



NMR Self–Service User Meeting



Overview

- **Training** of new users every Monday at 9am
 - For registration, write an email to **jakub.stocek@uochb.cas.cz** or call **496**
- Maintenance time every Monday 9-11am, Cleaning every Tuesday 9:00-9:30
- Abandoned samples now kept for only 5 weeks
 - Samples from the current week are kept in separate holders for Old 400 and PRODIGY for user convenience
 - All remaining samples from **previous week** are **moved into a beaker** with

corresponding date during maintenance time







- **Q:** Why is it not possible to measure **NOE experiments?**
- A: The required time for would be too long for the queue

- **Q:** Can I get experiments with **longer relaxation times**?
- A: Yes, they can be made available on **special request**

- **Q:** Is there an easy way to **find my sample** after removal?
- A: Find a way to uniquely mark your tubes or tube caps (e.g. colored marker)



- **Q:** Can I measure ¹⁵N related experiments?
- A: No, they take too long

- Q: What are the instrument specifications and experiment parameters?
- A: You can find everything on the IOCB intraweb under
 Science/Service Labs/NMR

- Feel free to contact us with any additional questions!

New PDF Guide





NMR self-service at IOCB

1. Instrumentation





"OLD" probehead: broad band 5mm measured nuclei: ¹H, ¹⁹F, ¹³C, ¹⁴P, ¹¹B sample changer: B-ACS for 120 samples lcon-Web sccss: <u>http://ob-a401:8015</u>

"PRODIGY" probehead: broad band 5mm nitrogen cooled cryo-probe measured nudei: ¹H, ¹³C, ¹³P, ¹³B sample changer: Sample Xpress for 60 samples lcon-Web access: <u>http://ob-s402:8015</u>

2. General policy

- All users must pass a training course before they obtain their own access to the spectrometer. The trainin
 course takes place every Monday at 9:00 a.m. Please, arrange an appointment at <u>NMR-400@uochb.cas.cz</u>.
- The samples are run on a first-come-first-serve basis with all experiments longer than 35 minutes (OLD) or 3 minutes (PRODIGY) running at night. Night experiments start at 6:30 p.m. (18:30) and end at 8:30 a.m. (8:30 Weekends are treated as night days with day experiments priority.
- Clean the bottom part of the NMR tube and the spinner with provided paper napkins moistened by ethans prior to placing the sample into the sample changer. Always use the calibration tool to ensure correct tub insertion depth in the rotor. Incorrectly inserted tubes can break when being dropped into the magnet.
- The experiments can be only submitted via the Linux workstations controlling the NMR spectrometers.
- Take your NMR tubes back ASAP after the measurement is finished. Samples that are not taken back will b
 discarded one month after the date of measurement.
- Users are asked to report damaged or improperly inserted sample tubes to the NMR Facility staff immediatel in order to avoid needless down time and equipment damage!
- There is maintenance time every Monday morning (9:00 11:00 a.m.) reserved for the NMR Facility staf Entering the spectrometer room during maintenance time is forbidden to ensure safety and prevent equipmer damage.
- In the case of spectrometer failure, contact the NMR staff at <u>NMR-400@uochb.cas.cz</u>.

3. Before you start

- Samples for the measurement must be prepared in the user's laboratory.
- Special precaution for "OLD": Your NMR tube must be at least 17.5 cm (6.9 inches) in length and must not be broken or cracked! If your tube is shorter than 17.5 cm, it will not be grabbed fully by the sample changer and may fall and break on the floor or on the top of the magnet. Tubes with chipped tops are weakened and may be broken by the robotic arm pincers which grab the tube very firmly close to the top. Any tube shorter than 17.5 cm or with a chipped top submitted to the sample changer will be removed.
- Your NMR tube must not have any label (paper, tape, sticker etc....) attached to it. This may stop the sample
 changer from working properly and may result in sample breakage and equipment damage. Tubes can be
 labeled on the glass or the cap with permanent marker. Any tube with an attached label submitted to the
 sample changer will be removed.
- Always use 0.5-0.6 ml of solvent and make sure your sample is dissolved. Unsuitable solvent levels or nonhomogeneous samples can lead to a bad shimming result and useless spectra. Moreover, using excess solvent increases the risk of equipment damage in case of a broken tube.
- Think of experiments you are going to submit and use the spectrometer time responsibly. Do not submit both ¹³C and APT for one sample. Do not submit more than 3 samples with complete set of experiments in one submission.

4. Accessing and handling your data

- Status of the sample changer (information, if your sample has been already measured) can be viewed on-line
 using lcon-Web service simply by typing http://ob-a401:8015 (OLD) or http://ob-a402:8015 (PRODIGY) into
 your web browser. Submitting of experiments through lcon-Web is forbidden.
- Immediately after the experiment is completed, the data are stored at the Linux workstation in the directory opt/Bruker/Data/data/usemame and automatically archived at the SAMBA server in the directory 147.231.122.7/nmruser/data/usemame (OLD) or 147.231.122.7/nmruser/PRODIGY/data/usemame (PRODIGY) accessible through the IOCB network. Please, backup your data! We do not take responsibility for your data!
- Mestrenova process your data
- The latest version of NMR processing software Mestrenova can be downloaded from Mestrelab Software web
 pages: http://mestrelab.com/download/mnova/.
- The Mestrenova license files can be found at the SAMBA server in the directory: 147.231.122.7/nmruser/MestrecLic

The updated intraweb page



ÚOCHB # Q Vyhledat **IOCB PRAGUE** Science Noticeboard Events Management Administration HR & Payrolls ITS Contacts Misc Documents Signpost / Ovod / Science / Service Labs / NMR Grants

Library ORCID For Ph.D. Students...

Mentoring

Service Labs

Analytical Laboratory

Postdoc Projects Lectures Nomination

ELIXIR CZ - Resources



Self-service on Bruker Avance 400 MHz NMR spectrometers

- ♦ Check a progress of sample changer Bruker Avance III™ HD 400 MHz ("Old")
- ◆ Check a progress of sample changer Bruker Avance III™ HD 400 MHz with cryoprobe ("Prodigy")
- Instruction manual for self-service measurement 📆

FAQ

1. Why we cannot measure NOE-type experiments?

Our goal is to keep the night queue shorter than one night as often as possible. Unfortunately NOESY or ROESY experiments are usually quite time-consuming. To be meaningful, these experiments would require on the 400 MHz spectrometers several hours of the spectrometer time that would lead to significantly longer night queues. An alternative to the 2D NOE experiments is the measurement of 1D differential NOE spectrum. However, this experiment requires a careful experimental setup for each sample, which is not possible in the self-service automation mode.

If you need any NOE-type experiment, we will be happy to measure it for you on other spectrometers of the NMR Spectroscopy group.

2. How should we describe the 400MHz spectrometers in methodological parts of publications? Please edit the following text according to your needs:

The NMR spectra were measured on a 400 MHz Bruker AVANCE III spectrometer (¹H at 400 MHz or 401 MHz (PRODIGY), ¹³C at 101 MHz, ¹⁹F at 377 MHz, ³¹P at 162 MHz) equipped with a liquid-nitrogen cryoprobe (PRODIGY). The spectra were referenced to the solvent signals $\delta(1H) = 7.26$ ppm (CHCl₃), $\delta(1H) = 3.31$ ppm (CHD₂OD), $\delta(13C) = 77.0$ ppm (CDCl₃), $\delta(13C) = 49.0$ ppm (CD₃OD), ...

3. What is the number of scans in the experiments?

Experiment	Old 400	PRODIGY
1H	32	16
13C_long, APT_long	1536	528
13C_short, APT_short	384	-
31Pdec	32	16
31Pnodec	34	32
19F_nodec	16	-
COSY	1 scan, 128 increments	1 scan, 128 increments

Check a progre Instruction ma

MS NMR Molecular Spectroscopy Facility Cytometry Virology Computational core facility Special Authorizations Animal Facility CAS Code of Ethics



- Write an email to nmr-400@uochb.cas.cz for 24/7 support
 - We have tools for **remote access**
 - A lot of issues can be fixed by us from home
- Who we are:
 - Martin Dračínský
 - Radek Pohl
 - Ondřej Socha
 - Jakub Štoček





NMR Self-Service User Related Issues



Common Issues – Sample Preparation



- Inappropriate sample volume
 - **Ideal volume is 600 μl**, acceptable levels between 500-800 μl
 - Lock/Shimming might be impossible with low volumes
 - Increased risk of equipment damage with high volumes
- Too much sample/bad solubility
 - Results in **bad or useless spectra**
 - Roughly 10% of samples are cloudy or show significant sedimentation
 - Pay attention to solubility and remember that more sample is not always better
- Improper use of the calibration tool
 - Always use it and push the tube to the bottom of the tool







Common Issues – Sample Preparation



- Not cleaning the bottom of the tube/rotor with ethanol
 - Accumulating contaminants clog the air nozzles responsible for sample rotation
 - Risk of probe contamination and damage
 - Meaintenance downtime can take up to half a day
- **Removing** samples prior to measuring **without deleting** experiments
 - Common in cases of unexpected machine downtime
 - Detecting **3 missing samples** in a row makes the queue **stop automatically**

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Common Issues – Sample Preparation



- Using **broken** tubes or rotors
 - High risk of equipment damage
- Using unusually **short tubes**
 - Causes sample load failure on Old 400
 - Dont try to fix broken tubes by making them shorter
- Not all rotors and tubes are created equal, avoid loose fit





Common Issues - Mess in the lab

- Samples are not being picked up
 - Over **150 samples per month** are **abandoned** and many more are retrieved late
 - Maintenance time takes longer
- Tubes are being left in rotor holders
 - High risk of sample damage, spillage and injury
- Users interchange samples between beakers
 - Samples are harder to find
 - Your samples can be discarded after two weeks if unfortunate
- Keep the self-service lab **nice and tidy** for other users









Common Issues – Experiment Planning



- Redundant experiments

- Measuring both **APT/¹³C** for one sample
- Measuring **full sets of 2D** experiments for a whole **batch of samples**
 - Very obvious that the user does not know what they are doing
- Doing this gives you **no valuable data** and **makes the queue longer for everyone**
- Choose experiments according to what you really need, not "what if"

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- Restarting automation on your own
 - **NEVER** do this!
 - There often is a **broken tube inside** of the machine
- IconNMR crashing
 - Avoid using special symbols in sample names
- Make sure to **backup your data**
 - Data on the SAMBA disk are automatically deleted after 3 years
 - We do not keep a data backup from the 400MHz spectrometers



Stop doing NMR!

- Magnets were meant for fridges! Not for nuclei!
- NUCLEAR magnetic resonance!!! If that's not TERRORISM I don't know what is!
- All this research and still no ¹²C NMR???
- Labelling with ¹⁵N and ²D is against GOD'S will and against NATURE ITSELF!!!!!
- Are you an organic chemist who wants to know what you synthesized? Yeah, there's a method for that called TASTING!!!!