INSTRUCTIONS FOR PERFORMING EXPERIMENTS BY BRUKER AVIII HD 300, AVIII 400 & 500 NMR SPECTROMETERS

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Interested in collecting and performing NMR analysis with your samples?

First, YOU MUST:

1) accomplish/pass a proper safety and NMR training in order to use NMR instruments on your own at the Department of Chemistry. Consult the NMR administrators for training (contact details below).

2) use the electronic booking system (infrabooking.jyu.fi) to reserve time for NMR instruments.

3) follow the guidelines provided in this document for the proper handling and setting up of NMR instruments for your samples.

These instructions are providing you with the **basic guidelines** how to perform NMR experiments using 300-500 MHz NMR systems at the Department of Chemistry. By following these guidelines, you're more likely to succeed with your measurements/data collection.

DO NOT carry out any unusual experiments without asking from the NMR administrators whether the system is compatible with such experiments, especially those having a great potential to harm the probehead e.g., using excessive radiofrequency (RF) power or non-typical temperatures.

Please consult the NMR administrators if you need assistance when setting up your experiments. Always ask before doing something blind-folded! You're liable of a proper and safe usage of NMR facility and its instruments. Violating these rules or using NMR instruments for non-standard activities without consulting NMR administrators will make you responsible for potential costs associated to repair or replacement of damaged goods/instrumentation.

Since October 2024, all research groups are responsible for providing deuterated NMR solvents and NMR tubes for their own research projects. Consult your PI for the availability of adequate deuterated solvents. Deuterated solvents for teaching purposes are provided from different sources.

Contact information of NMR administrators:

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1) Sample preparation

- Deuterated solvents are almost invariably required for NMR spectroscopy. The solubility of the sample should first be checked by using a non-deuterated solvent, since the deuterated ones are expensive.
- $CDCl_3$ should be used whenever possible, since it is the cheapest of the NMR solvents.
- The solvent should not be wasted – the solvent volume of 500 µL is sufficient. Also the solvent bottles should be closed tightly in order to prevent unnecessary evaporation. Of note: If you want to save your solvent consumption, you can use 3 mm OD NMR tubes where the volume is 180 µL. However, because the standard probeheads are 5 mm in diameter, the sensitivity will be lower per unit time due to lower filling factor. This does not pose to a problem in case you're not limited with the solubility, that is, you can concentrate your sample.
- Any precipitate should be filtered off.
- Immediately equip your sample with a tape label (name of the sample, solvent, date, name of the person).
- If unnamed/unmarked sample tubes are found in the NMR laboratory, they will be disposed.

Make sure that the spectrometer is free!

- If the spectrometer is in use, you can see it on the Acquisition information box of the status bar below the command line: Name/Expno: | Scan Exp: | Residual time: If the spectrometer is not in use, the text: No acquisition running. appears in the Acquisition information box (Fig. 1).
- Also note that previous user might have samples in the queue, while no active experiment is on-going. If no queued experiments exist, the spooler is empty (Fig. 1).

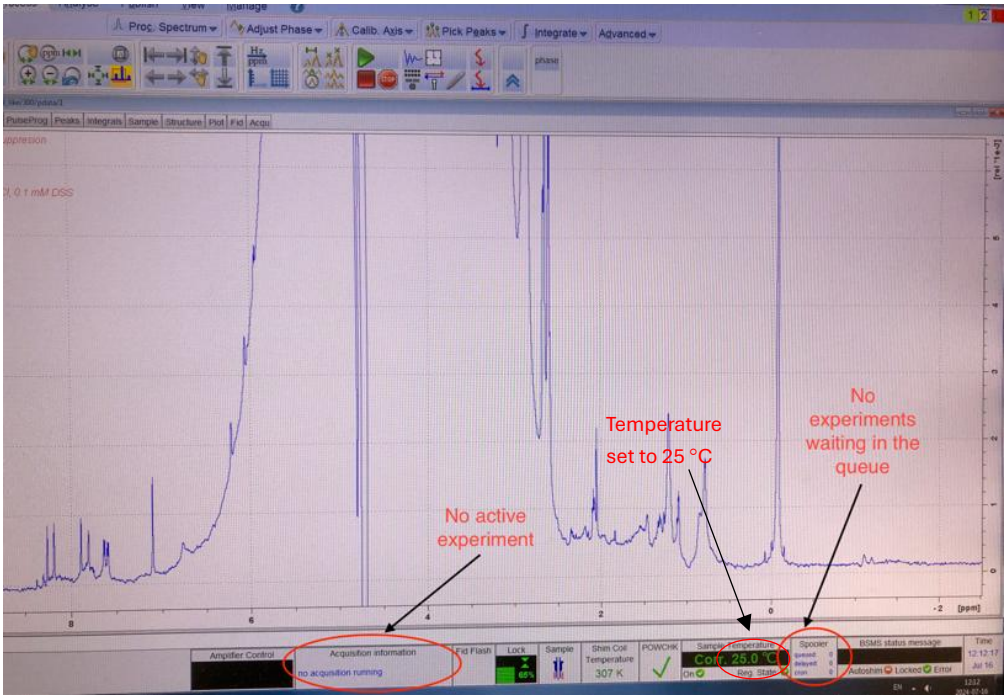


Fig. 1. *No active experiment or experiments in the queue/spooler*

- The measurement can be ended by typing "**halt [enter]**" on the command line.

Always ask before stopping any on-going measurement!!

2) *Replacement of the sample*

- Make sure that the temperature in the probehead is set correctly before inserting the sample as your sample may not tolerate possible elevated temperature used by the previous measurer (Fig. 2). Temperature for the probe/sample can be changed by typing "**edte [enter]**" (= '*edit temperature*'), which pops up a specific window for that (Fig. 2). Consult the NMR administrators if you need to use temperatures outside the range of 5-60 °C.

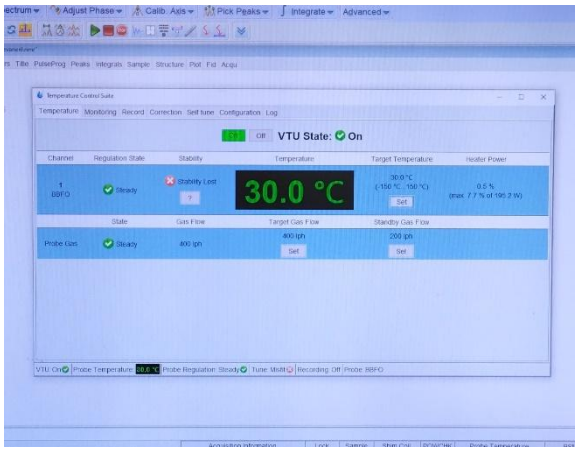
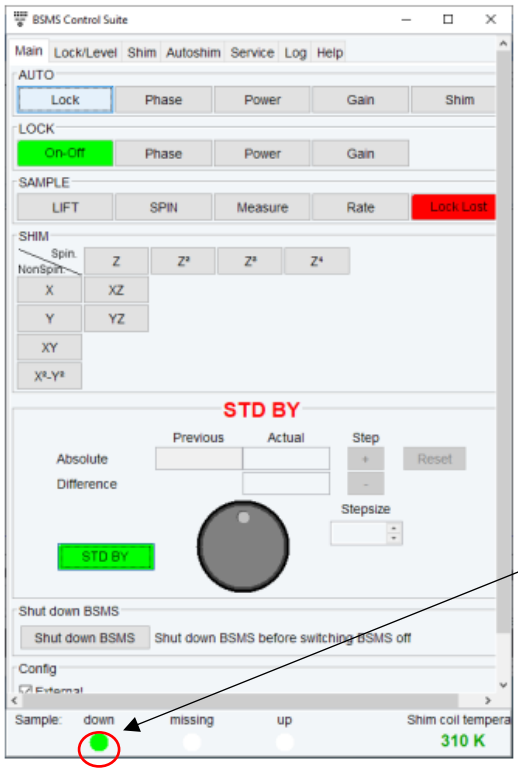


Fig. 2. *Control panel for setting the temperature. Do not change Probe gas or BCU settings before consulting the NMR administrator.*

- Using the left mouse button, press **"LOCK: On-Off"** in the digital BSMS control suite (upper green button in Fig. 3; turns red when lock is off). In the case of the 500 MHz NMR spectrometer either the BSMS keyboard (button LOCK) or the digital BSMS control suite can be used.



Shows the position of the sample (up, missing, down)

Fig. 3. *Digital BSMS control panel*

https://nmr.chem.ucsb.edu/docs/Bruker_NMR_Manuals/user_manual_topspin_ts40.pdf

- If it is "on/green", terminate spinning by clicking "**SPIN**". (Spinning is not in use in the 300 MHz NMR spectrometer.)
- Press "**LIFT**" (The sample tube rises. Red light next to the word "missing" is turned on).
    - You can also type **"ej [enter]"** on the command line to eject the sample.
- Pull the previous sample carefully out of the spinner and replace it with yours.

- DO NOT TOUCH THE UPPER PART OF THE SPINNER WITH YOUR FINGERS!! (Get hold of the NMR tube, not from the cap however, Fig. 4)

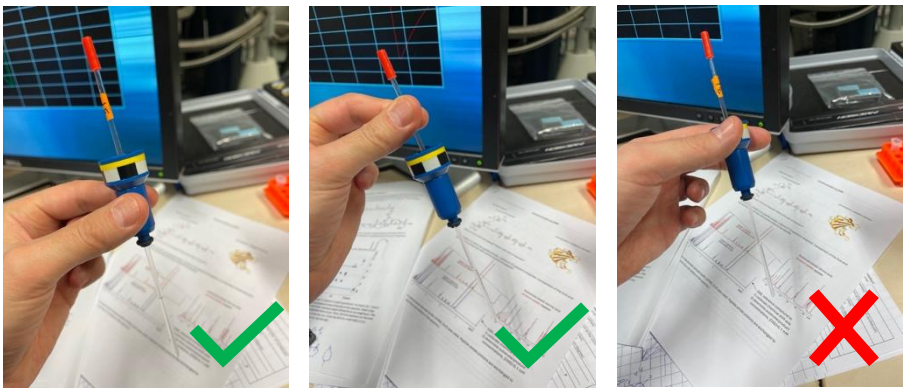


Fig. 4. *Right ways to hold the spinner or the NMR tube. Avoid touching the upper part of spinner*

- Transfer the NMR tube with the spinner to the sample gauge for measuring the correct placement of your sample.
- Adjust the height of the NMR tube using the sample gauge. The sample should be positioned symmetrically with respect to the RF coil diameter (Fig. 5). This is very important especially if you have a 'short' sample i.e. the sample volume smaller than 450 µl in 5 mm OD tube.

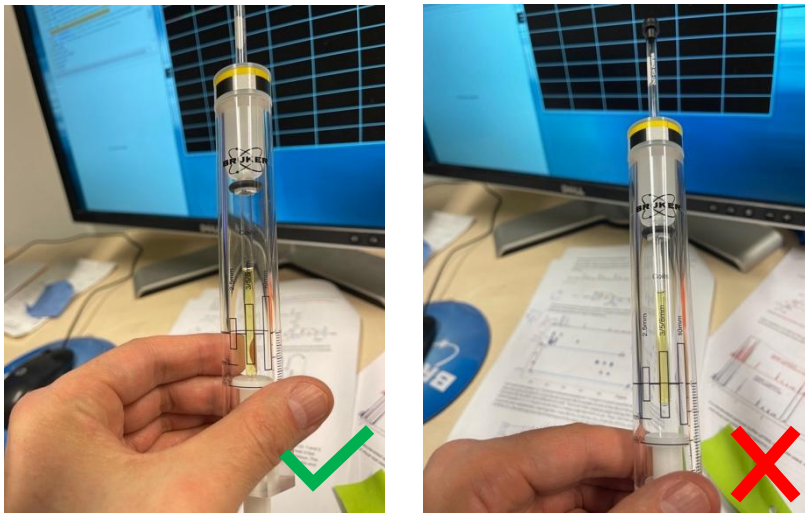


Fig. 5. *Adjusting the height of your sample for optimal performance. On the right the sample is not filling the coil area on the panel at right.*

- Take your NMR tube with the spinner on top of the magnet bore, where the samples will "float in the airflow". Do not release the sample before you sense the supporting airflow.
- Press "**LIFT**" (the sample smoothly lands into the magnet). Wait until the red "missing" light is turned off and the green "down" light is on (see Fig. 3).
    - You can also type **"ij [enter]"** on the command line to insert the sample.
- Sample is properly placed into magnet and the probehead therein if you see the sample icon in the main window under the command line (Fig. 6). If there is 

(question mark) in the sample icon slot, the sample is not properly set.

https://nmr.chem.ucsb.edu/docs/Bruker_NMR_Manuals/user_manual_topspin_ts40.pdf

Fig. 6. *Sample is in the magnet and correctly in the probehead.*

- If you need to, you can start spinning by clicking "**SPIN**" (Spinning is not in use in the 300 MHz NMR spectrometer). For 2D experiments, spinning should not be used, and it is not advised at 500 MHz NMR either.

3) Locking the sample

- Type "**lock [enter]**" on the command line and choose the appropriate solvent from the list e.g. Acetone, $CDCl_3$, DMSO or D_2O (Fig. 7).

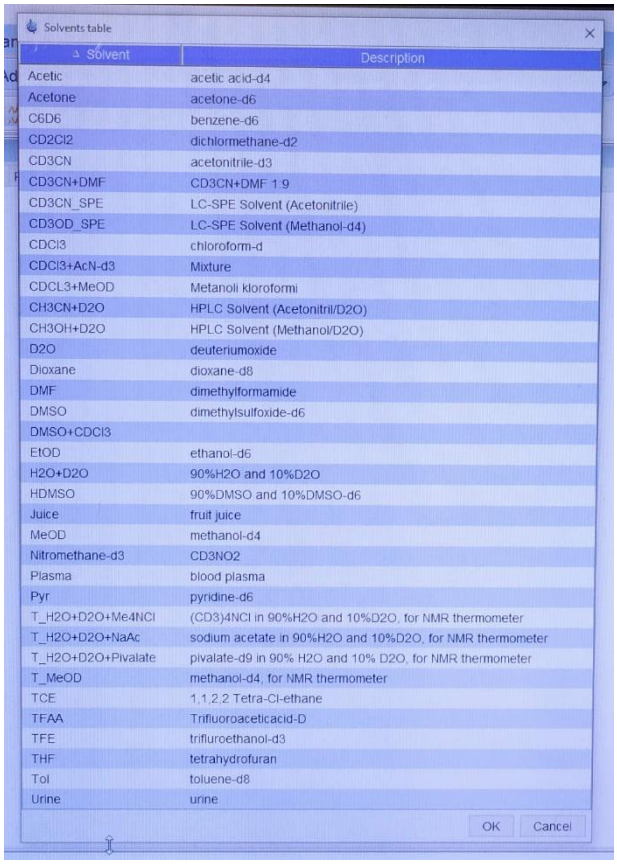


Fig. 7. *List of available deuterated solvents for the field locking. It is important to select the correct solvent used in your sample to get referencing right.*

- Wait until the text "lockn: done" appears under the command line (bottom left corner). The sample is now locked successfully.
- If you have problems in locking your sample, first check that you have enough solvent in the NMR tube and that the spinner is correctly positioned with the depth gauge. You may also try to change the solvent to another one, which contains more deuterium (e.g. DMSO or acetone, > 5% v/vo).
- If the previously mentioned issues are ok, you may try to read the premade shim files for the solvent you are using. Typing **"rsh [enter]"** in the command line opens the shim file directory, where you can select the most appropriate shim file for your sample. Read that before locking your sample in the case of very noisy lock signal/level.

4) Creating the measurement file

- Go to your own measurement directory and open a measurement file to ensure that your current measurement will end up in the correct directory.
- Create a new measurement file by pressing the combination **Ctrl + N** or type "**new"**
- Change the **NAME (**`filename`**)** and the **EXPNO (**`experiment number`**)** (if the experiment number in the ^{1}H measurement is 1, experiment numbers 101→ for the ^{13}C measurements and 201→ for the 2D measurements are commonly used) to correspond to your sample (Fig. 8).
- **Directory** is the path where your filename will be placed (`DIR/NAME/EXPNO/PROCNO`)
- Select the correct **Experiment** from the drop-down menu. You can filter experiments e.g., Oma_1H

  List of experiments available at 300, 400 and 500 MHz systems with different probes (see end of the for description of these experiments*):

  - **For the 300 MHz NMR with the 5 mm BBFO** use the following experiments:
    - JYU_1H, JYU_1H_solvent_suppression, JYU_13C-{1H}, JYU_DEPT135, JYU_19F, JYU_19F_Decoup, JYU_31P, JYU_31P-{1H}, JYU_d-NOE, JYU_DOSY_95, JYU_HH-COSY, JYU_FF-COSY, JYU_HHROESY, JYU_HMBC, JYU_HMQC, JYU_HSQC_edited, JYU_15N-1H_correlation
  - **For the 400 MHz NMR with the 5 mm BBFO probe**
    - JYU_1H, JYU_13C-{1H}, JYU_DEPT135, JYU COSY, JYU_HH-TOCSY, JYU_HHNOESY, JYU_171Y, etc.
  - **For the 500 MHz NMR with the Prodigy probehea**
    - JYU_1H, JYU_13C-[1H}, JYU_DEPT135, JYU_ JYU_2D_DOSY, JYU_DOSY_98%, JYU_DOSY_ JYU_HH-NOESY, JYU_HH-TOCSY JYU_HME JYU_15N-1H_correlation, etc.

Fig. 8. *Creating of the measurement file. Choose the correct directory ("**DIR**") and file name ("**NAME**") which will be placed under the **DIR**. Different experiment numbers (**EXPNO**s) will then be **DIR/NAME/EXPNO**.*

Experiment with the correct parameter setup can be obtained by clicking "Select" and choosing the experiment from the list, e.g. `JYU 1H`

- From **Parameters**:
  - **Set solvent** → select the correct solvent from the drop-down menu
  - **Execute 'getprosol'** (= '*get probehead and solvent dependent parameters*'); has to be performed after changing the probehead or the solvent. For 2D measurements don't select Execute 'getprosol').
- Change the **title** to correspond to your sample.

(*The text of the title can be of the following form:*

`name_of_the_sample`
`in CDCl`$_3$ `at 30 C`
^{1}`H NMR at 300 MHz/`^{13}`C NMR at 75 MHz`
`date name_of_the_operator`).

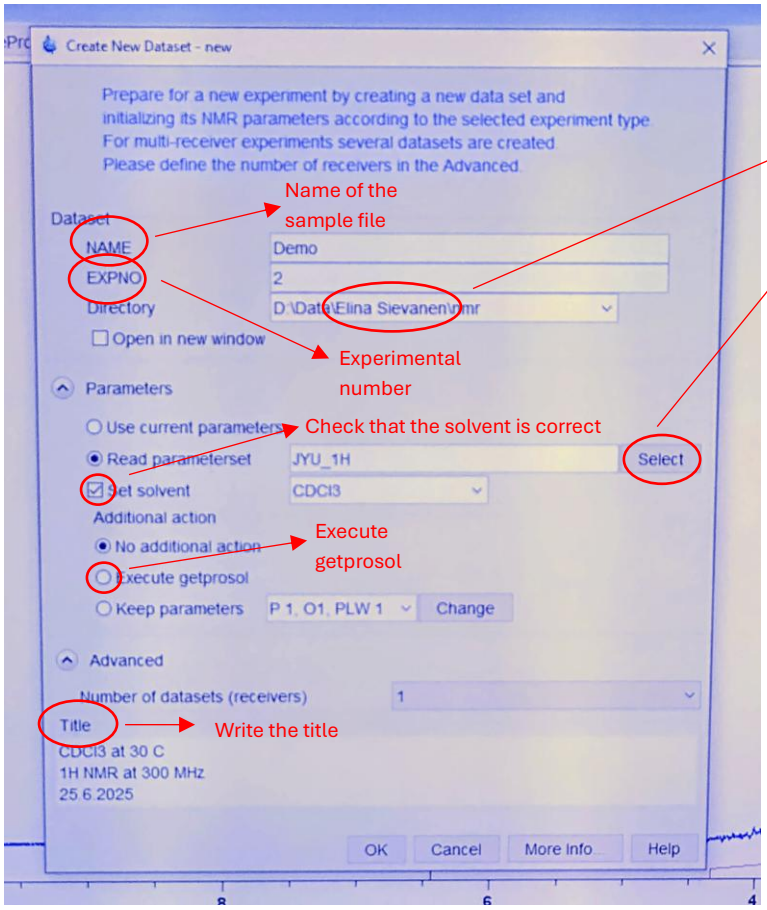


Fig. 8. *Creating of the measurement file. Choose the correct directory ("**DIR**") and file name ("**NAME**") which will be placed under the **DIR**. Different experiment numbers (**EXPNO**s) will then be **DIR/NAME/EXPNO**.*

Experiment with the correct parameter setup can be obtained by clicking "Select" and choosing the experiment from the list, e.g. `JYU_1H`

5) Tuning and matching the probehead

- Type "**atma [enter]**" (= '*automatic tuning and matching*': ensures that the coils in the probehead are tuned on the right frequency). Wait until the text "atma: finished" appears under the command line (bottom left corner).
- If no automatic tuning and matching is available for the probehead, please consult NMR administrator for the assistance unless you're experienced carrying out the tuning and matching manually.

6) Shimming (i.e. adjusting the homogeneity of the magnetic field, B_0)

- Type "**topshim [enter]**" to evoke the gradient shimming routine.
- Wait until the text "topshim: completed successfully" appears under the command line (bottom left corner).
- In the case of the 500 MHz NMR spectrometer, you may need to shim manually to obtain more satisfactory result. Shimming can be done by using either the BSMS keyboard or the digital BSMS control suite (cf. Fig. 3).
- You may want to initiate more sophisticated shimming protocol using "**topshim gui [enter]**". It launches the graphical user interface where you can choose more demanding shimming routine. You should ask for consultation from the NMR administrator unless you know what you're doing.
- If you experience problems in shimming, you may try to read the premade shim files for your solvent (look point 3) Locking). Typing **"rsh [enter]"**in the command line opens the shim file directory, where you can select the most appropriate shim file for your sample. Magnetic field

homogeneity depends on many factors, the tube, the solvent, the temperature, etc. Consequently, this does not fix the shims, but you can probably get into ok starting position.

7) Before the measurement

- Type ”**rga [enter]**” (= ‘*receiver gain adjustment*’) on the command line and wait until the text “rga: finished” appears under the command line.
- You may check the following parameters (and adjust them if needed):
    - ns (=number of scans): in ^{1}H experiments typically 4 or 8, in ^{13}C experiments ns = 8, td0 = 10k (or ns = 10 k).
    - Type **“expt [enter]”** (= ‘*experimental time*’) to find out for how long your experiment takes and change the number of scans accordingly.
- For 2D measurements check the spectral widths on AcquPars leaflet. For example, for the COSY experiment the preset spectral width (SW) is −1.25 - 8.75 ppm. If your sample has e.g. an aldehyde proton resonating at δ ~ 10 ppm you need to change the preset values of the spectral width. It can be done online SW (= spectral width). Also remember to update the center of the spectral area on row O1P. Do the change for both F2- and F1-axes.

8) Starting the experiment

- The experiment is started by typing ”**zg [enter]**” on the command line.
- If needed, the measurement can be halted by typing “**halt [enter]**” or by clicking “zg” in the pop-up window opened after typing ”**kill [enter]**” on the command line.

New experiment for the same sample (e.g. ^{13}C)? →

- Create a new experiment file by pressing the combination **Ctrl + N or** typing **new**
- Change the experiment number (if the experiment number in the ^{1}H measurement is 1, an experiment number 101 is commonly used for the ^{13}C measurement).
- Select the correct experiment from the drop-down menu.
- From Options: **Set solvent** to choose the correct solvent
- Check that **Execute ‘getprosol’** is chosen (in 1D experiments)
- Change the **title** of the measurement to correspond your experiment.
- Type ”**atma [enter]**”. Wait until the text “atma: finished” appears under the command line (bottom left corner).
- Continue from ***” 7) Before the measurement”***.

9) Ending

- Repeat ***” 2) Replacement of the sample”***. Insert the solvent tube into the magnet and **carry out the field locking routine**.
- **Remember to fill in the logbook.**

For washing the NMR tubes own guidelines are presented (file: *Instructions_for_washing_the_NMR_tubes.pdf*).

Following routines should be carried out on personal workstation especially during busy hours

Copying and processing the data on your own workstation

- Workstations at the Department of Chemistry contain software TopSpin suitable for spectral processing. You can also download the program to your own computer from:

  https://www.bruker.com/service/support-upgrades/software-downloads/nmr.html

- Copy the data to a USB memory. You can find your measurement files from C:\Data\your own measurement directory.
- On your own computer paste the data to C:\Bruker\TopSpin 3.2\examdata.
- Open the TopSpin software. You will see the data on Browser leaflet from where it can be processed.

10) Spectral editing

- The Fourier transformation is conducted by typing **"efp [enter]"** on the command line (for 2D measurements "**xfb [enter]**"). Phase is corrected by the command **"apk [enter]"** and the baseline by the command **"abs [enter]"** (for 2D experiments baseline is corrected by using command "**absb [enter]**". For homonuclear 2D correlation spectra symmetrization can be applied by command "**syma[enter]**" (however, this is not recommended since F1 → F2 and F2 → F1 magnetization transfer pathways are not necessarily identical).
- If the automatic phase correction does not give a satisfying result, the phase can be adjusted manually. Use the mouse to choose the Adjust Phase functionality from the Process menu. By using the left mouse button and dragging (up/down) on top of the icons PH0 and PH1 in the phase correction mode, you can adjust the phase. When you are pleased with the result, click the "save & enter" icon ).
- Expand the spectrum in the vicinity of the resonance signal of the solvent by dragging with the left mouse button. Select the **Calib. Axis** functionality from the Process menu, set the cursor on top of the signal, and select using the left mouse button. Insert the chemical shift value of the reference signal to the pop-up window (can be found e.g., from the "NMR Solvent Data Chart" booklet located in the NMR laboratory. CDCl3: $^{1}H\delta$ = 7.26 ppm, $^{13}C\delta$ = 77.0 ppm) and press [enter]. (For 2D measurements first project the previously measured 1D spectra to F2 and F1 axes by clicking the right mouse button on top of each axis, selecting "External projection", and finally choosing the correct measurement from the pop-up menu. Then decide the correlation peak that you will use for calibration. Expand the spectrum from the vicinity of the resonant frequency of the selected correlation peak area by dragging with the left mouse button down. Select the Calib. Axis functionality from the Process menu (equal to 1D processing), set the cursor in the middle of the contour chart of the signal (on top of the signal), and select using the left mouse button. Insert the corresponding chemical shift values from the 1D spectra.)
- Reset the whole spectrum on the screen (see functions of the icons below). Spread out the spectrum in the vicinity of the resonance signals of your sample and choose the **Integrate** functionality from the Process menu.

- In the integrate mode, first remove the integrals defined by the software as part of the abs command by clicking the icon:

  Then choose the integrate icon:

  and integrate the signals by dragging with the left mouse button.

- The default value for the first signal is 1.00. In the case you wish to change the value, click the right mouse button on top of the signal, choose calibrate from the pop-up menu, insert the desired value, and confirm by pressing [enter].
- When finished, leave the integrate mode by choosing "save & enter" icon 
- Select **Pick Peaks** functionality from the Process menu and further "Auto-pick All" from the drop-down menu, which opens by clicking the arrowhead on the frame of the Pick Peaks functionality. You can change the MI (= *minimum intensity*) value (0 → e.g., 0.3) on the ProcPars leaflet by choosing "Show/Modify Picking Parameters" from the drop-down menu, which opens by clicking the arrowhead on the frame of the Pick Peaks functionality.

  (When you move the cursor on top of an icon and wait for a while, the text describing the functionality of that icon appears on the screen.)

11) Printing

- Select **Plot Layout** functionality from the Publish menu.
- LAYOUT can be changed by clicking the arrowhead next to the text on the grey background and selecting "Open" from the drop-down menu.

^{1}H:	+/1D_H.xwp
^{13}C & ^{13}C DEPT-135:	+/1D_X.xwp
^{1}H,^{1}H COSY:	+/2D_hom.xwp
^{1}H,^{13}C HMQC & HMBC:	+/2D_2pro.xwp

- Edit the spectrum the way you wish. There are separate instructions for using the PlotEditor software.
- The active window can be printed by selecting **Print** functionality from the Publish menu or saved by selecting **Export as** from the File menu.

Appendix

List of **JYU_*** NMR experiments with premade parameters available

JYU_1H – *a basic one-dimensional (1D) ^{1}H experiment with a single 30 ° RF pulse.*

JYU_13C-{1H} – *a basic 1D ^{13}C experiment with ^{1}H decoupling during the acquisition. OBS! Keep the number of points in during the acquisition limited due to applied ^{1}H decoupling, which may damage the probehead.*

JYU_DEPT135 – *the ^{13}C multiplicity edited polarization transfer with ^{1}H decoupling during the acquisition. The ^{13}C resonance intensity is modulated by the number of attached protons → CH_2 and CH/CH_3 moieties are phased positive and negative signals, respectively. OBS! Keep the number of points in during the acquisition limited due to applied ^{1}H decoupling, which may damage the probehead.*

JYU_HH-COSY – *a classical 2D, magnitude mode ^{1}H-^{1}H COSY experiment. It shows correlations between protons 2- and 3-bonds apart.*

JYU_NOESY – *a classical 2D, magnitude mode ^{1}H-^{1}H NOESY experiment. It shows through space correlations between protons > 5-6 Å apart. Pay attention to zero-quantum correlations stemming from the COSY transfer.*

JYU_HSQC – *a classical 2D heteronuclear ^{1}H,^{13}C correlation experiment. You will see one-bond correlations between ^{1}H and ^{13}C nuclei. OBS! Keep the number of points in F_2 (^{1}H) -dimension limited due to applied ^{1}H decoupling, which may damage the probehead.*

JYU_HSQC_edited – *a more sophisticated 2D ^{1}H, ^{13}C correlation experiment. OBS! Keep the number of points in F_2 (^{1}H) -dimension limited due to applied ^{1}H decoupling, which may damage the probehead. Similar info to Oma_135DEPT in two-dimensional form i.e. methylene groups have different phase compared to methine/methyl groups.*

JYU_HMBC – *a 2D heteronuclear ^{1}H,^{13}C multiple-bond correlation experiment. HMBC shows correlations mostly between protons and carbons separated by two or three, and sometimes four and even five bonds. Pay attention to leaky one-bond artifacts sometimes present in the spectrum.*