Exercise at the Spectrometer

General

The script is written for Bruker spectrometers. The color code means, red for all commands and green for parameters in the TopSpin commando line. The script gives additional information to the recommended Bruker manuals: Beginner Guide, 1D and 2D Step-by-Step - Basic & - Advanced which can be found under the help button in TopSpin.

set up a 1D NMR experiment, general procedure:

- Temperature adjustment
- Load sample into the magnet
- create a new data set (e.g. *rpar PROTON all*)
- tuning the probe
- lock & shimming
- determine the acquisition parameters
- start the experiment
- processing
- plotting

Probe temperature

Before you load a new sample into the magnet check the actual temperature in *edte*. For cryogenic probe heads it is necessary that the gas flow is 670 l/h and the temperature calibration is active.

Load the sample into the magnet

Use the Bruker tool at the spectrometer to center the sample in the spinner around the active volume. For cryogenic probe heads the maximum sample depth is 21mm. The right orientation is shown in Figure 1, step 5. In addition to the standard NMR tubes for Bio-NMR in a 5mm probe head the following tubes can be used:

Tubes	Diameter	Sample volume	Coasts	Comments
High quality	5mm	550 µl	10 – 20 €	
High quality	3mm	180 µl	10 – 20 €	for lossy biological sample
Shigemi tube	5mm	280 µl	110 €	for expensive sample
Shape tube	oval tube	340 µl	260 €	for lossy biological sample

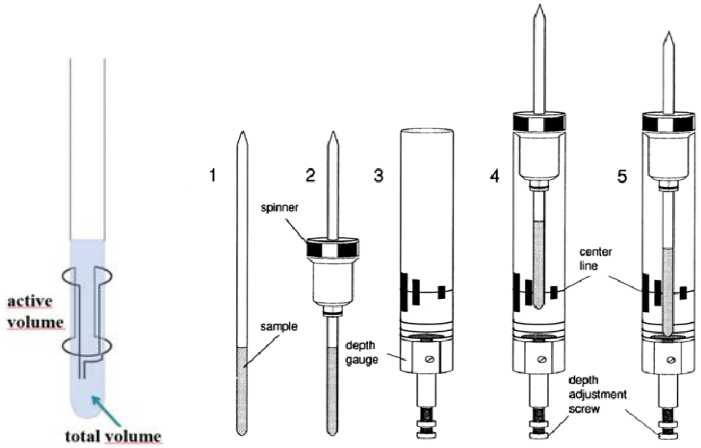


Figure1 from the Bruker manual beginner guide.

For Shigemi tubes the active volume between the bottom glass and the plunger glass should be centered. It is important to expel air bubbles from the sample, especially when Shigemi tubes are used.

Create a new data set

New or edc generate a new data set name. A dataset is defined by 5 parameters:

<Dir>:	The directory where the dataset is located
<User>:	The name of the TopSpin user who did the experiment
<Name>:	Identifier of the sample
<Expno>:	Contains the raw data of one experiment (= FID)
<Procno>:	Contains the processed data of one experiment (= spectrum)

The command rpar reads a parameter set (experiment) to the current dataset. When it is entered without arguments, rpar opens a dialog box with a list of the available parameter sets. The naming of the parameter sets corresponds to the name of the pulse sequences, where the first characters specify the type of experiment, e.g. DEPT, COSY, NOESY etc. Further properties of the pulseprogram are indicated by a two-character code, which is added to the name in alphabetical order. The Bruker naming convention can be checked in edcpul Pulprog.info. When using the parameter set to start NMR-experiments it is important to keep in mind that the pulseprogram is only one of the parameters.

Commands to change the data structure

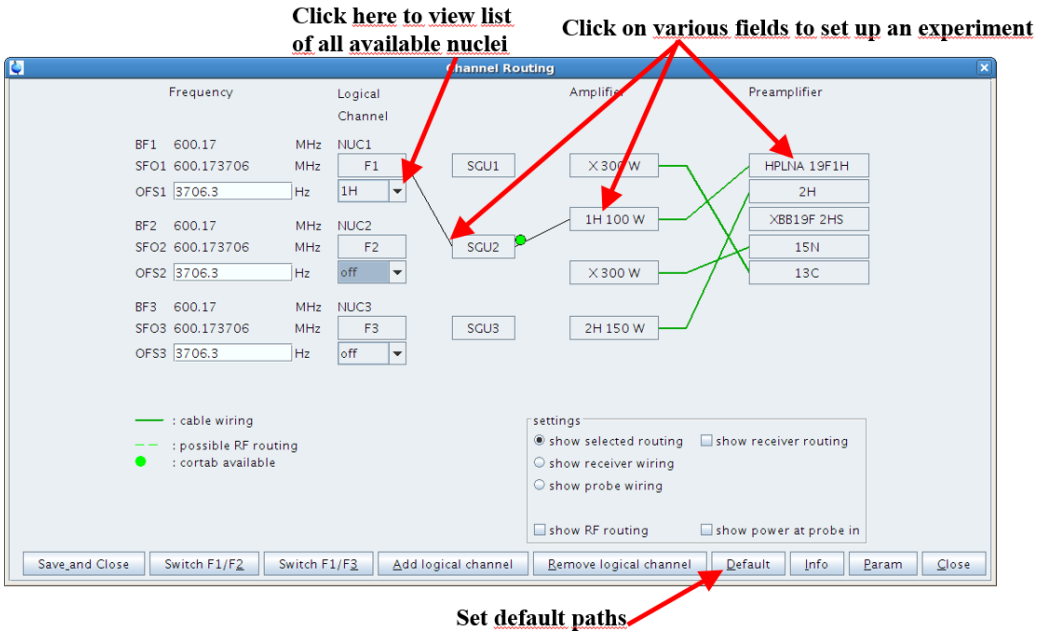
- *edc, new* — create a new data set
- *search* — find an existing data set
- *re expno* — with this command you change the expnos
- *rep procno* — with this command you change the procnos
- *wrp procno* — copy the processed data to a new procno
- *wrpa expno* — copy the FID and processed data to a new expno

tuning the probe

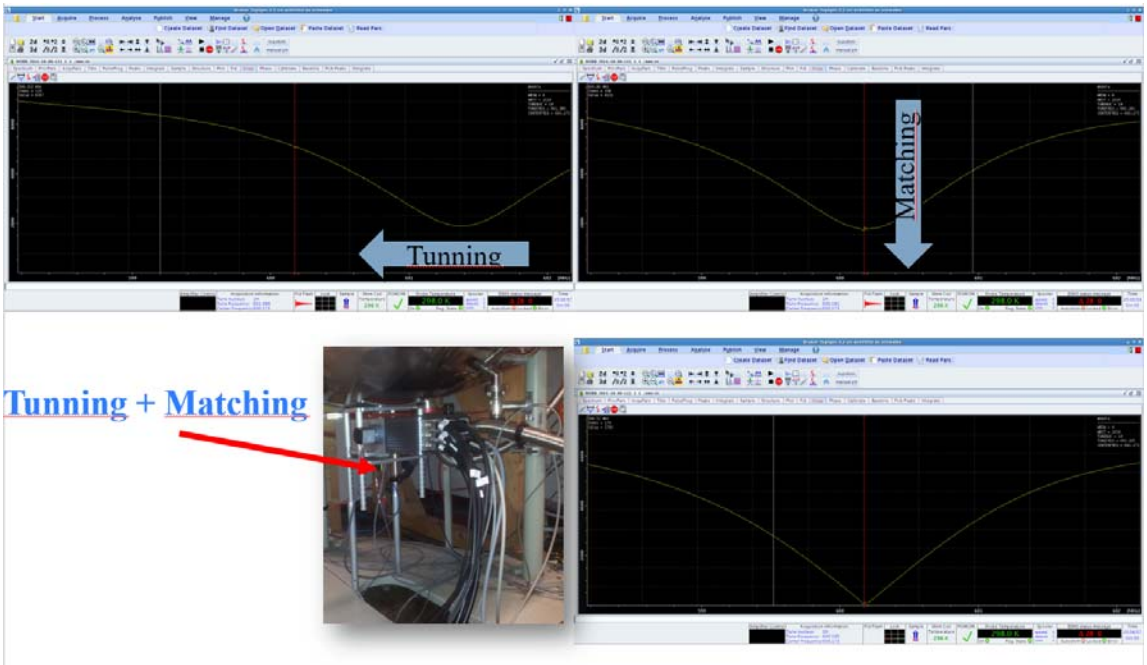
The probehead is a LC resonance circuit (like an old radio receiver) which needs to be adjusted to the frequency used. This procedure is called tuning & matching. While tuning means optimizing to the frequency, matching will minimize the reflected power.

Procedure for tuning and matching the probe head

- define the channels in edasp



- wobb start the wobble process
- Color code of T + M - screws:
  - yellow: ^{1}H Proton
  - blue: ^{13}C Carbon
  - red: ^{15}N Nitrogen
  - grey: ^{2}H Deuterium (only CryoProbe™)
- change the tuning und matching screws at the probe head to reach a minimum at the red central line.



- Automated tuning and matching:
    - Special probe head required: ATM
    - Commands: atm (fully automated) or atmm (manually)

- Procedure T + M of ^{2}H Deuterium (only for cryogenic Probes)
    - Generate a new data set
    - read parameter set for deuterium gradient shimming: rpar gradshim1d2h & wobb tune and match the probe
    - Afterwards change back to ^{1}H-1D-dataset and do ii for lock again
      (ii initializes the spectrometer. It is only a software command and can be used any time)

Lock and shimming

The magnetic field B_0 requires stabilization of the main field (LOCK) and control of the field homogeneity (SHIM).

LOCK

- continuously determines the frequency of ^{2}H signal of the solvent (deuterated solvents)
- adds a small extra field to the main field of the magnet to keep the overall field constant
- ^{2}H signal also used for shimming

SHIM

- additional coils outside the sample used for adjustment of the homogeneity of the B_0 field (e.g. Z, Z2, Z3, X, Y….)
- An NMR Spectrometer has <u>*cryo shims*</u> and <u>*room temperature* shims</u>

The lock channel can be thought as a ‚*complete independent second spectrometer within the main spectrometer*‘ (Larmor frequency: $\omega_0=\gamma B_0$). An additional field H_0 is created: ω_0 =

$\gamma(B_0+H_0)$. B_0 is not constant, but (B_0+H_0) can be held constant by control technology (field regulation). The lock signal is recorded by two quadrature channels in absorption and dispersion. The dispersion signal is used for field stabilisation.

LOCK Parameter

- LOCK gain
  The gain setting for the display only.
- LOCK Power
  The ^2H transmitter output power. Due to the different relaxation behavior of ^2H for individual solvents, the lock power has to be adjusted for each solvent. Too high lock power will result in an unstable lock signal due the saturation. To check if the look power is close to saturation, change the lock power of -1dB and decrease lock gain by -1dB. If the lock line is at same position as before, repeat the procedure.
- LOCK phase
  If the lock phase is not adjusted correctly, absorption and dispersion signals will be mixed. Non-pure phases will result in imperfect field homogenisation (shimming) and field shifts during experiment using pulsed field gradients.
- Regulation parameters
  - Loop Gain: how *strong* to react on field disturbance
  - Loop Time: how *fast* to react on field disturbance
  - Loop Filter: *smoothing* the lock signal to remove noise, low pass filter

  Wrong settings will result in unstable signal position of the lock, which can give suppression artifacts or additional noise around the signal in NMR-spectra. The regulation parameters and lock phase can be adjusted using an AU-program (Bruker C-program language) with the command xau set_loopval (after lock). The lists after running the AU-Prog give the option to select how strong the lock reacts on changes: lock.1: soft to lock.12 strong.

	1	2	3	4	5	6	7	8	9	10	11	12
Lock gain	119.3	115.4	110.2	107.2	104.1	99.7	96	92.6	89.6	86.0	83.9	82.2
Loop gain	-17.9	-14.3	-9.4	-6.6	-3.7	0.3	3.9	7.1	9.9	13.2	15.2	16.8
Loop time	0.681	0.589	0.464	0.384	0.306	0.220	0.158	0.111	0.083	0.059	0.047	0.041
Loop filter	20	30	50	70	100	160	250	400	600	1000	1500	2000

Table 1: Macros lock.x to set LOCK regulation parameters. Data from BSMS manual.

The command lock sets all the LOCK parameters and locks automatically on the selected solvent. The lock signal can be visualized in lockdisp. All LOCK parameters are listed in edlock.

The room temperature shims have to be optimized on every new sample to get optimal homogeneity and small line width of the NMR signal. The Bruker tool Topshim helps to adjust the shims, starting command: topshim gui. More information and tips for using Topshim:

- 1D Topshim optimizes only all the z-shims. The 1D Topshim can be used for all solvent. The program switches automatically to ^{1}H or ^{2}H gradient shimming depending on the selected solvent during the lock procedure.
- 3D Topshim adjusts all the shims and is only for water samples.
- for shimming of x/y shims in topshim 1D use the tune option
- Z6 works on ^{1}H (less on ^{2}H), check the water line in zgpr
- select parameters
  - for Shigemi tubes: shigemi
  - shimming Z7 & Z8: ordmax = 8
- 3D Topshim adjusts all the shims and is only for water samples.
  - parameter: ordmax = 5
  - use before and after 1D topshim, after manual adjustment of x + y shim

Determine acquisition parameters

Pulse calibration

AUTOMATIC

The automatic ^{1}H pulse calibration using a single scan stroboscopic nutation experiment (P.S.C. Wu & G. Otting, Magn. Reson. 176, 115-119 (2005), description: http://u-of-o-nmr-facility.blogspot.de/2010/03/fast-90-degree-pulse-determination.html) is implemented in Topspin as an AU-Program (Bruker c-program language): pulsecal (for a water sample) or pulsecal sn (for all the other solvents).

MANUAL

The quality of the automatically ^{1}H pulse calibration depends on the spectrometer hardware and software. For that reason, the pulse calibration should also check manual following the procedure below:

- read parameter set → rpar PROTON
- change pulse program to zg or zgpr → pulprog zg
- set the acquisition time to 100ms → aq 100m
- set number of scans to 1 → ns 1
- set number of dummy scans to 0 → ds 0
- set relaxation time delay to 1s → d1 1
- set receiver gain to 1 → rg 1
- set proton pulse first short to 0.1 µs → p1 0.1
- record a 1D spectrum → zg
- process the spectrum → efp

- phase correction with only 0 order → apk0
- select the region around the solvent peak or other strong signal → dpl
- Optimization of the 360° pulse using the macro popt (parameter optimization), fill up the new window:

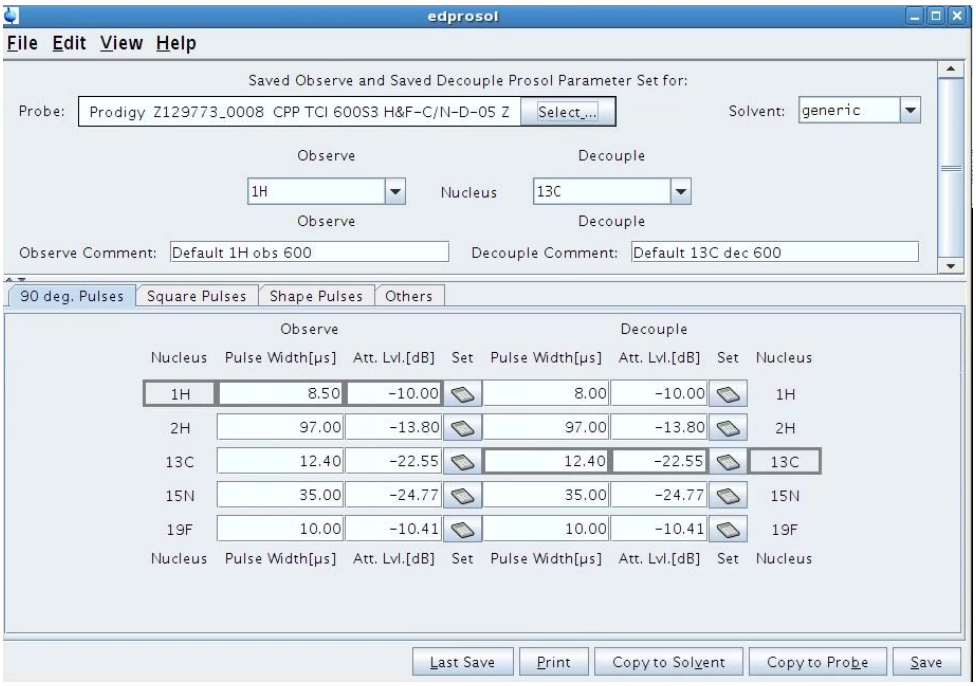
OPTIMIZE	GROUP	PARM.	OPTIMUM	START.	END.	NEXP	VARMOD	INC
Step by step	0	P1	ZERO	**a**	**b**	11	LIN	0.2

a = 4 times the pulse length from macro pulsecal, **b** = automatic

The result is stored in procno 999. The spectrum should show a zero crossing and the 360° pulse length is written in the title. If the zero crossing in the spectra is not obtained, change the popt parameter. Remember the ^{1}H-90° pulse length for the next experiments.

Prosol table

The prosol table is a list of standard pulse length and power level related to the Bruker pulseprogram library. The pulses are stored for each probe head, nucleus and channel. The prosol table can be opened with the command edprosol: new entries or change of entries can be done.



The translation between prosol table and the pulse sequence definition of pulses, power levels and delay is defined in the relation files. The definition is indicated on the top of the pulse sequence by the command: prosol relations=<name>, e.g. prosol relations=<triple>. If in the pulse sequence no file is specified, the program uses the default prosol relation file, compare

file:///...topspin3.5home-directory/prog/docu/english/xwinproc/html_pp/792436363.html. The relation files are stored in: ...TopSpin3.5home\conf\instr\spect\prosol\pulseassign. The command getprosol changes all pulses in the current data set to the values stored in the prosol table. In addition, the prosol table allows to recalculate the complete set of pulses for one nucleus, e.g. if the ^{1}H is different from the standard pulse length, put a new value in and press the calc. button. Please do not store the changes. Only the spectrometer admin should do this. A better option is to use the command line options: getprosol Nuc P90 PL90 or write the new value in a macro, e.g. edmac ubi.

New: parameter can also copy to other parameter sets by command copypars.

Commands for the acquisition

- eda — list all acquisition parameters
- ased — only parameters listed which are necessary for using the actual pulse sequence
- rga — automatic receiver gain adjustment
- zg — delete the old data and starts the experiment
- go — starts the experiment and add the data to the actual data set (works also in 2D)
- stop — stops the experiment; losing the 1D data
- halt — stops the experiment; store the 1D data on disk
- tr — transfer data from acquisition memory to the disk
- gs — pulsing of the pulse sequence without recording the data; for parameter optimisation

Commands for the 1D processing

- edp — setup processing commands
- ft — fourier transformation
- apk — automatic phase correction
- apks — automatic phase correction, faster
- abs — base line correction and automatic integration
- sref — referencing to solvent (solvent in eda)
- si — number of points for processing
  si = td * 0,5
- absf1, absf2 — high field and low field values for abs in [ppm]
- absg, absl — polynome used for abs, normal =5 and 3
- 2s nc_proc — internal scaling factor

A selection of useful standard directories in TopSpin for

- pulse program: /<TopSpin_home>/exp/stan/nmr/lists/pp
- shape pulses: /<TopSpin_home>/exp/stan/nmr/lists/wave
- au-program: /<TopSpin_home>/exp/stan/nmr/au/src
- Parameter set: /<TopSpin_home>/exp/stan/nmr/par
- User file were stored in additional user directory, e.g.
  /<TopSpin_home>/exp/stan/nmr/lists/pp/user

Small molecules

Start a 1D 1H Experiment

^{1}H 1D-NMR Experiment

For setting up a standard ^{1}H 1D-NMR experiment:

rpar PROTON all & do all steps described in the general part. A useful test sample for the small molecules is 100mg Cholesteryl Acetat in C_6D_6.

The PROTON parameter set use the pulse sequence zg30, where the 90° pulse is multiplied with 0.33. The smaller flip angle for the excitation pulse allows shorter relaxation delay (d1). Typical relaxation delays range from 1 – 5 times T_1. For best sensitivity choose the 'Ernst angle':

$$\cos \beta_{opt} = \exp(-TR/T_1)$$

(TR = pulse repetition time, TR = d1 + AQ; β_{opt}: optimum flip angle)

The consequences of too short relaxation delay are loss in sensitivity and artifacts in spectra. The pulse sequence with 30 degree pulses, e.g. zg30 minimized those problems. The pulse program code:

```
;zg30
;1D sequence
#include <Avance.incl>          load definition file for NMR experiments
1 ze                            reset all memory on CPU
2 30m                           delay 30m
  d1                            relaxation delay
  p1*0.33 ph1                   proton 90° pulse multiplied with 0.33 at phase ph1
  go=2 ph31                     acquisition period
  30m mc #0 to 2 F0(zd)
exit
ph1=0 2 2 0 1 3 3 1             phase cycle of pulse p1
ph31=0 2 2 0 1 3 3 1            phase cycle of receiver
```

0 is x axis
1 is y axis
2 is -x axis
3 is -y axis

All 1D/2D NMR pulse sequences are summarized in the TopSpin manual:

'Pulse Program Catalogue, 1D/2D'

Optimal Parameter for ^{1}H-1D-NMR Experiment

Acquisition time	aq	4s
Relaxation delay	d1	0.1
Spectral width	sw	20.0 ppm
Excitation frequency	o1p	7.0 ppm
Receiver gain	rg	use rga
Number of scans	ns	32
Number of dummy scans	ds	8

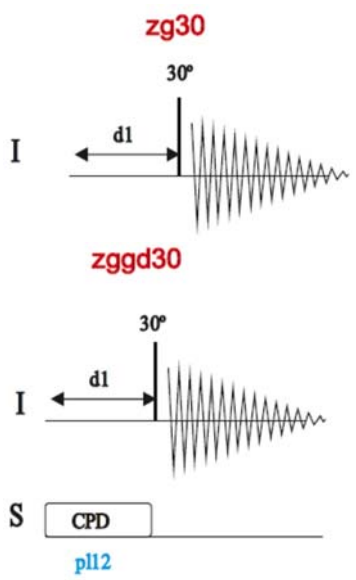
Through choosing the combination of acquisition time and spectral width, the time domain points TD will be automatically calculated in TopSpin, because the parameters depend on each:

AQ = TD * DW= TD/2SWH (DW = dwell time, SWH = spectral width in Hz)

For the 1D record a spectrum with large spectral width SW due the digital filtering will be neglected signals outside of spectral window. The combination of spectral width SW and excitation frequency o1p should be optimized for setting up a 2D-NMR spectra. The selected number of scans depends on the sample concentration.

^{13}C 1D-NMR and DEPT Experiment

The natural abundance of the NMR active carbon isotope ^{13}C is only 1 %. Therefore, is the sensitivity the main issue for ^{13}C. In ^{13}C-1D is the decoupling of ^{1}H necessary and results into two feature: simplify the multiplet structure and increase sensitivity due to Nuclear Overhauser Effect 'NOE'. The NOE depends also on the gyomagnetic ratio and can either be either negative or positive. For ^{13}C is the NOE positive and decoupling also during the relaxation delay is useful. The different possibilities are summarized in table 3.



Pulse program	Properties	Performance
zg zg30	No decoupling	No signal enhancement, coupled spectrum
zggd zggd30	‚Gate Decoupling': decoupling during relaxation delay	Signal enhancement due to NOE effect, coupled spectrum

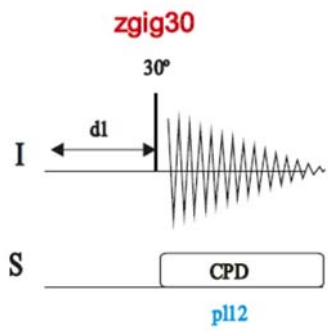
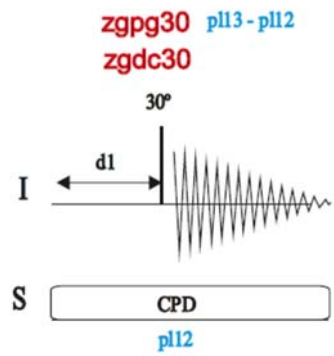
	zgig zgig30	‚Inverse Gate Decoupling‘: decoupling during acquisition	For quantitative analysis of ^{13}C-signals, decoupled spectrum
	zgdc zgdc30 zgpg zppg30	Decoupling during entire experiment PG: ‚Power Gate Decoupling‘ lower power during relaxation delay, pl13	For maximal signal intensity, decoupled spectrum

Table 3: pulse sequences for ^{13}C 1D-NMR, pp- figures from Bruker manual, ‘Pulse Program Catalogue, 1D/2D’.

Decoupling statements in the pulse sequence:

cw:	continuous wave decoupling
hd:	homo decouling
cpds1, … cpds8:	composite pulse decoupling with CPD sequence 1,… 8, synchronous mode
cpd1, … cpd8:	composite pulse decoupling with CPD sequence 1,… 8, asynchronous mode
do	switch decoupling off

Example: part of zgpg (F1-Channel: ^{13}C & F2-Channel: ^{1}H)

```
…….
 10u pl13:f2                 change power level on channel 2, soft decoupling power
 d11 cpd2:f2                 start decoupling
 DELTA
 4u do:f2                    switch decoupling off
 10u pl12:f2                 change power level on channel 2, strong decoupling power
 100m cpd2:f2                start decoupling program cpd2
 p1*0.33 ph1
 go=2 ph31
 30m do:f2 pl13:f2 mc #0 to 2 F0(zd)          switch decoupling off
exit
```

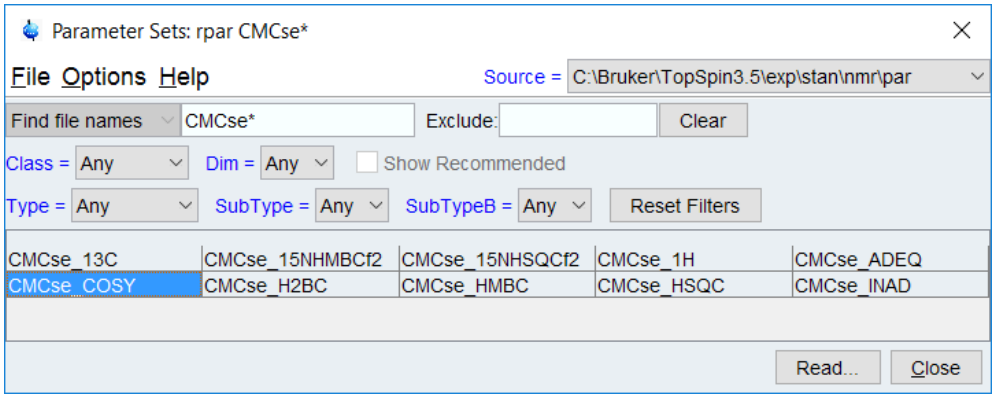
The most popular pulse sequence for carbon 1D is zgpg30, due maximal signal intensity. Do rpar C13CPD all (pp=zgpg30) and getprosol. Further optimization like pulse calibration are not necessary for hetero nucleus. Use maximum receiver gain and start the experiment.

Due the decoupling in a ^{13}C 1D-NMR the information how many protons bound to the carbons is neglected. Polarization transfer technique like DETP bring back this information. The DEPT starts on ^{1}H and transfer the magnetization to ^{13}C, which increase the sensitivity compared to the standard ^{13}C 1D-NMR. DEPT Experiment can easily start by:

rpar C13DEPT135 and getprosol (or better getprosol 1H P90 PL90, if you have calibrated the ^{1}H-pulse). The parameter set based on the pulse sequence deptsp135, which use a adiabatic composite pulse for refocusing. To compare how the sensitivity can profit from using adiabatic shape in pulse sequence generate a new data set and change the pulse sequence to dept135 and re- measure the DEPT.

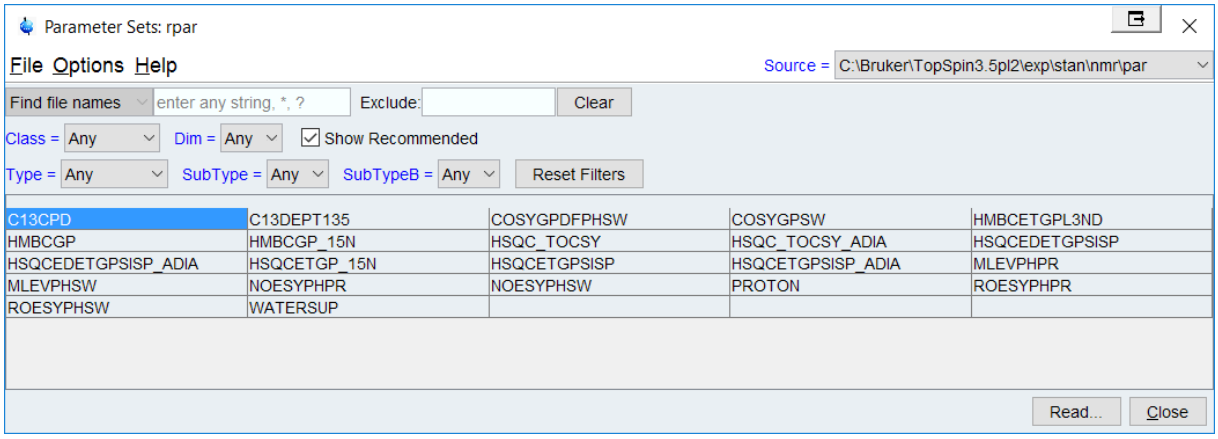
Standard 2D Experiments

A wide range of possible and useful pulse sequences for running a 2D COSY experiment are included in TopSpin. The important NMR experiments for analyzing small molecules is summarized in the data set for CMSse which use optimized pulse sequences. The command rpar CMCse* show all experiments



CMCse is an extra Bruker program for automatically analyzed NMR spectra of small molecules which need an additional license)

Or select the recommended data sets:



2D COSY

rpar CMSseCOSY

getprosol 1H P90 PL90

The parameter set based on pulse sequence cosygpmfppqf. A pulse sequence using gradient pulses for selection with multiple quantum filter according to gradient ratio.

The sample depending parameters are acquisition time (AQ), spectral widths (SW) and excitation frequency (o1p) should set in eda. For the indirect dimension the parameter SW1 = SW. The minimum value for TD1, number of points in the indirect dimension should in minimum set to 384, because the polarization transfer in COSY is happening during the t1 evolution period. That is also the reason why the receiver gain adjustment where doing in the ^{1}H 1D-NMR. Due the gradient selection with multiple quantum filter the experiment works only with 1 scan.

For suppression the zero quantum coherence by the z-filter (M.J. Thrippleton & J. Keeler, Angew. Chem. Int. Ed. 42, 3938-3941 (2003)) change:

- pulprog cosygpphzfzs
- 1 FNMODE States-TPPI
- gpz0 10
- NS 4

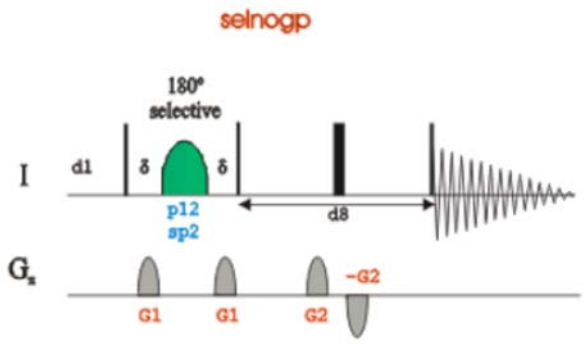
selective 1D-NOESY experiment

Introduction

This experiment consist of three parts:

- **Selective excitation** of the selected resonance using the SPFGE block.
- **Mixing period** consisting of the basic 90^0(1H)-delay-90^0(1H) block in phase polarization transfer to other spins via NOE. Purging gradients are usually applied during the mixing period in order to remove any residual transverse magnetization.
- **Proton detection** as usual.

Figure 5.21



Steps for setup a selective NOESY:

- record a ^{1}H 1D-Experiment

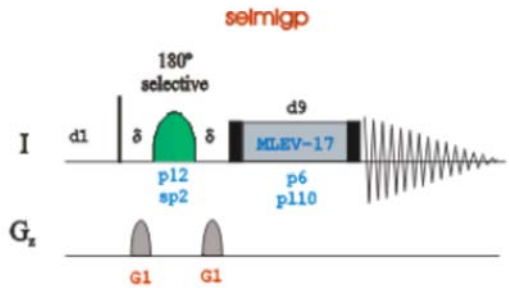
- select separated signals and determine the chemical shifts
- generate a new dataset
- rpar SELNOGP all
- getprosol 1H P90 PL90
- o1p = set to the chemical shift of the selected signal of step 2
- d8 = 300ms or better the T_1-time of the selective signal of step 2

For each selected signal a new EXPERIMENT must have recorded. Be aware that the sign between selected signals and the NOE-signals is the opposite. Instead of using the standard pulse sequence selnogp, use selnogpzs with better artefact suppression.

selective 1D-TOCSY experiment

- This experiment consist of three parts:
- **Selective excitation** of the selected resonance using the SPFGE block.
- **Mixing period** to achieve in phase polarization transfer to other spins. This is usually achieved by applying some isotropic mixing sequence like MLEV, WALTZ or DIPSI pulse trains. This in-phase transfer avoids possible cancellation when the coupling is poorly resolved.
- **Proton detection** as usual.

Figure 5.38



Steps for setup a selective TOCSY:

- record a ^{1}H 1D-Experiment
- select separated signals and determine the chemical shifts
- generate a new dataset
- rpar SELMLGP all and change pp to seldigp
- getprosol 1H P90 PL90
- o1p = set to the chemical shift of the selected signal of step 2
- d9 = 20ms for only one transfer step
- or set d9 = 80ms for more transfer step

For each selected signal a new EXPERIMENT must have recorded. The selected signal and the TOCSY-signals have the same sign. Instead of using the standard pulse sequence seldigp, use seldigpzs with better artefact suppression.

2D HSQC / H2BC

Compare the different HC-correlations:

Experiment A	Experiment B	Experiment C
rpar HSQCETGPSI	rpar CMCse_HSQC	rpar C1MCseH2BC

getprosol 1H P90 PL90
AQ, o1p, SW: optimized value from the 1D
o2p 75ppm
1 SW 150ppm
1TD 64
NS 4

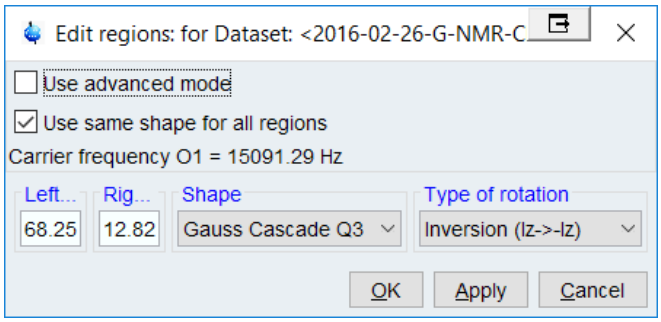
Compared the results, which HC correlation is the most useful experiment.

2D HMBC / sel HMBC
Set up:
rpar CMCse HMBC
getprosol 1H P90 PL90

The pulse sequence hmbcetgpl3nd uses three-fold low-pass J-filter to suppress one-bond correlations (D.O. Cicero, G. Barbato & R. Bazzo, J. Magn. Reson. 148, 209-213 (2001).

AQ, o1p, SW: optimized value from the 1D
o2p 100ppm
1 SW 250ppm
1TD 64
NS 8

The processing command for the HMBC is xfb and xf2m. For running a selective HMBC change the pulse sequence to shmbcctetgpl2nd and change the recommended gradient values. Start the shape tool (stdisp) in the ^{13}C 1D-data set and select a region of interest. Use the button in shape tool to find the right pulse length for the shape



Set the pulse length (p32) and offset (spoffs32) of the shape. In a second experiment set the offset of shape (spoffs32) to 0 and change the middle of the 13C-frequency with smaller spectral widths in F1. Compare all three HMBC spectra.

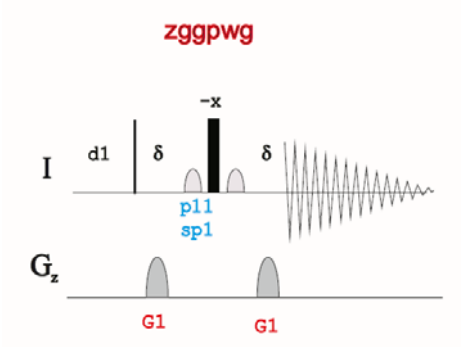
Bio-NMR

Start a 1D 1H Experiment

WATERGATE

rpar ZGGPWG all

getprosol 1H P90 PL90

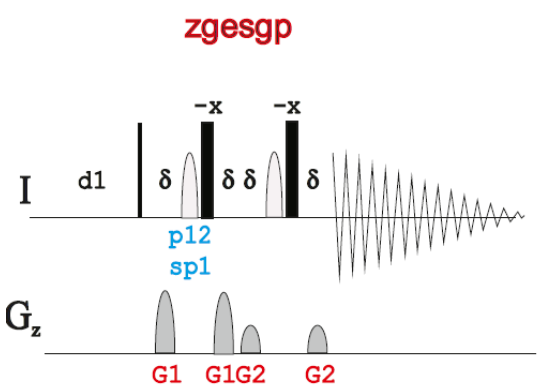



```
Pulse sequence code: zggpwg
prosol relations=<triple>
#include <Avance.incl>
#include <Grad.incl>

"p2=p1*2"
"d12=20u"

1 ze
2 30m
  d1
  10u pl1:f1
  p1 ph1
  50u UNBLKGRAD
  p16:gp1                first gradient dephases all the coherences, gpnam1=SINE.100, gpz1=20%, p16=1ms
  d16 pl0:f1             recovery delay, set power for F1-Channel to pl0
  (p11:sp1 ph2:r):f1     sel 90° pulse on water, p11 = 1ms, spnam1=Sinc1.1000, sp1 calculated from p1
  4u
  d12 pl1:f1             set power back to hard power of proton pulse
  (p2 ph3)
  4u
  d12 pl0:f1
  (p11:sp1 ph2:r):f1             second selective 90° pulse for water, the phase 2 can be optimized in gs-mode
  46u
  p16:                   gp1 second gradient re-phases all the resonances exceptto the on resonance solvent resonance

  d16
  4u BLKGRAD
  go=2 ph31
  30m mc #0 to 2 F0(zd)
Exit                                   (M. Piotto, V. Saudek, V. Sklenar, J.Biomol., 1992, 2, 661)

ph1=0 2
ph2=0 0 1 1 2 2 3 3
ph3=2 2 3 3 0 0 1 1
ph31=0 2 2 0
```

Excitation Sculpting

rpar ZGESGP all

getprosol 1H P90 PL90




```
Pulse sequence code: zgesgp
prosol relations=<triple>

#include <Avance.incl>

#include <Delay.incl>

"p2=p1*2"
"d12=20u"
"TAU=de+p1*2/3.1416+50u"
"acqt0=0"
baseopt_echo
        1 ze
        2 30m
        d12 pl1:f1 BLKGRAD
        d1
        p1 ph1
        50u UNBLKGRAD
        p16:gp1                 Start first gradient echo, p16=1ms, gpnam1=SINE.100, gpz1=31%
        d16 pl0:f1              recovery delay, set power for F1 to pl10=120Db
        (p12:sp1 ph2:r):f1              selective 180° pulse for water, spnam1=Sinc1.100, p12=2ms, sp1
calculated from p1
        4u
        d12 pl1:f1              set power F1 channel back to pl1
        p2 ph3                  non selective 180° pulse
        4u
        p16:gp1                 refocussing gradient
        d16
        TAU
        p16:gp2                 start second gradient echo, p16=1ms, gpnam2=SINE.100, gpz2=11%
        d16 pl0:f1
        (p12:sp1 ph4:r):f1              selective 180° pulse for water
        4u
         d12 pl1:f1
        p2 ph5                  non selective 180° pulse
        4u
        p16:gp2                 refocussing gradient
        d16
        go=2 ph31
        30m mc #0 to 2 F0(zd)
        4u BLKGRAD
        exit                                    (T.-L. Wang, A. J. Shaka, J.Mag.Res., 1995, A112)
ph1=0
ph2=0 1
ph3=2 3
ph4=0 0 1 1
ph5=2 2 3 3
ph31=0 2 2 0
```

More Water suppression sequences:

- zggpw5,
- zggpjrse, Jump-Return Echo, the best pulse sequence for analysis of RNA.
- zgesgppe, with perfect Echo, reduces the artifacts in 1D.

set up 2D ^{15}N-^{1}H correlation, 600 MHz

Setting up a 2D ^{15}N-^{1}H correlation NMR experiments, like HSQC, starts with loading the Bruker standard parameter set using the commands:

rpar <<PARAMETER SET>>
getprosol 1H P90 PL90

For ^{15}N- and ^{13}C-labeled Protein the ZGOPTNS –DLABEL_CN should be activated to enable the ^{13}C decouplingduring the ω_1-evolution period.

General starting parameters for a protein-project at 600 MHz are:

Aq (^{1}H) = 71ms
SW (^{1}H) = 12ppm
o1p (^{1}H) = 4.7ppm
d (d26) = 2.631ms (cnst4 = 95 Hz)
^{15}N dec.: pcpd3 = 220µs
o2p (^{13}C) = 101ppm
^{13}C dec.: p14 = 500µs

Aq (^{15}N) = 105ms
SW (^{15}N) = 40ppm
o3p (^{15}N) = 117ppm
CPD_prog = *grarp4*
adiabatic pulse = *Crp60,0.5.20.1*

These general parameters should be optimized for each protein-project and remembered for the 3D experiments below.

In addition, check the parameters below:

	HSQC	HMQC	TROSY	TROSY (sel. ^{15}N)
Parameter set	HSQCFPF3GPPHWG	SFHMQCF3GPPH	B_TROSYETF3GPSI	B_TROSYETF3GPSI
Pulse program	hsqcfpf3gpphwg	sfhmqcf3gpph	b_trosyetf3gpsi.2	b_trosyetf3gpsi.3
DS	32	32	32	32
NS	8	32	32	32
d1 [s]	1	0.2	0.2	0.2
^{1}H selective pulses	sp1 (90°pulse) Sinc1.1000 p11 → 1 ms	sp23 (120°pulse) Pc9_4_120.1000 p39 → 2.4 ms	sp26 (180°pulse) Repurp.1000 p42 → 1.4 ms	sp25 (90°pulse) Pc9_4_90.1000 p41 → 2.2 ms
		sp24 (180°pulse) Rsnob.1000 p40 → 0.8 ms	sp28 (90°pulse) Eburp2.1000 p43 → 1.7 ms	sp26 (180°pulse) Repurp.1000 p42 → 1.4 ms
			sp29 (90°pulse) Eburp2tr.1000 p43 → 1.7 ms	sp28 (90°pulse) Eburp2.1000 p43 → 1.7 ms
				sp29 (90°pulse) Eburp2tr.1000 p43 → 1.7 ms
^{15}N selective pulses				sp39 (180°pulse) Reburp.1000 p56 → 1.6 ms

				sp40 (180°pulse) Bip720,50,20.1 p56 → 0.5 ms[A]

Table2: Parameters for 2D ^{15}N-^{1}H correlation experiments. [A] 500 µs at all field strength

New option in Topspin 3.5: Setting ZGOPTNS to -DCALC_SP (or -DCALC_SP -DLABEL_CN for ^{13}C & ^{15}N labeled proteins) allows to automatically calculate all the band-selective proton pulses, based on cnst54 and cnst55 and the manually determined p1

cnst54: H(N) chemical shift (offset, in ppm), Protein: 8.5 RNA: 12.3
cnst55: H(N) bandwidth (in ppm), for both 4.8

Shape pulses
Calculation of radiofrequency field strength γB_1

- The magnetization vector precesses around the radiofrequency field B_1 according to

$$\omega = 2\,\pi\nu = \gamma B_1 \qquad (1)$$

- The angle precession q (in rad), is proportional to the pulse width p:

$$\theta = \gamma B_1 * \pi \qquad (2)$$

- For a 90° pulse ($\theta = \pi/2$) and Equation (1):

$$\gamma B_1 = 1/(4 * \pi) \qquad (3)$$

Types of shape pulses
- 90° pulses (excitation, $I_z \rightarrow I_y$)
- 180° pulses
  - Inversion pulses, $I_z \rightarrow -I_z$
  - Refocusing pulses, $I_y \rightarrow -I_y$
- Very narrow excitation
  - Solvent suppression (e.g. Sinc, Gauss, Square pulses)
- Excitation / Inversion of a region Ca, C'…
  - Gaussian Cascades
    - 180° pulses: Q3, G3
    - 90° pulses: Q5, Q5tr, G4, G4tr
- Broadband Inversion/ Refocusing
  - Inversion, $I_z \rightarrow -I_z$
    - Adiabatic pulses (e.g. smoothed Chirp pulses)
    - Broadband Inversion pulses (BIPs)
  - Refocusing, $I_x \rightarrow -I_x$ or $I_y \rightarrow -I_y$
    - Composite adiabatic pulses (e.g. Composite Chirp)

Nomenclature for shapes in Topspin:

- Standard shapes, e.g. Q5tr.1000 →
  - Shape (Q5 time reversed)
  - Number of points (1000)
- Adiabatic shapes, e.g. Crp60, 05,20.1

- Shape and sweep width (Chirp, 60 kHz)
- duration (0.5 ms)
- Smoothing and number of points (20% and 1000)

Instead of using band-selective pulses, for protein NMR selective rectangular pulses can be applied due the large chemical shift difference between CO and CA. The excitation profile with maximum on CA and minimum on CO is calculated as follows:

90° pulse:

$$\beta^{eff} = 4*\beta = 360° \Rightarrow (\omega_1^{eff})^2 = (4\omega_1)^2 = \omega_1^2 + (\Delta\Omega)^2 \Rightarrow \omega_1 = \Delta\Omega/\sqrt{15}$$

$$\boxed{\tau_{p(90°)} = \beta/\omega_1 = \pi/2 * \sqrt{15} / (2\pi*\Delta\Omega)}$$

180° pulse:

$$\beta^{eff} = 2*\beta = 360° \Rightarrow (\omega_1^{eff})^2 = (2\omega_1)^2 = \omega_1^2 + (\Delta\Omega)^2 \Rightarrow \omega_1 = \Delta\Omega/\sqrt{3}$$

$$\boxed{\tau_{p(180°)} = \beta/\omega_1 = \pi * \sqrt{3} / (2\pi*\Delta\Omega)}$$

Both equations can be implemented in a pulse sequence using cnst21 (CO chemical shift) and cnst22 (CA chemical shift) with the following definitions:

90°-pulse: "pxx=3.873*1000000/((cnst21-cnst22)*bf2*4)"
180° -pulse "pxx=1.732*1000000/((cnst21-cnst22)*bf2*2)"

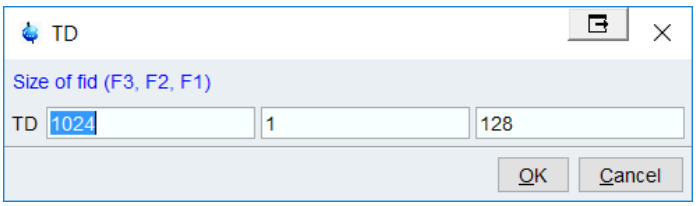
Setup 3D Experiment
First read a standard parameter set, e.g. for the protein backbone 3D experiments

rpar <<PARAMETER SET>>
getprosol 1H P90 PL90
set the optimized general parameters from the HSQC

- protein backbone 3D experiments based on WATERGATE: HNCOGPWG3D, HNCACOGPWG3D, HNCAGPWG3D, HNCOCAGPWG3D, HNCACBGPWG3D, HNCOCACBGPWG3D
- protein backbone 3D experiments based on Best-method: B_HNCOGP3D, B_HNCACOGP3D, B_HNCAGP3D, B_HNCOCAGP3D; B_HNCACBGP3D, B_HNCOCACBGP3D
- protein backbone 3D experiments based on Best-TROSY-Method: B_TRHNCOGP3D, B_TRHNCACOGP3D, B_TRHNCAGP3D, B_TRHNCOCAGP3D; B_TRHNCACBGP3D, B_TRHNCOCACBGP3D

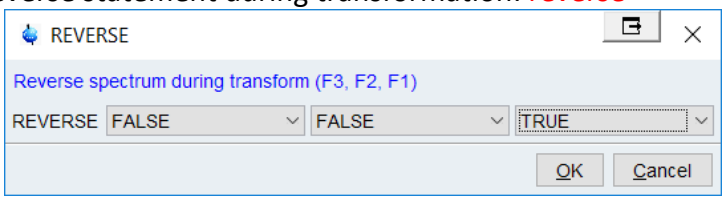
Which 3D methods is the best option for protein assignment depends on the size of the molecule. This can be experimentally tested by recording the carbon plane in a 3D HNCO. This

can be done by setting the number of points in the evolution period of ^{15}N (F2) to 1 and ^{13}C(F3) to 128, td.



Processing the 2D Plane in a 3D dataset.

- Checking reverse statement during transformation: reverse



Depends on the pulse sequence, hncogpwg3d: FALSE; b_hncogp3d.2 & b_trhncogp3d.2: TRUE

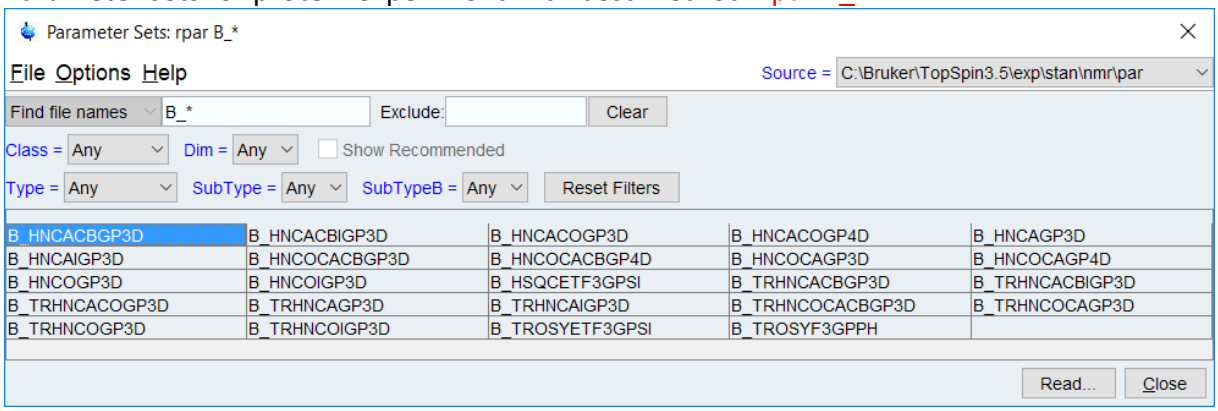
- 2D processing command FT: xfb
  - Enter a new PROCNO for 2D data: e.g. 13 (store the carbon-plane in procno 13 and ^{15}N plane in procno 23).
- 2D processing command for phase correction: apk2d
- Compare the 2D spectra:
  - read out the 1D projection, by rhpp 2 (read horizontal positive projection and store it in procno 2)

Additional Information

All implemented NMR-experiments in TopSpin are described in the manual 'pulse program catalogue BIO'.

In TopSpin a wide range of NMR experiments are implemented as parameter set which can easily use. A selection of important experiments follows below:

Parameter sets for protein experiment with best method: rpar B_*



Parameter sets for 4 dimensional NMR experiments: rpar *4D*

Parameter Sets: rpar *4D*

File Options Help — Source = C:\Bruker\TopSpin3.5\exp\stan\nmr\par

Find file names *4D* Exclude: Clear

Class = Any Dim = Any Show Recommended

Type = Any SubType = Any SubTypeB = Any Reset Filters

B_HNCACOGP4D	B_HNCOCACBGP4D	B_HNCOCAGP4D	CBCACONHGPWG4D	HBHACBCANHGPWG4D
HBHACONHGPWG4D	HNCACOGP2H4D	HNCACOGP4D	HNCACOGPWG2H4D	HNCACOGPWG4D
HNCOCAGP2H4D	HNCOCAGP4D	HNCOCAGPWG2H4D	HNCOCAGPWG4D	HSQCNOESYHSQCCCGP4D
HSQCNOESYHSQCCNGP4D	HSQCNOESYHSQCNCGP4D	HSQCNOESYHSQCNNGP4D	TRHNCOCAETGP2H4D	TRHNCOCAETGP4D
TRHNCOCAGP2H4D	TRHNCOCAGP4D			

Read... Close

Parameter sets for ^{13}C direct detection: rpar C_*

Parameter Sets: rpar C_*

File Options Help — Source = C:\Bruker\TopSpin3.5\exp\stan\nmr\par

Find file names C_* Exclude: Clear

Class = Any Dim = Any Show Recommended

Type = Any SubType = Any SubTypeB = Any Reset Filters

C_CACO	C_CACO_IA	C_CACO_S3	C_CAN_IASQ	C_CAN_MQ
C_CAN_MQ.2	C_CANCO_IA3D	C_CANCO_IA3D.2	C_CANCOI_IA3D	C_CBCACO_IA3D
C_CBCACO_S33D	C_CBCACON_IA3D	C_CBCANCO_IA3D	C_CCCO_IA3D	C_CCCO_S33D
C_CCCON_IA3D	C_CCFLOPSY16	C_CCFLOPSY16_CT	C_CCFLOPSY16_CTIA	C_CCFLOPSY16_IA
C_CCNOESY	C_CCNOESY_CT	C_CCNOESY2	C_COCA	C_COCA_IA
C_COCA_MQ	C_COCA_MQ.2	C_CON_IASQ	C_CON_MQ	C_CON_MQIA
C_CON_SQ	C_COSY	C_COSY_CT	C_COSY2_CT	C_HACACO_3D
C_HCACO_IA3D	C_HCACO_S33D	C_HCAN_IA3D	C_HCANCO_IA3D	C_HCANCOI_IA3D
C_HCBCA_IA3D	C_HCBCACO_IA3D	C_HCBCACO_S33D	C_HCBCAN_IA3D	C_HCCFLOPSY16_3D
C_HNCA_IA3D	C_HNCACO_IA3D	C_HNCACO_S33D	C_HNCO_IA3D	C_HNCOCA_IA3D
C_HNCOCA2_IA3D				

Read... Close

Parameter sets for nucleic acids: rpar NA_*

Parameter Sets: rpar NA_*

File Options Help — Source = C:\Bruker\TopSpin3.5\exp\stan\nmr\par

Find file names NA_* Exclude: Clear

Class = Any Dim = Any Show Recommended

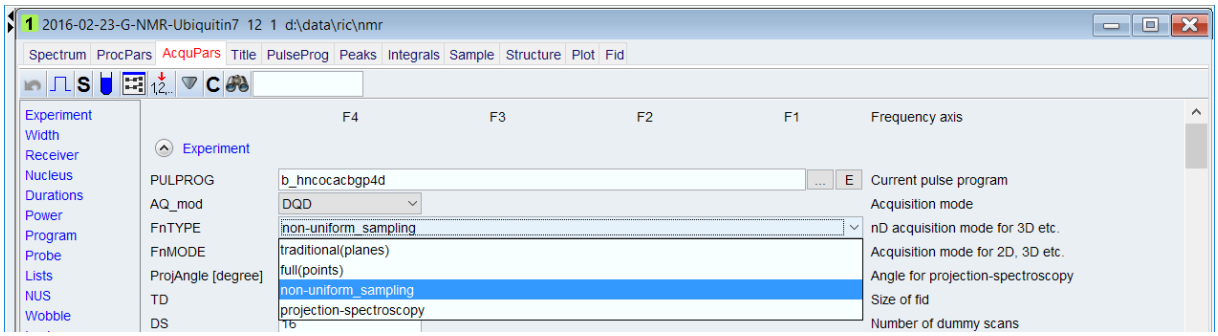
Type = Any SubType = Any SubTypeB = Any Reset Filters

NA_C6NOESYHSQCGP3D	NA_GHCCHFWDIGP3D	NA_H56C56C4N3H_3D	NA_H56C56C4N3H_3D2	NA_H5C5C4N3H_3D
NA_H68C68N19C42	NA_HCCHECGP3D	NA_HCCHFWDIECGP3D	NA_HCCHFWDIECGPJR3D	NA_HCCHFWDIGP3D
NA_HCCNHDIGPWG3D	NA_HCNCCHCOMQ3D	NA_HCNCCHDIMQ3D	NA_HCNCHGPJRPHSP	NA_HCNCHGPMQSP3D
NA_HCNETGPSI3D	NA_HCNETGPSISP3D	NA_HCNHGPPH19	NA_HCNMQ3D	NA_HCNMQGPPHPR
NA_HCNMQSP3D	NA_HCPDIETGP3D	NA_HCPDIETGPSI3D	NA_HCPDIGP3D	NA_HCPDIGPJR3D
NA_HCPETGPSI3D	NA_HCPQETGPSI.1	NA_HCPQETGPSI.2	NA_HNC6C5ETGPSI	NA_HNCCH3D
NA_HNNCOSYGPPHSPWG	NA_HNNCOSYGPPHWG	NA_HPCCOCTETF3GP3D	NA_HPCCOETF3GP3D	NA_HPCCTCO3D
NA_HPCOSYPHPR	NA_HPCTCO	NA_HPCTCOJR	NA_HPDI	NA_HPDICO3D
NA_HPDINO	NA_HPDINO19	NA_HPDINO193D	NA_HPDINO3D	NA_HSQCCTETGPSP.2
NA_HSQCETF3GPXY	NA_HSQCETGPJCSP	NA_HSQCF3GPJRPHXY	NA_HSQCF3GPNOPHXY	NA_HSQCFPF3GPPHWG
NA_NOESYGPPHPRXF	NA_NOESYGPPHXF19	NA_NOESYHSQCCTETGP3D	NA_NOESYHSQCF3GPWG3D	NA_PCCHCO
NA_PCCHDI	NA_PCCHDI2	NA_PFIDSETGPSI	NA_TRHCCHCO3D	NA_TRHCNCHMQSP3D
NA_TRHCNCHMQSPWG3D	NA_TRHCNCHMQSPWG3D2	NA_TRHCNET	NA_TRHCNETSI3D	NA_TRHCNPH
NA_TRHCNPH3D	NA_TRHNNCOSYGPPHSPWG	NA_XHCOCTETF3GP	NA_XHCOETF3GP	NA_ZGGPJRSE

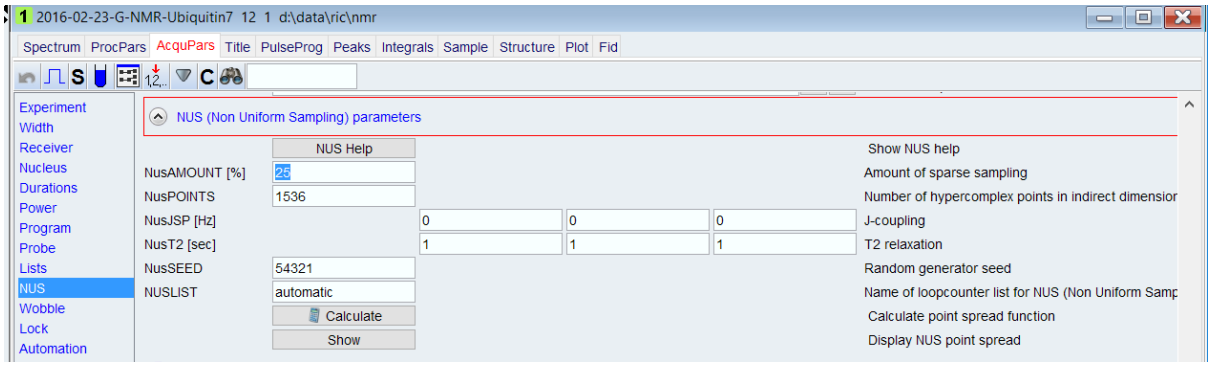
Read... Close

NUS – Non Uniform Sampling

The general concept of NUS is summarized in TopSpin manual 'NUS Parameters'. The NUS reduced for a multidimensional NMR-experiment the number of points for the indirect dimension by using algorithm. For using NUS in setup NMR-Experiments only minor changes of the parameter setup are necessary. First, you set up the 2D, 3D or 4D NMR experiments in the described way before. The NUS will be activated for the data set by selecting the parameter FnTYPE as non-uniform_ sampling in eda.



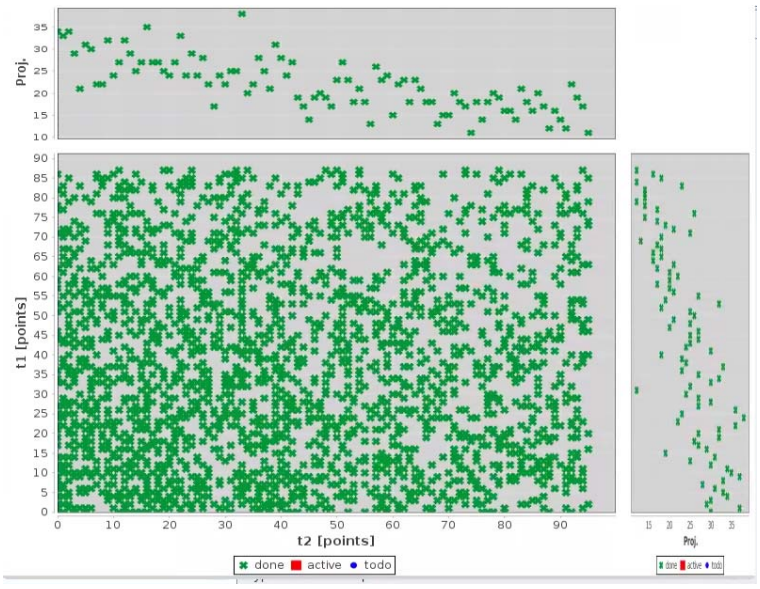
In the category NUS of eda the number NusAMOUNT [%] selected how many total points are recorded for the indirect dimension.



The choice of NusAMOUNT [%] compromised between as less as possible points and getting a multidimensional NMR spectrum without artefacts. From practical experiences is used as recommended values 50% for 2D NMR and 25% for 3D & 4D NMR. The parameter NusT2 [sec] weight the calculated points combination for the indirect dimension. If all NusT2 1 yields in equal distribution of all points. For **protein NMR experiment** the suggestion of NusT2:

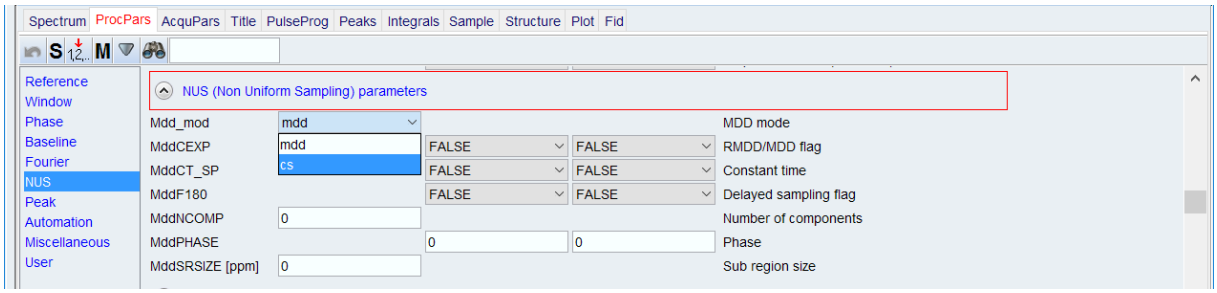
^{1}H(aliphatic):0.02 ^{15}N: 0.05 ^{13}C: 0.5,

which weight the points distribution more to beginning of the experiment, shown by press Show button. Result:



Processing of NUS data.

TopSpin recognized automatic if the date were recorded with NUS and do the processing like usually. For processing of NUS data two algorithm are implemented in TopSpin: **MDD** and **CS**. Both need an additional license for processing, but not for recording the data. Which algorithm are used can define by the parameter Mdd_mod in edp, showing below:



General, in the most cases the **CS** algorithm give a better result, but the processing takes longer. For that reason, optimized the other processing parameter with **MDD** and select **CS** for final processing. In TopSpin 3.5 the **CS** algorithm is included for free by processing 2D data.

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