Instructions to the NMR users

Contacting the NMR Administrator

When you contact the NMR Administrator for the first time, *i.e.* when you request the NMR training, please provide the following information in the email:

- Your FirstName LastName and the Aalto username.
- Name of your research group and group leader.
- Do you want to perform liquid-state or solid-state experiments (please note that the solid-state experiments are possible only for a couple of weeks per year)?
- Which instrument you want to use: AVIII400 or AVNEO400 (see descriptions below)? The final decision about the instrument is made by the NMR Administrator. The third spectrometer, AVNEO600, is operated by the NMR Administrator.
- What experiments you want to perform: the basic experiments such as ^{1}H and ^{13}C, and/or more advanced experiments such as 2D HSQC, ^{1}H with a solvent suppression, quantitative ^{13}C, *etc.*?

Before you contact the NMR Administrator, please ask your group members if they are also interested in receiving NMR training.

AVIII400 (400 MHz spectrometer). AVIII400 is equipped with an automatic sample changer with 60 sample positions. AVIII400 has less restricted reservation policy than AVNEO400, allowing long (>2h) experiments to be performed also during daytime. AVIII400 is typically used by researchers analyzing synthetic polymers, lignin, cellulose, and other wood-related biopolymers and compounds.

AVIII400 is equipped with a 5 mm liquid-state broadband probe allowing NMR experiments on various nuclei, the most typical being ^{1}H, ^{13}C, ^{19}F and ^{31}P. With AVIII400, a variety of 1D and 2D experiments can be performed. Examples of 1D experiments: ^{1}H, ^{13}C, ^{13}C DEPT, ^{19}F, ^{31}P. Examples of 2D experiments: ^{1}H,^{1}H COSY, ^{1}H,^{1}H NOESY, ^{1}H,^{13}C HSQC, ^{1}H,^{13}C HMBC.

AVIII400 can also be equipped with a 4 mm CP/MAS solid-state probe. Typical nuclei studied with this probe are ^{13}C and ^{31}P. The CP/MAS probe is changed based on requests only for short periods a couple of times per year.

AVNEO400 (400 MHz spectrometer). AVNEO400 is equipped with an automatic sample changer with 24 sample positions. The reservation policy is more restricted than with AVIII400 (max. 2h during daytime). AVNEO400 is typically used by researchers performing organic synthesis.

AVNEO400 is equipped with a 5 mm liquid-state broadband probe allowing NMR experiments on various nuclei, the most typical being ^{1}H, ^{13}C, ^{19}F and ^{31}P. With AVNEO400, a variety of 1D and 2D experiments can be performed. Examples of 1D experiments: ^{1}H, ^{13}C, ^{13}C DEPT, ^{19}F, ^{31}P. Examples of 2D experiments: ^{1}H,^{1}H COSY, ^{1}H,^{1}H NOESY, ^{1}H,^{13}C HSQC, ^{1}H,^{13}C HMBC. Due to a transceiver architecture, it is possible to run multi-receiver experiments with AVNEO400.

AVNEO600 (600 MHz spectrometer). AVNEO600 is equipped with a 5 mm helium-cooled TCI (^{1}H, ^{13}C, ^{15}N) liquid-state probe and an automatic sample changer (24 sample positions). Due to an unsurpassed sensitivity of the cryoprobe, AVNEO600 can be used in the NMR analysis of mass-limited samples. If higher concentration samples are available, AVNEO600 allows experiments to be run within a fraction of time in

comparison with 400 MHz spectrometers. In addition, the 600 MHz magnet provides more resolution than the smaller 400 MHz magnets. Please note that this spectrometer is not intended for routine NMR analysis of samples that can be measured with AVIII400 or AVNEO400. However, also routine samples can be analyzed if instrument is not heavily booked (please contact the NMR Administrator).

Examples of 1D experiments: ^{1}H, ^{13}C and ^{13}C DEPT. Examples of 2D experiments: ^{1}H,^{1}H COSY, ^{1}H,^{1}H NOESY, ^{1}H,^{13}C HSQC, ^{1}H,^{13}C HMBC, ^{1}H,^{15}N HSQC, ^{1}H,^{15}N HMBC. In addition to 1D and 2D experiments, a number of 3D experiments can be performed. Due to a transceiver architecture, it is possible to run multi-receiver experiments with AVNEO600.

General information

Researchers operate AVIII400 and AVNEO400 by themselves. Before you can operate the instrument, you have to go through training given by the NMR Administrator. After the training is finished (it will take about one to two hours), you will be designated as an authorized user and you will be able to reserve time on the instrument which you were trained at. There are separate training sessions for the liquid-state operation and for the solid-state operation (solid-state: AVIII400 only). ***AVNEO600 is operated by the NMR Administrator*** (more information below).

Please note that the NMR Administrator is not able to provide any NMR solvents or sample tubes. You should obtain these items from your group. However, there are two sets of CP/MAS tools/rotors for a loan. One set is located in the Department of Chemistry and Materials Science (Kemistintie 1) and the other set can be found in the Department of Bioproducts and Biosystems (Vuorimiehentie 1).

Only the basic operation of the spectrometer is included in the training, *i.e.* routine ^{1}H and ^{13}C measurements or basic CP/MAS operation. Training for the more advanced experiments, *e.g.* 2D experiments and ^{1}H with a solvent suppression, is provided by request. Typically, the interpretation of the spectra and the analysis of the data are not included in the training. If you need more assistance in NMR spectroscopy in your research, the NMR Administrators' name has to be included in the author list of the possible publication.

There is no democracy in the NMR room. If required, the NMR Administrator is authorized to override your reservation. Invoicing is based on reservations. Therefore, if for some reason you are not able to perform your experiments and cancel your reservation, please contact the NMR Administrator to avoid being charged for an unused reservation. The NMR Administrator does not handle invoicing.

AVNEO600 instructions

Prepare your samples as described below in section "Liquid-state sample preparation" and deliver sample tubes to the NMR Administrator with the following information:

- Your FirstName LastName and the Aalto username.
- Name of your research group and group leader.
- A brief description of samples to be measured.
- Required experiments.
- NMR solvents that have been used in the sample preparation.
- An estimation of the concentration of samples.
- Required experiment temperature (if other than 298 K).

You will be informed when you can transfer the data from the AVNEO600 PC to your own computer. Invoicing is based on the actual usage of the instrument.

Additional instructions

- ***Only acquisition and initial processing of the NMR data is allowed with the NMR PC.*** Do not create/save picture files, PDF documents, *etc.* In addition, do not create new file folders (other than TopSpin/IconNMR operation requires). Use your own PC for further data processing and analysis. Bruker TopSpin processing-only software is freely available for the academic users. Contact IT for the details.
- 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.***
- Keep reservations as short as possible. Do not reserve the instrument "just in case". Long measurements should be done during night time or weekends.
- Do not change TopSpin/IconNMR window position/size.
- Do not remove tools, test equipment or manuals from the NMR room.
- Do not modify standard library pulse sequences, parameter files, system configuration files, standard AU programs *etc.* except under direct instruction of the NMR Administrator. In addition, do not make any changes in the hardware configuration.
- If you run experiments without the automation, *i.e.* if you are using the TopSpin interface, please remove your sample from the magnet before the next user enters the NMR room. Do not leave your sample tubes/rotors in the NMR room.
- Contact the NMR Administrator if you want to change the sample temperature more than ±10 K.
- If you change the sample temperature, please return the temperature back to the default value of 298 K before the next user starts to operate the instrument.
- Always sign the NMR logbook before leaving the NMR room.

Liquid-state sample preparation

- **400 MHz spectrometers (AVIII400 and AVNEO400):** for ^{1}H NMR, 1-5 mg of the sample is sufficient to obtain a decent S/N in short experiment time. For high molecular weight samples, more concentrated solutions are sometimes recommended. However, too concentrated solution leads to lower resolution due to saturation and/or increased viscosity. For ^{13}C NMR, *ca.* five times the concentration is recommended. For 2D measurements, the sample should be concentrated enough to achieve an acceptable S/N ratio. As a rule of thumb, 25 mg of the sample is sufficient for nearly everything, including ^{1}H,^{13}C HMBC experiment. With samples of only 1-5 mg, the homonuclear ^{1}H,^{1}H experiments (*e.g.* COSY, NOESY) are still feasible, but those involving ^{13}C (*e.g.* HSQC, HMBC) may take overnight to complete. Recommended sample tube: **Wilmad 527-PP-7** or **527-PP-8**; **Norell 507-HP-7** or **507-HP-8**.
- **600 MHz spectrometer (AVNEO600):** this instrument provides considerable increase in S/N in comparison with the 400 MHz spectrometers: over 16-fold increase in S/N for ^{1}H detected experiments and *ca.* 6-fold increase in S/N for ^{13}C detected experiments. The increase in the S/N ratio by a factor of *ca.* 4 leads to a possible reduction in experiment time of 16-fold or a reduction in required sample concentration by a factor of 4. Recommended sample tube: **Wilmad 535-PP-7** or **535-PP-8**; **Norell S-5-600-7** or **S-5-600-8**. Recommended tube cap (can only be used with Wilmad Precision 535-PP tubes): **Wilmad 5 mm PTFE NMR Cap** (especially when using $CDCl_3$).
- Always use deuterated solvents. The solvent should be dry, *i.e.* free from water (unless the solvent is 90% H_2O/10% D_2O).

- Sample solution height must be ***at least 4 cm*** for 5 mm NMR tubes. However, solution height more than 5 cm is not recommended.
- Always use clean and dry sample tubes that are free of scratches. Keep the bottom half of the sample tube clean and do not touch it with your bare hands. Use lint free tissue to clean the outer surface of the sample tube. ***The above is especially important if samples are going to be measured using AVNEO600.*** Dirty sample tubes will contaminate the probe. Scratched tubes could break inside the probe.
- The sample solution should be clear with no precipitate. If there is precipitate or particles in the solution, the sample should be filtered. A small plug of fresh medical cotton wool at the neck of a Pasteur pipette will do the trick. However, it is necessary to pre-rinse the pipette with a little amount of the solvent to be used to flush out any loose fibers.
- ***Do not use marker pen to write directly on the NMR tube wall.*** Instead, write the sample information on a paper/plastic label and secure the label to the tube by wrapping with clear, adhesive tape (e.g. Scotch tape).
- Proper cleaning procedures for NMR tubes: https://www.wilmad-labglass.com/Support/NMR-and-EPR-Technical-Reports/Proper-Cleaning-Procedures-for-NMR-Sample-Tubes/.

Solid-state sample preparation

- A video demonstrating how to pack and handle a CP/MAS rotor properly: https://www.youtube.com/watch?v=bNFJj2g0UjI.
- Always wear gloves when handling the sample rotors.
- Reserve at least *ca.* 200 mg of sample for the CP/MAS analysis. If your sample is not fine powder, grind it with the Bullet/ball mill grinder that is used to prepare IR samples. Alternatively, use electric coffee grinder to prepare a powder sample. If necessary, use liquid nitrogen to cool your sample before grinding. The cooling step helps to obtain powder with sufficient fineness level. The sample must be finely ground before it is packed into the rotor.
- The sample rotors are very expensive. The rotors are not disposable – they must be recycled. When the experiment temperature is 20-60 °C, use rotors, which have a clear cap.
- Confirm that the rotor and cap are clean.
- Weight the rotor before and after the use. This way you can determine whether you have cleaned it properly after the use.
- Pack your sample (weight *ca.* 100-200 mg) into the rotor in small batches using the funnel and packing tool. If you pack the sample on hard surface, use a bent paper hand towel as a cushion under the funnel and rotor. Packing must be done in sufficiently small batches to make the sample sufficiently compact.
- Use the red markings in the packing tool to see when the rotor is fully packed. Do not leave rotor less than full – there is a risk of rotor explosion during spinning.
- Use the cap set tool to correctly position the rotor cap. Handle caps with care – the blades damage easily if you handle them with too much force. Always check first that the cap or blades are not damaged. Do not use defective caps.
- Wipe off dust and grease from the rotor with lint free tissue.
- If the rotor has no laser-etched semicircle for the spinning speed control, use a Sharpie pen to draw a semicircle to the bevel in the rotor bottom. Let the marking dry well before inserting the rotor to the magnet.
- If you do not need the sample anymore, remove the cap with the cap removal tool and clean the rotor with bore bit of the packing tool.
- Return rotors and tools back where you took them.

About the magnet and the security

Large attractive forces may be exerted on magnetic materials or equipment in proximity to the NMR magnet, which is always at field. The force may become large enough to move the equipment uncontrollably towards the NMR magnet. Small pieces of equipment (*e.g.* tools, bolts and nuts) may therefore become projectiles. Large equipment (*e.g.* gas bottles) could cause bodies or limbs to become trapped between the equipment and the magnet. The closer to the magnet, the larger the force. The larger the equipment mass, the larger the force.

Due to the very effective shielding of the superconducting coil, the effects of the magnetic stray field are minimized. Nevertheless, keep in mind that directly above and directly below the magnet, the stray field is very high and the attractive forces on magnetic items are very strong.

The operation of medical electronic implants, such as cardiac pace makers, may be affected by the magnetic field. Other medical implants, such as aneurysm clips, surgical clips or prostheses, may contain ferromagnetic materials and therefore would be subjected to strong attractive forces near to the NMR magnet. Items such as watches and phones may be magnetized and irreversibly damaged. In addition, information encoded magnetically on credit cards may be irreversibly corrupted.

Note that moving magnetic materials affects the magnetic field disturbing the measurements. Note also that there is a possibility for (unlikely) event of the magnet quenching, where up to 100 m^3 of helium gas may evolve over a period of several minutes. Personnel should evacuate the area in such a situation since helium gas can displace oxygen in the room. A quench warranting evacuation would be obvious by the noise of the erupting gas and clouds of vapor. In addition, the oxygen meter will alarm.

Briefly: do not bring any magnetic objects near the NMR magnet. Before going close to the magnet, empty your pockets and take off your watch.