

KJM 5280

VII

¹H-NMR metabolomics

TopSpin 3.5

AVIIIHD800

Version 1.0

© I. C. Hvinden and F. Rise Crude and unfinished manual May 2018 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 also not 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		X
Prepare for a new experiment by creating a initializing its NMR parameters according to For multi-receiver experiments several data Please define the number of receivers in the	new data set and the selected experiment type. sets are created. e Options.	
NAME	Matabolomics	
EXPNO	1	
PROCNO	1	
O 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		
	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\AVIIIHD800\data\tnmr\nmr								
Spectrum ProcPa	Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu							
տ Л S 🔰 🖼 1.2,	. ▼ C #	Probe: CP TCI	800S4 H-C/N-D-05 Z					
Experiment Width								
Receiver	PULPROG	zg30	E Current pulse program					
Nucleus	AQ_mod	DQD -	Acquisition mode					
Durations	TD	65536	Size of fid					
Program	DS	2	Number of dummy scans					
Probe	NS	16	Number of scans					
Lists	TD0	1	Loop count for 'td0'					
Wobble Lock	⊘ Width							
Automation	SW [ppm]	20.0312	Spectral width					
Miscellaneous	SWH [Hz]	16025.641	Spectral width					
Routing	AQ [sec]	2.0447233	Acquisition time					
rtouting	FIDRES [Hz]	0.489064	Fid resolution					
	FW [Hz]	4032000.000	Filter width					

1 ICH_W47_D98 2 1 D:\uio\AVIIIHD800\data\tnmr\nmr											
Spectrum I	Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu										
ю Л S 📕	м Л S U 🖼 📩 🛡 C 🚜 Probe: CP TCI 800S4 H-C/N-D-05 Z										
Experiment Width Experiment											
Receiver		PULPROG		zg				E	Curr	ent p	oulse program
Nucleus		AQ_mod		DQD		-			Acqu	uisitio	on mode
Durations		TD		65536					Size	of fic	t
Program		DS		2					Num	ber o	of dummy scans
Probe		NS		16					Num	ber o	of scans
Lists		TD0		1					Loop	o cou	int for 'td0'
Wobble Lock		⊗ Width									
Automation		SW [ppm]		20.0312					Spec	ctral	width
Miscellaneou	US	SWH [Hz]		16025.64	11				Spec	ctral	width
Routing	1	AQ [sec]		2.044723	33				Acqu	uisitio	on time
louing		FIDRES [Hz]		0.489064	1				Fid r	resol	ution
		FW [Hz]		4032000	.000				Filte	r wid	th

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 ¹H¹³C 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.

🖕 Parameter Sets: rpar *nn*			E S	
File Options Help			Source = C:\Bruker	\TopSpin3.5pl6\exp\stan\nm(\par\user) -
Find file names v hnn*	Exclude: Clea	ar		\smile
Class = Any Dim = Any	Show Recommended			
Type = Any • SubType = Any • S	SubTypeB = Any Reset Filters			
DIPSI2ESGPPH.nn	DIPSI2ESGPPH.nnNUS	HSQCETGPSISP2.2_ADIA.nn	HSQCETGPSISP2.2_ADIA.nnNUS	JRES.nn
JRES.nnNUS	singlescanwater.nn	ZGESGP.nn		

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

🖕 Parameter Sets: rpar "nn"						
File Options Help			Source = C:\Bruke	er\TopSpin3.5pl6\exp\stan\nmr\par\user -		
Find file names v *nn*	Exclude: Cl	ear				
Class = Any Dim = Any	Show Recommended					
Type = Any SubType = Any SubTypeB = Any Reset Filters						
DIPSI2ESGPPH.nn	DIPSI2ESGPPH.nnNUS	HSQCETGPSISP2.2_ADIA.nn	HSQCETGPSISP2.2_ADIA.nnNUS	JRES.nn		
JRES.nnNUS	singlescanwater.nn	ZGESGP.nn				

ZGESGP.nn is the proton experiment used for statistics.

🖕 Parameter Sets: rpar *nn*				X
File Options Help			Source = C:\Bruke	er/TopSpin3.5pl6/exp\stan\nmr\par\user -
Find file names v *nn*	Exclude:	Clear		
Class = Any Dim = Any	Show Recommended			
Type = Any SubType = Any	SubTypeB = Any Reset I	Filters		
DIPSI2ESGPPH.nn	DIPSI2ESGPPH.nnNUS	HSQCETGPSISP2.2_ADIA.nn	HSQCETGPSISP2.2_ADIA.nnNUS	JRES.nn
JRES.nnNUS	singlescanwater.nn	ZGESGP.nn		

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.

Se Parameter Sets: rpar *nn*				X			
File Options Help			Source = C:\Bruker	r\TopSpin3.5pl6\exp\stan\nmr\par\user -			
Find file names v *nn*	Exclude: Cle	ar					
Class = Any Dim = Any	Show Recommended						
Type = Any SubType = Any	Type = Any SubType = Any SubTypeB = Any Reset Filters						
DIPSI2ESGPPH.nn	DIPSI2ESGPPH.nnNUS	HSQCETGPSISP2.2_ADIA.nn	HSQCETGPSISP2.2_ADIA.nnNUS	JRES.nn			
JRES.nnNUS	singlescanwater.nn	ZGESGP.nn					

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.

2 Matabolomics 3 1 D:\uio\AVIIIHD800\data\froderi\nmr							
Spectrum ProcPa	ars AcquPars Title F	PulseProg Peaks Integr	rals Sample Structure	Plot Fid Acqu			
т Л S 🔰 🖽 1,2,	🖙 Л S 🚽 🖼 📩 🛡 С 🚜 О1 Probe: CP TCI 800S4 H-C/N-D-05 Z						
Experiment Width	 Experiment 						
Receiver	PULPROG	zgesgp	E	Current pulse program			
Nucleus	AQ_mod	DQD -		Acquisition mode			
Durations	TD	32768]	Size of fid			
Program	DS	4		Number of dummy scans			
Probe	NS	128		Number of scans			
Lists	TD0	1		Loop count for 'td0'			
Wobble Lock	🐼 Width						
Automation	SW [ppm]	16.0250		Spectral width			
Miscellaneous	SWH [Hz]	12820.513		Spectral width			
Routing	AQ [sec]	1.2779520		Acquisition time			
routing	FIDRES [Hz]	0.782502		Fid resolution			
	FW [Hz]	4032000.000		Filter width			

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

Z Matabolomics 4 1 D:\uio\AVIIIHD800\data\froderi\nmr								
Spectrum ProcPa	Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu							
ю Л S 🔰 📰 เวื	n S 🗑 🖼 🛃 🛃 🖉 C 🚜 Probe: CP TCI 800S4 H-C/N-D-05 Z							
Experiment Width Receiver	 Experiment 	F2	F1	Frequency axis				
Nucleus Durations Power Program Probe Lists NUS Wobble Lock Automation Miscellaneous	PULPROG AQ_mod FnTYPE FnMODE TD DS NS TD0 TDav	dipsi2esgpph DQD	States-TPPI •	Current pulse program Acquisition mode nD acquisition mode for 3D etc. Acquisition mode for 2D, 3D etc. Size of fid Number of dummy scans Number of scans Loop count for 'td0' Average loop counter for nD experiments				
User	Width							

The top of the eda or AcuPars section of a DIPSI2ESGPPH.nnNUS parameter set containing the dispsi2esgpph pulse program.

Z Matabolomics 3 1 D:\uio\AVIIIHD800\data\froderi\nmr							
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu							
т Л S 🔰 🖼 1,2,	▼ C #3	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				
Durations	BF1 [MHz]	800.0300000	Basic transmitter frequency				
Power Program	Solution						
Probe	Nucleus 3						
Wobble	e 🕑 Nucleus 4						
Automation	Nucleus 5						
User	Nucleus 6						

Z Matabolomics 4 1 D:\uio\AVIIIHD800\data\froderi\nmr						
Spectrum ProcPa	rs AcquPars Title P	ulseProg Peaks Integ	rals Sample	Structure	Plot Fid	Acqu
տ 🎵 S 📕 🖼 12.	▼ C #3	Probe: CP TC	1800S4 H-C/	N-D-05 Z		
Experiment	NUS (Non Unifo	rm Sampling) paramete	rs			
Receiver		NUS Help			Show NU	JS help
Nucleus	NusAMOUNT [%]	25			Amount	of sparse sampling
Durations	NusPOINTS	128			Number	of hypercomplex points in indirect dimension
Power	NusJSP [Hz]		0		J-couplin	ng
Program	NusT2 [sec]		1		T2 relaxa	ation
Lists	NusSEED	54321			Random	generator seed
NUS	NUSLIST	automatic			Name of	loopcounter list for NUS (Non Uniform Sampling)
Wobble		Calculate			Calculat	e point spread function
Lock		Show			Display	NUS point spread
Miscellaneous						
User	WBSW [MHz]	4.0000000			Wobble	sweep width
Routing	WBST	1024			Number	of wobble steps
	Lock					
	LOCNUC	2Н 🔻			Lock nuc	cleus
	SOLVENT	D2O_salt -]		Sample :	solvent
	 Automation 					
	AUNM	au_prof.nn		E	Acquisiti	on AU program
	PYNM			E	Acquisiti	on PYTHON program
	EXP	DIPSI2ESGPPH.nnNUS	8		Experime	ent performed
	TUBE_TYPE				Type of	used sample tube
	Miscellaneous					
	GRDPROG			E	Gradient	t program
	CHEMSTR	none			Molecule	e 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?