

**Training Material for remote access to NMR
Facilities**

NMR Data Acquisition

Provided by Remote-NMR (R-NMR):

Moving NMR infrastructures to remote access capabilities



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1. Preamble

General guidelines and standard operating procedures are developed in the course of the R-NMR project. This file does not provide the final solution, but is the state of the discussion as of June 2023.

Remote NMR access has always been an option, but it has gained particular significance during the COVID-19 pandemic when travel is greatly restricted. During this period, the ability to access research facilities remotely is especially valuable.

When users access a research infrastructure, their interaction with the scientific and technical staff for support and guidance is often just as important as the actual instrument time. These interactions contribute to the success of the measurements. Consequently, we are currently developing new methods to maintain productive interpersonal communication through remote access procedures.

User support can encompass various aspects, ranging from providing suggestions and advice during the initial planning stages of measurements to assisting with experimental setup.

Additionally, we offer training on specific areas of interest to users.

However, the level of assistance and supervision required varies depending on the expertise of the visiting scientist using the requested technology remotely.

In this file we provide training material- not particularly tethered towards remote NMR but applicable also for local NMR measurements. So far SOPs for **liquid state NMR** and **metabolomics** are provided.

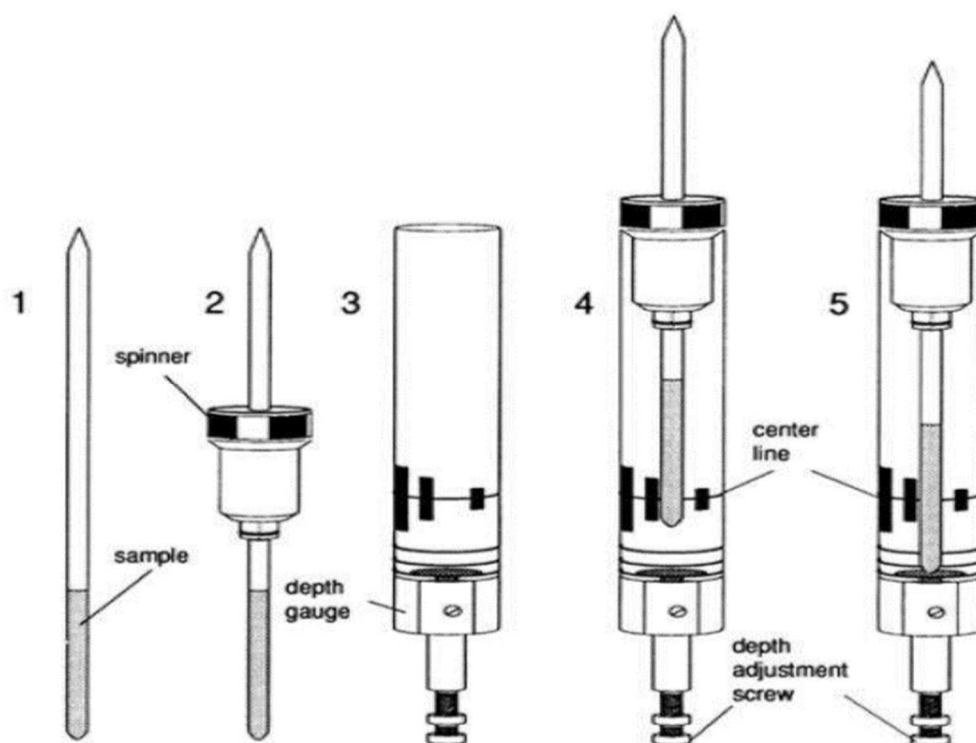
2. SOP for liquid state NMR

All NMR spectra and procedures are dedicated to a spectrometer, which is controlled from a PC with windows 7 installed:

1. Open the Topspin software from the desktop of the PC controlling the spectrometer
2. Open or make a dataset. To do this just select it from the browser menu at the left side of the screen and drag and drop it to the main window of the software (or right click on it and choose display). Someone can make a new directory if it is needed. You can add it by right clicking and choosing “Add new data dir...” menu.

3. The desired sample temperature can be set by typing edte at the command line at the bottom of the screen. At the window opens press set and choose the desired temperature (NOTE: Be carefull about the Temperature range capacity of the operating spectrometer!). Wait until the temperature stabilize.

4. Note for NMR facility managers: Insert the sample into the magnet either manually or by using the automated sample case (if existed). NMR tube containing sample should be held in a plastic/ceramic spinner (5mm, 3mm etc.). Hold the sample by the top, place sample tube in the spinner and the spinner in the sample depth gauge. Push or pull the sample tube so that the depth of the sample above and below the center line of the sample depth gauge is equal. However, never exceed the lower limit (position of the adjustable white platform, which should be at about 21mm) as this can damage the probe as well as the sample. Put the sample in any position of the sample case and push the blue button on it to switch the sample until the green button turn on. Then push the green button to lift the sample and place it inside the magnetic field into the probe.





5. Lock signal can be seen in the lock display window. Open the window by typing in the command line `lockdisp`. Type `lock` (or `ll`) at the command line to choose the appropriate solvent from the pop up solvent table window and wait until the lock finished message to appear at the bottom of the software window. If the spectrometer fails to lock, this may indicate that your sample does not include any deuterated solvent.
6. It is necessary to “Tune” the RF coil to the correct value to yield the correct resonant frequency for the magnet field strength. This can happen by adjusting two variables called “tune” and “match”. Someone must tune and match all nucleus. This can be done automatically typing in the command line `atma` (or `aa` to do it manually). To do any of the above procedure a dataset of parameters should be open.
7. To create dataset someone can type `edc` to a previously used dataset and save it in a different directory or exp number or can type `new` and choose to create a new from scratch by typing all the necessary parameters like Name, Expno, Dir, User, etc. and then press OK. This will take you to the new created dataset.
8. Shimming is a process in which minor adjustments are made to the magnetic field until uniform magnetic field is achieved around the sample. This is difficult to do manually but can be done using automatic procedures. Type `topshim gui` (or `tg`) to open the `topshim` window. Choose the optimization parameters for the sample and press the start button at the bottom of the window. After the procedure finishes the lock display will stabilize the lock signal in the middle.
9. From the BSMS Control suite someone can adjust from the main tab the parameters of the lock. Choose the appropriate GAIN level and PHASE of the LOCK and don’t forget to press the STD BY button when the adjustment finishes (or use the AUTO GAIN and PHASE button for automatically adjustment).
10. Determine the 90 degree proton pulse at high power. This can be done automatically typing at the command line `pulsecal` and wait for some seconds. Calibrated pulselenghts for a hard pulse and soft pulse will be shown in a new pop up window. Note down these values.
11. To setup your experiment of interest, use `edc` to create a new data set and then use `rpar` to read a standard parameter set for the experiment. Choose any suitable standard parameter set from the list which is appropriate for your purpose and click OK. This step will ensure that all the necessary standard parameters are copied for the experiment.



12. Type edasp and check that the routings are OK. If you don't know the correct routing press the default button at the bottom of the window and then press save and close. If you have doubts, ask an NMR specialist of the spectrometer.
13. Use the command "getprosol 1H <pulselength> <power>". Substitute the values of the proton hard pulse length and power level you got from step 2 above. All the other 1H pulses and their power levels will be calculated (as well as for all other nuclei) and stored in the current dataset.
14. The carrier positions for each of the dimensions of your experiments have to be set up. These parameters determine the center of the spectrum in any dimension of it in ppm. These parameters can be changed by typing o1p, o2p, o3p and choosing the appropriate value of ppm for each one. If you are not familiar with it ask an NMR specialist.
15. After setting the center, someone have to set the desired spectral width in units of ppm. Type sw and choose the desired values of the spectral width of all dimensions.
16. Change the number of sample points in each dimension if you want to increase the resolution of the spectra. Type td and fill with the desired number of datapoints in each dimension. Typically, a multiple of 64 used (128,256,512,1k etc.).
17. Change the number of scans and dummy scans if you want to increase the signal to noise ratio of the spectra. Type ns and use the desired number of scans (look the pulse program reference). For the dummy scans (the scans needed to equilibrate the sample before start measurement) type ds and use a reasonable number.
18. Receiver gain is a very important parameter to match the amplitude of the fid to the dynamic range of the digitizer. If the FID is clipped at the top or bottom of the display then the receiver gain should be reduced. If the gain is too low and not utilizing a suitable (25-40 %) part of the dynamic range of the digitizer, it should be increased. Type rg to put manually the desired value or use the automatic calculation of the value by typing rga.
19. To start acquisition type zg.
20. After an FID acquired someone should process it to have the spectra. For 1D spectra type efp, for 2D xfb and 3D ft3d. After that phasing and baseline correction are useful for the better presentation of the spectra. External users may request access through one of routes the available listed on the website of each facility, choosing the one that best fits with their necessities. Otherwise, they should contact the facility manager.

Parameters and Pulse Programs suitable for analysis of protein structure and dynamics

				F3			F2			F1				
Experiment Name	Pulse program	NS	DS	SW ppm	O1P ppm	TD	SW ppm	O1P ppm	TD	SW ppm	O1P ppm	TD	O2P ppm	O3P ppm
¹ H-1D	zgesgp_f3ig.ga	128	8							14	4.701	2048		76
¹ H- ¹⁵ N HSQC	hsqcetfpgpsi2	4	32				40	120	256	18	4.701	2048		120
3D-HNHA	hnhsgp3d	16	16	14	4.701	2048	14	4.701	80	35	117	40		117
3D-NOESY ¹⁵ N (d8=120msec)	noesyhsqcfpf3gpsi3d	16	16	14	4.701	2048	36	117	48	14	4.701	256		117
3D-TOCSY ¹⁵ N (d9=80msec)	mlevhsqcetf3gp3d	8	8	14	4.701	2048	39	117	48	14	4.701	128		117
2D- ¹ H- ¹ H NOESY (d8=120msec)	noesyegpph	64	16				14	4.701	2048	14	4.701	2048		
2D- ¹ H- ¹ H TOCSY (d9=80msec)	mlevesgpph	64	16				14	4.701	2048	14	4.701	1024		
3D-CBCANH	cbcanhgpwg3d	16	32	14	4.701	1024	38	117	40	62	39	96	39	117
3D-CBCAcoNH	cbcaconhgpwg3d	8	32	14	4.701	1024	38	117	40	62	39	96	39	117
3D-HNCA	hncagpwg3d	8	32	14	4.701	1024	38	117	40	38	55	80	55	117
3D-HNcoCA	hncocagpwg3d	32	16	14	4.701	1024	38	117	40	38	55	80	55	117
3D-HNCO	hncogpwg3d	32	8	12	4.701	1024	38	117	40	18	176	64	176	117
3D-HNcaCO	hncacogpwg3d	32	8	12	4.701	1024	38	117	40	18	176	64	176	117
3D-CBCAcoNH selective	cbcaconhgpwg3d.2	16	16	14	4.701	1024	38	117	44	62	39	64	39	117
3D-HBHAcoNH	hbhaconhgpwg3d	32	16	14	4.701	1024	38	117	40	8	4.701	112	39	117
3D-hCCH TOCSY	hcchdigp3d2	32	16	14	4.701	1024	80	39	48	80	39	128	39	117
3D-NOESY ¹³ C aliphatic	noesyhsqcetgp3d	16	16	12	4.701	2048	80	40	48	12	4.701	256	40	102
3D-NOESY ¹³ C aromatic	noesyhsqcetgp3d	16	16	14	4.701	2048	39	127	32	14	4.701	160	127	102
¹ H- ¹³ C HSQC	hsqcetgpsp	16	32				14	4.701	2048	160	80	512	80	



				F3			F2			F1				
Experiment Name	Pulse program	NS	DS	SW ppm	O1P ppm	TD	SW ppm	O1P ppm	TD	SW ppm	O1P ppm	TD	O2P ppm	O3P ppm
Relaxation Experiments For Mobility Analysis of Biomolecules														
¹ H- ¹⁵ N HSQC T1 relaxation	hsqct1etf3gpsi	16	16				13.5	4.701	2048	40	120	256		120
¹ H- ¹⁵ N HSQC T1 relaxation (trosy-version)	hsqct1etf3gpsi	16	16	14	4.701	2048	44	120.0	128	10	4.701	10	101.0	120.0
For T1 relaxation measurement at least 9 different delays should be used for D7(msec) : 7, 18, 40, 85, 150, 230, 350, 500, 680, 900														
¹ H- ¹⁵ N HSQC T2 relaxation	hsqct2etf3gpsi	16	16				13.5	4.701	2048	40	120	256		120
¹ H- ¹⁵ N HSQC T2 relaxation (trosy-version)	hsqct2etf3gpsi	16	16	14	4.701	2048	44.0	120.0	128	10	4.701	10	101.0	120.0
For T2 relaxation measurement at least 9 different delays should be used for D20(msec) : 18, 32, 50, 68, 86, 100, 120, 150, 190, 240														
HeteroNOE	hsqcnoef3gpsi	32	16				13.5	4.701	2048	40	120	512		120
HeteroNOE (trosy-version)	hsqcnoef3gpsi	32	16	14	4.701	2048	44	120.0	128	10	4.701	2	101.0	120.0



3. SOP for Metabolomics by NMR spectroscopy

1. Experimental Design:
 - Define the objectives and research questions of the metabolomics study.
 - Identify the sample types, treatment groups, and replicate requirements.
 - Consult with the NMR facility manager or an experienced metabolomics expert for guidance on experimental design.

2. Sample Preparation:
 - Follow established protocols for sample collection, extraction (if needed), and processing.
 - Ensure proper labeling and documentation of sample information, including treatment conditions, sample names, and any relevant metadata.

3. Quality Control:
 - Implement or ask for the availability of standard quality control measures to monitor the integrity of samples and detect any potential issues.
 - Include appropriate control samples, such as blanks or pooled quality control samples, to assess instrument stability and data reproducibility.

4. Experimental Setup:
 - Communicate with the NMR facility manager to discuss the specific requirements and constraints of the NMR instrument.
 - Provide detailed information about the samples (hazardness), experimental parameters (e.g., pulse sequences, relaxation delays), and acquisition settings to the facility manager.

5. Spectral Data Acquisition:
 - Acquire NMR spectra remotely using the established software and protocols (e.g., IconNMR).
 - Ensure adherence to the scheduled time slot and promptly notify the facility manager of any unexpected issues or interruptions.



6. Data Processing:

- Retrieve the acquired spectral data and perform initial processing, including baseline correction, phase correction, and referencing, using appropriate software tools.
- Apply consistent processing steps to all the NMR spectra in each dataset to maintain data integrity and samples' comparability.

a. Metabolite Library: NMR spectra of authentic metabolite/biomarker standards

Metabolites were selected based on their importance within metabolic pathways and their detection potential by NMR. As a general protocol, the selected metabolites first should be, diluted with the appropriate NMR solution buffer. The quantity of a standard NMR solution buffer for measurements using a 5mm NMR probe is 500 μ L of a 200 mM sodium phosphate buffer (Na_2HPO_4), containing 1 mM of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) or 3-(trimethylsilyl)-2,2,3,3-tetradeuteriopropionic acid (TMSP-d₄) as a NMR standard. Then, the corresponding solutions of metabolites (10 mM) should be mixed with 500 μ L solution of H₂O : D₂O, 90 : 10 %, containing an amount of 0.4% NaN₃ as a bacteriostat. The pH of the NMR buffer should be adjusted to 7.4 ± 0.05 by addition of small (μ L) volumes of 1 M HCl or NaOH as required. All standard solutions should be stored at refrigerator 4-8 oC until analysis. The typical characteristics of each biomarker solution are a) a nominal metabolite concentration of 5 mM, b) NMR solvents constitution of H₂O : D₂O, 90 : 10 % and c) chemical and quantification shift standard was 1 mM of internal standard (DSS or TMSP).

For each metabolite standard, NMR spectra can be recorded using a total of six NMR spectra per metabolite at the appropriate pH and Temperature, including 1D NOESY, ¹³C-NMR, 2D ¹H J-resolved, 2D ¹H-¹H COSY and ¹H-¹³C HSQC. These experiments provide the standard set of NMR spectra usually analyzed in NMR metabolomics studies. They provide adequate information about the signal multiplicity and coupling patterns, with different water suppression methods and different acquisition parameters.



- 1 1D 1H NOESY: a standard pulse sequence (noesygppr1d; Bruker). Suggestion: use 16 scans, 32k size of FID, a spectral width of 15.0 ppm, an acquisition time of ~1.5-1.6 s, and a relaxation delay of 2.0 s.
- 2 13C-NMR: a standard pulse sequence (zgig; Bruker). Suggestion: use 512 scans, 64k size of FID, a spectral width of 202.0 ppm, an acquisition time of 0.9 s, a relaxation delay of 2.0 s.
- 3 2D 1H J-resolved: standard pulse sequence (jresgpprqf; Bruker). Suggestion: use 8 scans, 80 and 16k (F1/F2) size of FID, a spectral width of 0.11 and 16.0 ppm (F1/F2), an acquisition time of 0.5 and 0.7 s (F1/F2), a relaxation delay of 2.0 s.
- 4 2D 1H-1H COSY: standard pulse sequence (cosygpprqf; Bruker). Suggestion: use 4 scans, 256 and 8k (F1/F2) size of FID, a spectral width of 11.0 and 11.0 ppm (F1/F2), an acquisition time of 0.5 s, a relaxation delay of 2.0 s.
- 5 2D 1H-1H TOCSY: standard pulse sequence (mlevph; Bruker). Suggestion: use 8 scans, 256 and 2k (F1/F2) size of FID, a spectral width of 11.0 and 11.0 ppm (F1/F2), an acquisition time of 0.2 s, a relaxation delay of 2.0 s.
- 6 2D 1H-13C HSQC: standard pulse sequence (hcqcetgpsisp2; Bruker). Suggestion: use 4 scans, 128 and 2k (F1/F2) size of FID, a spectral width of 200.0 and 11.0 ppm (F1/F2), an acquisition time of 0.1 s, a relaxation delay of 2.0 s.

Additionally, for NMR metabolomics measurements of biological fluids, there are four standard pulse sequences that are commonly used, with variations based on the metabolite concentration, sample's total salinity, as well as the protein and lipid content of the samples. Here, we report five biological sample types and the NMR sequences that are being applied.

For Plasma & Serum samples

- 1 1D 1H NOESY: a standard pulse sequence (noesygppr1d; Bruker). Suggestion: use 32 scans, 98k size of FID, a spectral width of 13.0 ppm, an acquisition time of ~5.4 s, and a relaxation delay of 4.0 s, mixing time 0.01 s.
1. 1D 1H CPMG: a standard pulse sequence (cpmgpr1d; Bruker). Suggestion: use 32 scans, 73k size of FID, a spectral width of 13.0 ppm, an acquisition time of 4 s, a relaxation delay of 4.0 s



2. 2D 1H J-resolved: a standard pulse sequence (jresgpprqf; Bruker). Suggestion: use 4 scans, 40 and 12k (F1/F2) size of FID, a spectral width of 0.11 and 16.0 ppm (F1/F2), an acquisition time of 0.5 and 0.3 s (F1/F2), a relaxation delay of 2.0 s.

3. 1D 1H Diffusion-edited: a standard pulse sequence (ledbpgppr2s1d; Bruker). Suggestion: use 32 scans, 98k size of FID, a spectral width of 13 ppm, an acquisition time of 3 s, a relaxation delay of 4.0 s, diffusion time 0.119 s, a square gradient strength: 80% of the maximum (53.5 G cm⁻¹) a square gradient length of 1.5 ms

For Urine samples

1 1D 1H NOESY: a standard pulse sequence (noesygppr1d; Bruker). Suggestion: use 64 scans, 65k size of FID, a spectral width of 15.0 ppm, an acquisition time of ~3.11 s, and a relaxation delay of 2.0 s, mixing time 0.01 s.

2 2D 1H J-resolved: a standard pulse sequence (jresgpprqf; Bruker). Suggestion: use 4 scans, 40 and 12k (F1/F2) size of FID, a spectral width of 0.11 and 16.0 ppm (F1/F2), an acquisition time of 0.5 and 0.3 s (F1/F2), a relaxation delay of 2.0 s.

For cell extracts or cell media

1 1D 1H NOESY: a standard pulse sequence (noesygppr1d; Bruker). Suggestion: use 128-256 scans, 11k size of FID, a spectral width of 11.0 ppm, an acquisition time of 3.5 s, and a relaxation delay of 4.0 s, mixing time 0.01 s.

2 1D 1H CPMG: a standard pulse sequence (cpmgpr1d; Bruker). Suggestion: use 128-256 scans, 73k size of FID, a spectral width of 13.0 ppm, an acquisition time of 4 s, a relaxation delay of 4.0 s

3 2D 1H J-resolved: a standard pulse sequence (jresgpprqf; Bruker). Suggestion: use 4 scans, 40 and 12k (F1/F2) size of FID, a spectral width of 0.11 and 16.0 ppm (F1/F2), an acquisition time of 0.5 and 0.3 s (F1/F2), a relaxation delay of 2.0 s.