



KJM 5300

VII

^1H -NMR metabolomics

TopSpin 3.5

AVIIIHD800

Version 0.9

© I. C. Hvinden and F. Rise
Crude and unfinished manual
December 2017

The goal of this document is to enable users to be able to obtain NMR–metabolomics data on the 800 MHz NMR instrument at the UiO NMR Center. Interpretation of spectra is not (yet) covered. Statistical treatment of the data is neither covered (yet). A database at Ohio State University, which can help you with identification of individual molecules in the metabolomics samples, is mentioned in this document, but the use is not (yet) covered. In order to understand the content of this document the reader need to know how to perform standard nmr-experiments on Bruker nmr-spectrometers.

New...

Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type.
For multi-receiver experiments several datasets are created.
Please define the number of receivers in the Options.

NAME: Matabolomics

EXPNO: 1

PROCNO: 1

☐ Use current parameters

☒ Experiment: PROTON [Select]

Options

☒ Set solvent: D2O_salt

☒ Execute 'getprosol'

☐ Keep parameters: P 1, O1, PLW 1 [Change]

DIR: D:\uio\AVIIIHD800\data\froderi\nmr

☐ Show new dataset in new window

Receivers (1,2, ...16): 1

TITLE: [Empty text area]

OK Cancel More Info... Help

Run a regular proton experiment. Rpar PROTON all or do as above.

1 ICH_W47_D98 2 1 D:\uio\AV\IH800\data\tnmr\nmr

Spectrum ProcPars **AcquPars** Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu

Probe: CP TCI 800S4 H-C/N-D-05 Z

Experiment	Experiment	
Width	PULPROG	zg30 ... E Current pulse program
Receiver	AQ_mod	DQD Acquisition mode
Nucleus	TD	65536 Size of fid
Durations	DS	2 Number of dummy scans
Power	NS	16 Number of scans
Program	TD0	1 Loop count for 'td0'
Probe	Width	
Lists	SW [ppm]	20.0312 Spectral width
Wobble	SWH [Hz]	16025.641 Spectral width
Lock	AQ [sec]	2.0447233 Acquisition time
Automation	FIDRES [Hz]	0.489064 Fid resolution
Miscellaneous	FW [Hz]	4032000.000 Filter width
User		
Routing		

1 ICH_W47_D98 2 1 D:\uio\AV\IH800\data\tnmr\nmr

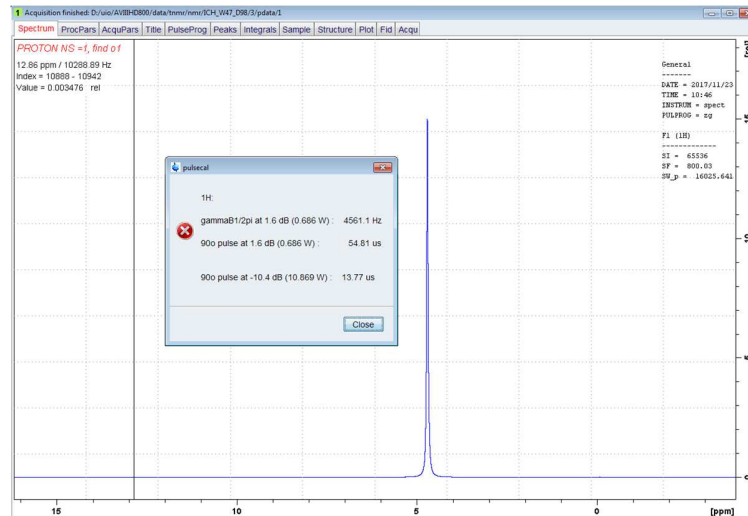
Spectrum ProcPars **AcquPars** Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu

Probe: CP TCI 800S4 H-C/N-D-05 Z

Experiment	Experiment	
Width	PULPROG	zg ... E Current pulse program
Receiver	AQ_mod	DQD Acquisition mode
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Program	TD0	1 Loop count for 'td0'
Probe	Width	
Lists	SW [ppm]	20.0312 Spectral width
Wobble	SWH [Hz]	16025.641 Spectral width
Lock	AQ [sec]	2.0447233 Acquisition time
Automation	FIDRES [Hz]	0.489064 Fid resolution
Miscellaneous	FW [Hz]	4032000.000 Filter width
User		
Routing		

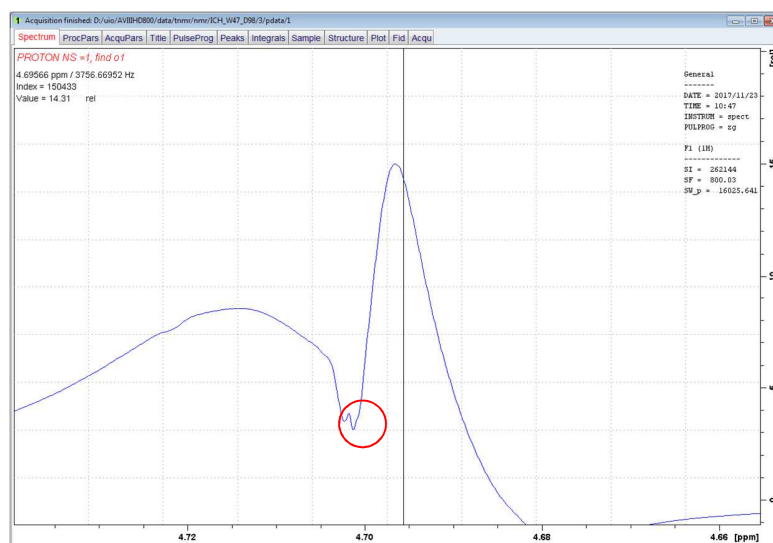
Change the pulse program from zg30 to zg and change ns to 1 and ds to 0.

Acquire the spectrum. Run pulsecal.

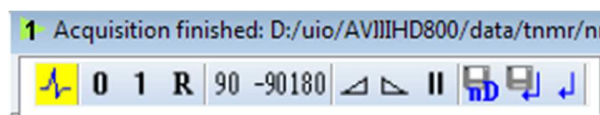


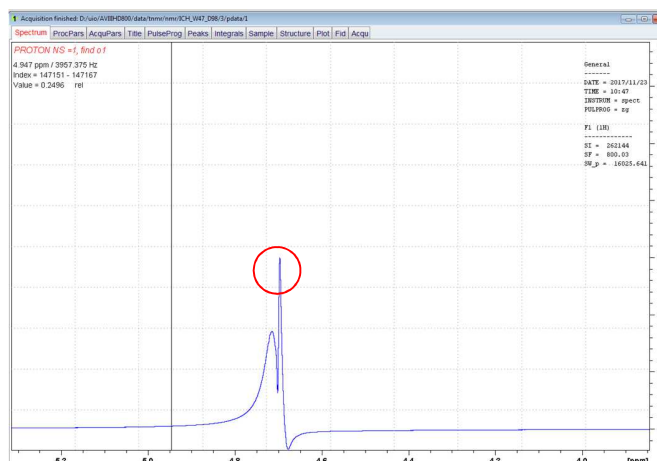
Multiply the new p1 with 4 and enter it by typing p1 and enter the 4 times larger number. (In the example here, 4 times 13.77).

Acquire the spectrum.



Go to Phasing and invert 180 degrees.





Zoom in to the sharp peak. That is the exact O1 value to be used in the metabolomics experiments. Write down this number (in Hz).

You will not get this result if lock is misadjusted, or the sample is badly shimmed, or if the temperature is not adjusted and has not reached stable temperature. **The sample must have been in the magnet for at least 15 minutes, preferably 20, before shimming and acquisition can start.** If a suitable solvent is available in the list of lock solvents (*e.g.* h2o_d2o_salt, then the standard o1 often works just as well as the one you find manually. A suitable lock solvent will need only small adjustments during tuning and matching. If it is far off, try a different lock solvent. In addition, you might be in more need of finding o1 yourself.

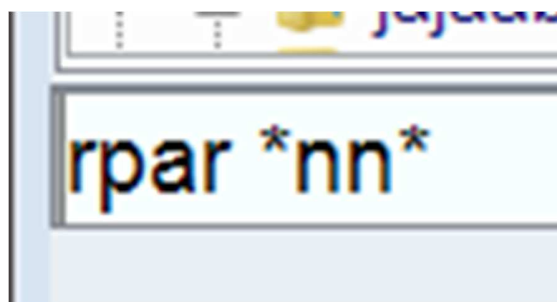
Finally, if the magnet is drifting a lot, you might have to refresh/find again the o1 value.



The three experiments which are routinely used at UiO are shown here, taken from the left pane of the TopSpin interface. The experiments are either manually run in TopSpin or in automation using ICONNMR.



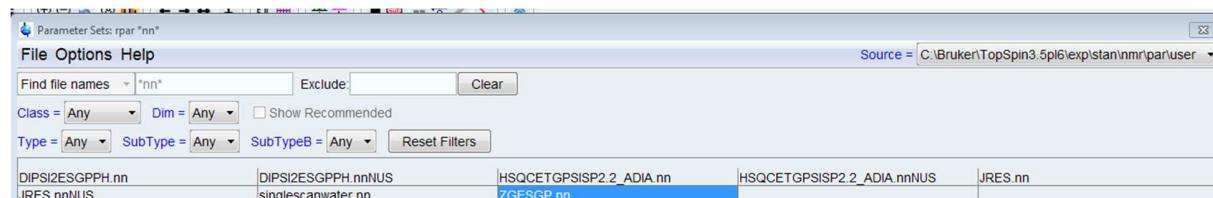
For more extensive studies a $^1\text{H}^{13}\text{C}$ HSQC experiment is added. This 2D experiment is used in structural confirmation/elucidation of individual metabolites. As usual the main problem for newcomers is to figure out what rpar files to use. Nils Nyberg from Bruker has made some experiments that are well suited for NMR-metabolomics



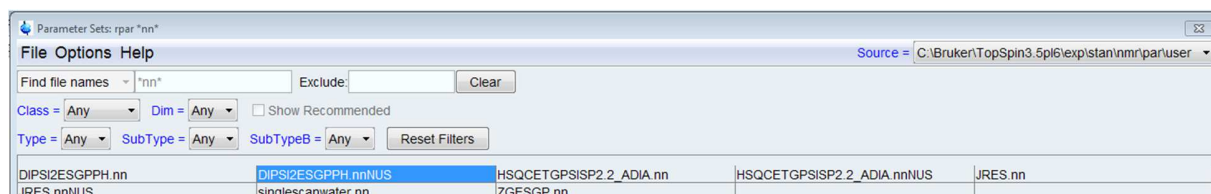
Rpar *nn* brings up all parameter files with nn or NN in the names, *i.e.* those made by Nils Nyberg.



Observe the line at the top right in the screenshot. \par\user is the location of the *nn* files.



ZGESGP.nn is the proton experiment used for statistics.



DIPSI2ESGPPH.nnNUS is the TOCSY experiment we use. NUS stand for Non Uniform Sampling. The experiment time is cut to ¼ by using 25 % NUS. The computer will calculate the missing FIDs based on how the acquired FIDs “look” when the spectrum is Fourier transformed. The Fourier transformation can take up to 15 minutes. The DIPSI/TOCSY experiment is used online with a database at Ohio State University when the investigator needs to check or determine the molecular identity behind NMR resonances or peaks. A direct link to the 2D page is here: (<http://spin.ccic.ohio-state.edu/index.php/toccata2/index>). A useful manual is found here: (http://spin.ccic.ohio-state.edu/database/toccata_protocol.pdf). Please observe that you need a Linux computer or a dual boot computer to use this database, if you want to upload the files they ask for. The programs for making these files work best on Linux. Otherwise you have to manually read the peaks from TopSpin or MNova and write it into the search bar on the webpage.



HSQCETGPSISP2.2_ADIA.nnNUS is the HSQC experiment used in NMR-metabolomics at the UiO NMR center.



Please note that you might only see the pulse programs in the left pane of TopSpin and not the complete parameter names.

Matabolomics 3 1 D:\uio\AVIIIHD800\data\froderi\nmr

Spectrum ProcPars **AcquPars** Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu

Probe: CP TCI 800S4 H-C/N-D-05 Z

Experiment

Width

Receiver

Nucleus

Durations

Power

Program

Probe

Lists

Wobble

Lock

Automation

Miscellaneous

User

Routing

Experiment

PULPROG zgesgp E Current pulse program

AQ_mod DQD Acquisition mode

TD 32768 Size of fid

DS 4 Number of dummy scans

NS 128 Number of scans

TD0 1 Loop count for 'td0'

Width

SW [ppm] 16.0250 Spectral width

SWH [Hz] 12820.513 Spectral width

AQ [sec] 1.2779520 Acquisition time

FIDRES [Hz] 0.782502 Fid resolution

FW [Hz] 4032000.000 Filter width

The top of the eda or AcquPars section of a zgesgp experiment.

Matabolomics 4 1 D:\uio\AVIIIHD800\data\froderi\nmr

Spectrum ProcPars **AcquPars** Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu

Probe: CP TCI 800S4 H-C/N-D-05 Z

F2 F1 Frequency axis

Experiment

Width

Receiver

Nucleus

Durations

Power

Program

Probe

Lists

NUS

Wobble

Lock

Automation

Miscellaneous

User

Routing

Experiment

PULPROG dipsi2esgpph E Current pulse program

AQ_mod DQD Acquisition mode

FnTYPE non-uniform_sampling nD acquisition mode for 3D etc.

FnMODE States-TPPI Acquisition mode for 2D, 3D etc.

TD 2048 1024 Size of fid

DS 32 Number of dummy scans

NS 16 Number of scans

TD0 1 Loop count for 'td0'

TDav 0 Average loop counter for nD experiments

Width

The top of the eda or AcquPars section of a DIPSi2ESGPPH.nnNUS parameter set containing the dipsi2esgpph pulse program.

Matabolomics 3 1 D:\uio\AVIII\HD800\data\froderi\nmr

Spectrum ProcPars **AcquPars** Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu

Probe: CP TCI 800S4 H-C/N-D-05 Z

Experiment	O1 [Hz]	3760.14	Transmitter frequency offset
Width	O1P [ppm]	4.700	Transmitter frequency offset
Receiver	SFO1 [MHz]	800.0337601	Transmitter frequency
Nucleus	BF1 [MHz]	800.0300000	Basic transmitter frequency
Durations			
Power	▼ Nucleus 2		
Program			
Probe	▼ Nucleus 3		
Lists			
Wobble	▼ Nucleus 4		
Lock			
Automation	▼ Nucleus 5		
Miscellaneous			
User	▼ Nucleus 6		

Matabolomics 4 1 D:\uio\AVIII\HD800\data\froderi\nmr

Spectrum ProcPars **AcquPars** Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu

Probe: CP TCI 800S4 H-C/N-D-05 Z

^ NUS (Non Uniform Sampling) parameters

NUS Help Show NUS help

NusAMOUNT [%] 25 Amount of sparse sampling

NusPOINTS 128 Number of hypercomplex points in indirect dimension

NusJSP [Hz] 0 J-coupling

NusT2 [sec] 1 T2 relaxation

NusSEED 54321 Random generator seed

NUSLIST automatic Name of loopcounter list for NUS (Non Uniform Sampling)

Calculate Calculate point spread function

Show Display NUS point spread

^ Wobble

WBSW [MHz] 4.0000000 Wobble sweep width

WBST 1024 Number of wobble steps

^ Lock

LOCNUC 2H Lock nucleus

SOLVENT D2O_salt Sample solvent

^ Automation

AUNM au_prof.nn Acquisition AU program

PYNM Acquisition PYTHON program

EXP DIPSI2ESGPPH.nnNUS Experiment performed

TUBE_TYPE Type of used sample tube

^ Miscellaneous

GRDPROG Gradient program

CHEMSTR none Molecule file for structure display (pdb, xyz, ...)

Examples of spectra will follow:

The OHIO database states that DSS must be used as an internal calibrant, but from experience of the authors of this manual, TSP works fine as well. However, it is *strongly* recommended to confirm the identity of the suggested molecule with 1D spectra and J resolved spectra. In other words, does the suggested molecule have the coupling shown in the J resolved spectra and do 1D databases agree with the 2D Ohio database?