Implementing ultrafast 2D NMR experiments on a Bruker Avance Spectrometer

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Important remarks

- Ultrafast 2D NMR has been patented by the Weizmann Institute of Science. Its use for commercial purposes requires a licence from the Weizmann Institute.
- 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 following protocol has been published in *Annual Reports* (in press).

Introduction

This document describes the implementation of four major ultrafast 2D NMR experiments: COSY, TOCSY, DQS and HSQC, 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:

General principles of ultrafast 2D NMR:

- 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.
- B. Gouilleux, L. Rouger, P. Giraudeau, *Ultrafast Multi-dimensional NMR: Principles and Recent Applications*, in: *EMagRes*, John Wiley & Sons, Ltd, 2007.
- B. Gouilleux, L. Rouger, P. Giraudeau, *Ultrafast 2D NMR : Methods and applications*, Annual Reports (in press).

…and to go further :

- B. Shapira, A. Lupulescu, Y. Shrot, L. Frydman, *Line shape considerations in ultrafast NMR*, J. Magn. Reson 166 (2004) 152-163.
- P. Giraudeau, S. Akoka, *Sources of sensitivity losses in ultrafast 2D NMR*, J. Magn. Reson. 192 (2008) 151-158.
- P. Giraudeau, S. Akoka, *Sensitivity losses and line shape modifications due to molecular diffusion in continuous encoding ultrafast 2D NMR experiments*, J. Magn. Reson. 195 (2008) 9-16.
- P. Giraudeau, S. Akoka, *A new gradient-controlled method for improving the spectral width of ultrafast 2D NMR exepriments*, J. Magn. Reson. 205 (2010) 171-176.
- P. Giraudeau, S. Akoka, *Sensitivity and lineshape improvement in ultrafast 2D NMR by optimized apodization in the spatially-encoded dimension*, Magn. Reson. Chem. 49 (2011) 307-313.
- M. Pathan, B. Charrier, I. Tea, S. Akoka, P. Giraudeau, *New practical tools for the implementation and use of ultrafast 2D NMR experiments*, Magn. Reson. Chem. 51 (2013) 168-175.
- L. Rouger, B. Charrier, M. Pathan, S. Akoka, P. Giraudeau, *Processing strategies to obtain clean interleaved ultrafast 2D NMR spectra*, J. Magn. Reson. 238 (2014) 87-93.

- B. Gouilleux, L. Rouger, B. Charrier, I. Kuprov, S. Akoka, J.-N. Dumez, P. Giraudeau, *Understanding J-Modulation during Spatial Encoding for Sensitivity-Optimized Ultrafast NMR Spectroscopy*, ChemPhysChem. 16 (2015) 3093–3100.
- L. Rouger, B. Gouilleux, M. Pourchet-Gellez, J.-N. Dumez, P. Giraudeau, *Ultrafast double-quantum NMR spectroscopy with optimized sensitivity for the analysis of mixtures*, Analyst. 141 (2016) 1686–1692.

Please also note that all experiments described below are based on the continuous phase-encoding excitation scheme proposed by P. Pelupessy (J. Am. Chem. Soc. 2003), which was found the best compromise between sensitivity and resolution limitations.

The latest version of this protocol includes an automated parametrization of UF experiments from conventional 2D NMR acquisition parameters. This automation not only handles the acquisition of single-scan experiments, but also calculates interleaving and processing parameters (including spatial apodization). UF data processing has also been updated to include an automatic calibration of the spectrum.

1 Description of the attached files

The following files are attached to this document: please copy them to the indicated folder:

Pulse programs:

echograd: gradient and chirp pulse calibration
to be placed in \Bruker\TOPSPIN\exp\stan\nmr\lists\pp\user

ufcosy: acquisition of UF-COSY spectra
to be placed in \Bruker\TOPSPIN\exp\stan\nmr\lists\pp\user

uftocsy: acquisition of UF-TOCSY spectra
to be placed in \Bruker\TOPSPIN\exp\stan\nmr\lists\pp\user

ufdqs: acquisition of UF-DQS spectra
to be placed in \Bruker\TOPSPIN\exp\stan\nmr\lists\pp\user

ufhsqc: acquisition of UF-HSQC spectra
to be placed in \Bruker\TOPSPIN\exp\stan\nmr\lists\pp\user

Pulse shape:

Chirp-15-11: example of Chirp pulse for ^{1}H
to be placed in \Bruker\TOPSPIN\exp\stan\nmr\lists\wave\user

AU program and associated text files:

ufset: AU program for automatic settings
to be placed in \Bruker\TOPSPIN\exp\stan\nmr\au\src\user

UFfeatures1H.txt: text file of the spectrometer characterization for ^{1}H UF experiments
to be placed in \Bruker\TOPSPIN\exp\stan\nmr\au\src\user

UFfeatures13C.txt: text file of the spectrometer characterization for ^{13}C UF experiments

to be placed in \Bruker\TOPSPIN\exp\stan\nmr\au\src\user

Python programs (Topspin 2.0 and later versions):

ufproc: processing for UF spectra
\Bruker\TOPSPIN\exp\stan\nmr\py\user

ufcalauto: automatic calibration of UF spectra
\Bruker\TOPSPIN\exp\stan\nmr\py\user

ufcal: manual calibration of UF spectra
\Bruker\TOPSPIN\exp\stan\nmr\py\user

ufsym: symmetrization of UF spectra
\Bruker\TOPSPIN\exp\stan\nmr\py\user

2 General recommendations

- Do not start anything before reading the literature suggested above! Otherwise you may lose a lot of time…
- 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 50% ethanol, 50% D_2O.
- For studying more complex samples, a recommended solvent is DMSO-d_6, as its high viscosity limits sensitivity losses due to molecular diffusion effects.
- Avoid high temperatures (it increases diffusion losses).
- A probe equipped with *z*-gradients (at least) is necessary.
- No sample rotation.
- Gradient amplifier on. If the red « error » light is on, reset the gradient amplifier before starting.
- Run a preliminary 1D ^{1}H experiment, tune and match, lock and shim, and calibrate the pw90.

3 Preliminary calibrations

Preliminary calibrations, designed to characterize hardware performances, are thereafter used to automatically parametrize UF experiments.

3.1 Dispersion induced by the gradients

Preliminary calibrations must be done on a sample with a limited number of resonances, for example ethanol (10%) in D2O. Run a preliminary 1D ^{1}H experiment, tune and match, lock and shim, and calibrate the 90° pulse angle (pw90).

The echograd pulse sequence (Figure 1a) is used for the calibration of the gradients and chirp pulse. The chirp pulse is first inactivated to calibrate the gradients. It leads to a spin echo which, after FT and phasing, shows a profile of the sample. In a second experiment, the selective pulse is activated for calibration.

Create a new experience by copying the previous one (1D ^{1}H spectrum), set the pulse program (echograd), and run the automatic parametrization macro: **ufset**. Adjust the receptor gain, and run the acquisition. The FID should look like Figure 1b. Delay d5 should be adjusted to shift the echo approximatively in the center of the acquisition window. The processing parameters have also been automatically parametrized by **ufset**. The apodization window used is Gaussian, and the center of the latter (parameter GB in Topspin) must be set according to the position of the echo, i.e., 0.5 if the echo perfectly centered in the acquisition window (default value). Process the FID, by Topspin commands **GM**, **FT** and phase the spectrum starting by first order phase correction. Due to the phase dispersion induced by the gradients, a huge first order phase correction is necessary. A symmetric image can then be obtained via a small zero order phase correction. The resulting profile should look like Figure 1d. Measure the width at the base of the dispersion profile (ΔF, in Hz – as indicated in Figure 1d); it corresponds to the frequency dispersion induced by a 10% gradient. The corresponding gradient amplitude value (in T/m) can be calculated as $\frac{2\pi \cdot \Delta F}{\gamma \cdot L}$, where γ is the gyromagnetic ratio for ^{1}H nucleus and L is the length of the sensitive volume. Call your customer service to know the exact value of L for your probe (generally around 1.8 cm). However, knowing the exact value of L is not necessary to perform the UF experiments.

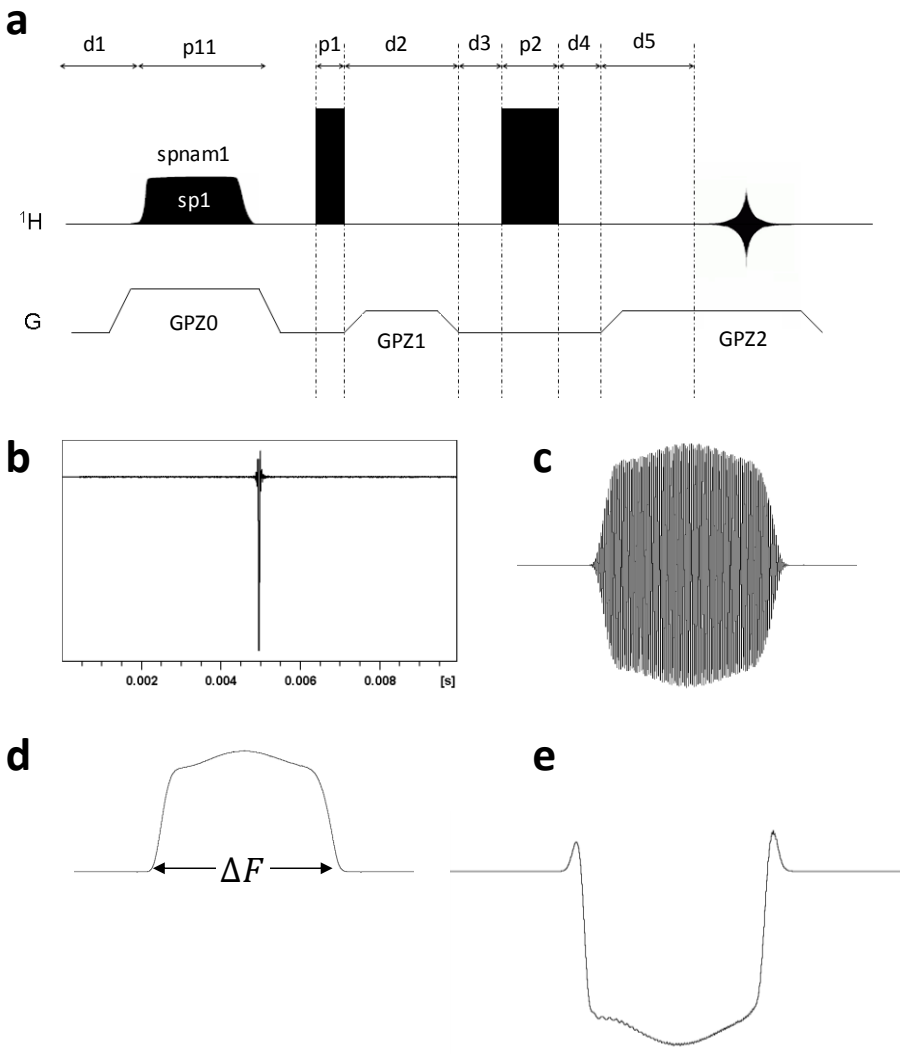


Figure 1. (a) echograd pulse sequence; (b) FID obtained, centered in the acquisition window; dispersion profile obtained after FT, (c) before and (d) after phase correction; (e) dispersion profile inversed by the calibrated chirp pulse.

3.2 Calibration of the encoding pulses

Create a new experience by copying the previous one, in order to calibrate the chirp pulses used for spatial encoding. The pulse shape used for this calibration is UF-chirp-15-11 (already set by **ufset**). The GPZ0 gradient must induce a frequency dispersion equivalent to the chirp pulse bandwidth (11 kHz in this case). This value can be calculated thanks to the last experiment. Adjust the power of the chirp pulse (sp1) to obtain an effective inversion (as Figure 1e). Signal must be processed with the **GFP** command. 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.

3.3 Description of the parameters

The parameters described below are already set by the **ufset** program, excepting GPZ0.

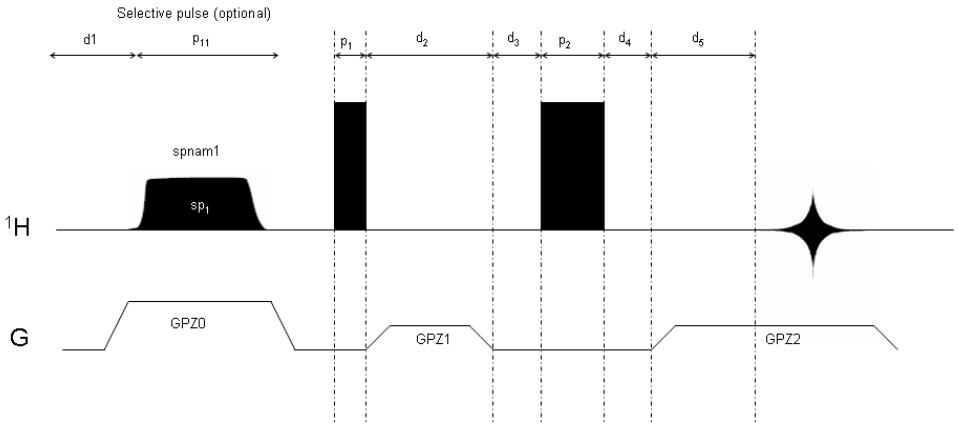


Figure 2. echograd pulse sequence

Acquisition parameters:

pulprog: echograd	**AQMOD:** qsim (Avance I) or DQD (Avance III)
NS: 1	**DS:** 0
SW: 250 ppm	**AQ:** 10 ms
RG: as determined on a classical zg experiment	**O1:** middle of the chemical shift range
pl1: hard pulse power	**p1:** 90° ^{1}H pulse (carefully calibrated)
sp1: encoding pulse power (120 dB for the gradients calibration, then calibrated)	**p11:** duration of the encoding pulse (15 ms here) **spnam1:** encoding pulse name
p2: 180° ^{1}H pulse (set by pulse program to 2*p1)	**d1:** 1-5 T_1
d2: 10 ms	**d3:** 1 ms
d4: 1 ms	**d5:** 5 ms
GPZ0: gradient amplitude active during encoding pulse (0% during gradient calibration, then set as indicated in 3.2)	**GPZ1 = GPZ2:** 10%

Processing parameters:

WDW: GM	**LB:** -20 Hz
GB: 0.5 (centred on the position of the echo)	

3.4 Spectrometer features file

Open the file \Bruker\TOPSPIN\exp\stan\nmr\au\src\user\UFfeatures1H.txt in a text editor. This file is used by the **ufset** program to automatically parametrize UF experiments. Indeed, many parameters are hardware-dependent. The first line will be modified thereafter. On the second line, indicate the frequency dispersion induced by a 100% gradient (in Hz): ten times the value measured above. This value is used in the **ufset** program to calculate all gradient amplitudes in the pulse sequence according to the desired spectral widths. On the third line, indicate the calibrated power of the chirp pulse (in dB). Save and close the file.

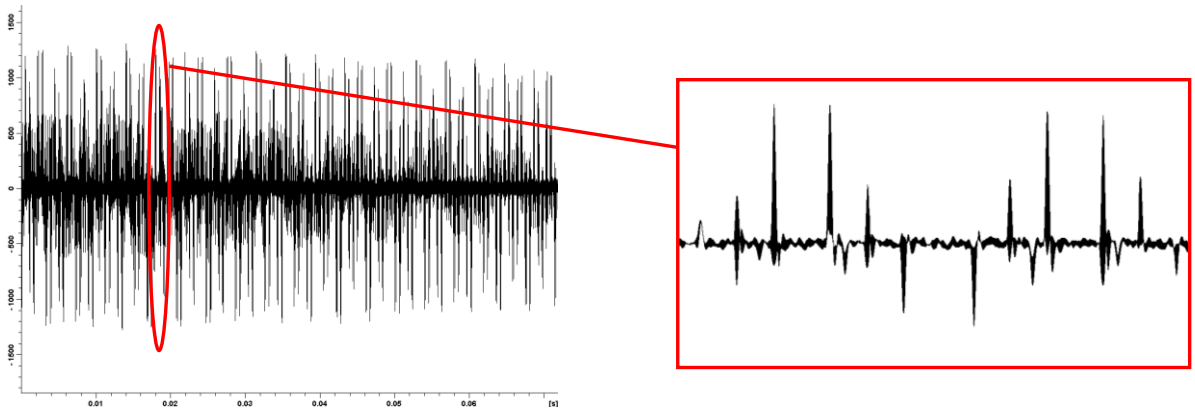
4 Implementation of UF-COSY experiments

4.1 Implementation via automated parametrization

As for the preliminary calibrations, a simple sample such as ethanol in D_2O may be used for this implementation.

Create a conventional COSY experiment, by the use of a parameter file (**rpar**), and adjust the following parameters to the sample: offset, spectral widths (note the spectral width in the indirect dimension as SW_{th}), pulse durations and powers. One scan and two dummy scans are recommended for the first experiment, but these values can be adjusted as needed. Then set the pulse program to ufcosy. Run the automatic settings program **ufset**. The latter calculates all the necessary experimental parameters to run this experiment in an ultrafast manner, and directly set them in the Topspin experiment. The execution of the program ends with a message displaying the effective spectral width in the conventional dimension, an eventual modification of the recovery delay if the latter was too short for ultrafast experiments, or warnings concerning the experiments duration. Ultrafast settings are now applied to this experiment. Note that the command "**ufset r**" allows to retrieve the initial conventional parameters.

Adjust the receptor gain, and run the acquisition. You should hear some noise due to the fast-alternating acquisition gradients. The resulting FID should look like this (here for a 100 mM ethanol sample in DMSO-d_6/D_2O 4:1):



Processing parameters have also been calculated and modified by the **ufset** program. Process the data with the **ufproc** command. The latter lasts a dozen of seconds while nothing must be done before the apparition of the "ultrafast processing done" message. A few options (arguments) are associated with this processing program:

- "**ufproc shon**" performs the same processing but adds a correction of shearing effects
- "**ufproc +**" performs the whole processing without adding the positive and negative acquisition datasets.

- "**ufproc help**" opens a window with a list of all possible options.

The spectrum thus obtained is automatically calibrated, thanks to the features indicated in the file UFfeatures1H.txt. Although, as the first parameter of this file has not been adapted to the spectrometer, the automatic calibration is still inaccurate. Calibrate the spectrum with the manual calibration program (**ufcal**), then measure the effective spectral width in the ultrafast dimension (SW_{obs}). Change the first line of the UFfeatures1H.txt file to $2 \cdot SW_{th}/SW_{obs}$. The automatic calibration will be more accurate for the next experiments.
On any other sample, a new ultrafast experiment can be created, acquired and processed following the same procedure as above, after calibration of the 90° pulse angle in a 1D ^{1}H experiment.

4.2 Description of the parameters

The parameters described below are already set by the **ufset** program.

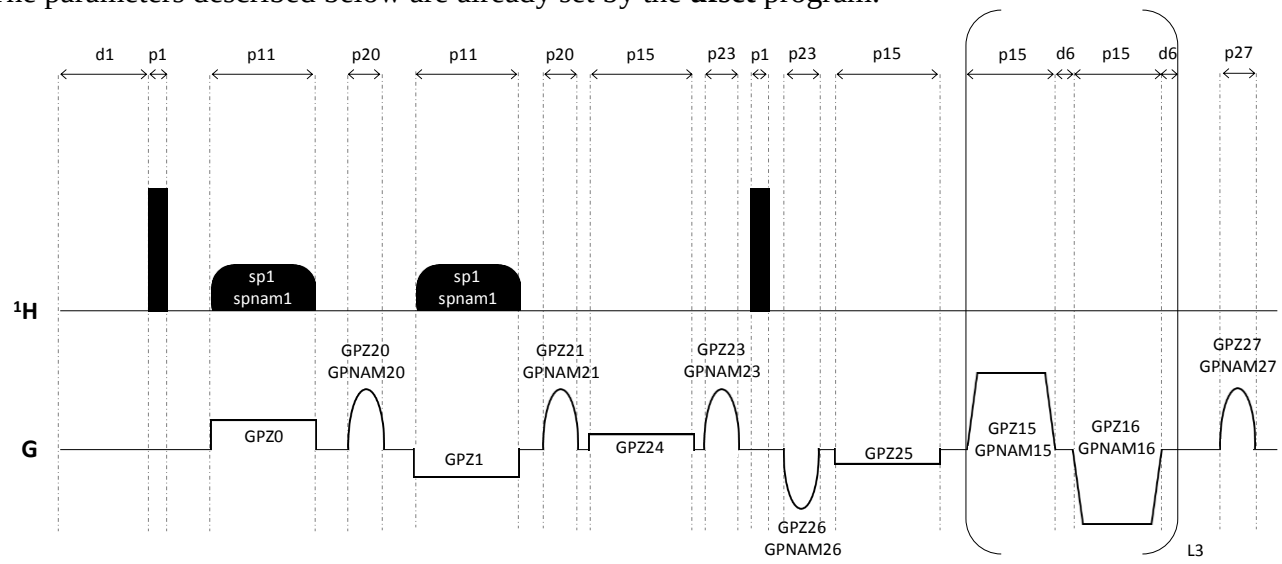


Figure 3. ufcosy pulse sequence

Acquisition parameters for a single-scan experiment:

pulprog: ufcosy

PARMODE: 2D

NS: 1

TD (F2): 128k (points acquired during the train of gradients, must be of the form 2^n)

TD (F1): 1 (number of interleaved scans)

SW (F2/F1): no importance

DIGMOD: analog or digital (for Avance I or III)

L3: 128 (number of acquisition gradients pairs)

L4: 2 (number of dummy scans)

pl1: hard pulse power

p11: duration of the encoding pulse (15 ms here)

sp1: encoding pulse power (120 dB for the gradients calibration, then calibrated)

p23 = p26: duration of coherence-selection gradients for mixing, typically 1 ms

d6: gradients commutation delay (20 µs)

p27: purge gradient duration (5 ms)

AQMOD: qsim (Avance I) or DQD (Avance III)

FnMODE: QF

DS: 0 (not used to performed dummy scans)

FW: should approximately match the frequency dispersion induced by acquisition gradients

AQ: automatically calculated

DW: 0.55 µs

DE: 6.5 µs

NBL: 1

d1: 5 s

p1: 90° ^{1}H pulse (carefully calibrated)

spnam1: encoding pulse name

p15: duration of the acquisition gradients, calculated by pulse program

p20 = p21: duration of coherence-selection gradients for spatial-encoding, typically 1.2 ms

p24: folding gradients duration, equal to p15

GPZ27: purge gradient (80%)

GPZ0 = -GPZ1: encoding gradient, set so that the induced frequency dispersion is equal to the encoding pulse bandwidth	**GPZ25:** pre-phasing gradient, set to –GPZ15/2 to center the peaks in the UF dimension **GPZ24:** folding gradient (0%)
GPZ15 = -GPZ16: acquisition gradients (maximum 80%)	**GPNAM15 = GPNAM16:** SMSQ10.32
GPZ20 = GPZ21 = GPZ23 = -GPZ26: coherence selection gradients (80%)	**GPNAM20 = GPNAM21 = GPNAM23 = GPNAM26:** SINE.100

Processing parameters:

F2	F1
WDW: GM	**WDW:** SINE
LB: -170 Hz (to be adjusted according to the acquisition gradients duration) **GB:** 0.5 (centred on the position of the echo)	**SSB:** 0

The resulting spectral widths can be increased by the use of interleaving. To double the spectral width in the conventional dimension, double TD(F1). To double the spectral width in the UF dimension, halve L3 and double TD(F1).

5 Parametrization of UF-TOCSY and UF-DQS experiments

5.1 Implementation via automated parametrization

Once the preliminary calibrations are performed and the UF-COSY experiment implemented, the **ufset** program can be used to automatically parametrize UF-TOCSY or UF-DQS experiments. Such experiments can thus be acquired using the same procedure as for UF-COSY experiments, indicating the desired pulse program (uftocsy or ufdqs, respectively) prior to the application of the **ufset** program.

It is important to understand that in the case of UF-DQS, the spatial encoding is carried out at DQ frequencies. As a consequence, the DQ spectrum is detected in the spatially-encoded dimension. Finally, the DQS spectrum obtained is tilted by 90° compared to a conventional DQS spectrum, *ie* the DQ spectrum appears in F_2 and the SQ spectrum in F_1.

5.2 Description of the parameters of an UF-TOCSY experiment

The parameters described below are already set by the **ufset** program.

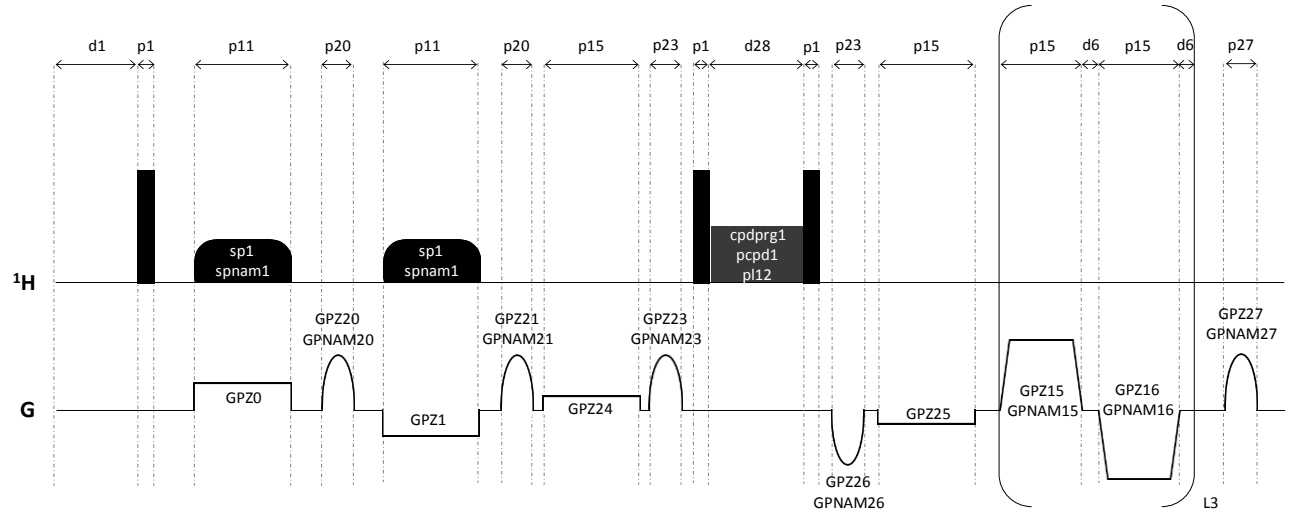


Figure 4. uftocsy pulse sequence

Acquisition parameters for a single-scan UF-TOCSY experiment:

pulprog: uftocsy

pl12: power for a 90° hard pulse of 40 µs (calculated via *calcpowlev* and calibrated on a 1D spectrum)

CPDPRG1: MLEV16

PCPD1: 40 µs (recommended value)

d28: spin-lock duration, multiple of PCPD1, typically 80 ms

The other parameters are similar to the equivalent UF-COSY experiment.

5.3 Description of the parameters of an UF-DQS experiment

The parameters described below are already set by the **ufset** program.

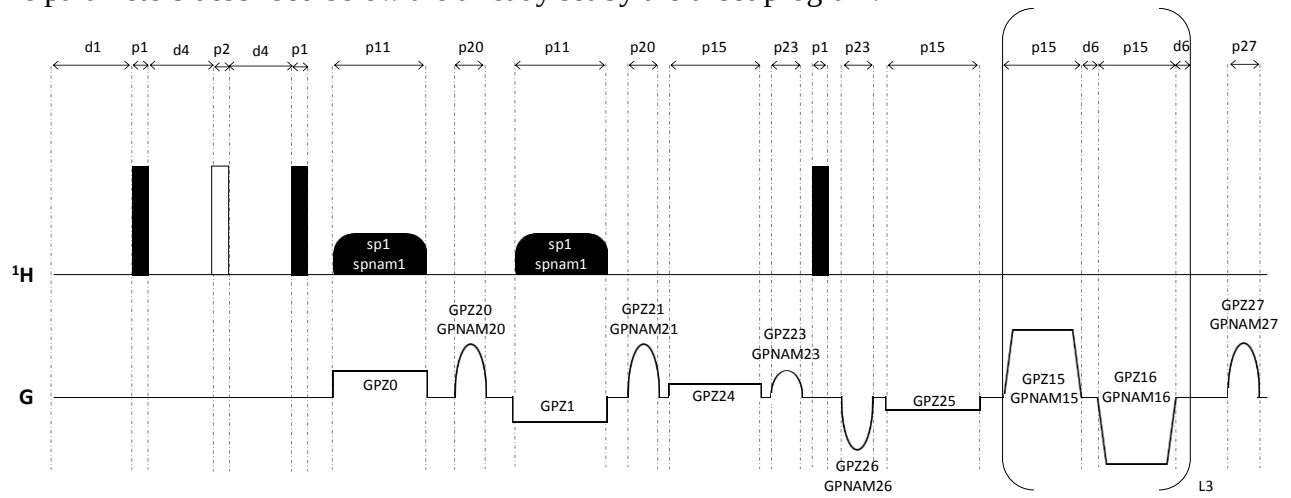


Figure 5. ufdqs pulse sequence

Acquisition parameters for a single-scan UF-DQS experiment:

pulprog: ufdqs

p0: 60° ^{1}H hard pulse

CNST1: mean ^{1}H-^{1}H J-coupling constant

GPZ23: 40%

Note that the spectral width in the UF dimension (DQ chemical shifts) should be doubled compared to the equivalent UF-COSY experiment, using interleaving. The other parameters are similar to the equivalent UF-COSY experiment.

6 Implementation of UF-HSQC experiments

6.1 Implementation via automated parametrization

The implementation of the UF-COSY experiments must be done prior to this procedure. A sample such as concentrated ibuprofen in acetone-d_6 is recommended. Preliminary settings include 1D ^{1}H and ^{13}C experiments, and calibration of the pw90 for each channel.

It is important to understand that in the case of HSQC, the spatial encoding is carried out at the ^{13}C frequency. As a consequence, the ^{13}C spectrum is detected in the spatially-encoded dimension, but it is detected on the ^{1}H channel! Finally, the HSQC spectrum obtained is tilted by 90° compared to a conventional HSQC spectrum, *ie* the ^{13}C spectrum appears in F_2 and the ^{1}H spectrum in F_1. Another difference is that the spatial encoding scheme is split into four pulses, to ensure a symmetrical encoding with perfect ^{1}H decoupling. Finally, the pulse sequence has been optimized with strong coherence-selection gradients to remove the signal arising from ^{1}H bound to ^{12}C.

Open the file \Bruker\TOPSPIN\exp\stan\nmr\au\src\user\UFfeatures13C.txt in a text editor. The first line will be modified thereafter, as well as the second one if needed. On the third line, indicate the power of the ^{13}C chirp pulse (in dB). In a first approximation, the latter can be considered equal to the ^{1}H one. The frequency bandwidth encoded during the spatial encoding step is usually five times larger than the desired spectral width. However, if the maximal ^{13}C spectral width needed (SW_{UF}^{max} - in Hz) is higher than $0.04 \cdot G_a^{max}$ (where G_a^{max} is the frequency dispersion – in Hz – induced by a 100% gradient, as indicated in the UFfeatures1H.txt file), then encoding gradients will not reach the required amplitude. In this case, change the second line of the UFfeatures13C.txt file to $\frac{0.2 \cdot G_a^{max}}{SW_{UF}^{max}}$. Save and close the file.

Create a conventional HSQC experiment, by the use of a parameter file (**rpar**), and adjust the following parameters to the sample: average coupling constant (cnst2), offsets, spectral widths (note the spectral width in the indirect dimension as SW_{th}), pulse durations and powers… Eight scans and two dummy scans are recommended for the first experiment, but these values can be adjusted as needed. Then set the pulse program to ufhsqc. Run the automatic settings program **ufset**.

Adjust the receptor gain, and run the acquisition. Process the data with the **ufproc** command. The spectrum thus obtained is automatically calibrated, thanks to the features indicated in the files UFfeatures1H.txt and UFfeatures13C.txt. Although, as the first parameter of this latter file has not been adapted to the spectrometer, the automatic calibration is still inaccurate. Calibrate the spectrum with the manual calibration program (**ufcal**), then measure the effective spectral width in the ultrafast dimension (SW_{obs}). Change the first line of the UFfeatures13C.txt file to $2 \cdot SW_{th}/SW_{obs}$. The automatic calibration will be more accurate for the next experiments.

On any other sample, a new UF-HSQC experiment can be created, acquired and processed following the same procedure as above, after calibration of the 90° pulse angles in 1D ^{1}H and 1D ^{13}C experiments.

6.2 Description of the parameters

The parameters described below are already set by the **ufset** program.

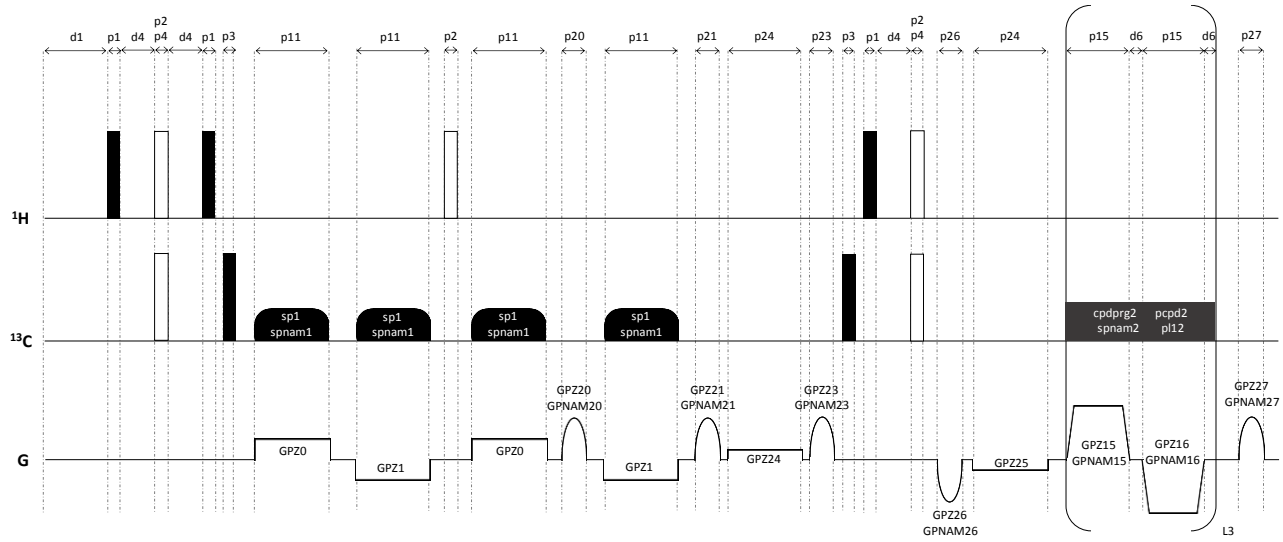


Figure 6. ufhsqc pulse sequence

Acquisition parameters for a single-scan experiment:

pulprog: ufhsqc

O1: set to the middle of the ^{1}H range

NS: 2*n

pl2: ^{13}C hard pulse power

p11: duration of the encoding pulse (5 ms here)

sp1: encoding pulse power (120 dB for the gradients calibration, then calibrated)

p23: 1 ms

CPDPRG2: p5m4sp180

pl12 = sp15: decoupling power corresponding to $\gamma B_1 = 6.45\ kHz$

NUC2: ^{13}C

O2: set to the middle of the ^{13}C range

CNST2: mean ^{1}H-^{13}C J-coupling constant

P3: 90° ^{13}C pulse (carefully calibrated)

spnam1: encoding pulse name

p15: duration of the acquisition gradients, calculated by pulse program

p26: 500 µs

PCPD2: 1024 µs

SPNAM15: caWURST-250-123k-1024-2

The other parameters are similar to the reference UF-COSY experiment. As mentioned above, the resulting spectral widths can be increased by the use of interleaving. To double the spectral width in the conventional dimension, double TD(F1). To double the spectral width in the UF dimension, halve L3 and double TD(F1).

7 Important remarks

Acquisition. The procedures described here were implemented successfully on several Bruker spectrometers (from 400 to 700 MHz). However, depending on your spectrometer, additional artefacts may appear on the spectrum, due to several reasons including possible instabilities in the gradient amplifier. We may already have solutions to get rid of these artefacts, so please send your data to patrick.giraudeau@univ-nantes.fr. We will have a look at it and try to provide solutions.

Processing. Send us any errors you may get with this processing (if possible with the corresponding datasets), it will greatly help us to improve it!

What about other pulse sequences? This protocol has been written for a limited number of experiments. We currently do not have such a standardized protocol for other experiments, but we can

provide other pulse programs such as DQF-COSY, J-res, etc. We are also open for new collaborations to design new UF experiments on demand.