a Bruker instrument can only be processed with TopSpin if a separated Bruker NUS license is not available. The data processing using Bruker software requires prior knowledge of spectral phasing parameters, which are often not available until data are processed. Several iterations with a heavy user intervention are needed to produce a high-quality 2D NUS spectrum. The NUS data processing with a Bruker software takes up to tens of minutes, almost as long as the data acquisition time itself. Therefore it is not practical to adopt the NUS version of 2D experiments as a generalized method in an open access laboratory.

All of these have been changed when the newest version of MNova software (9.1.x or higher) was released recently. The new MNova NMR software includes a NUS data reduction and reconstruction package, which works really fast without a need of user's intervention. MNova NUS processing is done fully automatically ("drag and drop" approach) and a total 2D NUS processing time is just a few seconds.

The current version of MNova NUS package works well for 2D COSY, HMQC, HSQC, and HMBC. It does not work well for NUS NOESY dataset at the moment.

References:

(1) K. G. Hyberts, H. Arthanari, G. Wanger, *Top Curr. Chem*. **2012**, 316, 125 – 148. And references therein.