PRACTICAL CONSIDERATIONS IN ULTRAFAST 2D NMR

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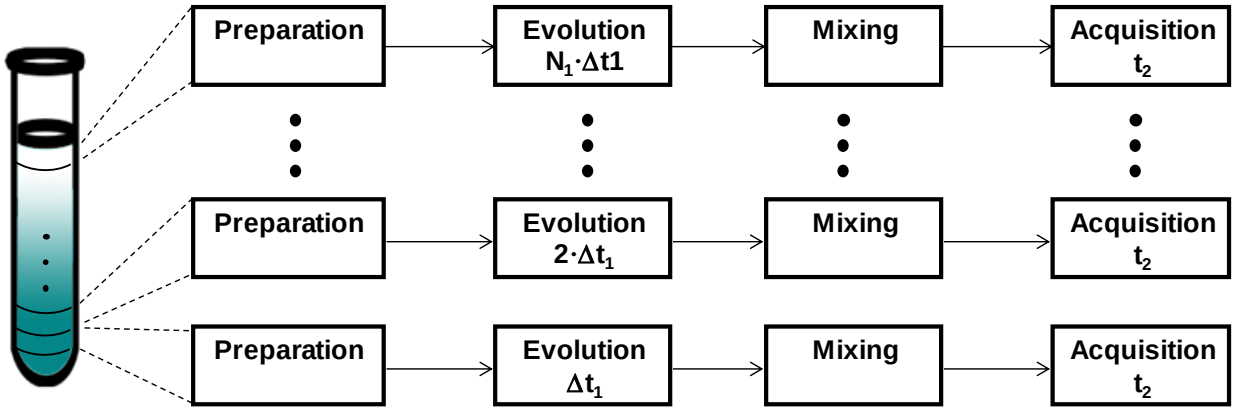
Institut Universitaire de France

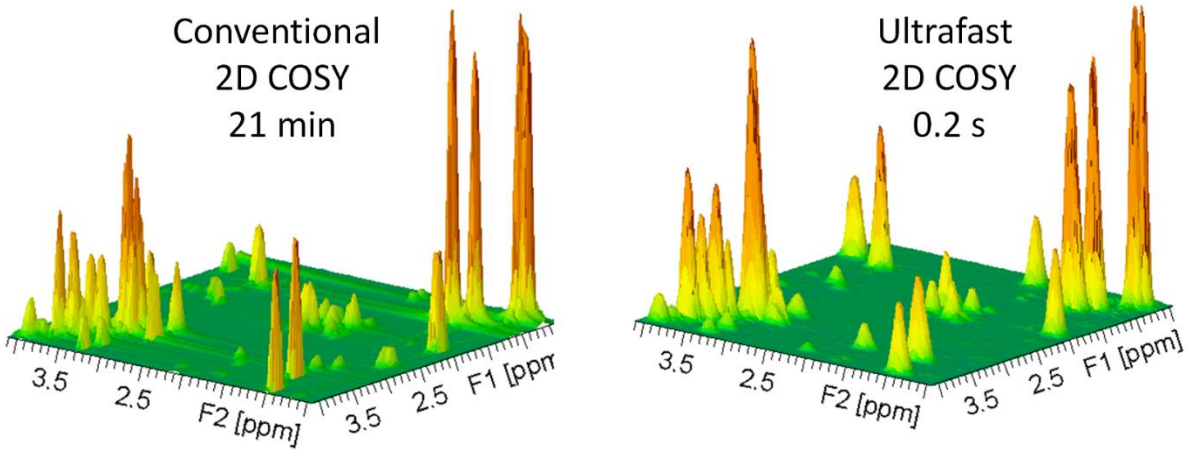
SMASH Workshop, Atlanta, 10/09/2014

OUTLINE

- **What** is UF 2D NMR ?
- **When** using (or not) UF 2D NMR ?
- **How** to implement/use UF 2D NMR ?

Ultrafast 2D NMR: Basic Principle

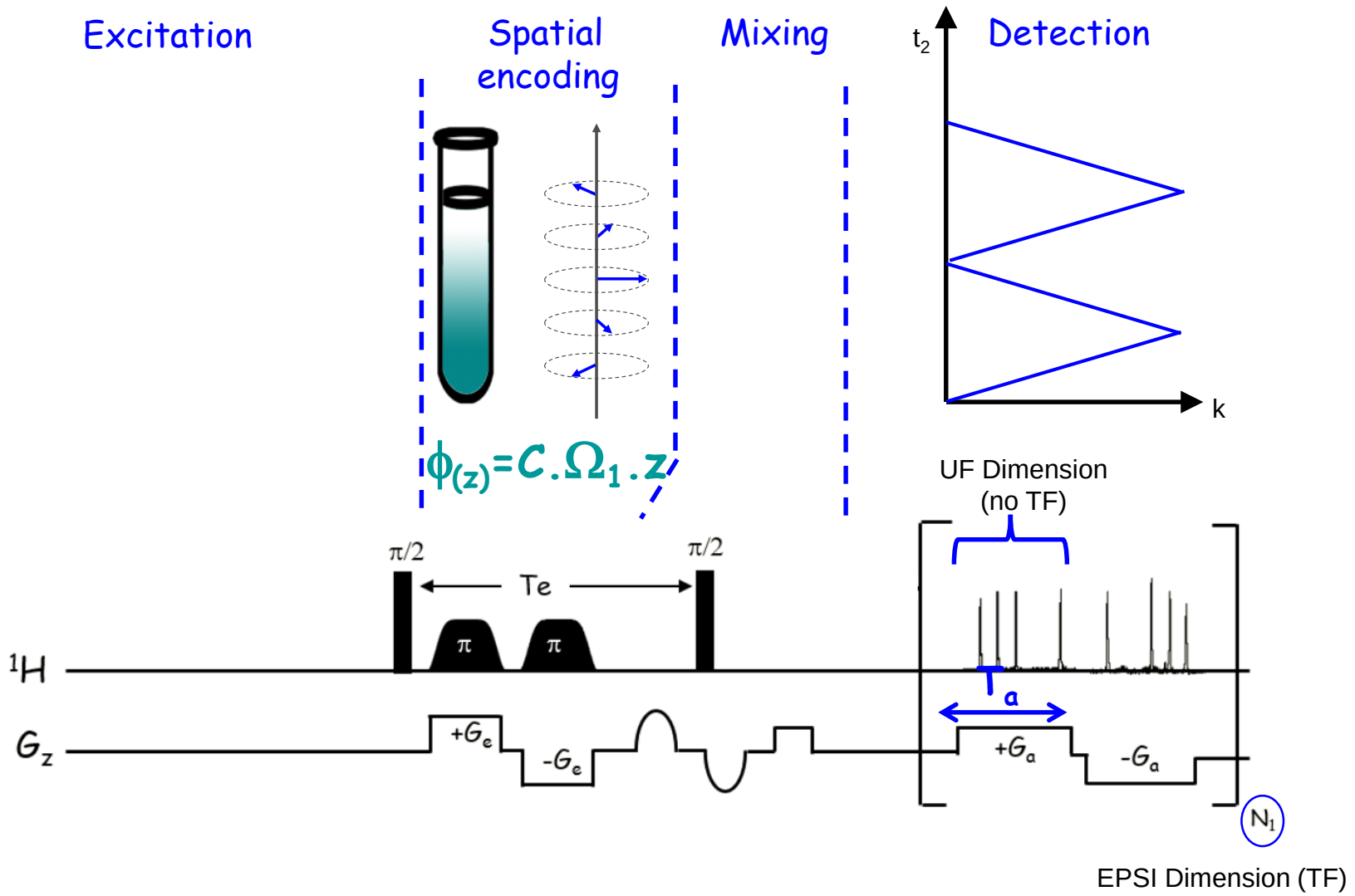




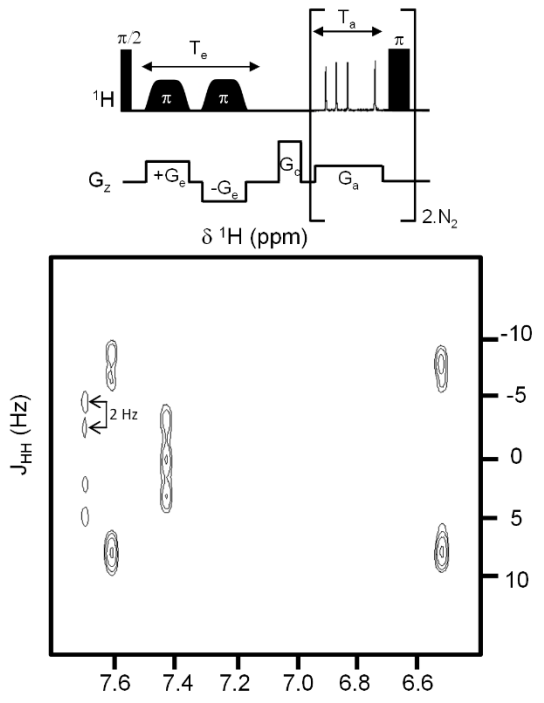
L. Frydman, T. Scherf, A. Lupulescu, PNAS 2002

P. Giraudeau, L. Frydman, Ann. Rev. Anal. Chem. 2014.

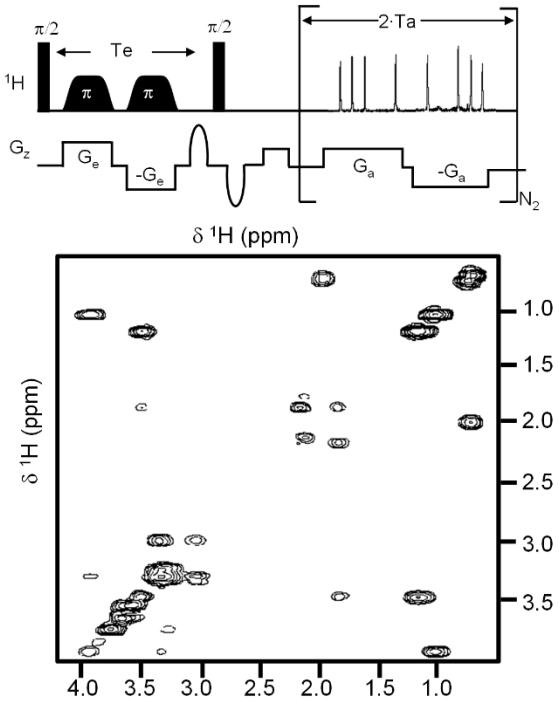
Ultrafast 2D NMR: Basic Principle



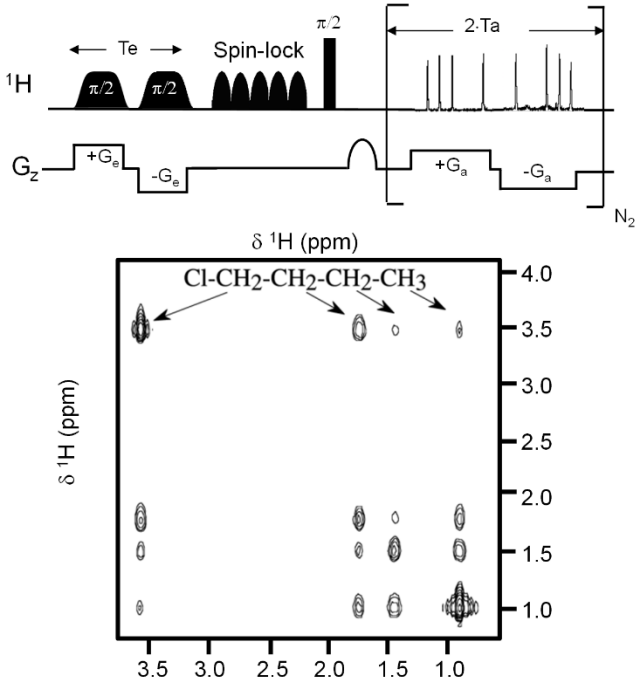
Ultrafast 2D NMR: Examples



Jres



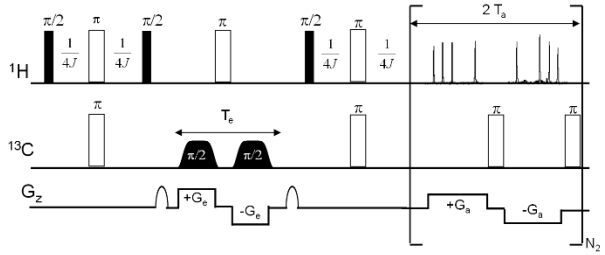
COSY

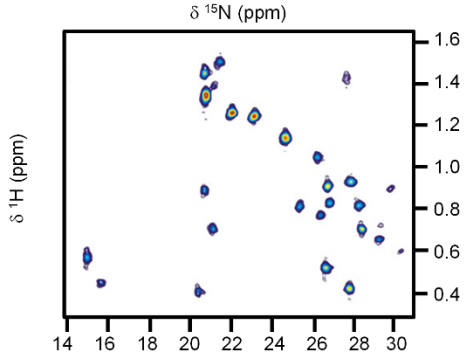


TOCSY

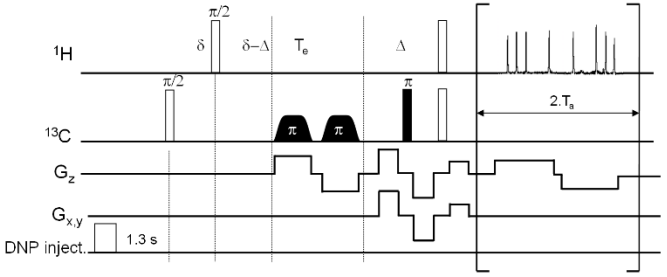
P. Giraudeau, L. Frydman, Ann. Rev. Anal. Chem., 2014.

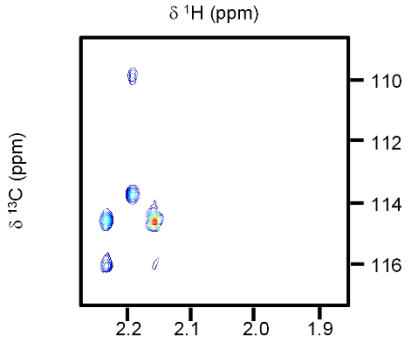
Ultrafast 2D NMR: Examples





HSQC





HMBC

P. Giraudeau, L. Frydman, Ann. Rev. Anal. Chem., 2014.

When using UF ?

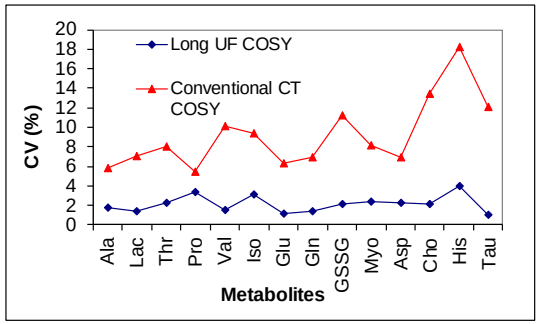
- **Save time** (if SNR is sufficient)
- Monitor **fast reactions/dynamics** in real time for which 1D is not sufficient
- **Hyphenated techniques** that need single-shot acquisitions (dissolution DNP, on-line HPLC-NMR…)
- **Quantitative analysis** of complex mixtures (better precision with hybrid techniques based on UF)
- Speed up acquisitions with **higher dimensionality** (fast nD)
- **Have fun** with exciting developments in spatial encoding ☺

When using UF ?

There are many cases where a single scan is not needed!

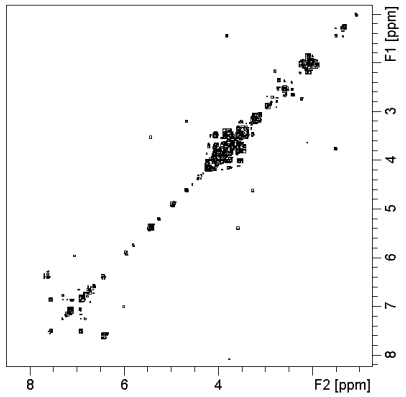
Hybrid techniques (UF with several scans) are often more interesting!

To improve **sensitivity**
(Multi-scan Single Shot)



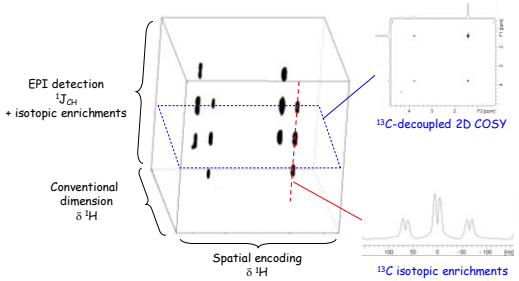
A. Le Guennec *et al.*, *Anal. Chem.* 2012

To increase **spectral widths**
or to improve **resolution**
(interleaved acquisitions)



L. Rouger *et al.*, *JMR* 2014

To increase
the **dimensionality**
(fast hybrid 3D)



P. Giraudeau *et al.*,
ChemPhysChem 2012

When using UF ?

Recent reviews on applications of UF

Analytical applications in general

Annu. Rev. Anal. Chem. 2014. 7:129–61

Ultrafast 2D NMR:
An Emerging Tool in
Analytical Spectroscopy

Patrick Giraudeau[1] and Lucio Frydman[2]

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[2]Department of Chemical Physics, Weizmann Institute of Science, 76100 Rehovot, Israel; email: lucio.frydman@weizmann.ac.il

Quantitative applications

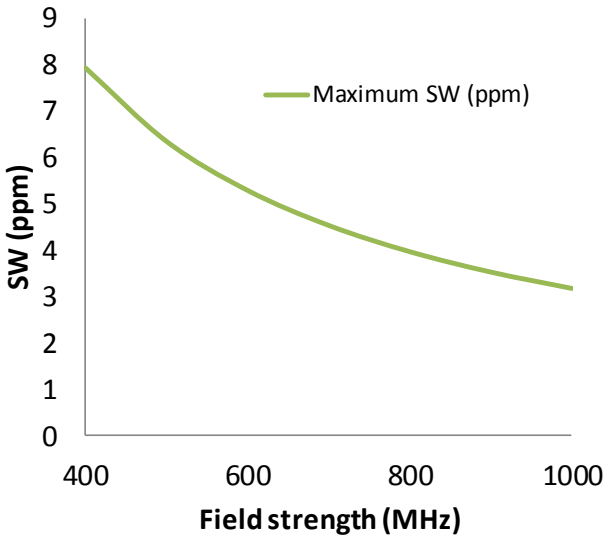
Magn. Reson. Chem. 2014. 6:259-272

Spectral Widths

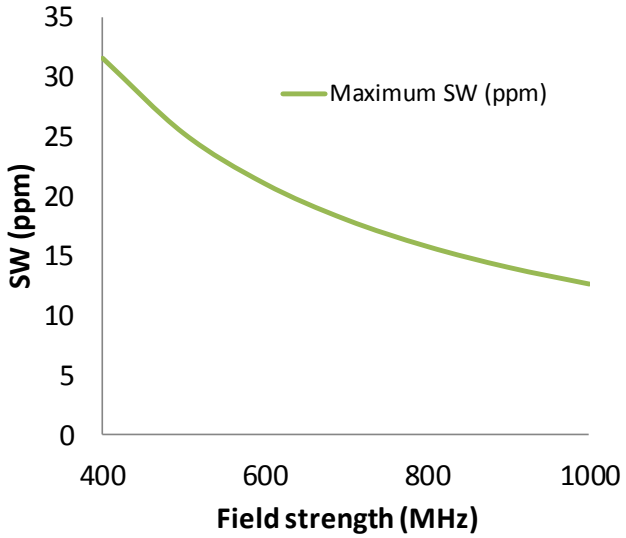
For a single-scan experiment with a standard gradient system (50 G/cm) and a 5 mm probe

$$\gamma \cdot G_a \cdot L = \frac{2 \cdot SW_1 \cdot SW_2}{\Delta\nu_2}$$

Single-scan ^{1}H COSY



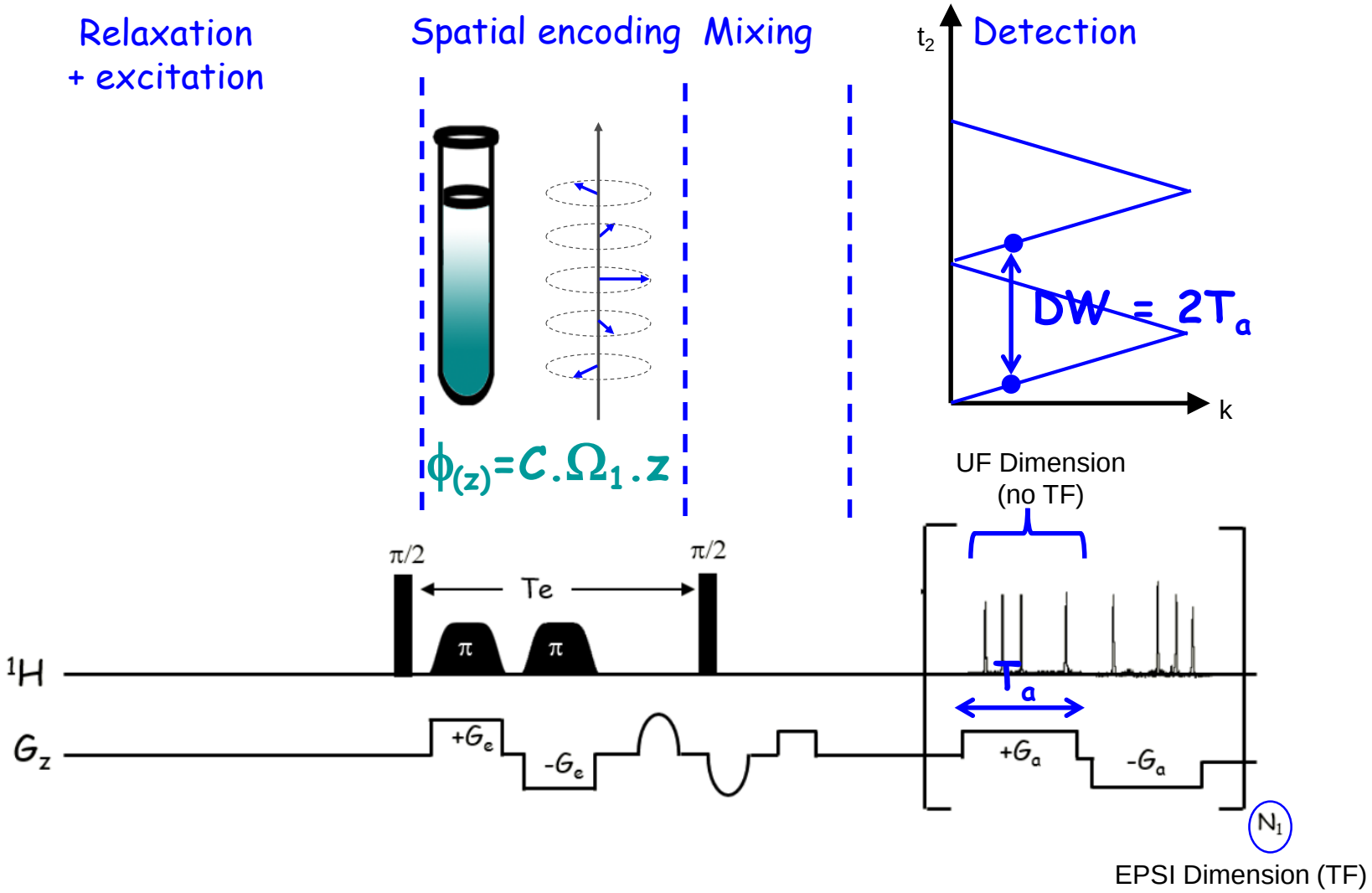
Single-scan ^{1}H-^{13}C HSQC



BUT these values can greatly increase with interleaved acquisitions !

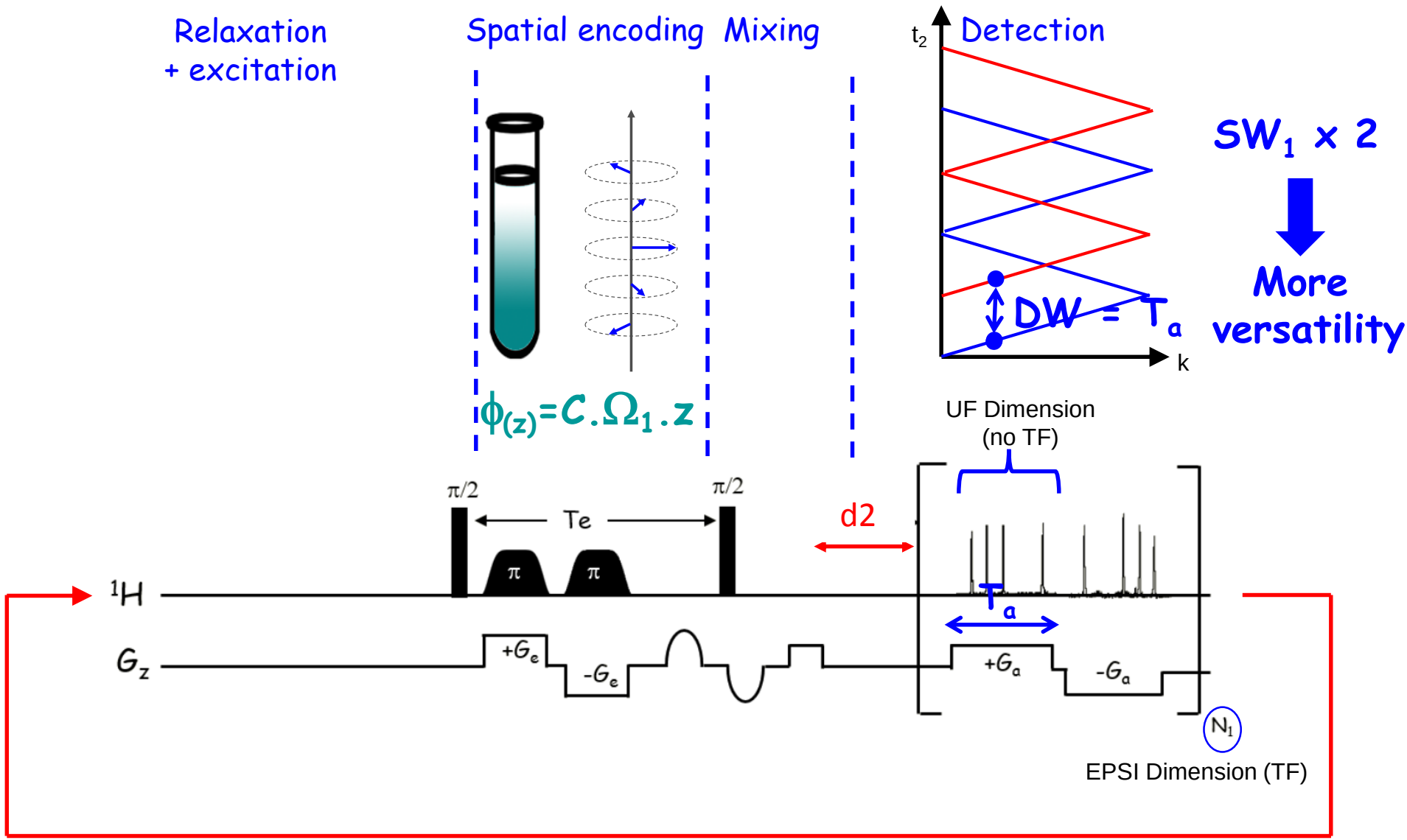
P. Giraudeau, L. Frydman, Ann. Rev. Anal. Chem., 2014.

Spectral Widths – interleaved acquisitions



L. Rouger *et al.*, JMR 2014

Spectral Widths – interleaved acquisitions



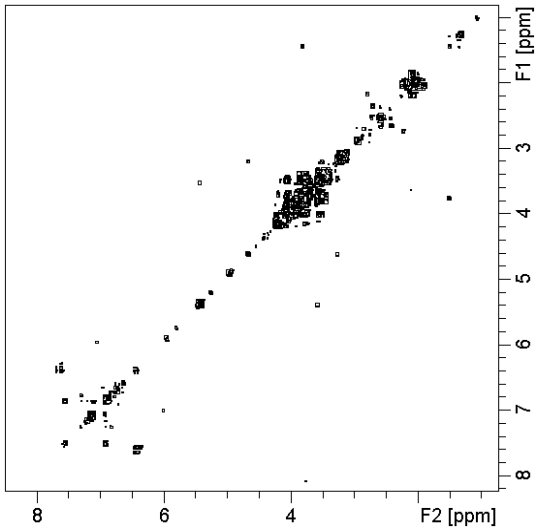
L. Rouger *et al.*, JMR 2014

Spectral Widths – interleaved acquisitions

EXAMPLES @ 500 MHz

PULSE SEQUENCE	SWs in 1 scan	SWs in 4 interleaved scans (10-20 seconds)
COSY	4 x 4 ppm	8 x 8 ppm
^{1}H-^{13}C HSQC	35 x 4 ppm	70 x 8 ppm

EXAMPLE @ 700 MHz

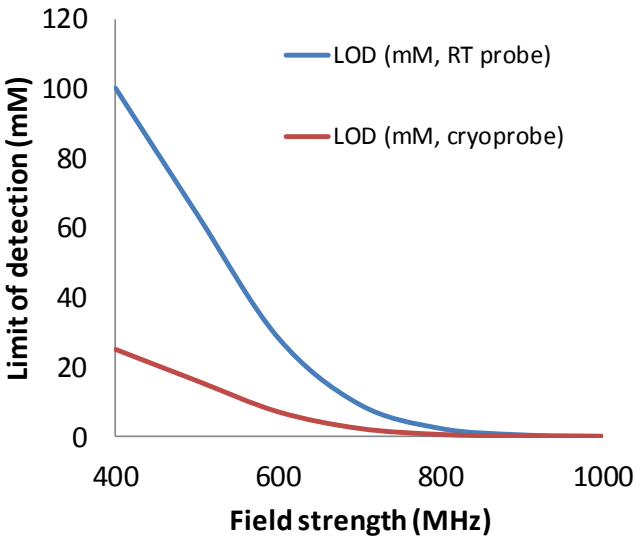


COSY of a corn extract
700 MHz - 80 seconds
(16 interleaved scans)

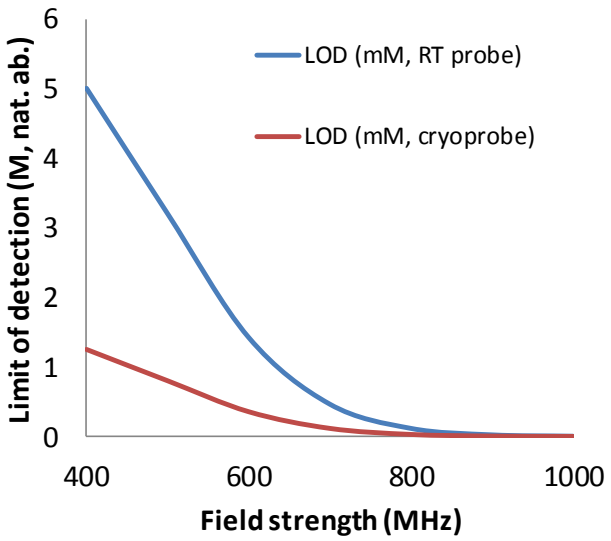
Sensitivity

For a single-scan experiment with a standard gradient system (50 G/cm) and a 5 mm probe

Single-scan ^{1}H COSY



Single-scan ^{1}H-^{13}C HSQC



Signal averaging is possible:

-SNR increases with $NS^{1/2}$ as in conventional NMR

-No t_1 noise in UF experiments

P. Giraudeau, L. Frydman, Ann. Rev. Anal. Chem., 2014.

Sensitivity

EXAMPLES @ 500 MHz with cryoprobe

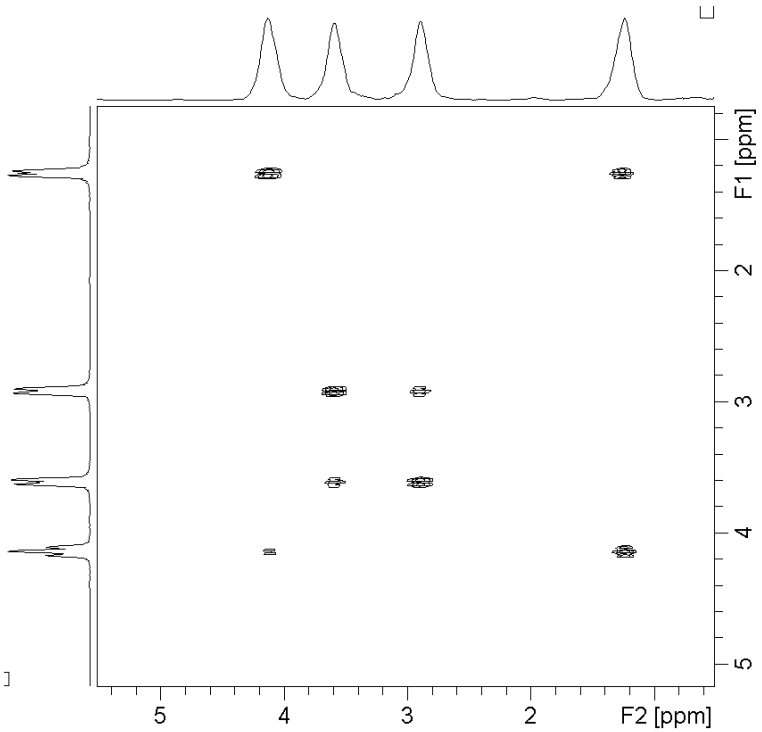
TARGET	Possible in 1 scan	Possible in a few scans	Not currently possible with UF
Record a **homonuclear 2D** (COSY, TOCSY, Jres…) of a **diluted small molecule** (10 mg, MW = 300 g/mol)	✓		
Record a **heteronuclear 2D** (HSQC, HMBC…) of a **concentrated small molecule** (100 mg, MW = 300 g/mol)	✓		
Record a **heteronuclear 2D** (HSQC, HMBC…) of a **diluted small molecule** (10 mg, MW = 300 g/mol)		✓	
Record a **COSY of a biological extract**		✓	
Record an **HSQC of unlabeled biological material**			✗
Record an **HSQC of a labeled protein**		✓	

Resolution

Ultrafast dimension: $\Delta\nu^{1/2} \approx 40$ Hz

Conventional (EPSI) dimension $\Delta\nu^{1/2} \approx 10$ Hz

(equivalent to a conventional 2D with $TD_1 = 128$)



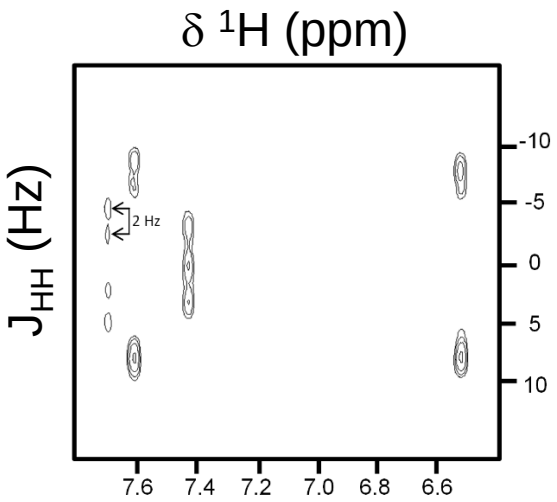
- F_1 resolution can improve with linear prediction
- Coupling patterns are visible

Coupling patterns are not visible, but it is not a matter of resolution: UF spectra are homodecoupled in F_2.

Resolution

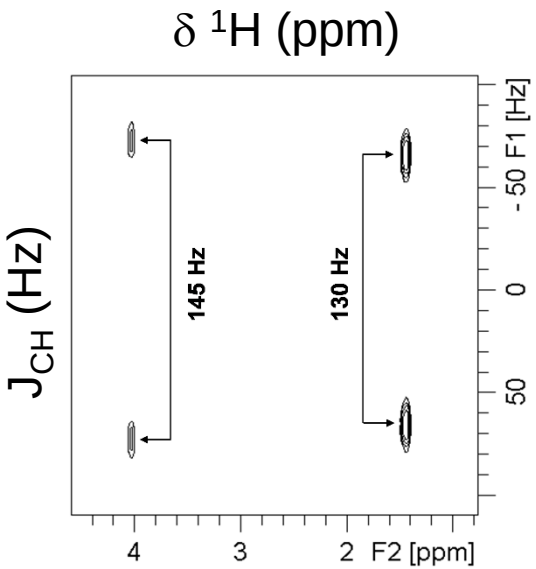
MEASUREMENT OF COUPLING CONSTANTS

Jres



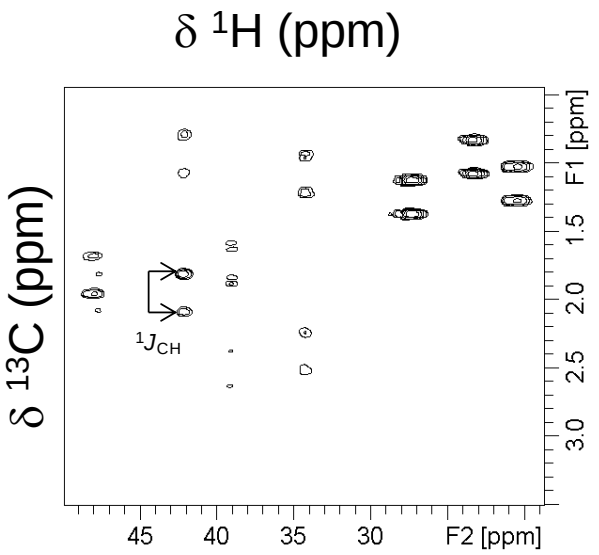
JMR 2007

Hetero Jres



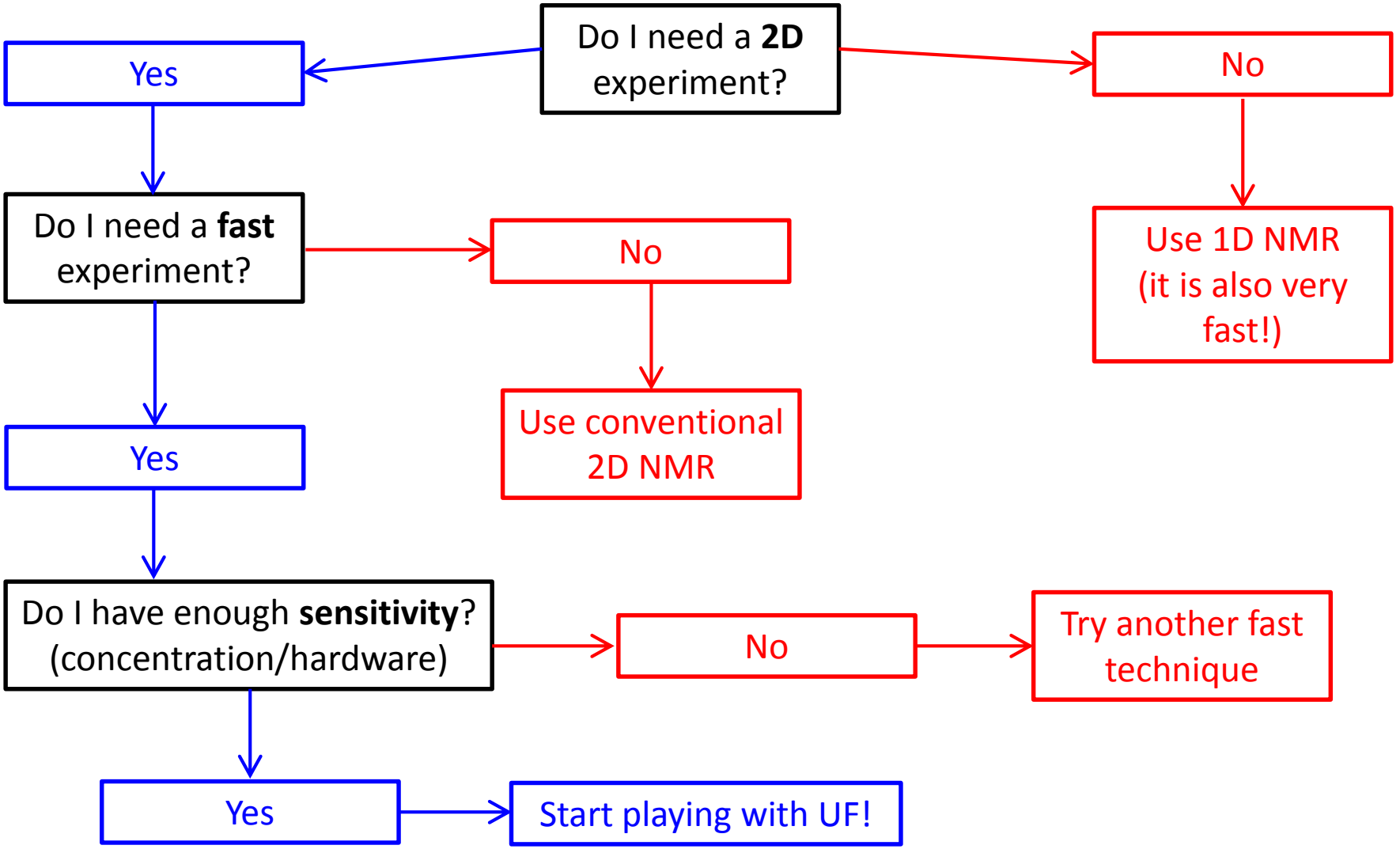
JMR 2012

Coupled HSQC



MRC 2012 – Coll. C. Thiele (Darmstadt)

Questions to ask before using UF 2D NMR



Technical requirements

- **Probe with z-gradient (cryoprobe OK)**
- **Chirp pulses**
- **Good ADC (smallest DW < 1 µs)**

Human requirements

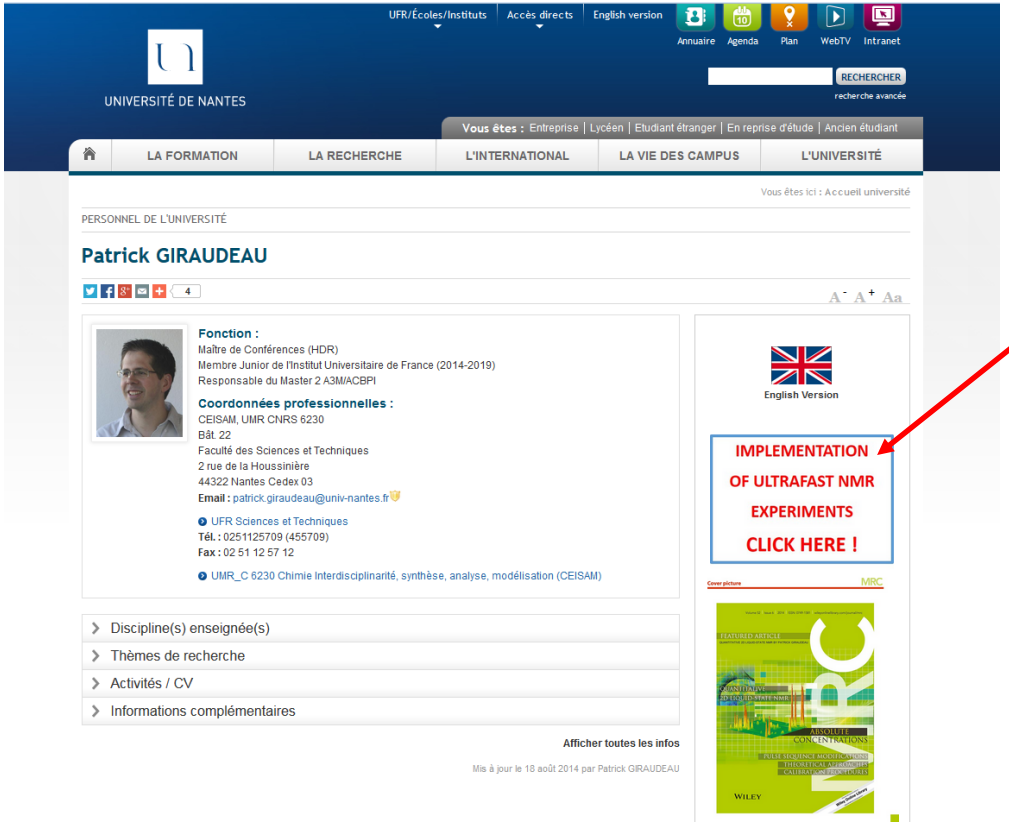
- **Take some time to read papers**
- **Implement the experiments step-by-step**

Available on our website

- **Pulse sequences (Bruker)**
- **Processing routines (Jython programs fully integrated in Topspin)**
- **Step-by-step implementation protocol**
- **Eveything is free…as far as you don't make money with it**
- **We also provide technical assistance to solve troubleshooting issues.**

How to download the material

- **Go to http://www.univ-nantes.fr/giraudeau-p**



How to download the material

Accueil ► Cours ► P-EBSI

Aperçu des sections

Elucidation de Biosynthèse et Spectrométries Isotopiques

EBSI est une équipe de recherche du laboratoire CEISAM (UMR 6230, Université de Nantes-CNRS)

Les activités de recherche d'EBSI concernent le développement et l'application de la chimie analytique aux problèmes bio-organiques. Notre équipe a développé une forte expertise dans l'analyse des isotopes stables et de leur application à l'étude de phénomènes chimiques et physiques. Elle est maintenant reconnue pour ses compétences concernant les mesures de rapports isotopiques dus au fractionnement isotopique naturel (défini comme étant la discrimination entre isotopes légers et isotopes lourds au cours de réactions chimiques ou biochimiques et de processus physiques.)
Ces études sont menées afin d'approfondir notre connaissance du comportement des isotopes stables, lesquelles sont à la base d'une grande gamme d'applications de méthodes isotopiques telles que le traçage, l'authentification, le métabolisme, les mécanismes réactionnels.

En savoir plus

1

Implementation of Ultrafast 2D NMR experiments

The protocol, pulse sequences and processing program to implement ultrafast 2D NMR experiments on your spectrometer are described below. Please read carefully the protocol first.

Important remark: Ultrafast 2D NMR has been patented by the Weizmann Institute of Science, Israel. Its use for commercial purposes requires a licence from the Weizmann Institute.

Please first download the protocol and files below, and carefully read the protocol!

PROTOCOL TO IMPLEMENT ULTRAFAST EXPERIMENTS ON YOUR SPECTROMETER
Files attached to the protocol for download

2

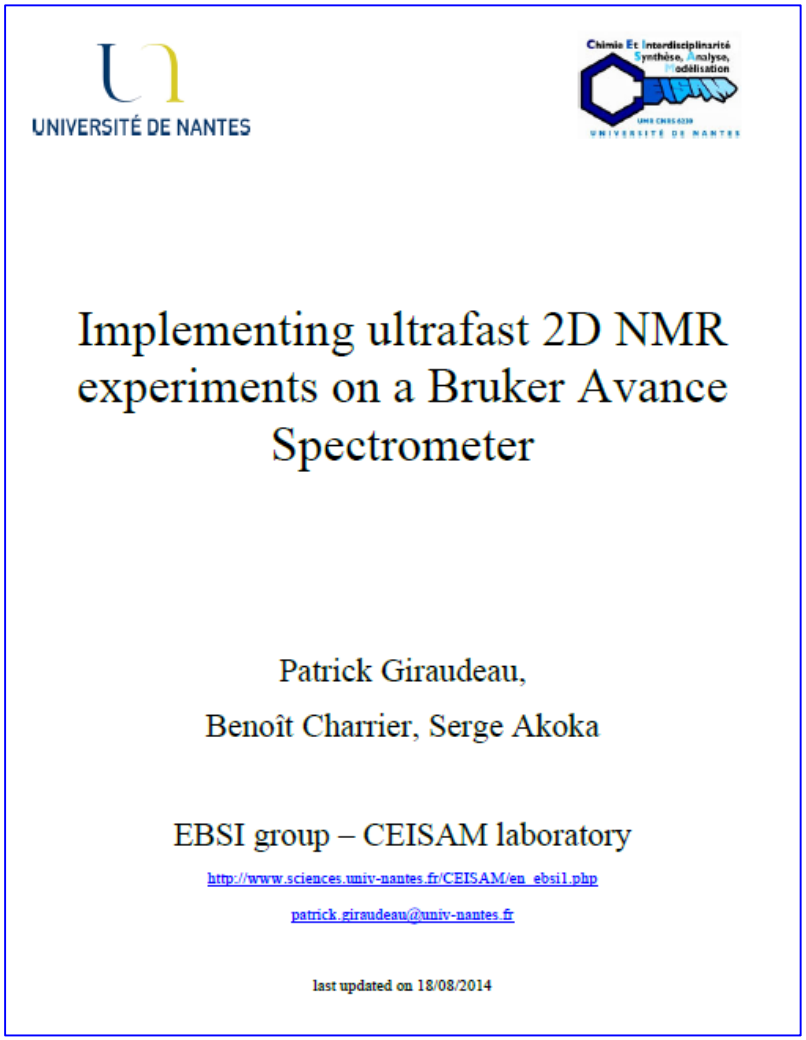
Additional resources for Ultrafast 2D NMR experiments

An interactive web page for the determination of ultrafast experimental parameters (for COSY and HSQC) is available at the following address:

http://www.sciences.univ-nantes.fr/CEISAM/ebsi/ultrafast/uf_acq.html

Below, you can download the ppt presentations from the 1st international symposium on ultrafast 2D NMR that was held in Santiago de Compostela in September 2013.

The implementation protocol



UNIVERSITÉ DE NANTES

Implementing ultrafast 2D NMR experiments on a Bruker Avance Spectrometer

Patrick Giraudeau,

Benoît Charrier, Serge Akoka

EBSI group – CEISAM laboratory

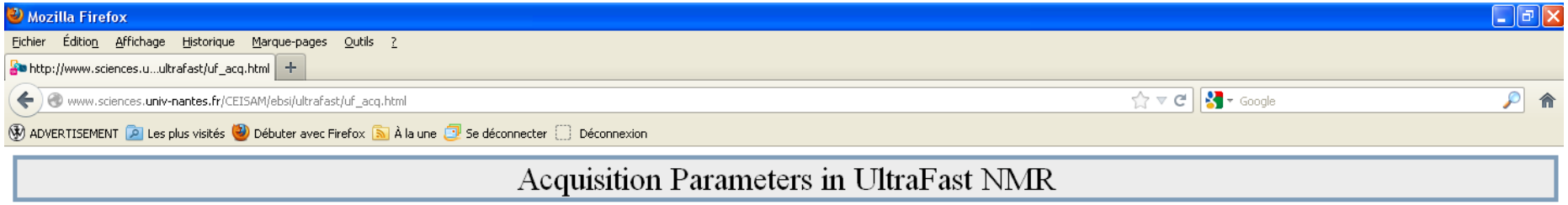
http://www.sciences.univ-nantes.fr/CEISAM/en_ebsi1.php

patrick.giraudeau@univ-nantes.fr

last updated on 18/08/2014

- Gradient and chirp pulse calibration
- Setting the UF COSY step-by-step
- Setting the UF COSY in the general case
- Setting the Jres
- Setting the HSQC
- Setting the interleaved experiments (COSY and HSQC)

Web interface to help setting the parameters



Acquisition Parameters in UltraFast NMR

Implementation of Ultrafast 2D NMR experiments:

The protocol, pulse sequences and processing program to implement ultrafast 2D NMR experiments on your spectrometer are available for download here:
http://madoc.univ-nantes.fr/course/view.php?id=24710

Please first read carefully the protocol and download the files. Once the ultrafast experiments are implemented on your spectrometer, use the following pages to set up your acquisition parameters:

Type of experiment:

Pulse sequence : UFCOSY
UFHSQC

Troubleshooting
You can here download the pages to run on a local computer.

QUANTUM project: Quantitative Ultrafast Analysis by 2D NMR To Unravel Metabolic complexities 2011-2014 (ANR grant 2010-JCJC-0804-01)

Important remark: Ultrafast 2D NMR has been patented by the Weizmann Institute of Science, Israel. Its use for commercial purposes requires a licence from the Weizmann Institute.

Web interface to help setting the parameters

Mozilla Firefox

Fichier Édition Affichage Historique Marque-pages Outils ?

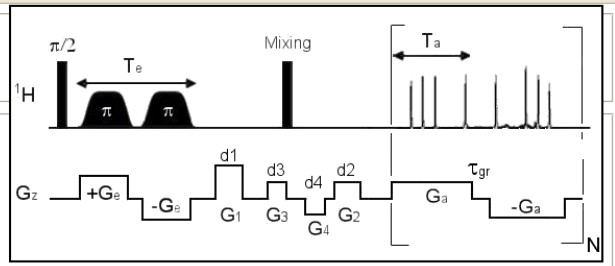
http://www.sciences.u...ltrafast/uf_cosy.html

www.sciences.univ-nantes.fr/CEISAM/ebsi/ultrafast/uf_cosy.html

ADVERTISEMENT Les plus visités Débuter avec Firefox À la une Se déconnecter Déconnexion

Spectrometer parameters:

Lp :	16	Length of proton coil (mm)
Gmax :	0.816	Maximum strength of gradient coil (T/m)



Input parameters:

SW1 :	1900	Spectral width in Conventional dimension F1(Hz) (Maximum value : 2812.93 Hz)
SW2 :	1900	Spectral width in Ultrafast dimension F2 (Hz)
O1 :	1403.96	Offset in Conventional dimension F1 (Hz)
O2 :	1403.96	Offset in Ultrafast dimension F2 (Hz)
Setup folding parameters	☐	
ν:	1403.96	Center of the unfolded region (Hz)
ν^f:	-2000	Center of the folded region (Hz)

Advanced parameters :

Calculate

Output parameters:

G1 = -13.24 %
G2 = 11.71 %
Ga = 63.25 % (Amplitude of acquisition Gradient)
Ta = 243.158 µs (Duration of acquisition Gradient)
Ge = 1.71 % (Amplitude of excitation Gradient)
Δν = 9.500 kHz (Linear sweep of adiabatic pulse)
FW has to be upper than 351.62 kHz
dw (dwell time) = 0.514 µs , *this theoretical dwell time will be slightly adjusted by the spectrometer (final dw).*
You should enter that final dw as an advanced input parameter and run again the calculation to finely adjust all output parameters.

Topspin parameters for pulsprog = uf_cosy:

d1:	d24 = 5ms	G1:	gpz24 = -13.24	N:	L3 = 128
d2:	d25 = 5ms	G2:	gpz25 = 11.71	TD2	= 131072
d3:	p23 = 1ms	G3:	gpz23 = 80.00	TD1	= 1
d4:	p26 = 1ms	G4:	gpz26 = -80.00	O1	= 1403.96
τgr:	d6 = 20µs	Ga+:	gpz2 = 63.25	dw	= 0.514µs
Ta:	d20 = 243.158µs	Ga-:	gpz3 = -63.25	SW1	= 1900.0Hz
		Ge+:	gpz0 = 1.71	Design a chirp pulse with:	
		Ge-:	gpz1 = -1.71	Δν	= 9.500 kHz
				p11 (Te/2)	= 15ms

CONCLUSION

- **UF is extremely rewarding if you are ready to spend some time on it.**
- **Do not expect miracles.**
- **If it does not work: send an e-mail! We are happy to help.**

ACKNOWLEDGEMENTS

The EBSI group

Special thanks to Serge Akoka & Benoît Charrier

Funding sources

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ANR

IUF