A very short introduction to operation of Bruker AVANCE NEO 400 MHz

General instructions

- Only acquisition and initial processing of the NMR data is allowed with the NMR PC. Use your own PC for further data processing and analysis.
- Do not create/save picture files, PDF documents, *etc.* In addition, do not create new file folders.
- ***Do not change TopSpin/IconNMR window position/size.***
- Use the WinSCP program to transfer the NMR data to your own PC. ***The use of the USB devices is strictly forbidden***.
- ***Do NOT put the NMR PC into sleep mode or lock it.***
- The NMR room door must be kept locked at all times.

About the sample

- Sample solution height must be ***at least 4 cm*** for 5 mm NMR tubes.
- Always use clean and dry sample tubes, which are free of scratches.
- Keep the bottom half of the sample tube clean and ***do not touch it with your bare hands***.
- The sample solution should be clear with no precipitate.
- High ion concentration (salts) could cause tuning and matching (ATM) problems.
- ***Do not use marker pen to write directly on the NMR tube wall***.

Inserting sample tube(s) into the sample changer (SampleCase)

1. Wipe the bottom half of the sample tube with a lint free tissue.
2. Insert the sample tube into the spinner. ***Do not touch the spinner with your bare hands***.
3. Use the sample depth gauge to adjust the sample to a correct height.
4. Once more, wipe the bottom half of the sample tube with a lint free tissue.
5. Insert the sample tube with a spinner into SampleCase to the left side of the red sample overlength latch (gently – do not press the tube). Check the holder position number.

Running experiments using IconNMR

1. Click on the **Change User** button (

) in the upper right corner in the IconNMR window and select your username from the popup window. Check the *User* next to the **Change User** button.
2. Double click the **Holder** number of the first sample.
3. Click inside the **Name** box and give a name for your experiment(s).
4. Click on the **No.** (experiment number) box. The experiment number automatically increments to the next available number if a data set already exists with that name and experiment number.
5. Click on the down arrow next to the **Solvent** box and select the solvent.
6. Click on the down arrow next to the **Experiment** box and select the experiment. **N** is a normal experiment *i.e.,* only one experiment is created. **C** is a composite experiment, which is a series of two or more experiments combined under one name.

7. Click on the **Edit specific parameters** button (**Par** column) to change parameters. If parameters are changed, the icon of the button will change to an exclamation mark. Normally, only the number of scans (**NS**) should be changed. New **NS** must be a multiple of the default value. NOTE: if experiment time is not shown, change **NS** to itself (*e.g.,* 8 -> 8) or to a new value.
8. Click on the **Title** button (**Title/Orig** column) and edit the default title. Click on the **Set Title** button inside the Title window.
9. If you want to perform additional experiments on the same sample, click on the **Add** button and continue from step 6. NOTE: modified title will be copied to the next experiment. If you want to see the default title, delete the modified title, and select the experiment again.
10. If you have several samples, double click on the respective **Holder** numbers, and create experiments as described above.
11. When all the experiments have been created, click on the first line of the **Holder** number of the first sample (selected line will be highlighted).
12. If you have several samples, hold down the **Ctrl** key and click on the first line of the respective **Holder** numbers (selected lines will be highlighted).
13. Click on the **Submit** button. If the run does not start, click on the **Start** button () in the upper left corner of the IconNMR window. Enter the holder position number for the **First sample** and click on the **Start** button inside the Start window. NOTE: the total experiment time will be shown at the bottom right corner of the IconNMR display (*Busy until*).

Checking spectra using TopSpin

Double-click on the experiment entry in the *Preceding Experiments* pane in IconNMR. This will open TopSpin in the respective data set. NOTE: data set in TopSpin can be opened by left-click-holding the desired experiment number and dragging it into the spectra display.

Transferring data using WinSCP

See the separate instructions.

Before leaving the NMR room

1. Press the blue button in SampleCase (rotate carousel clockwise) and remove samples. Put spinners back in the grey box.
2. Minimize your folder in the TopSpin data browser.
3. Click on the IconNMR icon in the Windows Taskbar (to put the IconNMR window on the top) and delete experiment setup entries in IconNMR. The **Delete** button deletes selected (highlighted) experiment setup entries. It does not remove any spectral information from the hard disk.
4. Leave TopSpin and IconNMR open – IconNMR on the top.
5. Fill in the logbook.

Do not make changes in the acquisition parameters unless you know what you are doing!!!

About the 1D experiments

1D spectra are calibrated automatically. However, automatic calibration fails sometimes. Therefore, calibration should be checked in TopSpin (parameter **SR** = 0 in PROCPARS tab -> no calibration has been done).

If required, spectral width (parameter **SW**) and transmitter frequency offset (middle point of the spectrum, parameter **O1P**) can be changed in IconNMR. Avoid too narrow **SW** (*e.g.,* ^{1}H **SW** < 5ppm).

The only acquisition parameter that can be changed in the UDEFT parameters sets is **NS**. Do not change any other UDEFT acquisition parameter. In addition, processing parameter **LB** (*line broadening for em*) must be at least 2Hz (default).

The relaxation delay **D1** must be at least 60s (default) in the quantitative ^{1}H parameter sets using ^{13}C decoupling (**an_1H_***_13C_dec**). However, **D1** can be > 60s. It is not allowed to change any other acquisition parameter, except **NS**, in these parameter sets.

About the 2D experiments

All 2D experiments are composite experiments in which F2 projection is measured before the 2D experiment. F2 projections (typically ^{1}H) are added automatically to all 2D spectra. If the 2D parameter set name contains “sw”, a fixed spectral width(s) is used. Otherwise, the F2 spectral width will be optimized automatically based on the signal region. In the ^{1}H,^{1}H correlation experiments, also the F1 projection is optimized and both projections are added automatically to the 2D spectra. In the ^{1}H,^{13}C/^{15}N correlation experiments, the F1 (^{13}C/^{15}N) spectral width is always fixed and no F1 projection is measured or added automatically.

The default TD(F2) is 1K for most of the 2D experiments. If higher resolution is required in this dimension, TD(F2) can be changed in IconNMR (parameter **2TD**). The new value of **2TD** must be a multiple of the default value (typically 2K). However, avoid too long acquisition times in the HSQC experiments. Contact the NMR Administrator for further information.

The default TD(F1) is 128 for most of the 2D experiments. If higher resolution is required in this dimension, TD(F1) can be changed in IconNMR (parameter **1TD**). The new value of **1TD** must be a multiple of the default value (typically 256). NOTE: the experiment time will be doubled if **1TD** 128 -> 256.

If required, F2 and F1 spectral widths (parameters **SW** and **1SW**, respectively) and F2 and F1 transmitter frequency offsets (middle points of the spectrum, parameters **O1P** and **O2P**, respectively) can be changed in IconNMR. Perform the following steps if F2/F1 spectral widths are to be changed in ^{1}H,^{1}H correlation experiments: (1) change the parameter **SW**, (2) click on OK, (3) open the parameter edit again, (4) copy **SW**, (5) paste it to **1SW** and (6) click on OK. NOTE: only fixed **SW** and/or **1SW** can

be changed. Avoid too narrow spectral widths. Contact the NMR Administrator for further information.

The processing parameter *Size of real spectrum* (parameter **SI**) can be changed in PROCPARS tab in TopSpin. Typical default combination used in the 2D parameter sets is SI(F2) = 1K; SI(F1) = 1K. More data points can be added, for example, by applying SI(F2) = 2K; SI(F1) = 2K. Perform the following steps if **SI** has been changed (excluding COSY and HMBC datasets): (1) select the ***Process*** tab in TopSpin and (2) click on **Proc. Spectrum** button to process the data (includes FT, phase correction and baseline correction). In the case of the COSY and ^{1}H,^{15}N HMBC datasets, instead of clicking the **Proc. Spectrum** button, (1) enter TopSpin command *xfb* to perform FT and (2) enter TopSpin commands *abs2* and *abs1* to perform baseline correction. In the case of the ^{1}H,^{13}C HMBC datasets, (1) enter TopSpin commands *xfb* and *xf2m* to perform FT and (2) enter TopSpin commands *abs2* and *abs1* to perform baseline correction.

Calibration of the F2 projection is transferred to the 2D spectrum. Check that the calibration matches that of the 1D spectrum (parameter **SR** = 0 in PROCPARS tab -> no calibration has been done). Check also the F1 calibration.

Intensity levels of the 2D spectra are calculated automatically. Despite of that, intensity levels should be checked in TopSpin. Scroll the middle mouse button to change the intensity and click on the **Save the contour levels to disk** button ( ).

Adding F1 projections to the 2D spectra in TopSpin

F1 projections (^{13}C) must be added manually to the 2D ^{1}H,^{13}C correlation spectra (1D spectra must be measured separately):

1. Right-click inside the F1 projection area of the 2D data window (left side of the 2D spectrum).
2. Select *External Projection*.
3. Enter the *EXPNO* and *PROCNO* values of the 1D spectrum for this projection and click on OK.

About the 1D water suppression parameter sets

an_1H_H2O_noesygppr1d: 1D NOESY with presaturation during relaxation delay and mixing time and spoil gradient. Decent water suppression; does not suppress the analyte signals around the water signal as much as the soggy experiments; may suppress signals from exchangeable protons.

an_1H_H2O_zggppeso: 1D experiment using soggy with gradients and perfect echo. Excellent water suppression; may strongly suppress analyte signals around the water signal (*ca.* ±0.8ppm from water); does not suppress signals from exchangeable protons. NOTE: lock solvent must be H_2O+D_2O or H_2O+D_2O_salt.

an_1H_H2O_zggppeso_pr: 1D experiment using soggy with gradients, perfect echo and presaturation during relaxation delay. Best water suppression; may strongly suppress analyte signals around the

water signal (*ca.* ±0.8ppm); may suppress signals from exchangeable protons. NOTE: lock solvent must be H_2O+D_2O or H_2O+D_2O_salt.

NOTE: Above parameter sets work only if the water signal is close to 4.7ppm *i.e.*, when D_2O contains H_2O. Otherwise, parameter set **an_1H_H2O_noesygppr1d_comp** should be used. This composite parameter set allows determination of position of the water signal from normal ^1H spectrum before the water suppression experiment is performed while sample stays in the magnet. Contact the NMR Administrator for further information.

Opening IconNMR

NMR software should be always left open. However, if IconNMR has been closed, it can be opened by entering **icon** in the TopSpin command line and selecting your username from the popup window.