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:

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Diameter</th>
<th>Sample volume</th>
<th>Coasts</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>High quality</td>
<td>5mm</td>
<td>550 µl</td>
<td>10 – 20 €</td>
<td></td>
</tr>
<tr>
<td>High quality</td>
<td>3mm</td>
<td>180 µl</td>
<td>10 – 20 €</td>
<td>for lossy biological sample</td>
</tr>
<tr>
<td>Shigemi tube</td>
<td>5mm</td>
<td>280 µl</td>
<td>110 €</td>
<td>for expensive sample</td>
</tr>
<tr>
<td>Shape tube</td>
<td>oval tube</td>
<td>340 µl</td>
<td>260 €</td>
<td>for lossy biological sample</td>
</tr>
</tbody>
</table>
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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: \(^1\text{H}\) Proton
    - blue: \(^{13}\text{C}\) Carbon
    - red: \(^{15}\text{N}\) Nitrogen
    - grey: \(^2\text{H}\) Deuterium (only CryoProbe™)
  - change the tuning und matching screws at the probe head to reach a minimum at the red central line.
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### Automated tuning and matching:
- Special probe head required: ATM
- Commands: atm (fully automated) or atmm (manually)

### Procedure T + M of ²H 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 ¹H-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 ²H signal of the solvent (deuterated solvents)
- adds a small extra field to the main field of the magnet to keep the overall field constant
- ²H 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 *cryo shims* and *room temperature shims*

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: $\omega_0 =$
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\( \gamma (B_0 + H_0) \). \( B_0 \) 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 \(^2\text{H}\) transmitter output power. Due to the different relaxation behavior of \(^2\text{H}\) 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 to the saturation. To check if the lock 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`.

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

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

<table>
<thead>
<tr>
<th>OPTIMIZE</th>
<th>GROUP</th>
<th>PARM.</th>
<th>OPTIMUM</th>
<th>START.</th>
<th>END.</th>
<th>NEXP</th>
<th>VARMOD</th>
<th>INC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step by step</td>
<td>0</td>
<td>P1</td>
<td>ZERO</td>
<td>a</td>
<td>b</td>
<td>11</td>
<td>LIN</td>
<td>0.2</td>
</tr>
</tbody>
</table>

$a = 4$ 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.

![Prosol Table Image]

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
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The relation files are stored in: ...

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, absI** 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\(_6\)D\(_6\).

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’:

\[
\cos \beta_{\text{opt}} = \exp(-TR/T_1)
\]

(TR = pulse repetition time, TR = d1 + AQ; \(\beta_{\text{opt}}\): 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:

```plaintext
;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
```

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:

\[ AQ = TD \times DW = TD/2 \text{SWH} \]  
\( (DW = \text{dwell time}, \text{SWH} = \text{spectral width in Hz}) \)

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 \( \omega_{1p} \) should be optimized for setting up a 2D-NMR spectra. The selected number of scans depends on the sample concentration.

**13C 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 gyromagnetic 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.

<table>
<thead>
<tr>
<th>Pulse program</th>
<th>Properties</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>zg</td>
<td>No decoupling</td>
<td>No signal enhancement, coupled spectrum</td>
</tr>
<tr>
<td>zg30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>zggd</td>
<td>,Gate Decoupling’: decoupling during relaxation delay</td>
<td>Signal enhancement due to NOE effect, coupled spectrum</td>
</tr>
<tr>
<td>zggd30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>zgig</th>
<th>zgig30</th>
<th>'Inverse Gate Decoupling': decoupling during acquisition</th>
<th>For quantitative analysis of $^{13}$C-signals, decoupled spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>zgdc</td>
<td>zgdc30</td>
<td>Decoupling during entire experiment PG: 'Power Gate Decoupling' lower power during relaxation delay, pl13</td>
<td>For maximal signal intensity, decoupled spectrum</td>
</tr>
<tr>
<td>zgpg</td>
<td>zppg30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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
d100m cpd2:f2 star decoupling program cpd2
p1*0.3 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.
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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

Or select the recommended data sets:
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**2D COSY**

rpar CMSseCOSY

getprosol 1H P90 PL90

The parameter set based on pulse sequence `cosyppmppqf`. 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 1H 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 cosypphzhfs`
- `1 FNMODE States-TPPI`
- `gpz0 10`
- `NS 4`

**selective 1D-NOESY experiment**

This experiment consist of three parts:

- **Selective excitation** of the selected resonance using the SPFGE block.
- **Mixing period** consisting of the basic 90°(1H)-delay-90°(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.

Steps for setup a selective NOESY:

- record a 1H 1D-Experiment
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- 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.

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**
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Compare the different HC-correlations:

<table>
<thead>
<tr>
<th>Experiment A</th>
<th>Experiment B</th>
<th>Experiment C</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpar HSQCETGPSI</td>
<td>rpar CMCse_HSQC</td>
<td>rpar C1MCseH2BC</td>
</tr>
<tr>
<td>getprosol 1H P90 PL90</td>
<td>AQ, o1p, SW: optimized value from the 1D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o2p 75ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 SW 150ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1TD 64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS 4</td>
<td></td>
</tr>
</tbody>
</table>

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

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


- 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 13C 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.
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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 p1:f1
p1 ph1
50u UNBLKGRAD
p16:gp1
p16=1ms
d16 p0:f1
(p11:sp1 ph2:r):f1
4u
d12 p1:f1
(p2 ph3)
4u
d12 p0:f1
(p11:sp1 ph2:r):f1
gs-mode
46u
p16:

solvent resonance

d16
4u BLKGRAD
go=2 ph31
30m mc #0 to 2 F0(zd)
Exit
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

first gradient dephases all the coherences, gpnam1=SINE.100, gpz1=20%,
recovery delay, set power for F1-Channel to p10
sel 90° pulse on water, p11 = 1ms, spnam1=Sinc1.1000, sp1 calculated from p1
set power back to hard power of proton pulse

second selective 90° pulse for water, the phase 2 can be optimized in
gs-mode

gp1 second gradient re-phases all the resonances except to the on resonance

(M. Piotto, V. Saudek, V. Sklenar, J.Biomol.,
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
d16 pl0:f1
(p12:sp1 ph2:r):f1
calculated from p1
4u
d12 pl1:f1
p2 ph3
4u
p16:gp1
d16
TAU
p16:gp2
d16 pl0:f1
(p12:sp1 ph4:r):f1
4u
d12 pl1:f1
p2 ph5
4u
p16:gp2
d16
go=2 ph31
30m mc #0 to 2 F0(zd)
4u BLKGRAD
exit

ph1=0
ph2=0 1
ph3=2 3
ph4=0 0 1 1
ph5=2 2 3 3
ph31=0 2 2 0

Start first gradient echo, p16=1ms, gpnam1=SINE.100, gpz1=31%
recovery delay, set power for F1 to pl10=120Db
selective 180° pulse for water, spnam1=Sinc1.100, p12=2ms, sp1

set power F1 channel back to pl1
non selective 180° pulse
refocussing gradient

start second gradient echo, p16=1ms, gpnam2=SINE.100, gpz2=11%
selective 180° pulse for water
non selective 180° pulse
refocussing gradient

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More Water suppression sequences:
- zgppw5,
- zgppjrze, Jump-Return Echo, the best pulse sequence for analysis of RNA.
- zggesgppe, 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 decoupling during the $\omega_1$-evolution period.

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

- $A_q (^1H) = 71$ms
- $A_q (^{15}N) = 105$ms
- $SW (^1H) = 12$ppm
- $SW (^{15}N) = 40$ppm
- $o1p (^1H) = 4.7$ppm
- $o3p (^{15}N) = 117$ppm
- $d (d26) = 2.631$ms (cnst4 = 95 Hz)
- $^{15}$N dec.: pcpd3 = 220µs
- $^{13}$C dec.: p14 = 500µs
- 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:

<table>
<thead>
<tr>
<th>Parameter set</th>
<th>HSQC</th>
<th>HMQC</th>
<th>TROSY</th>
<th>TROSY (sel. $^{15}$N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse program</td>
<td>HSQCFPF3GPPHWG</td>
<td>SFHMQCF3GPPH</td>
<td>B_TROSYETF3GSPSI</td>
<td>B_TROSYETF3GSPSI</td>
</tr>
<tr>
<td>DS</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>NS</td>
<td>8</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>$d1$ [s]</td>
<td>1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>$^1$H selective pulses</td>
<td>sp1 (90°pulse) Sinc1.1000 p11 $\rightarrow$ 1 ms</td>
<td>sp23 (120°pulse) Pc9_4_120.1000 p39 $\rightarrow$ 2.4 ms</td>
<td>sp26 (180°pulse) Repurp.1000 p42 $\rightarrow$ 1.4 ms</td>
<td>sp25 (90°pulse) Pc9_4_90.1000 p41 $\rightarrow$ 2.2 ms</td>
</tr>
<tr>
<td></td>
<td>sp24 (180°pulse) Rsnob.1000 p40 $\rightarrow$ 0.8 ms</td>
<td>sp28 (90°pulse) Eburp2.1000 p43 $\rightarrow$ 1.7 ms</td>
<td>sp26 (180°pulse) Repurp.1000 p42 $\rightarrow$ 1.4 ms</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sp29 (90°pulse) Eburp2tr.1000 p43 $\rightarrow$ 1.7 ms</td>
</tr>
<tr>
<td>$^{15}$N selective pulses</td>
<td></td>
<td></td>
<td></td>
<td>sp39 (180°pulse) Repurp.1000 p56 $\rightarrow$ 1.6 ms</td>
</tr>
</tbody>
</table>

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

New option in Topspin 3.5: Setting \texttt{ZGOPTNS} to -\texttt{DCALC_SP} (or -\texttt{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

\textbf{Shape pulses}

Calculation of radiofrequency field strength $\gamma B_1$

- The magnetization vector precesses around the radiofrequency field $B_1$ according to
  \[ \omega = 2 \pi v = \gamma B_1 \]  
  (1)

- The angle precession $q$ (in rad), is proportional to the pulse width $p$:
  \[ \theta = \gamma B_1 \ast \pi \]  
  (2)

- For a 90° pulse ($\theta = \pi/2$) and Equation (1):
  \[ \gamma B_1 = 1/(4 \ast \pi) \]  
  (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
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- 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 to 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:

\[ \beta_{\text{eff}} = 4*\beta = 360^\circ \Rightarrow (\omega_{\text{eff}})^2 = (4\omega_{\text{1}})^2 = \omega_{\text{1}}^2 + (\Delta \Omega)^2 \Rightarrow \omega_{\text{1}} = \Delta \Omega/\sqrt{15} \]

\[ \tau_{p(90^\circ)} = \beta/\omega_{\text{1}} = \pi/2 * \sqrt{15} / (2\pi*\Delta \Omega) \]

180° pulse:

\[ \beta_{\text{eff}} = 2*\beta = 360^\circ \Rightarrow (\omega_{\text{eff}})^2 = (2\omega_{\text{1}})^2 = \omega_{\text{1}}^2 + (\Delta \Omega)^2 \Rightarrow \omega_{\text{1}} = \Delta \Omega/\sqrt{3} \]

\[ \tau_{p(180^\circ)} = \beta/\omega_{\text{1}} = \pi * \sqrt{3} / (2\pi*\Delta \Omega) \]

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, HNOCAGPWG3D,
  HNCACBGPWG3D, HNOCACBGPWG3D

- protein backbone 3D experiments based on Best-method:
  B_HNCOGP3D, B_HNCACOGP3D, B_HNCAACP3D, B_HNOCAGP3D;
  B_HNACBG3P3D, B_HNOCACBG3P3D

- protein backbone 3D experiments based on Best-TROSY-Method:
  B_TRHNCOGP3D, B_TRHNCACGP3D, B_TRHNCAP3D, B_TRHNCOCAP3D;
  B_TRHNCACBG3P3D, B_TRHNCOCACBG3P3D

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.
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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.

![Image showing TD settings](image1)

Processing the 2D Plane in a 3D dataset.

- Checking reverse statement during transformation: reverse
  
  ![Image showing reverse statement](image2)

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

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

Parameter sets for nucleic acids: rpar NA_*
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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:

\[
\begin{align*}
1^\text{H}(\text{aliphatic}): 0.02 & & 15^\text{N}: 0.05 & & 13^\text{C}: 0.5,
\end{align*}
\]

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 data 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.