CEISAM, Nantes Université, CNRS

Implementation protocol for the ultrafast COSY experiment
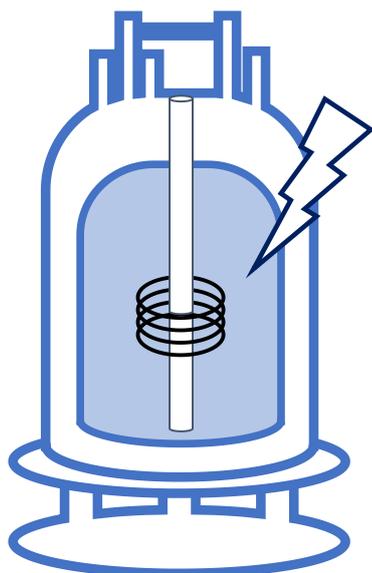
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Important remarks:

- If the use of this document leads to interesting new results, we would be happy to be informed about it. In case it leads to publishable work, we would appreciate if this protocol could be referred to in the experimental part or in the acknowledgments.
- The CEISAM and the authors of this protocol accept no liability for any hardware damage caused by the use of ultrafast experiments.

This protocol is based on the following existing protocols, which are also recommended:

- "OPTIMIZATION AND PRACTICAL IMPLEMENTATION OF ULTRAFAST 2D NMR EXPERIMENTS"; Luiz H. K. Queiroz Júnior et al.; *Quim. Nova*; 2013; Vol. 36, No. 4, 577-581
- "Implementing ultrafast 2D NMR experiments on a Bruker Avance Spectrometer" protocol; 2015 et 2017 versions; Patrick Giraudeau, Benoît Charrier, Serge Akoka, Laetitia Rouger
- Chapter "Ultrafast 2D NMR: Methods and Applications"; Gouilleux et al. ; *Annual Reports on NMR Spectroscopy*; 2018 ; Vol. 93

1 Introduction

This document describes the implementation of the ultrafast COSY experiment on Bruker spectrometers. Please note that some of the procedures described below are still under optimization. Other homo- or heteronuclear experiments can be easily implemented, either by the advanced user himself, or on demand.

Ultrafast 2D NMR experiments rely on principles that are quite different from conventional 2D NMR. **Before implementing the corresponding experiments, it is therefore recommended to understand the principles of ultrafast 2D NMR**. Reading the following papers is highly recommended:

- L. Frydman, T. Scherf, A. Lupulescu, The acquisition of multidimensional NMR spectra within a single scan, Prod. Natl. Acad. Sci. USA 99 (2002) 15858-15862.
- L. Frydman, A. Lupulescu, T. Scherf, Principles and features of single-scan two-dimensional NMR spectroscopy, J. Am. Chem. Soc. 125 (2003) 9204-9217.
- P. Pelupessy, Adiabatic single scan two-dimensional NMR spectroscopy, J. Am. Chem. Soc. 125 (2003) 12345-12350.
- M. Gal, L. Frydman, Ultrafast Multidimensional NMR: Principles and Practice of Single-scan Methods. In Encyclopedia of NMR, Grant, D. M.; Harris, R. K., Eds. Wiley: New-York, 2009; Vol. 10.
- P. Giraudeau, L. Frydman, Ultrafast 2D NMR: an emerging tool in analytical spectroscopy, Annu. Rev. Anal. Chem 7 (2014) 129-161.
- C. Lhoste, B. Lorandel, C. Praud, A. Marchand, R. Mishra, A. Dey, A. Bernard, J-N. Dumez, P. Giraudeau, Ultrafast 2D NMR for the analysis of complex mixtures, Prog. Nucl. Magn. Reson. Spectrosc. 130-131 (2022) 1–46.

2 Description of the attached files

Pulse sequences to be placed in \Bruker\TOPSPIN\exp\stan\nmr\lists\pp\user	
JNDechograd	Pulse program for gradient and chirp pulse calibration
iufcosy-pr	Pulse program for the interleaved ultrafast COSY experiment, including a solvent pre-saturation block

Pulse, to be placed in \Bruker\TOPSPIN\exp\stan\nmr\lists\wave\user	
Chirp-15-11	Example of Chirp pulse for 1H
Chirp-15-15	Example of Chirp pulse for 1H
Processing programs, to be placed in \Bruker\TOPSPIN\exp\stan\nmr\py\user	
ufproc.py	Python processing program for generating ultrafast spectra
ufcal.py	Python processing program for calibrating chemical shifts in ultrafast spectra
ufsym.py	Python processing program for symmetrizing ultrafast spectra

3 General recommendations

- For optimal results and in order to avoid wasting time, do not start anything before reading the literature suggested
- If you get into trouble (get error messages, do not obtain the expected result, etc.), send us an e-mail (patrick.giraudeau@univ-nantes.fr). There are significant chances that we can help to solve your problem.
- Start implementing ultrafast experiments with a very simple sample, typically 10% H_2O in D_2O.
- A probe equipped with z-gradients (at least) is necessary
- Gradient amplifier on. If the red « error » light is on, reset the gradient amplifier before starting

4 Gradient and chirp pulse calibration

4.1 Preliminary settings

Start the implementation with a very simple sample, constituted of **10% H_2O in D_2O**. It will allow a better visualization of the gradient echo profiles, since it contains only one resonance.

Run a preliminary 1D 1H experiment, tune and match, lock and shim, and calibrate the ^1H 90° hard pulse.

4.2 Dispersion induced by the gradients

Before running a 2D ultrafast experiment, it is necessary to know the frequency dispersion induced by your gradients. To do so, run the "JNDechograd" (see Figure 1) experiment which leads to an excitation profile of your sample. It will also allow you to calibrate the 180° chirp pulses.
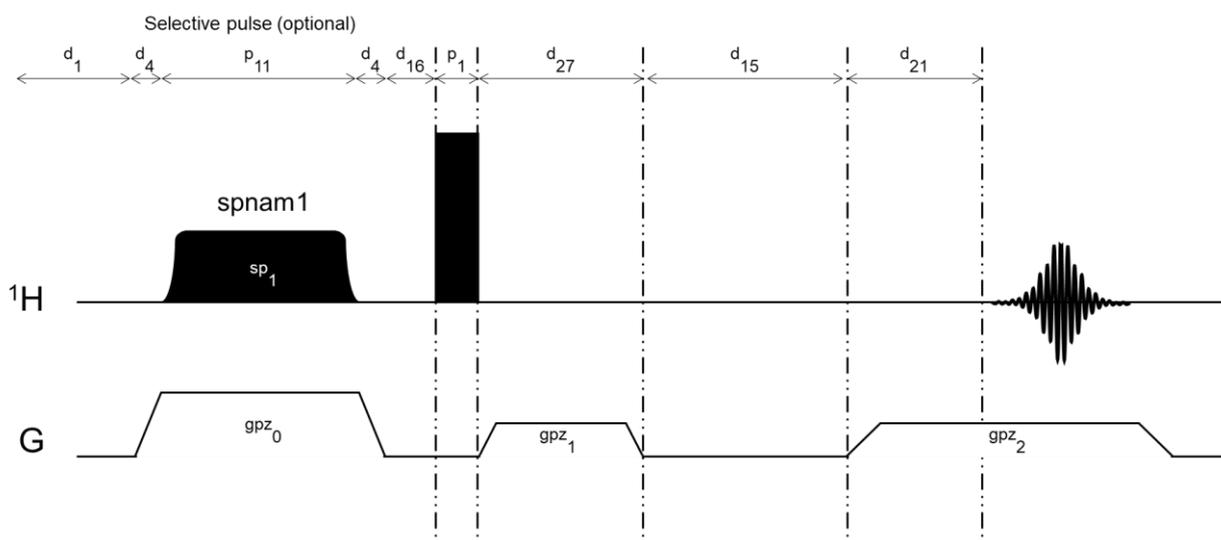

Figure 1. *"JNDechograd" pulse sequence*

4.2.1 Acquisition parameters

Create a new experiment by copying a classical ^{1}H experiment (zg). Use 298K as a working temperature and set the following parameters:

pulprog: echograd4
AQMOD: qsim (DQD on Avance III and ulterior console versions)
NS: 1
DS: 0
SW: 250 ppm
AQ: 10 ms
RG: as determined on a classical ^{1}H experiment (zg)
o1: Value corresponding to the middle of the aimed chemical shift range (3.5 ppm for instance)
pl1: ^{1}H hard pulse power
p1: Previously calibrated value, corresponding to a 90° ^{1}H hard pulse
sp1: 120dB (deactivates the selective pulse)
p11: 100 μs (not used for the moment but otherwise the acquisition will not start)
p2: 180° ^{1}H pulse (automatically set as p2 = 2*p1)
d1: 1-5.T1 (not important for the moment as only 1 scan is performed)
D4 = 50 μs
D15 = 1 ms
D16 = 200 us
D21 = 200 μS
D 27 = 5 ms
GPZ0: 0 % (no importance in this experiment as selective pulse is inactivated)
GPZ1: 10%
GPZ2: -10%

Launch the experiment and inspect the obtained FID. This should look like the one from Figure 2 :
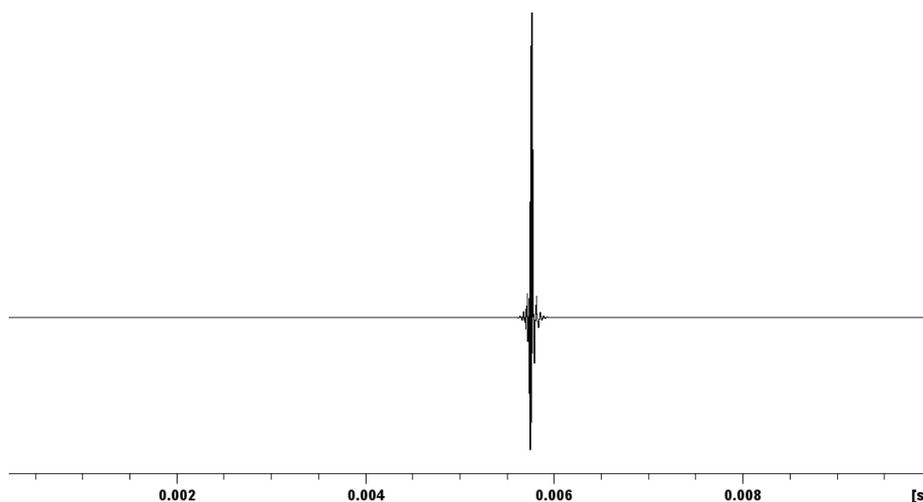

Figure 2. FID obtained from the "JNDechograd" experiment

Using the cursor, measure the distance between the maximum of the observed echo and the center of the window (located at "0.005" on Figure 3). Adjust the d27 value as a consequence and make a new acquisition, in order to observe the echo in the middle of the acquisition window (see example on Figure 3).
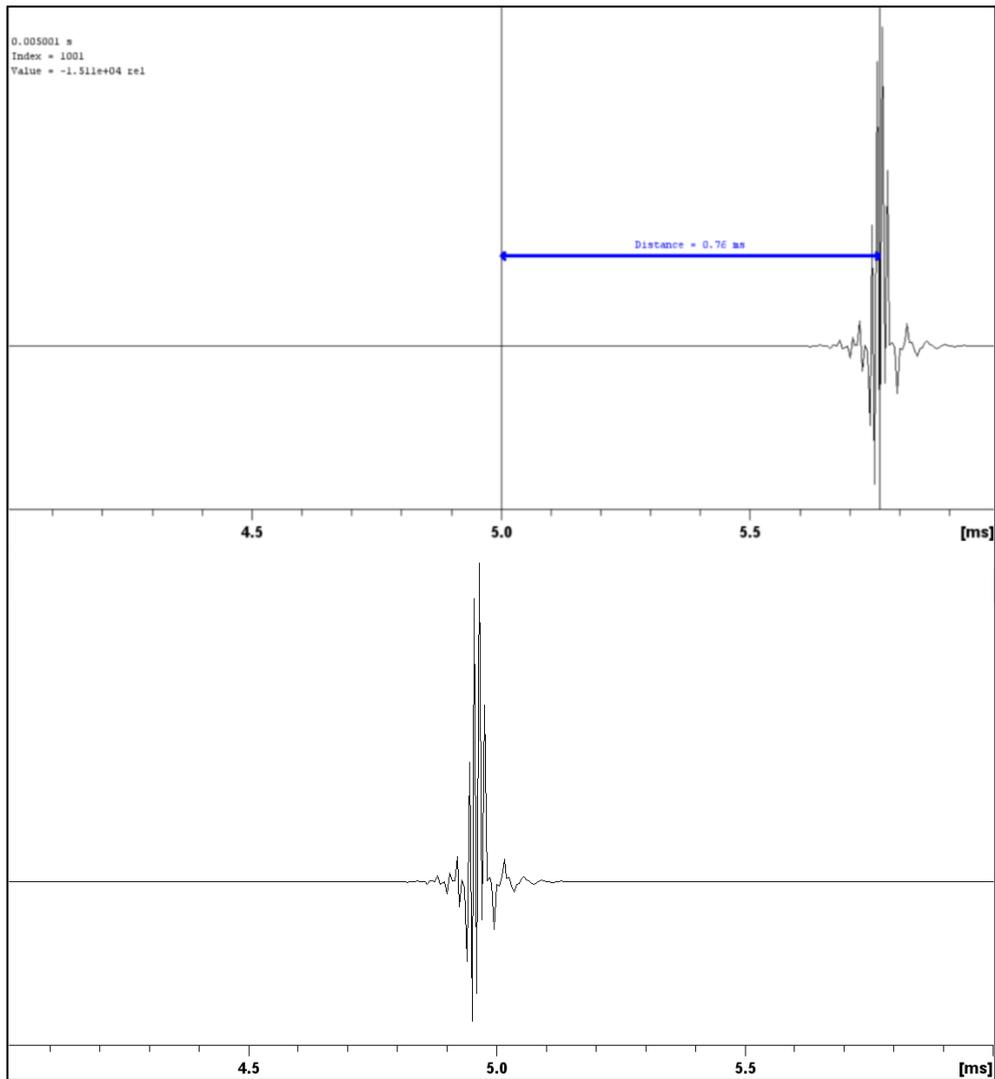
Figure 3. FID before (top) and after (bottom) correction of the d27 value. Here, the measured distance between the center of the acquisition window and the maximum of the echo is +0.76 ms, hence the d27 value was corrected to d27 = 5.8 ms

4.2.2 Processing parameters

Process the FID with a gaussian function:

WDW: GM

LB: -20 Hz

GB: 0.5

Use the *gm* and *ft* commands then manually phase the obtained spectrum, starting with a first order phase correction. Do not forget to display the complete spectral width. A huge ph1 phase correction is necessary because of phase dispersion induced by the gradients. Then a small zero order phase correction should be done to obtain a symmetric image. An example can be seen on Figure 4.
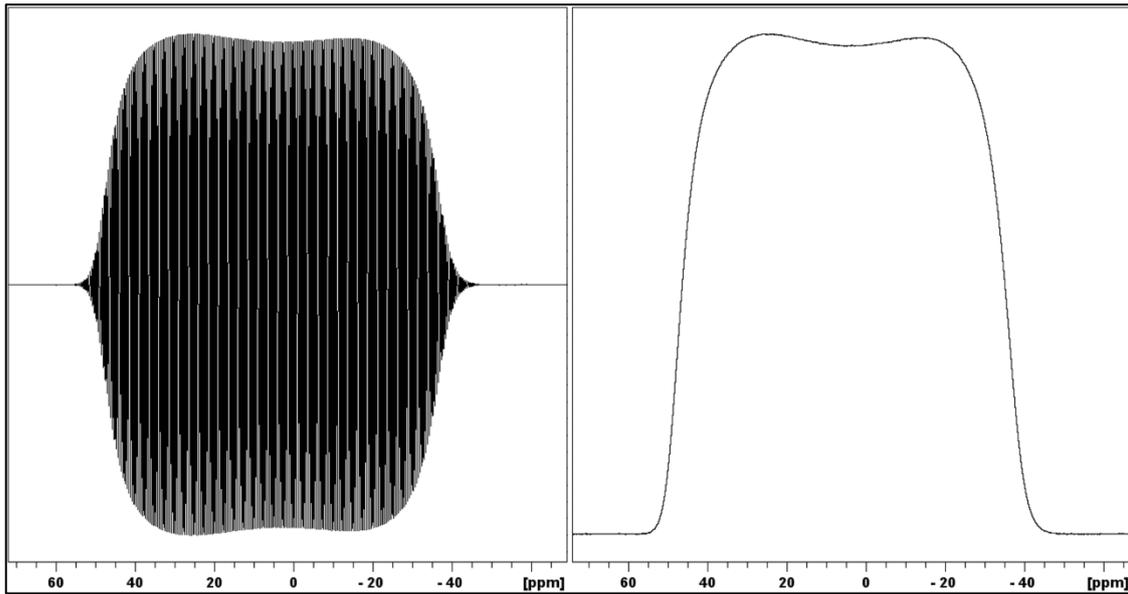
*Figure 4. Obtained spectrum before (left) and after (right) phase correction. Here a 150 000° first order phase correction was performed. Acquisition was performed on a 500 MHz spectrometer.*

On the phase spectrum, using the cursor, measure the width of the dispersion profile at half-height, which corresponds to the frequency dispersion induced by the gpz1 gradient (10%, using the parameters detailed above) and note this value. An example can be seen on Figure 5.
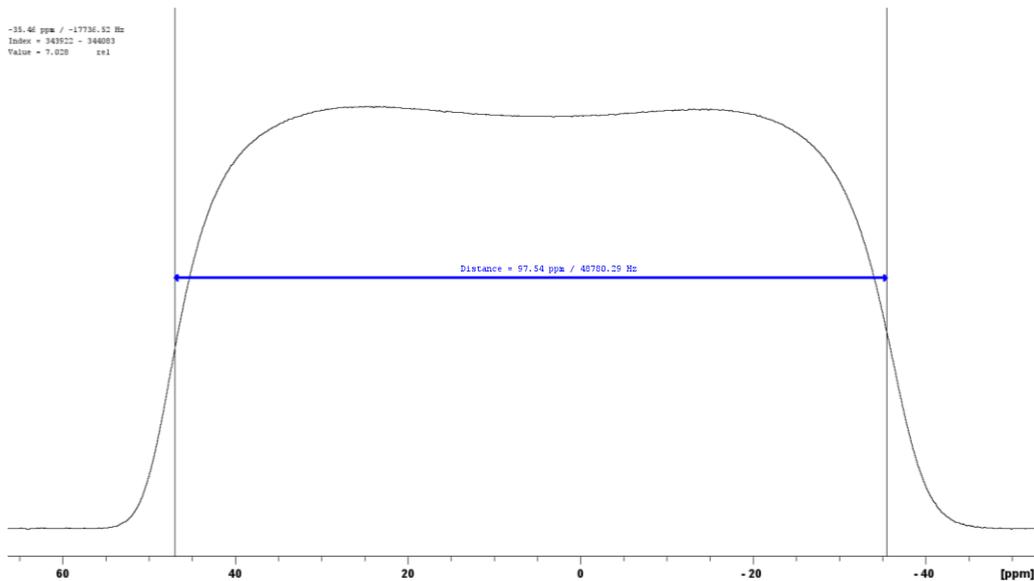
Figure 5. Example of the frequency dispersion (induced by the gpz1 gradient) measurement. Here the measured value is 48780 Hz when using gpz1 = 10%. Acquisition was performed on a 500 MHz spectrometer.

4.3 Calibration of chirp pulses

4.3.1 Creating the chirp pulse

The ultrafast COSY uses 180° chirp pulses. Such pulses can be created using the topspin *shapetool*. To do so, type the **stdisp** command. Then, create a new pulse using the ⬜ button and select *Adiabatic shape/Smoothed Chirp* (see screenshot on Figure 6).
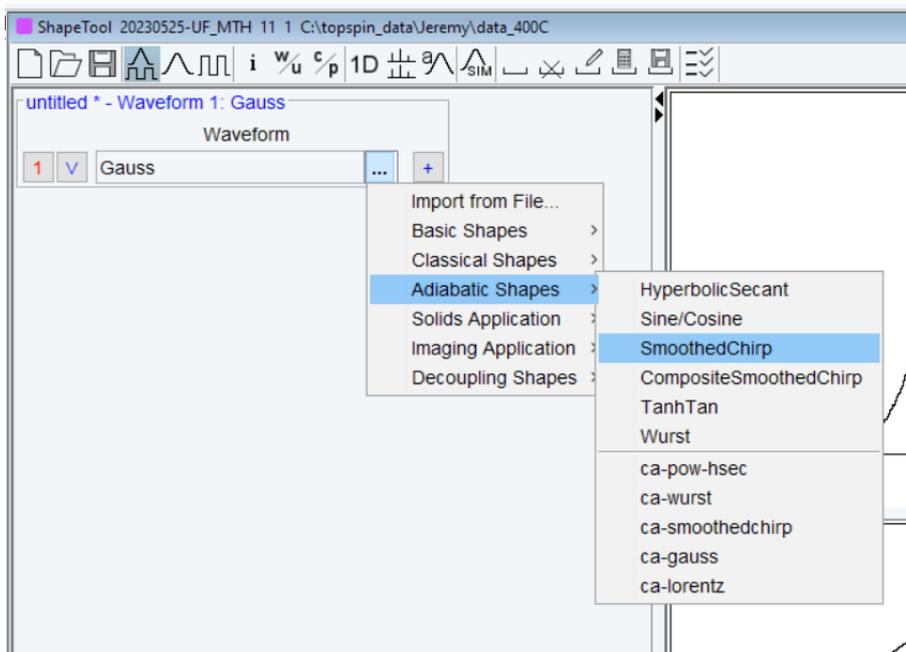
Figure 6. Creating a new "smoothed chirp" using the shapetool

Show all parameters thanks to the [V] button then enter the following parameters:

"Size": Sufficient number of points to obtain smooth phase variations and to ensure that the phase change between two points does not exceed 180° (1000 points for instance). To verify this, click on the [ʷ/u] option and inspect the (unwrapped) phase, which should be parabolic (see Figure 7)

**"Sweep width"**: This value must be equal to the frequency dispersion induced by the excitation gradients that you want to use for the ultrafast COSY experiment, in order to allow spatial encoding. To determine the aimed frequency dispersion, **the chosen value should be far larger than the frequency range than one wishes to encode**. Indeed, the sweep width should be efficient enough to encode all regions of the tube and all regions of interest from the spectrum, in order to ensure that all regions of the tube undergo the pulses and thus contribute to the signal. Empirically, **a chirp pulse using a frequency sweep width five times larger than the frequency range of interest** is advised*. However, avoid using a frequency sweep that is too wide, associated with a strong gradient, because this could lead to a loss of sensitivity due to diffusion effects**.

For instance, in order to encode a ^{1}H range from 0 to 7.5 ppm (corresponding to a 3000 Hz range on a 9.4T spectrometer), one should use a chirp pulse with a frequency sweep width equal to 5*3000 = 15 000 Hz. This corresponds to the attached chip pulse named "Chirp-15-15".

"Pulse length": This value should match the length of the pulse used for ultrafast COSY, 15 ms here

"% to be smoothed": Use 5% here

Untick the two options: "**Low to high field**" and "**Smoothing** (-π/2 → +π/2)"

Save the new chirp pulse in the wave/user folder. On Figure 7, a screenshot depicts an example of a chirp pulse created with the shapetool, corresponding to the "Chirp-15-15".

*: For more details, read "Frequency-swept pulses for ultrafast spatially encoded NMR"; Dumez; 2021; Journal of Magnetic Resonance

**: For more details, read "Sensitivity losses and line shape modifications due to molecular diffusion in continuous encoding ultrafast 2D NMR experiments"; Giraudeau, Akoka; 2008; Journal of Magnetic Resonance
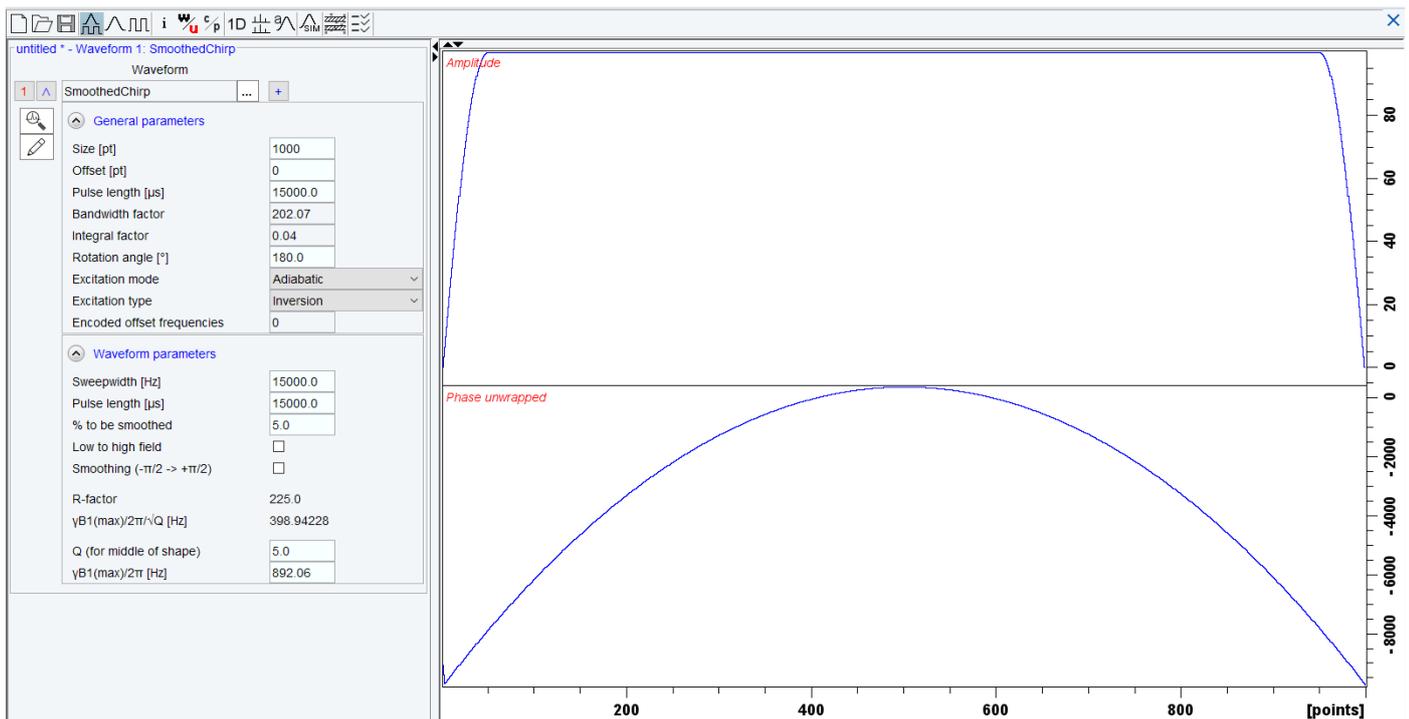
Figure 7. Example of chirp pulse created with the Shapetool

4.3.2 Optimization of the chirp pulse power

Copy the previous experiment and change the following parameters.

spnam1: Name of the chirp pulse previously created (for example "Chirp-15-15")

sp1: Power of the chirp pulse. This power will be optimized in order to obtain an inversion of the profile (see below). Avoid too high powers which could damage the probe.

P11: Same duration as the selected chirp pulse, 15 ms here

gpz0: Excitation gradient strength. This value should approximately match the frequency width swept by the chirp. Here, use the measurement performed earlier (see 4.2.2) and adapt the value.

In the example described above, if the measured frequency dispersion was 48780 Hz for 10% gradient strength and the desired dispersion is 15 000 Hz (to match the used chirp pulse), one must perform the following cross-multiplication: gpz0 [%] = (10*15 000) ÷ 48780 = 3.08%

Perform an acquisition and process the spectrum using the *gfp* command and repeat the experiment by changing sp1 until an inversion of the profile is observed. There is no need for an accurate value of sp1 as adiabatic inversion is efficient over a wide power range. However, residual oscillations may appear if sp1 is too small. The sweep width of the adiabatic pulse should be adjusted to excite the whole sample. The gpz0 can also be slightly adjusted for optimal results.

An example of an inversion profile can be seen on Figure 8.
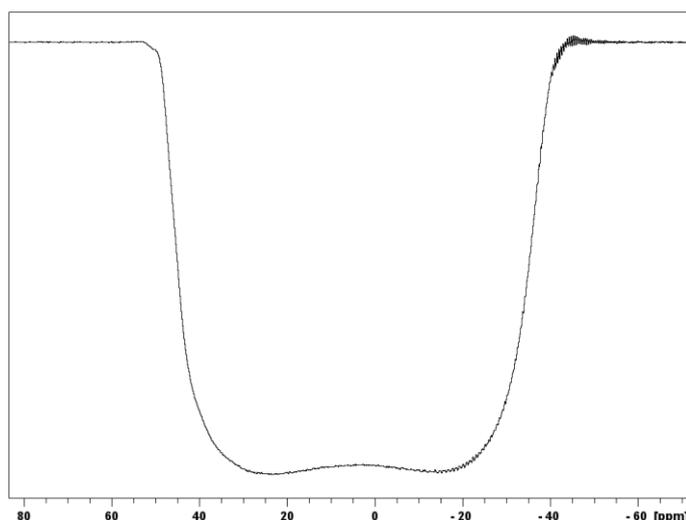
Figure 8. Example of an inversion profile. Acquisition was performed on a 500 MHz spectrometer.

5 Ultrafast COSY experiment on a simple sample

For the first ultrafast COSY experiment, it is advised to use a simple sample, such as a tube containing **10% ethanol in D$_2$O**. Perform preliminary settings on a 1D experiment before ultrafast acquisition: Tuning/matching, lock (D$_2$O), shims and optimization of the 90° ^{1}H hard pulse.

5.1 First 2D spectrum

Create a new experiment from a classical 1D experiment then, in the acquisition parameters, use "***iufcosy-pr***" as a pulse program. Switch to 2D parameters using the ⬓ button. Then, open a second ^{1}H channel in the edasp (*edasp* command, set F2: ^{1}H, then click on "default" and "save and close").

Set the acquisition parameters as follows:

	F$_2$	F$_1$
pulprog	*iufcosy-pr*	
PARMODE	2D	
AQmod	Qsim (consoles Avance I et II) or DQD (Avance III and ulterior consoles)	
FnMODE	QF	
TD	128 k (must be a power of 2)	1
NS	1	
DS	0 (this value is not taken into account with this sequence. Here, the dummy scans are set with the L4.) L4 = 2	
SW	No need to set; and do not pay attention to the indicated values, they do not make sense for ultrafast experiments. The explanation for the calibration of SW is detailed later in this is document	

ND_010	1 (not relevant for Avance III and ulterior consoles)	
AQ	automatically calculated from TD*DW	No need to set
FW	Should approximately match the frequency dispersion induced by acquisition gradients (80%). Thus, for a 48780 Hz dispersion with a 10% gradient (see 4.2.2), use 8*48780 = 390240 Hz	
RG	See below	
DW	0.55 us	
DE	6.5 us	
DIGMOD	analog (Avance I and II) digital (Avance III and ulterior) After setting DIGMOD, check the values of DW and FW, they may have been modified automatically!	
NBL	1	
L3	128	
O$_1$	Center of the aimed spectral range (for instance, 2.5 ppm for a 5*5 ppm spectrum)	
O$_2$	Water pre-saturation frequency (H_2O). Not used at this stage, but should be optimized for more complex samples	

- **Pulses**

pl1: hard pulse power on f1 channel

p1: duration of 90° hard pulse (previously calibrated)

pl9 : presaturation power. 1000 db (0 W) at first. Should be optimized for more complex samples

p11: shaped pulse duration, 15 ms here

p10 (duration of the spoiler gradient) = 10 ms

sp1: shaped pulse power. Value optimized above (see 4.3.2)

spnam1: name of the previously created chirp pulse (see 4.3.1)

p23 and **p26**: duration of coherence selection gradients for mixing, 1 ms.

p20 and **p21**: duration of coherence-selection gradients for spatial-encoding, soit 800 µs

- **Delays**

d1: recovery delay (not important if NS=1 with no interleaving). Typically, use values equal to or higher than 5 s (for instance d1 = 5 s) in order to limit the instrumental strain and leave a sufficient resting time for the gradient coil between two acquisitions

d6: delay allowing gradient switching. 20 us

p15: duration of the acquisition gradients. Automatically calculated by the pulse program, with p15+d6 = DW*TD2/(2*L3)

d24 and **d25**: duration of pre- and post-acquisition gradients. Here, d25 = p15, as defined by the pulse prog. For **D24**: 500 µs

- **Gradients**

GPNAM15 = **GPNAM16** = SMSQ10.32

GPNAM10 = SINE.100

GPNAM23 and **GPNAM** 26 = SINE.100

GPNAM20 and **GPNAM 21** = SINE.100

GPZ0: Strength for the positive excitation gradient. It should be set so that the frequency dispersion induced by the gradient is at least five times the chemical shift range. Value set previously (4.3.2)

GPZ1: Strength for the negative excitation gradient. GPZ1 = **- GPZ0**

GPZ15: Strength of the positive detection gradient (depends on the spectral width to be observed in the ultrafast dimension). Start with GPZ15 = 80%

GPZ16: Strength of the negative detection gradient (GPZ16 = **- GPZ15**). GPZ16 = **-** 80%

GPZ24 and **GPZ25**: Strength of pre- and post-mixing gradients: can be modified to adjust the signal position along the ultrafast dimension and to adjust "pseudo-aliasing" in the ultrafast dimension (see Giraudeau, JMR, 2010). Start with GPZ24 = 0% and GPZ25 = - 20 %, then change the values.

GPZ23 and **GPZ26**: Strength of coherence-selection gradients for mixing. GPZ26= **- GPZ23**. GPZ23 = 80%. Gpz26 = - 80%

GPZ20 and **GPZ21**: Strength of coherence-selection gradients for spatial-encoding. Here, GPZ20 = GPZ21 = 80%

GPZ10 = 80% (spoiler gradient to remove transversal magnetization residues)

The GPZ15 and GPZ16 gradients can be adjusted in order to observe the desired spectral width in the ultrafast dimension (SW_{UF}). SW_{UF} will increase when the strength of the gradients is increased. In theory, GPZ16 = - GPZ15. However, if there is a small offset in the gradient amplifier, this will result in data shearing. Even if this shearing effect can be corrected by the processing routine up to a certain degree, a correction of the shearing can be required during acquisition if it is too strong. To do so, acquisition gradients should be slightly adjusted. For instance, if GPZ15 = 80%, try GPZ16 = -79.99 % or -80,01%. Once the offset is known, it remains stable for a given gradient amplitude.

Before acquisition, use the *rga* in order to automatically set the necessary gain. **Caution**, when using this command for any ultrafast experiment, double check all parameters to make sure that they are correctly set, in

order to avoid any hardware damage, in particular check that D1 is sufficient and AQ below 100 ms (See **6.1 Important precautions when acquiring ultrafast spectra**).

Launch the acquisition using the *zg* command.

5.2 Processing

5.2.1 Generating the 2D spectrum

After acquisition, in order to generate the spectrum, the usual commands under topspin for 2D (xfb, xf2, xf1 etc.) will not work. Instead for ultrafast 2D data, a dedicated Python program called "**ufproc**" is used, allowing automatic processing.

Before executing it, set the following processing parameters (procpars):

- Apodization along F1: SINE function with SSB=0 (You can use other functions)

- Apodization along F2: With certain probes and hardware configurations, the gradients appear to be non-uniform over the sample length L. In ultrafast 2D NMR, it leads to asymmetric wiggles in the ultrafast dimension. This limitation is fully described in literature (P. Giraudeau et S. Akoka, Magn. Reson. Chem. 49 (2011), 307-313) and can be compensated by employing an apodization procedure in the spatially-encoded dimension. To do so, use the following additional parameters in F2:

   -WDW = GM

   -LB = -170 Hz (test several values to see the effect on lineshape – you need to re-run ufproc each time). When this value is too high (in absolute value), very wide peaks will appear. When this parameter is too low (in absolute value), asymmetric distortions of the peaks will be observed. Here the goal is to eliminate these distortions without broadening the peaks too much.

   -GB = 0.5

The other parameters will be set by the automation (including automatic *zero-filling*). Type the *ufproc* command to launch the processing and accept suggestions made by the program (regarding SI value). Processing takes a few tens of seconds and is divided in multiple steps. Please be patient and don't do anything until the "ultrafast processing done" message appears on the screen.

The first ultrafast spectrum can have an unexpected shape (spectral aliasing, non-optimal spectral window etc.). Then, it is advised to perform optimizations thanks to the modifications of multiple parameters, as described in part **6**.

5.2.2 Calibration of the chemical shifts

When the ultrafast spectrum is generated, chemical shifts are not calibrated and wrong values appear (see Figure 9). To correct this, it is then possible to use another Python program, allowing a manual calibration of the chemical shifts, by typing *ufproc*. Follow the instructions given by the dialog box. Click on "*close*", click on a signal for which the chemical shifts in F1 and F2 are known and enter the appropriate values in the dialog box. Repeat these steps for a second signal. This will result in a correction of chemical shifts in both dimensions. To facilitate this procedure, it is advised to use signals from the diagonal and refer to the corresponding chemical shift values from a 1D ^{1}H spectrum. Moreover, it is advised to perform this step **only after** making sure that the spectral window is correctly set (See part **6**), in order to avoid wrong interpretation.
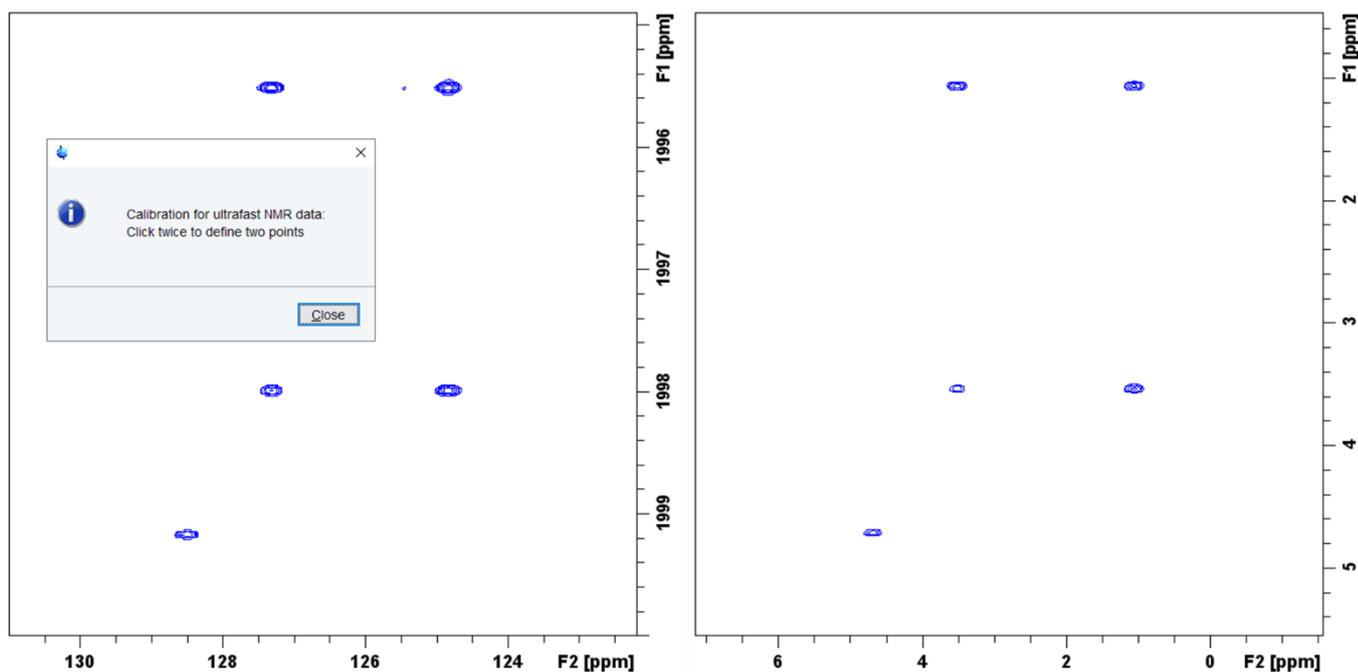
Figure 9. Ultrafast COSY spectrum (with interleavings) from an ethanol sample before (left) and after (right) calibration of the chemical shifts. Here acquisition was performed on a 400 Mhz spectrometer.

5.2.3 Symmetrizing ultrafast spectra

If needed, ultrafast spectra can be symmetrized, *e.g.* for facilitating interpretation. To do so, use the dedicated Python program by typing the ***ufsym*** command and follow instructions from the dialog box: use the cursor (by maintaining left-click) in order to draw the diagonal of the spectrum then release. Then click on "*Do symmetrization*". If needed, the diagonal can be corrected by clicking on the "*redraw diagonal*" button.

5.2.4 Other options available from the ufproc program

The ufproc program features multiple options, which are detailed when typing the ***ufproc help*** command. Among these, some are particularly useful for routine or while performing optimizations:

- ***ufproc inter***: Option to specify that the data were acquired in interleaved mode (See below). On certain configurations, this can save time by avoiding the apparition of a dialog box where the user must indicate whether or not interleaved acquisition was performed (by clicking on the appropriate button)
- ***ufproc shon***: Command which allows a slight correction of the shearing effects, thus improving the spectra when such effects are present
- ***ufproc abson***: Applies baseline correction according to the parameters detailed in PROCPARS
- ***ufproc +***: Allows the processing of the data from the sole positive gradients. This can be useful when edge effects are present, where intense peaks on the edge of the spectrum (after ***ufproc***) could lead to an apparent duplication of the signals along the ultrafast dimension (F2)
- ***ufproc rot***: Applies F1/F2 rotation to the spectrum

6 Optimizations of the spectrum

In order to obtain exploitable 2D ultrafast data, multiple optimizations can be performed, as described hereafter. For these, it is advised to start with a simple sample (10 % ethanol in D_2O for instance) so as to easily identify the signals and the effects of the optimizations, before moving on to more complex samples. Once optimized on a specific configuration (spectrometer, console, probe), the acquisition parameters can be used as such on a new sample with no further modifications, with a few exceptions detailed in a dedicated section (6.8).

6.1 Important precautions when acquiring ultrafast spectra

To avoid damaging the probe and the gradient coil, it is **vital**, whatever the optimizations performed, to stick to the following recommendations. We remind you that the CEISAM as well as the authors of this protocol accept no liability for any hardware damage caused by the use of ultrafast experiments.

- **AQ** duration **should not be higher than 100 ms**, in order to limit the constraint on the gradient coil.
- When performing multiple ultrafast 2D experiment or when using interleaving, dummy scans or multiple scans, the inter-scan delay **D1 must be set to a value equal or bigger than 5 seconds**, to ensure sufficient rest for the probe between two scans.
- **Total duration** of ultrafast experiments (multiple scans **or** multiple experiments in a row) **should not exceed 20-25 min**. After such duration, the hardware should be left to rest for around twenty minutes before the next ultrafast experiment. In practice, it is advised to alternate between ultrafast and more conventional experiments (*e.g.* a ^{1}H 1D acquisition of a similar duration)

6.2 Solvent suppression (pre-saturation)

The *iufcosy-pr* pulse program uses a solvent pre-saturation block. When required, for instance in the context of a metabolomic sample containing a water peak, solvent suppression can be carried out. When performing optimizations on a simple sample (10 % ethanol in D_2O), it is advised not to use it (set pl9 = 0W).

To use solvent suppression, the pre-saturation frequency can be set using the **O2** parameter (in Hz) and the power can be set using **pl9** (plw9 or pldb9).

Before an ultrafast experiment, these parameters can be optimized on a preliminary *zgpr* experiment, using a similar D1 (5 seconds, by default), DS =2 and NS =2 (minimum). Note that with this pulse sequence, pre-saturation is performed on the carrier frequency *i.e.* O1.

6.3 Interleavings

The *iufcosy-pr* pulse program allows performing interleaved acquisitions. The number of interleaved scans can then be set thanks to the **TD F1** parameter, which must be a 2^n value

Interleaving consist in an incrementation of the pre-acquisition delay, following: **(2 x Ta) / n_i**, with Ta : Acquisition time, ni: number of increments.

In the *iufcosy-pr* pulse program, the increment is **in2 = [2 x (p$_{15}$ + d6)]/TD$_{F1}$**, with TD F1 corresponding to the number of increment (= n_i) and (p15 + d6) = Ta

For the first experiment with interleaving, repeat the previous ultrafast acquisition but using TD F1 = 2 and D1 = 5s. Since two scans are now acquired, the experiment time is now doubled. After acquisition, the spectrum can be processed with *ufproc*. When processing interleaved acquisition data, on certain configurations (hardware and software), a dialog box might appear and ask to confirm whether the data includes interleaved acquisition. Then, clik on "*interleaved*" and the processing program will run normally. In this case, in order to prevent this dialog box from popping up, it is possible to directly write the *ufproc inter* command (see details in section 5.2.4)

On the new spectrum, the spectral width along F1 – called conventional dimension – is now doubled (see Figure 10, middle).

Interleaving can also be used for doubling spectral width along the F2 dimension F2 – called ultrafast dimension. To do so, while doubling TD F1, two options are available: either multiply by two the number of points from the FID (TD F2 = 256k) or divide by two the number of acquisition loops (L3 = 64). **The latter is strongly advised**, since the former will double acquisition duration, thus adding constraint to the gradient amplifier, with a risk of exceeding the recommended acquisition time (100 ms).

Hence, repeat the previous experiment with TD F1 = 4 et L3 = 64. The new spectral width in the ultrafast dimension should be twice larger than previously observed, as depicted on Figure 10 (right).
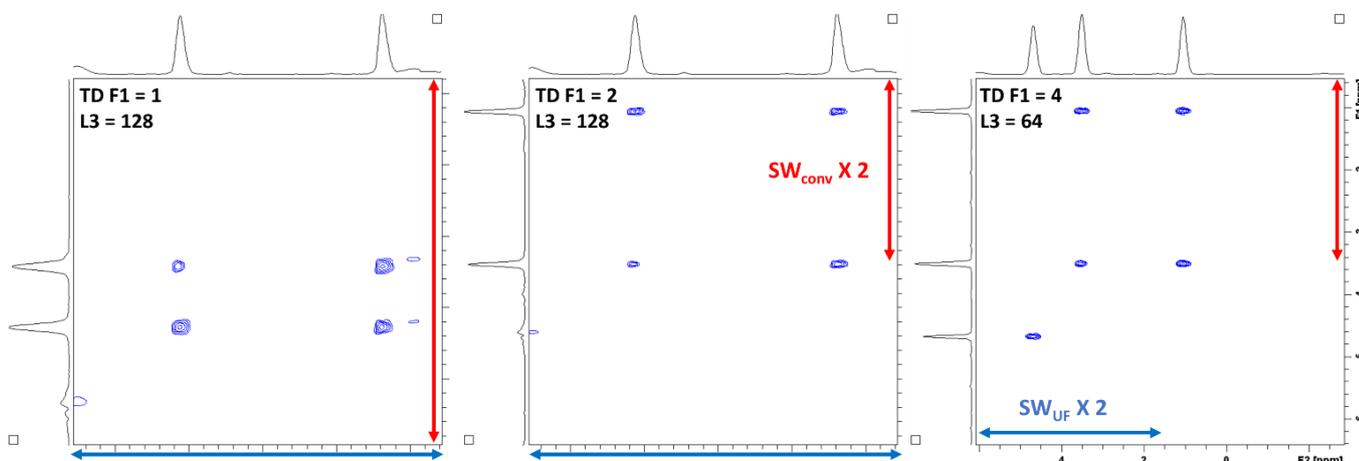

Figure 10. Effect of interleaved acquisitions on ultrafast COSY spectra from a 10% ethanol in D₂O sample, acquired on a 600MHz spectrometer. Left: acquisition without interleaving (TD F1 = 1; L3 = 128), the spectrum is aliased in the conventional dimension (F1). Middle: acquisition with 2 interleaved scans (TD F1 = 2; L3 = 128), one can observe the doubling of the spectral width in the conventional dimension (SW_{conv}). Right: Acquisition with 4 interleaved scans and half the number of acquisition loops (TD F1 = 4; L3 = 64), one can observe the doubling of the spectral width in the ultrafast dimension (SW_{UF}). On the right spectrum, the chemical shifts could be calibrated (using **ufcal***)*

6.4 Centering of the spectral window

A crucial parameter to be optimized when acquiring ultrafast 2D NMR data is the carrier frequency **O1**. As for any COSY spectrum, a change of the O1 frequency will shift the entire spectrum in both dimension (F1 and F2), *i.e.* along the diagonal. For ultrafast 2D NMR, this will be particularly helpful in order to move the entire spectral window and prevent edge effects: signals too close to the edge of the spectrum can be distorted (see Figure 11) and in certain cases, the processing program can struggle to align signals from negative and positive gradients, resulting in an apparent duplication of the spectrum.

Hence, the carrier frequency (O1 or o1p) should be carefully adapted in order to center all signals of interest.
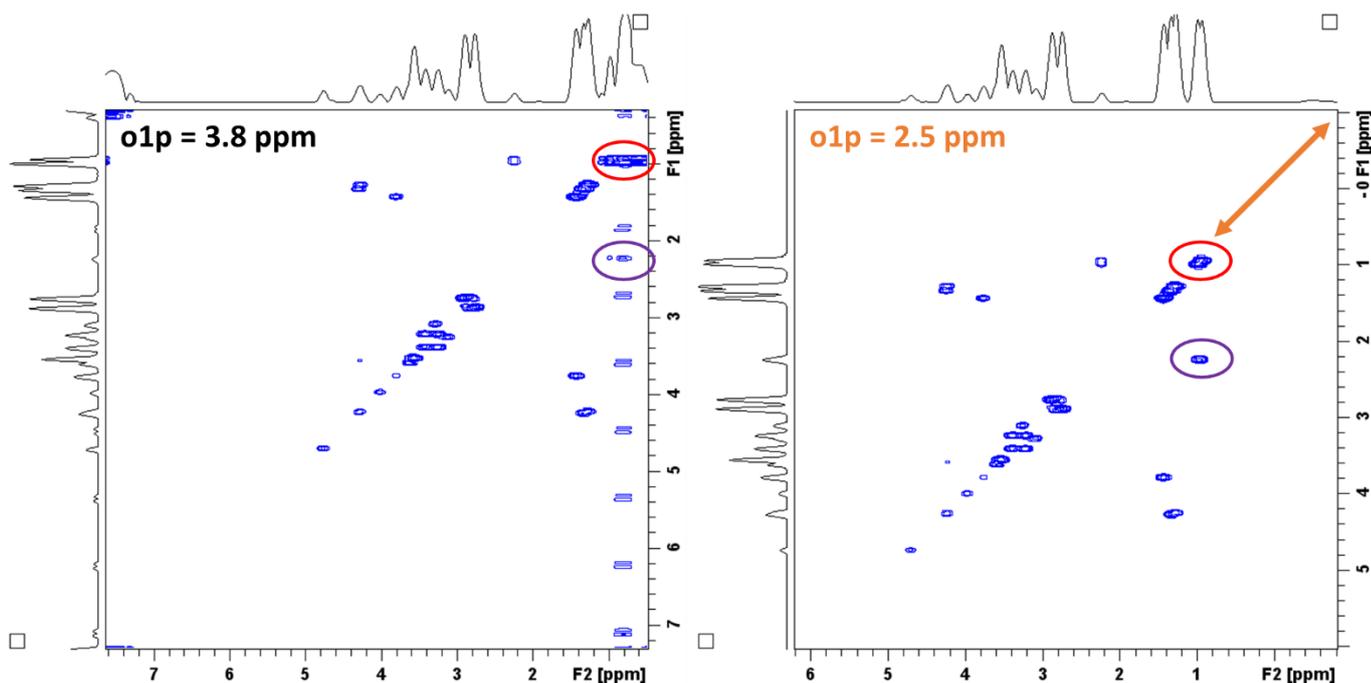
Figure 11. Results of the change of the carrier frequency (O1 or o1p) on the edge effects of a UF COSY spectrum acquired from a metabolite mixture (700 MHz). On the left (o1p = 3.8 ppm), the signals at 0.9 ppm (red and purple circles) are too close to the limit of the spectral window and are distorted due to edge effects. On the right (o1p =2.5 ppm), the centering of the spectrum allows observing all signal of interest, with no edge effect.

With the *iufcosy-pr* pulse program, a pre-phasing gradient (gpz25) can also be used in order to start refocusing the signals before the train of acquisition gradients. Effectively, a slight change of the **gpz25** parameter will then shift the whole spectrum along the ultrafast dimension only, *i.e.* along F2. The result can be observed on Figure 12.

With an appropriate optimization of **O1** and **gpz25**, it is then possible to adequately shift the spectral window, so as to observe all signals of interest while preventing edge effects.

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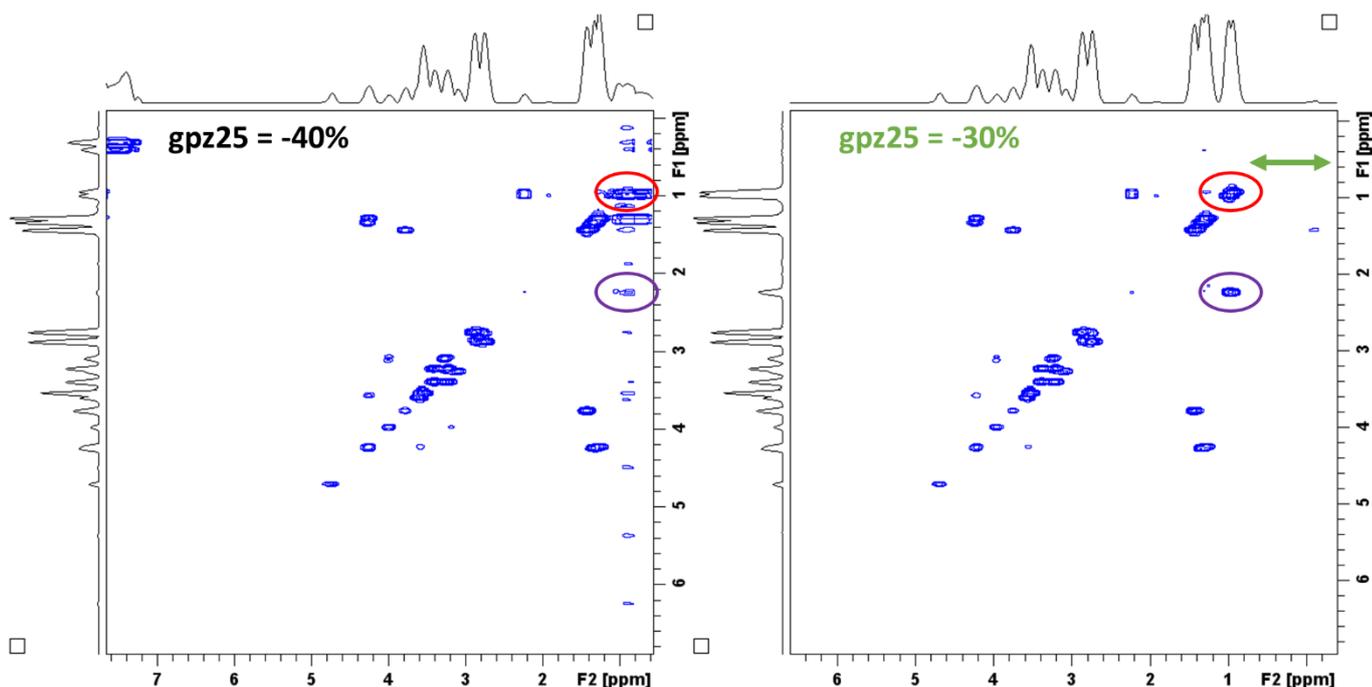
Figure 12. Results of the change of the pre-phasing gradient (gpz25) on the edge effects of a UF COSY spectrum acquired from a metabolite mixture (700 MHz). On the left (gpz25 = -40%), the signals at 0.9 ppm (red and purple circles) are too close to the limit of the spectral window and are distorted due to edge effects. On the right (gpz25 = -30%), the shifting of the spectrum allows observing all signals of interest, with no edge effect.

6.5 Optimization of the spectral window

Ultrafast 2D NMR is characterized by a set of compromises, in particular regarding the spectral width in or the other dimension. This section describes ways to optimize the parameters of this pulse sequence to attain a consistent spectral window, allowing the simultaneous observation of a large number of signals, while minimizing the constraint on the instrument.

6.5.1 Spectral width in the conventional dimension: SW$_{conv}$

As a way to increase the spectral width in the F1 dimension, called the conventional dimension (SW$_{conv}$), the number of interleaved acquisitions can be adapted. This can be done by changing **TD F1**, and must be **2^n** value, as stated in 6.3.

With interleaved acquisitions, SW$_{conv}$ is proportional to n_i / (2 x Ta). Therefore, if the number of interleavings n_i (*i.e.* TD F1) is doubled, then SW$_{conv}$ is doubled as well. Accordingly, since twice as many acquisitions are performed, the global experiment time is also doubled. Hence, it is essential to check that the total acquisition time does not exceed the **20-25 min** limit.

6.5.2 Spectral width in the ultrafast dimension: SW$_{UF}$

In order to increase the spectral width in the F2 dimension, called ultrafast dimension (SW$_{UF}$), one must consider that with ultrafast NMR, SW$_{UF}$ is proportional to Ga x Ta, with Ga: Strength of the acquisition gradients and Ta: duration of the acquisition gradients. To limit the instrumental strain caused by the gradients (*i.e.* limiting Ga), **Ta** can be modified instead.

Furthermore, with the ***iufcosy-pr*** pulse program, the duration **Ta** corresponds to the p15 value and **AQ = 2 x L3 x (p15+d6)** = **TD$_{UF}$ x DW$_{UF}$**, with L3: number of acquisition loops and d6: duration allowing gradient switching.

Consequently, as a way to increase SW$_{UF}$, it is then possible to change TD$_{UF}$, *i.e.* TD F2, but this requires to double this value at least, since it must be a 2^n value. **Instead, it is recommended to slightly increase the DW** value (i.e.

DW_{UF}). **Caution**, when doing so, **double check the effect on** the **AQ** value, which **should not exceed 100 ms**, to avoid damaging the instrument.

Additionally, since SW_{conv} is proportional to n_i / (2 x Ta), the increase of the Ta value (cause by an increase of DW) will reduce the spectral width in the conventional dimension (while increasing SW_{UF}). Therefore, an adequate compromise should be found.

6.6 Improving resolution in Conv dimension

If the resolution is found to be insufficient in the conventional dimension, this can be improved by simultaneously doubling L3 and TD F2. However, doing so will double AQ as well, so double check that **AQ does not exceed 100 ms**.

6.7 Optimization of the apodization

In addition to the acquisition parameters, processing parameters can also be adjusted for optimal results, in particular regarding apodization in the ultrafast dimension (F2).

In the processing parameters (PROCPARS), try multiple values for **LB**, aiming for thinner peaks in the UF dimension without a significant degradation of the signal-to-noise ratio (S/N). Each time, perform peak processing with *ufproc* and extract the rows that contain the signals of interest using the *rsr xxx* command (with xxx: index of the row to be extracted) and measure the S/B with the dedicated topspin interface (*.sino* command). Inspect the change in S/N when modifying LB, by reducing the latter (in absolute value).

Typically, a compromise will have to be found between peak shape, spectral resolution and S/N. This compromise will depend on the complexity and the concentration of the sample of interest: if the sample features low concentrated compounds, then S/B might be favored over resolution.

The shape of the peaks will mainly depend on the gradient profile, which is hardware specific (gradient coil etc.). Therefore, once the apodization has been optimized for an ultrafast experiment, the processing parameters can be applied as such on any analyzed sample.

6.8 Parameters to be optimized on a new sample

After a full optimization of the acquisition and processing parameters on a given configuration (spectrometer, console, probe), they can be saved and re-used for future experiments. For a new sample, it is not necessary to perform a complete optimization once again and only the following parameters will need to be adjusted:

- **P1 / pl1** (or pldb1) couple
- Presaturation parameters: **O2** frequency associated with the **pl9** (or pldb9) power
- Carrier frequency **O1**: may need to be adapted in order to move the spectral window along the diagonal of the spectrum
- Number of scans **NS**: When required, it is possible to accumulate signal up to a certain extent, using multiple scans (NS = 1*n) while ensuring that the **total experiment time does not exceed 20-25 min**
- Number of Dummy scans: In order to include **dummy scans** to the experiment, change the **L4** parameter, which replace the DS parameters for the *iufcosy-pr* sequence (DS **does not work** with this pulse program)
- Gain (**RG**): to be adapted according to the concentration of the new sample (using *rga*, but only after double-checking acquisition parameters are valid)