DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY
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User's Guide 2D NMR on DRX400

NMR LAB APPLICATIONS NOTES

2D NMR on DRX400

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General

Since the 2D experiments described in this document are the pulsed field gradient enhanced or gradient-accelerated experiments, usually sample is NOT spinning during the data acquisition. Before 2D dataset is acquired, always obtain a 1D ^{1}H survey spectrum, which will be used for determining the minimum spectrum width for 2D experiments. Always obtain the best magnetic filed homogeneity by adjusting the shims z^1, z^2, and z^3. Since the sample is not spinning, one should also shim on x and y. Make sure the gradient amplifier (inside the DRX400 consol) is on. General comments about sample preparation and sample insertion are given below:

- Use clean and dry sample tubes
- Use medium to high quality sample tubes
- Always filter the sample solution
- Always use the same sample volume or solution height: 5mm tubes 0.5ml or 5cm
- Use the sample depth gauge to adjust the sample depth: 2.0 cm
- The sample tube should sit tightly inside the spinner
- Turn on lift air to insert the sample into the magnet
- Wipe the sample tube clean before inserting into magnet

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^{1}H Survey Spectrum

The detailed method of obtaining a ^{1}H survey spectrum has been described in the DRX400 user's guide. We outline here the major steps of 1D NMR data acquisition as the following:

- Insert the sample as described before in the DRX400 User's Guide
- Create a new dataset by type `new` or `edc` in the command line of TopSpin software, fill in appropriate information
- Type `rpar PROTON all` and hit Enter
- Type `gpro` to load appropriate acquisition parameters into the current dataset
- If BBO probe is installed, you can tune the probe by typing `atma` and wait until the auto tuning and match finished.

  NOTE: Make sure the sample is NOT spinning when you do the probe tuning
- Spin the sample by click on **SPIN** button on the BSMS keypad
- Lock spectrometer frequency by typing `lock` followed by selecting appropriate solvent from the solvent list
- Shimming on the sample by adjusting shim values of z1 and z2, and/or z3.
- Prior to data acquisition, adjust the receiver gain by an auto routine with a command `rga`
- Start the data acquisition by typing `zg`

After data acquisition finished, examine the 1D proton spectrum carefully and estimate a spectral width by allowing 0.5 ppm on each side of proton peaks in the spectrum. For example, if all NMR resonance appeare between 0.5 and 7.0 ppm, the spectral width (SW) of 2D dataset should be set to 7.5 ppm ranging from 0.0 to 7.5 ppm. The spectral center frequency (o1p) for the 2D dataset should be set to 3.75 ppm [(7.5 + 0.0)/2].

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Prepare 2D Dataset and Data Acquisition

The common 2D experiments are listed in Table 3.1 with their name, the acquisition parameter file name, and the related comments. These experiments are best for samples with non-aqueous solvent such as CDCl3, DMSO, and C6D6. Prior to a 2D experiment, stop sample spinning and adjust the shim values of z, x, y, xz, and yz to maximize the level of lock signal. If you are doing a homo-nuclear 2D experiments, you do not need to tune the probe because you have already done so in the previous ^{1}H survey experiment. If you are doing a hetero-nuclear 2D experiment, you should check probe tuning before a 2D data acquisition starts. The procedure will be described below.

- Create a new dataset by typing `edc` or `new` then fill in appropriate information

- Load the acquisition parameter file by typing `rpar` *filename* `all` and you may select *filename* from the second column of Table 3.1. For example, for a COSY experiment, type `rpar COSYGPSW all` to retrieve the corresponding acquisition file into the current dataset.

- Type `gpro` to load correct acquisition parameters into the acquisition file in the current dataset.

- If you would like to do a hetero-nuclear 2D experiments, such as HMQC, HMBC, or HSQC, you should check the probe tuning. To do so, type `atma` and wait for the wobble curve appearing in the automatic tuning and match (ATM) GUI. Make necessary adjustments by clicking on the directional buttons until the wobble curve moves to an optimum location. In the file menu, select **Save the Position** and then **Exit**.

NOTE: If the wobble curve is too far away from the optimum position, stop tuning by exiting the ATM without saving the position. Report the problem to the NMR staff immediately

- Type `eda` and a window of 2D acquisition parameter will appear, which allows one to edit acquisition parameters. Under the column F2, change SW and o1p to the values determined previously with a 1D ^{1}H survey spectrum. For homo-nuclear 2D experiments such as COSY, TOCSY, and NOESY, SW in F1 dimension must be set exactly the same as SW of F2 dimension. For hetero-nuclear 2D experiments such as HMQC, HSQC, and HMBC, SW in F2 dimension and o1p must be set according to the survey 1D ^{1}H spectrum. It is not necessary to change SW (F2) and o2p

- Make sure the number of dummy pulses (ds) is set to 8.

- Set number of scans (ns) to 2 for COSY, TOCSY, HMQC, and HSQC experiments and to 4 for HMBC experiment, respectively.

- Type `expt` to calculate the total acquisition time before starting an acquisition, you may adjust the amount of time to fit your allowance by changing the number of scans (ns). Always choose the values of ns according to the rule specified by the pulse program.

- Set receiver gain by typing `rga`

- Start the data acquisition with command `zg`

- To view 2D spectrum while data acquisition is in progress, typing `xfb`

NOTE: It is normal to observe the lock level changes during the acquisition for the experiments with gradient pulses to accelerate the measurement

Table 3.1 Common 2D Acquisition Parameter Files

Experiment	File Name	Comments
COSY	COSYGPSW	COSY experiment with gradient pulse
TOCSY	MELVPHSW	TOCSY experiment with mlev spin-lock
HMQC	HMQCGP	HMQC experiment with gradient pulse
HSQC	HSQCGP	HSQC experiment with gradient pulse
HMBC	HMBCGP	HMBC experiment with gradient pulse
NOESY	NOESYPHSW	NOESY without gradient
NOESY	NOESYGPSW	NOESY with gradient pulse

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Data Processing

In this section, the general instructions of post-acquisition data processing will be provided. It is recommended to transfer 2D dataset to an NMR server for further processing. The workstations located in 045BRL can be used to access the data on the NMR server and can be used to process data with the Bruker TopSpin software installed on these workstations.

- Command `edp` allow you to edit processing parameters. Often, SI (F1) and SI (F2) should be set to 1024, respectively. For a COSY spectrum, window functions for F1 and F2 should be set to **SIN** with **ssb** being set to 0. For other 2D experiments, choose **QSIN** as the window functions with ssb of 2.

- With appropriate processing parameters, one can view processed 2D spectrum by typing `xfb`, the command `xfb` does the Fourier Transform for both dimensions

- For COSY and HMBC, the 2D data was collected in magnitude mode, no further phase correction is needed. For other 2D datasets a complicated spectral phase correction must be done. Please refer to Bruker's user's menu for detailed instructions

- 2D spectrum is often plotted with ***xwinplot*****,** a subprogram of TopSpin software for spectral plotting. Keep in mind that you can always get help by a built-in help function in the TopSpin program. Other software, such as Mnova is also recommended for data processing and spectrum plotting