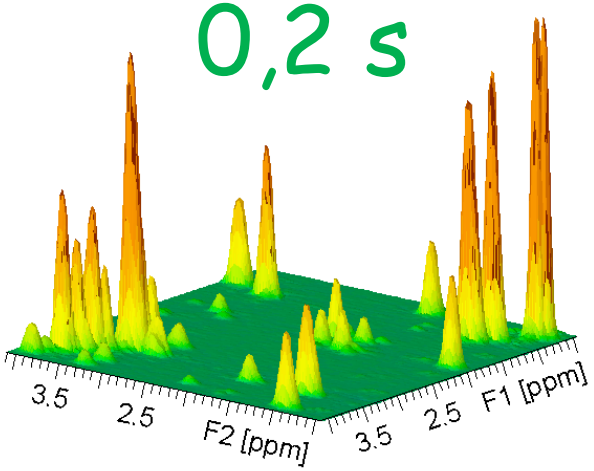
Analytical Ultrafast 2D NMR



Patrick GIRAUDEAU

CEISAM, Université de Nantes, CNRS, Nantes, France

Chimie Et Interdisciplinarité Synthèse, Analyse, Modélisation
UMR CNRS 6230
UNIVERSITÉ DE NANTES

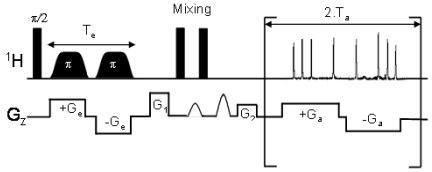
UNIVERSITÉ DE NANTES

cnrs dépasser les frontières

Analytical ultrafast 2D NMR

Main subjects in our research group

NMR method development



-High precision quantitative pulse sequences

-Quantitative nD methods

-Ultrafast (UF) NMR

-Localized spectroscopy

Analytical aspects

-Robustness

-Linearity

-Accuracy

Quantitative NMR methods

Quantitative applications

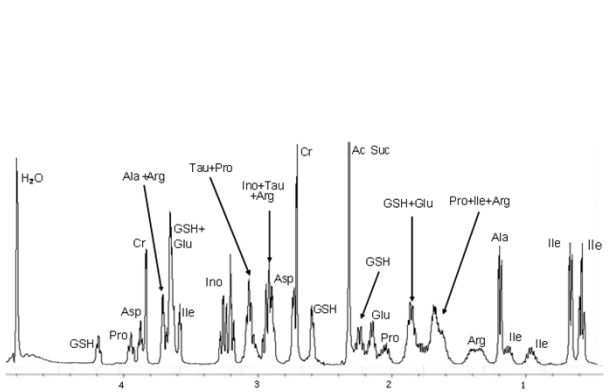
-Quantitative analysis of biological samples (understanding metabolism)

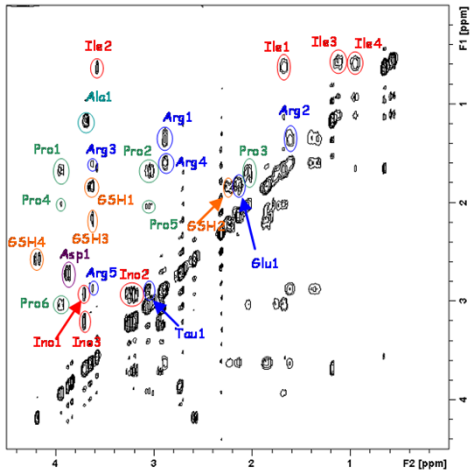
-Isotopic analysis @ natural abundance (authentication of food, drugs…)

-Isotopic analysis in enriched media (fluxomics)

Analytical ultrafast 2D NMR

Quantitative 2D NMR



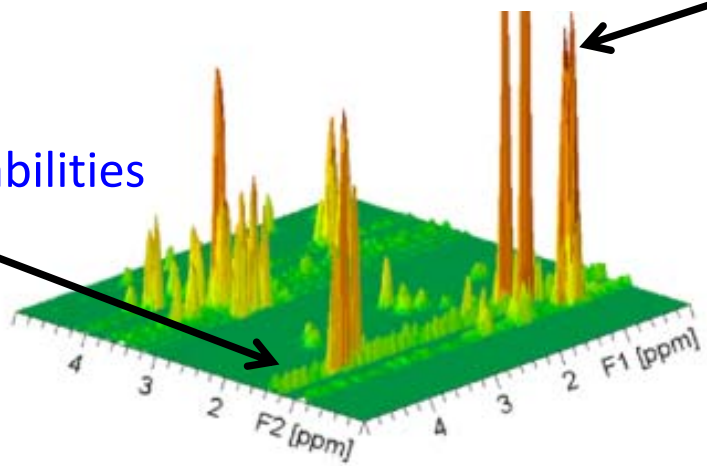


Unambiguous quantification
with higher precision
thanks to reduced overlap

Challenges

Long experiment duration

-timetable constraints
-instable samples
-sensitivity to hardware instabilities



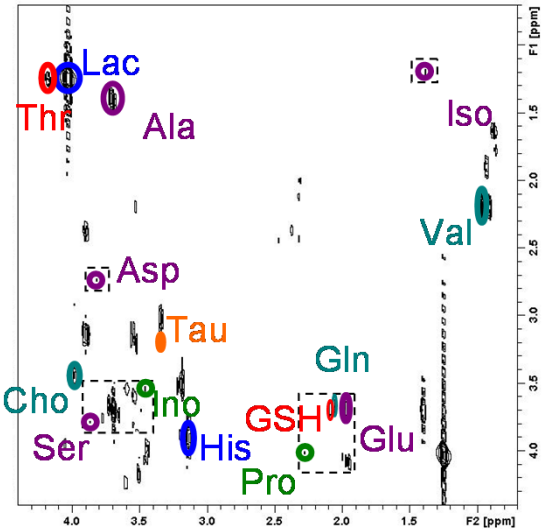
2D response
is peak dependant (T_2, J…)

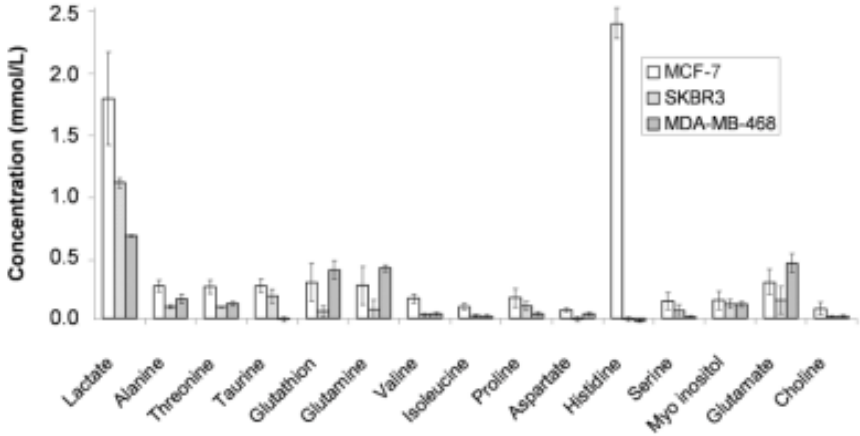
Calibration is needed !

P. Giraudeau and S. Akoka, *Adv. Bot. Res.* 2013

Analytical ultrafast 2D NMR

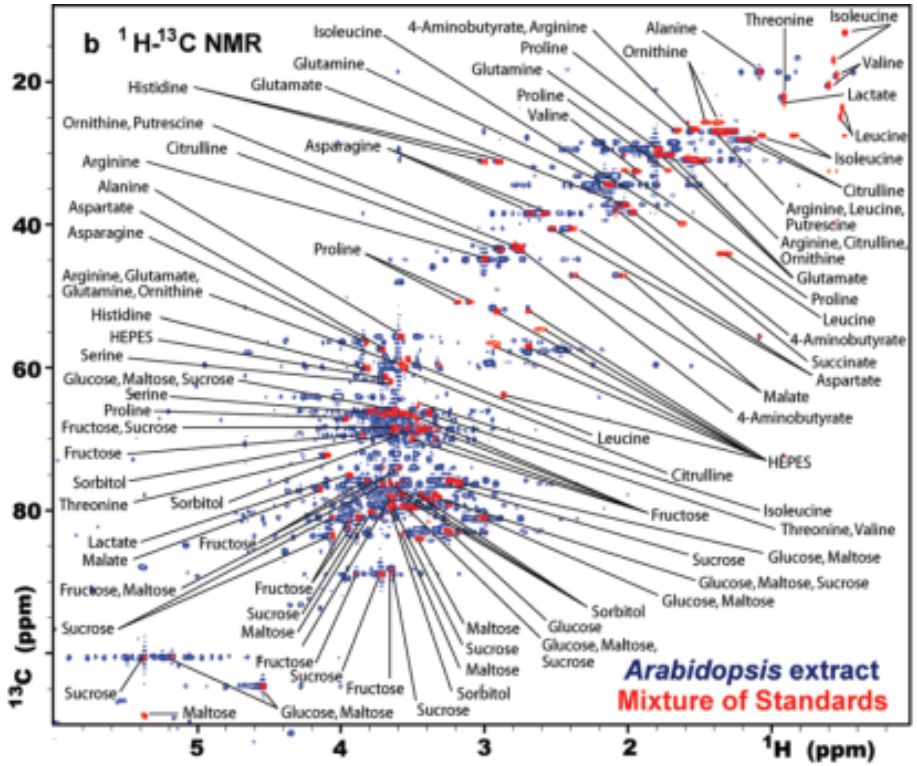
Quantitative 2D NMR on metabolic samples





Determination of metabolite concentrations
in breast cancer cell extracts
via a standard addition procedure
and a ^{1}H INADEQUATE experiment

E. Martineau, I. Tea, S. Akoka, P. Giraudeau, *NMR Biomed.* 2012.

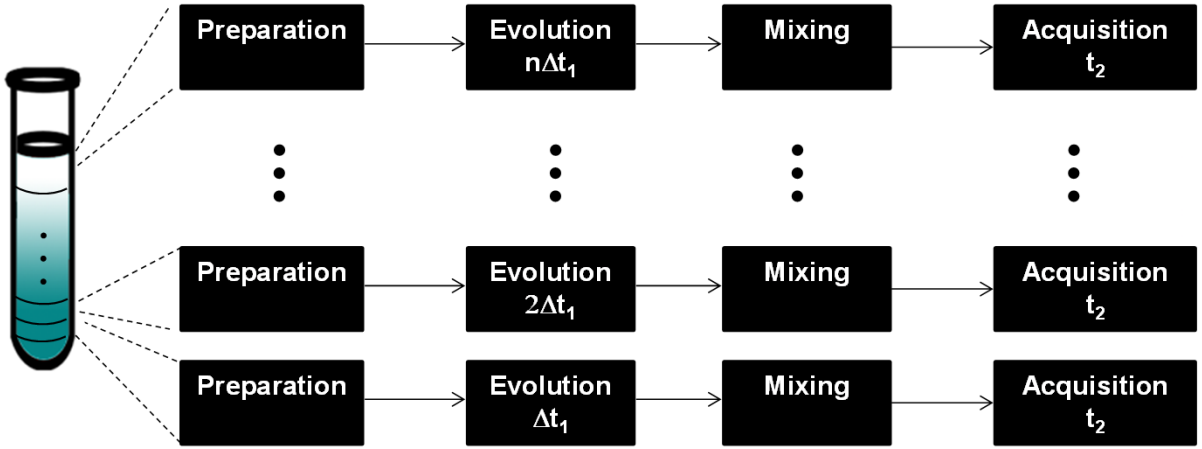


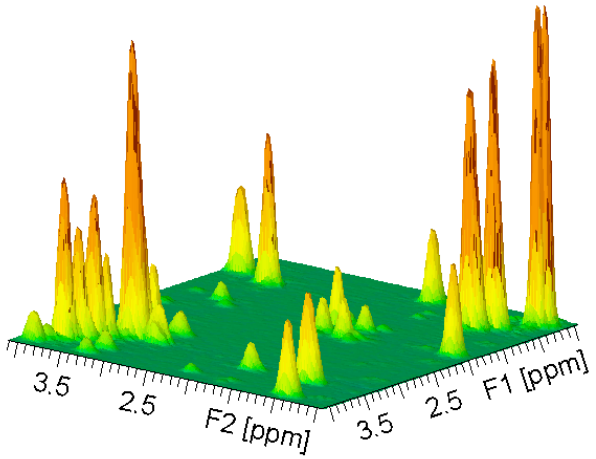
Determination of metabolite concentrations
in plant tissue extracts
via a calibration procedure
and a ^{1}H-^{13}C HSQC experiment

I. A. Lewis, S.C. Schommer, B. Hodis, K.A. Robb,
M. Tonelli, W.M. Westelr, M.R. Sussman, J.L. Markley
Anal. Chem. 2007

Analytical ultrafast 2D NMR

Ultrafast 2D NMR for quantitative analysis?





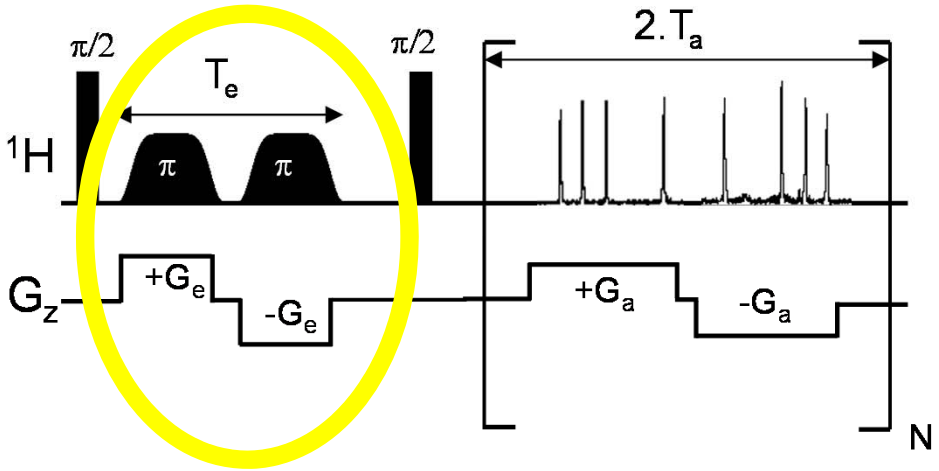
1. Increasing the analytical performance
2. Applications in quantitative metabolomics
3. Applications in fluxomics
4. Other recent analytical applications

Limitations of UF 2D NMR

Compromise SW/resolution

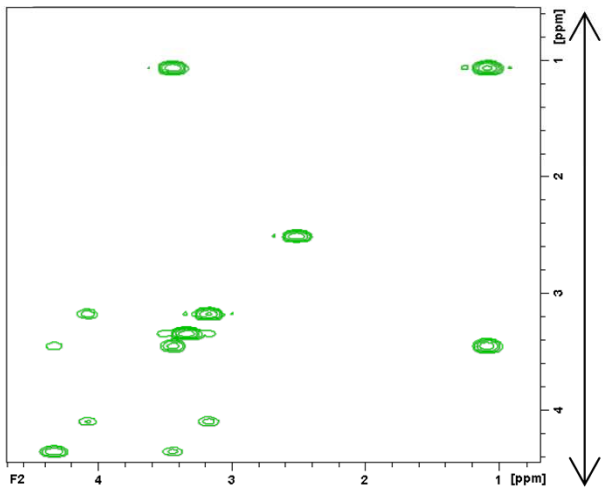
$$\gamma \cdot G_a \cdot L = \frac{2 \cdot SW_1 \cdot SW_2}{\Delta\nu_2}$$

Compromise Sensitivity/Resolution



Ultrafast dimension: SW_2

$\Delta\nu_2$: Peak width - F_2



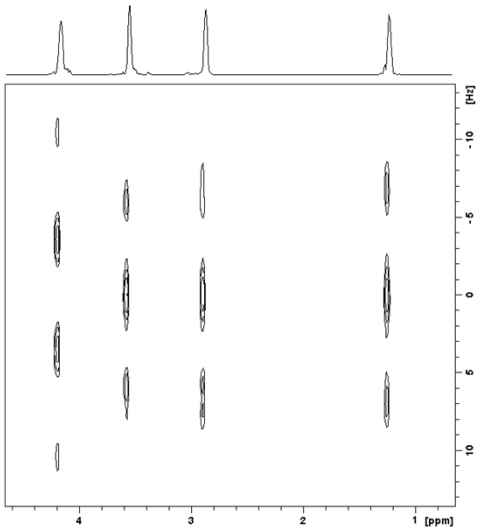
Conventional dimension: SW_1

$\Delta\nu_1$: Peak width - F_1

P. Giraudeau, S. Akoka, *J. Magn. Reson.* 192 (2008) 151

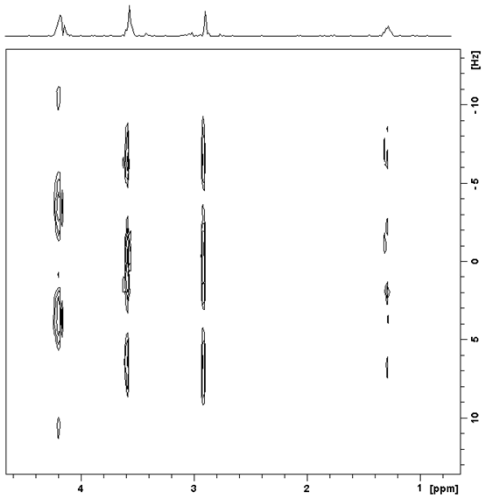
Increasing the analytical performance

Reducing diffusion effects – multi-echo encoding



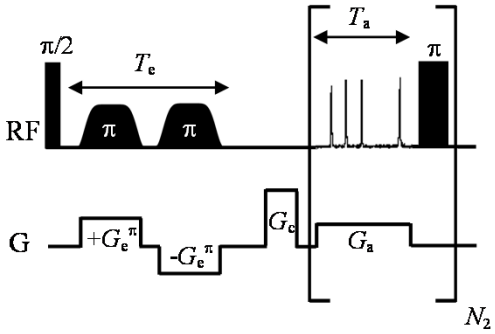
UF Jres, Te = 60 ms

Δν = 17 1 Hz



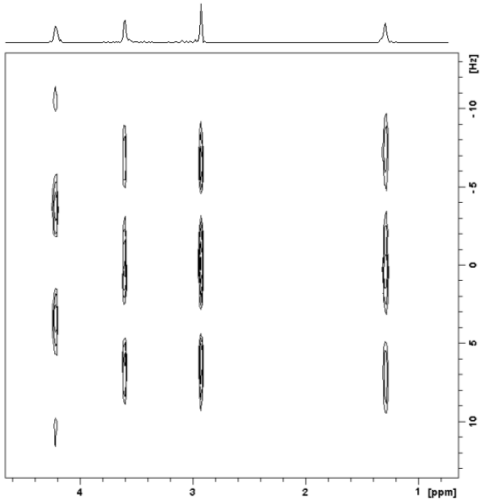
UF Jres, Te = 120 ms

Δν = 13 1 Hz



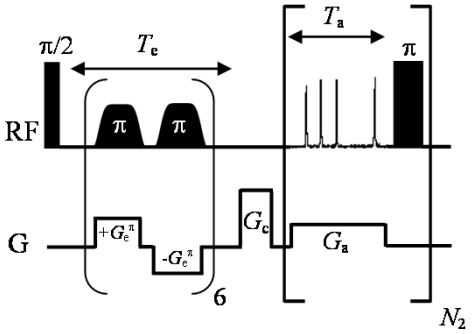
Better resolution

Sensitivity losses (diffusion)



UF Jres multi-écho, Te = 120 ms

Δν = 13 1 Hz



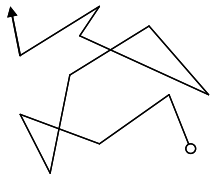
Better resolution

Better sensitivity

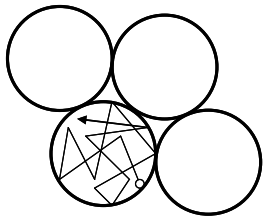
P. Giraudeau, S. Akoka, *J. Magn. Reson.* 2008

Increasing the analytical performance

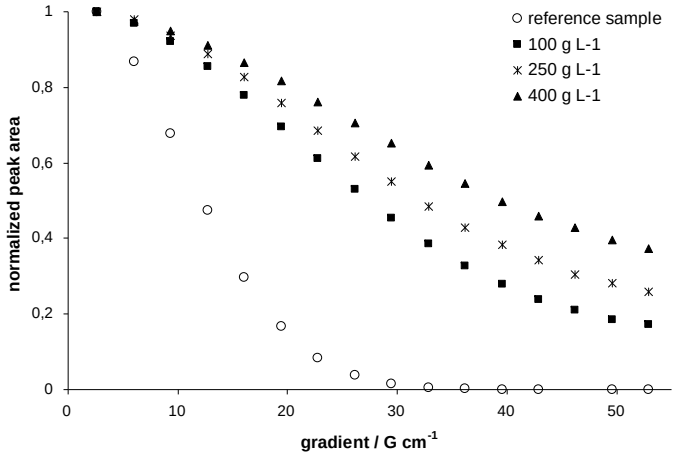
Reducing diffusion effects – sample preparation



Free diffusion

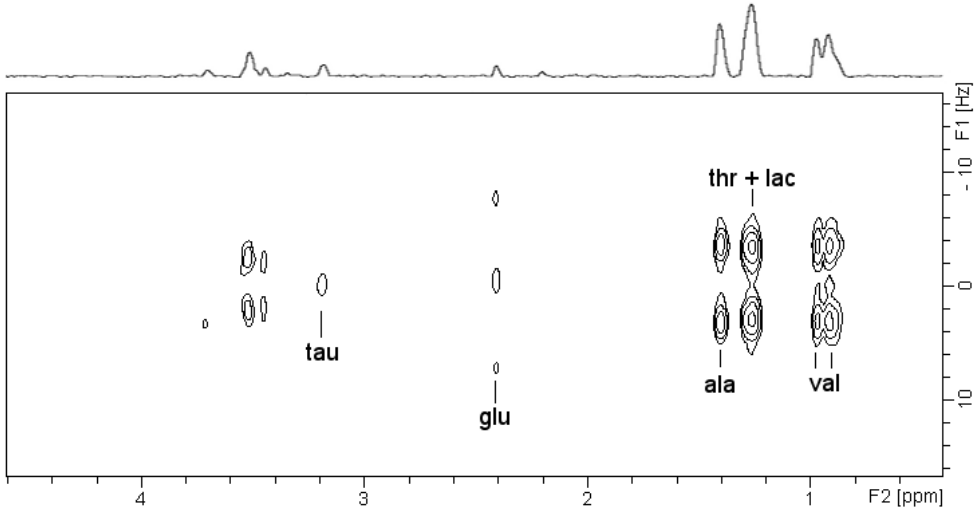


Restricted diffusion
In liposomes

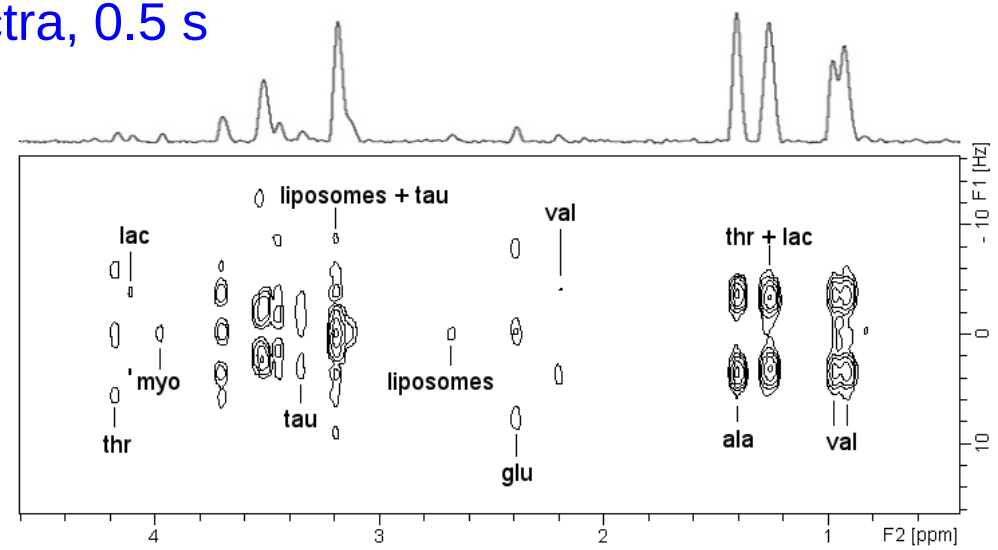


Diffusion curves - DSTE

UF Jres spectra, 0.5 s



Metabolic sample in D_2O

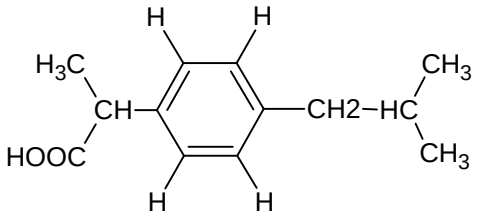


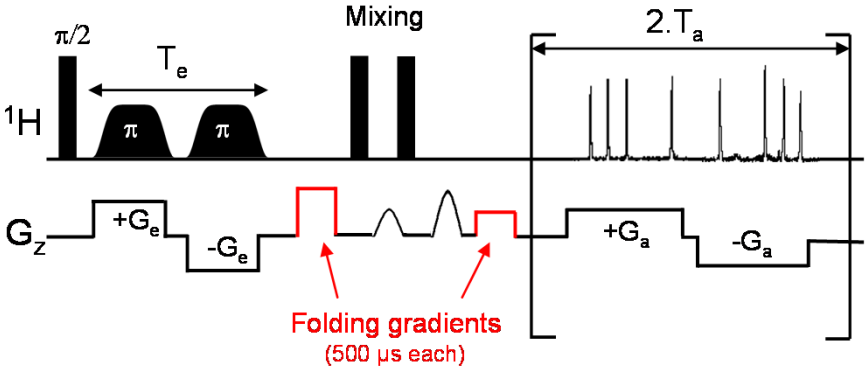
Metabolic sample in D_2O + liposomes

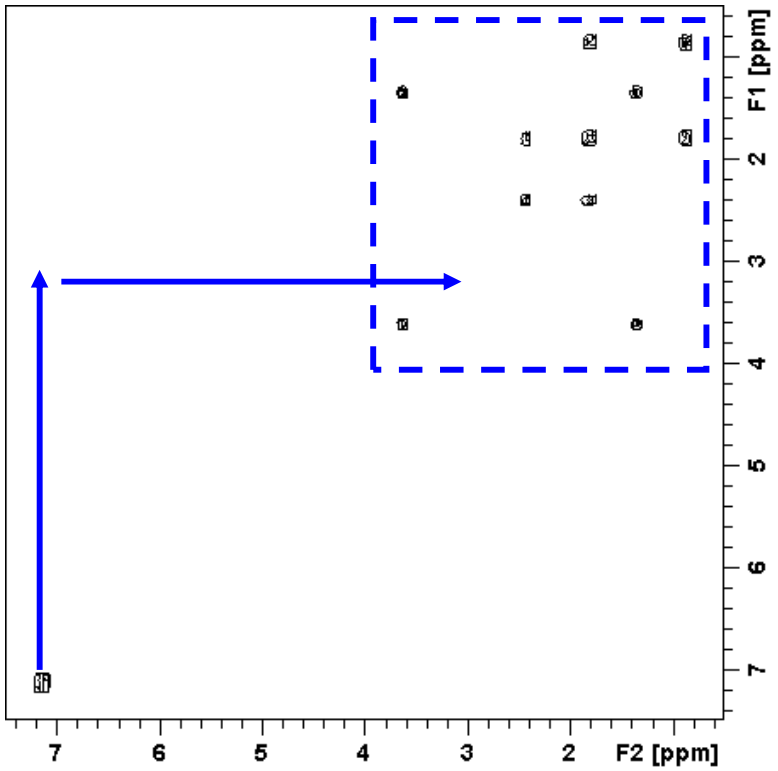
L. Rouger, D. Loquet, S. Massou, S. Akoka, P. Giraudeau, *ChemPhysChem* (2012)

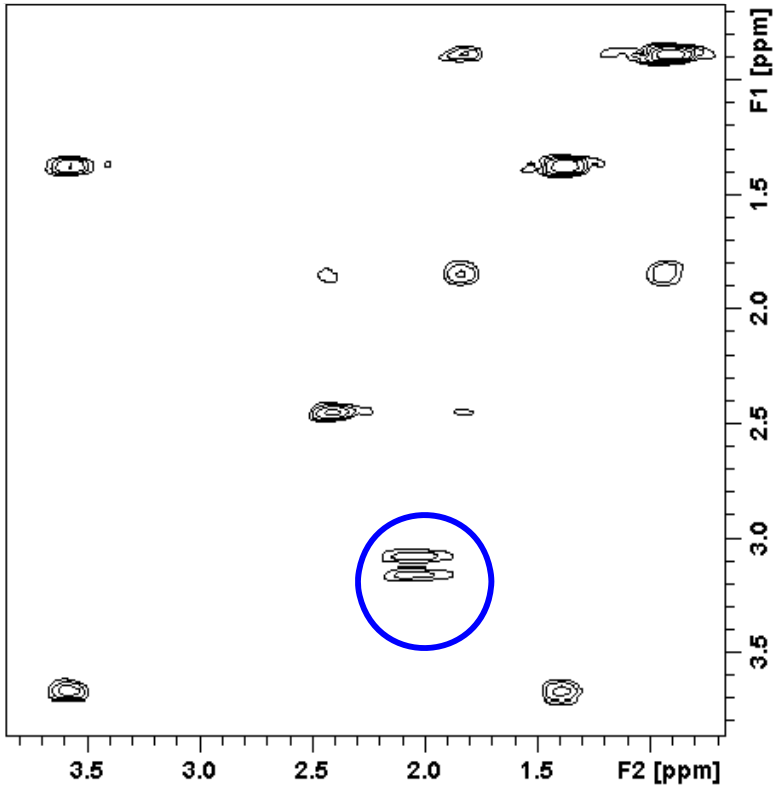
Increasing the analytical performance

Increasing the spectral range - folding









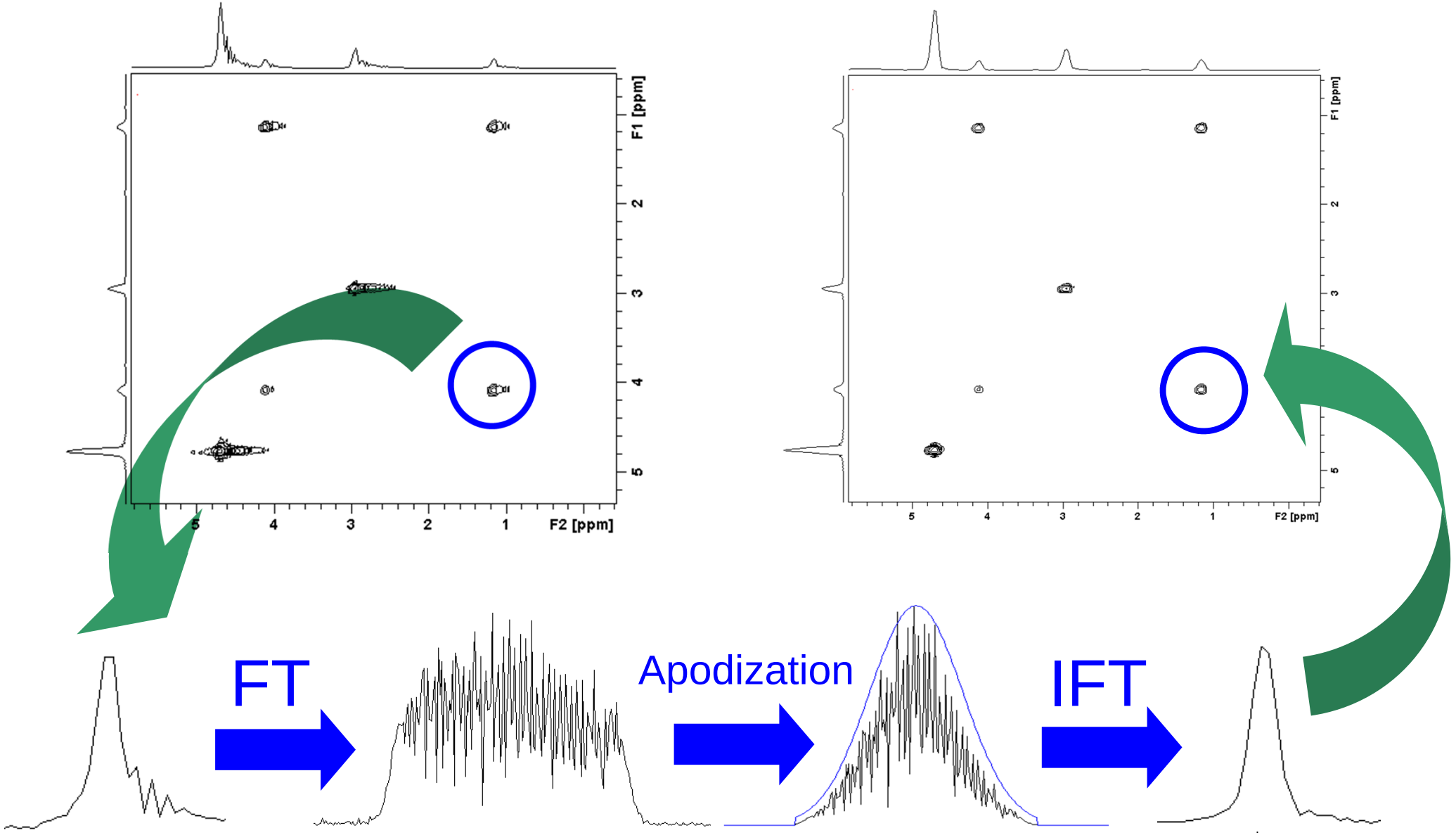
Conventional DQF-COSY – 13 min

Ultrafast folded DQF-COSY – 0.2 s

P. Giraudeau, S. Akoka, *J. Magn. Reson.* 2010

Increasing the analytical performance

Improving the lineshape by processing



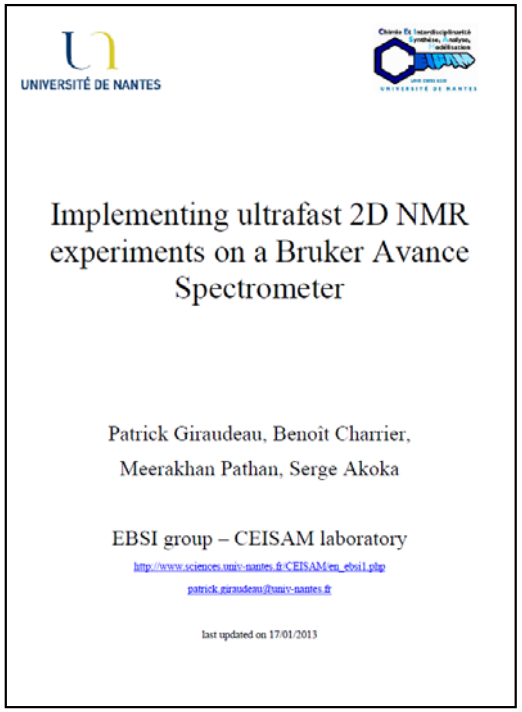
P. Giraudeau, S. Akoka, *Magn. Reson. Chem.* 2011

Increasing the analytical performance

Making UF 2D NMR more accesssible

Dedicated webpage including:

- -pulse sequences
- -implementation protocol
- -processing routine
- -web interface for parameter setting



UNIVERSITÉ DE NANTES

Implementing ultrafast 2D NMR experiments on a Bruker Avance Spectrometer

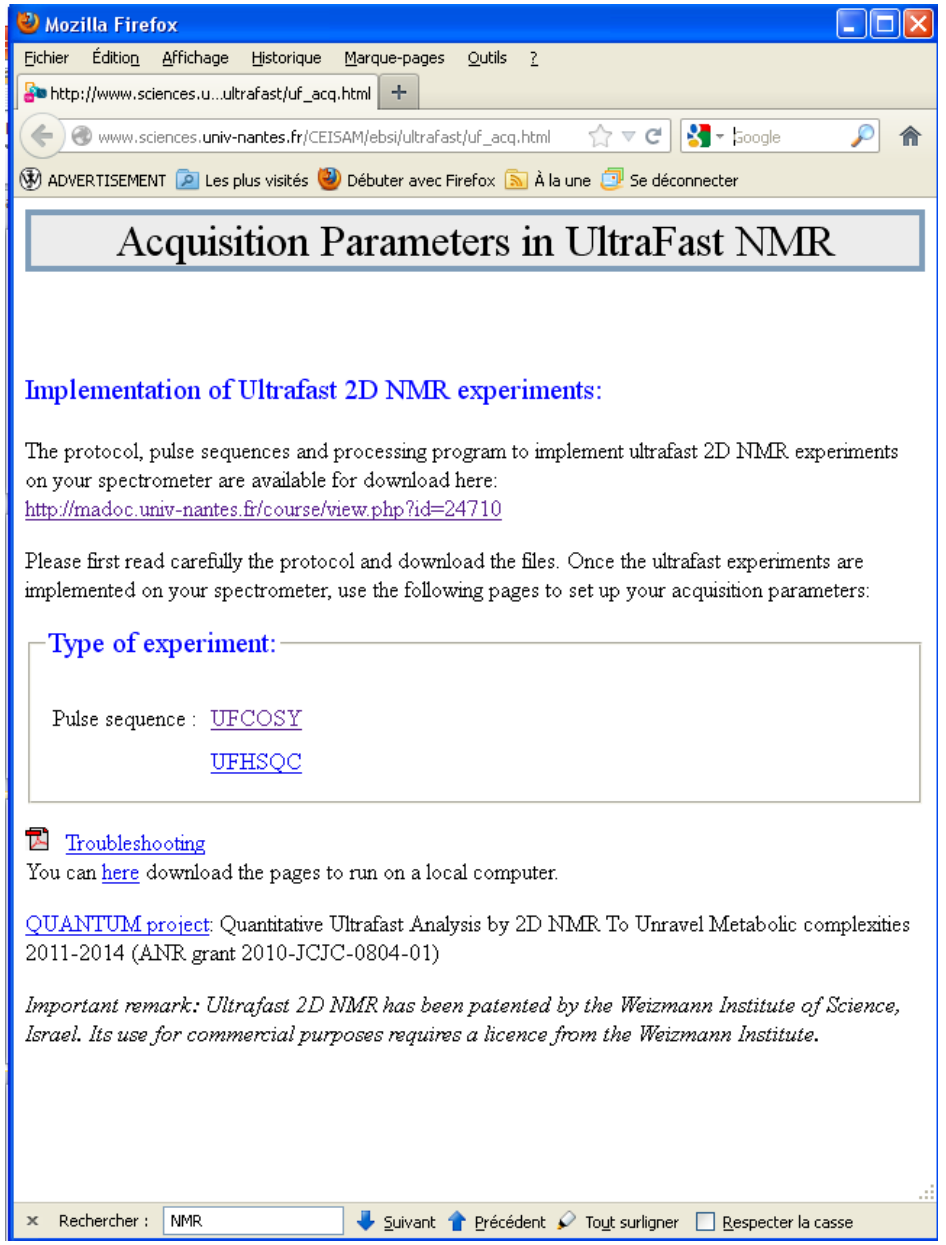
Patrick Giraudeau, Benoît Charrier, Meerakhan Pathan, Serge Akoka

EBSI group – CEISAM laboratory

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

patrick.giraudeau@univ-nantes.fr

last updated on 17/01/2013



Mozilla Firefox

Fichier Édition Affichage Historique Marque-pages Outils ?

http://www.sciences.u...ultrafast/uf_acq.html

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

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

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.

Rechercher : NMR Suivant Précédent Tout surligner Respecter la casse

M. Pathan, B. Charrier, I. Tea, S. Akoka, P. Giraudeau, *Magn. Reson. Chem* 51 (2013) 168

Increasing the analytical performance

2006 2007 2008 2009 2010 2011 2012 2013

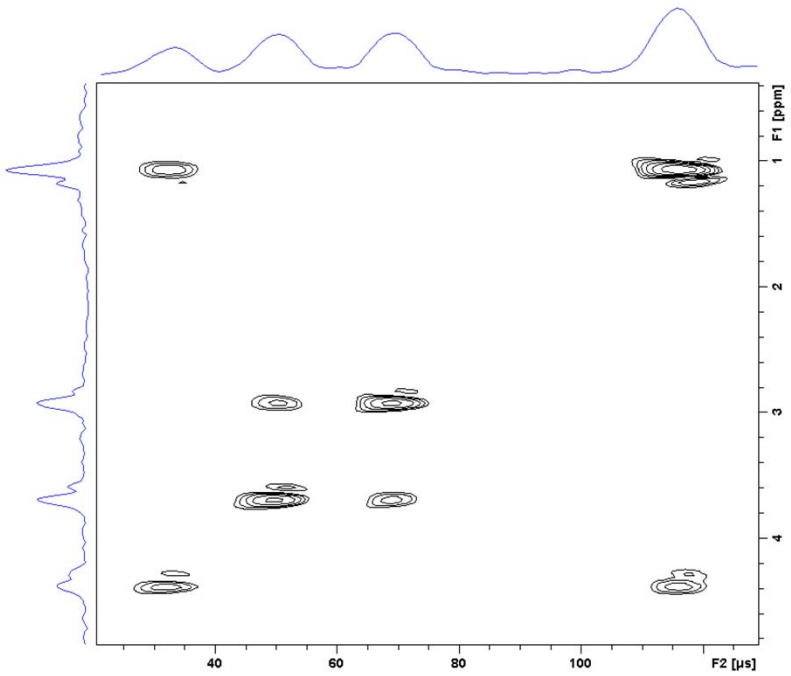
1st ultrafast experiment at CEISAM

Sensitivity and resolution improvements

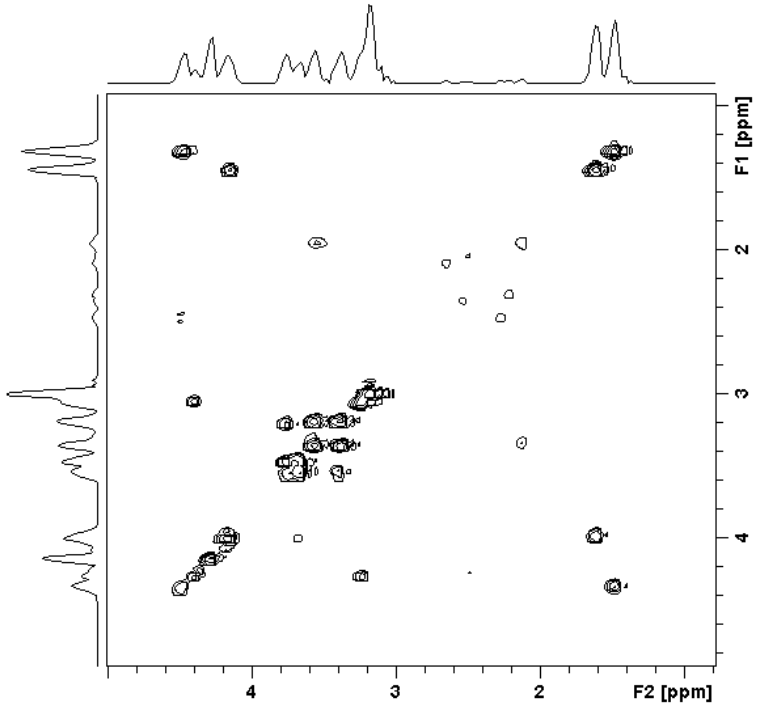
Spectral width improvements

Lineshape improvement

Automated acquisition and processing



UF COSY 0.1 s – model sample



UF COSY 0.1 s – metabolites

P. Giraudeau, S. Akoka, *Magn. Reson. Chem.* 2011

Applications to quantitative metabolomics

Context: NMR metabolomics

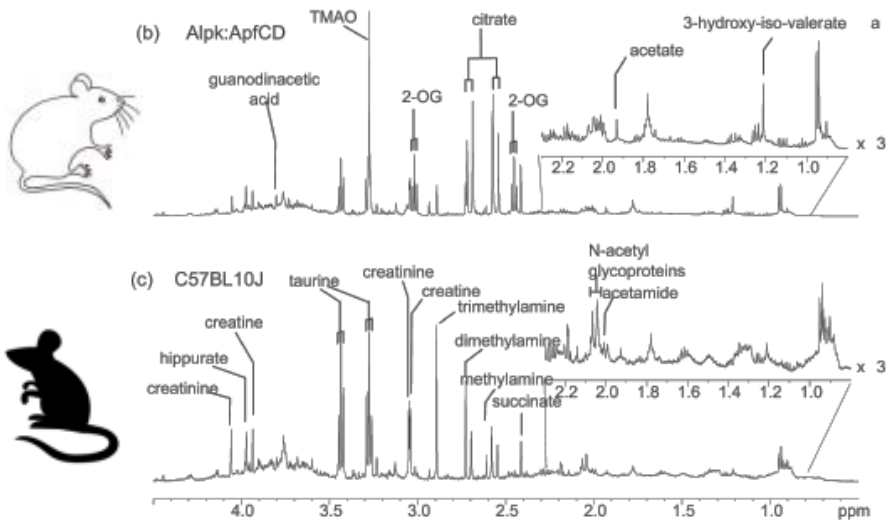
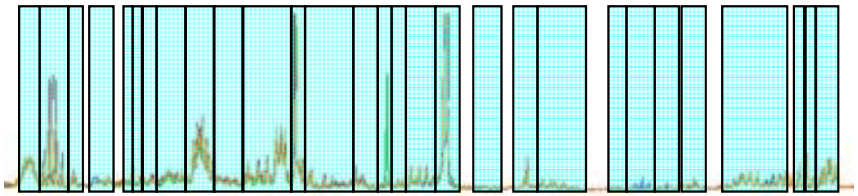
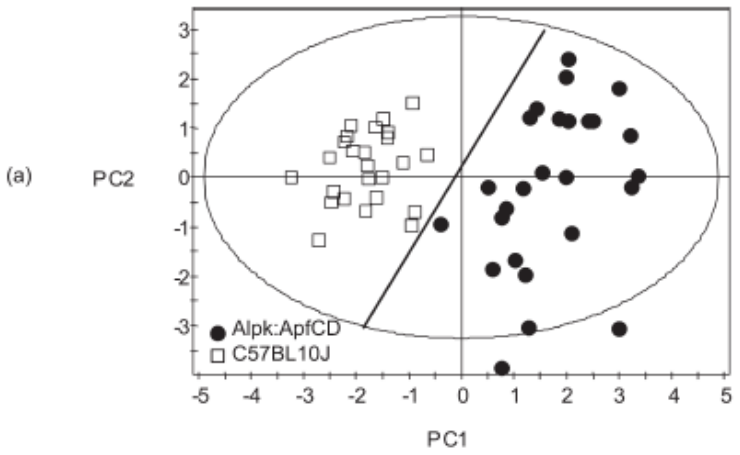


Figure 4. (a) PC scores plot derived from the 500 MHz ^{1}H NMR spectra of urine samples from (b) Alpk:ApfCD (white) mice and (c) C57BL10J (black) mice. 2-OG, 2-oxoglutarate; TMAO, trimethylamine-*N*-oxide

Spectral decomposition (bucketing)



Statistical treatment



Individual quantification is difficult due to overlap

Bollard *et al. NMR Biomed* 18 (2005) 143-162

Applications to quantitative metabolomics

Ultrafast quantitative 2D NMR ?

Anal. Chem. **2009**, *81*, 479–484

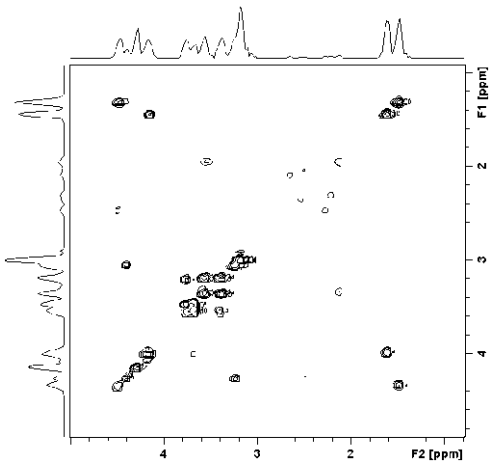
Evaluation of Ultrafast 2D NMR for Quantitative Analysis

Patrick Giraudeau,* Gérald S. Remaud, and Serge Akoka

Université de Nantes, CNRS, CEISAM UMR 6230, B. P. 92208, 2 Rue de la Houssinière, F-44322 Nantes Cedex 03, France

→ **High repeatability and linearity**

What about metabolic mixtures ?



UF COSY 0.1 s
metabolites

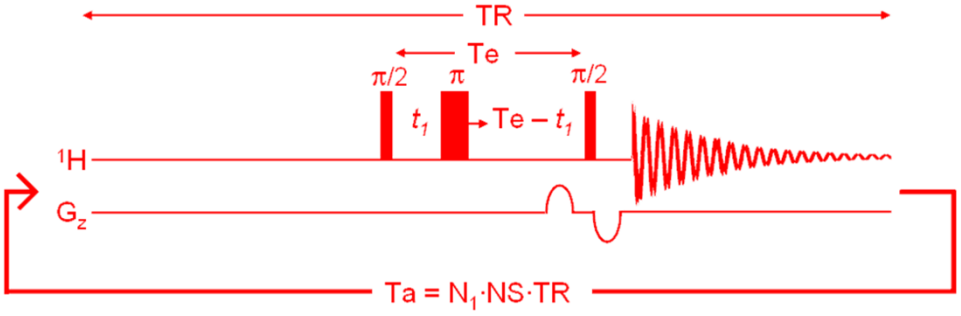
LOQ (1 scan) = 10 mM (500 MHz cryo)

↓

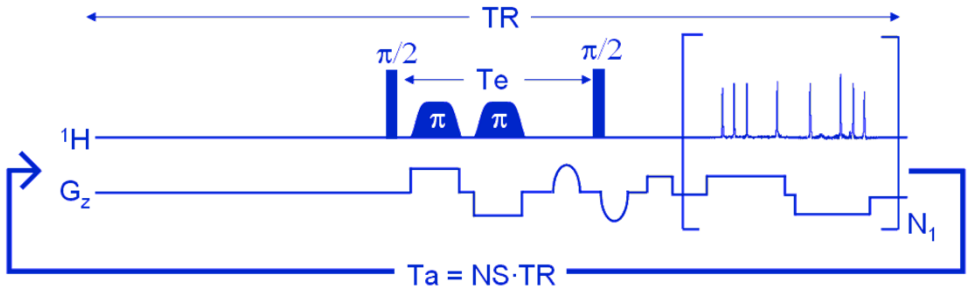
Signal averaging necessary !!!

Applications to quantitative metabolomics

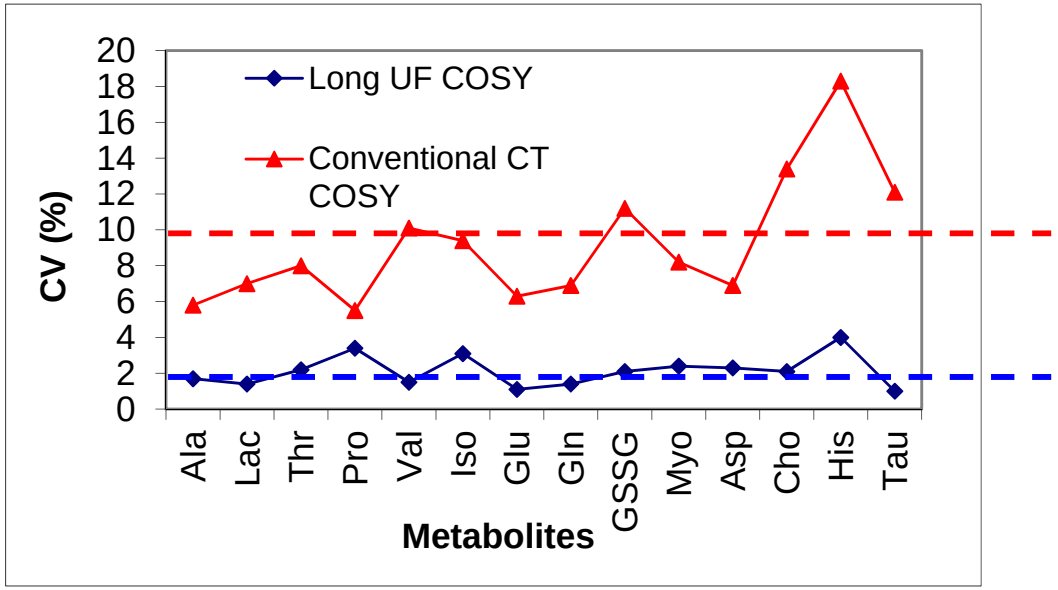
« Long-ultrafast » vs conventional 2D NMR



Conventional CT COSY



« Long ultrafast COSY »



Repeatability of
Conventional CT COSY
vs
« Long ultrafast COSY »

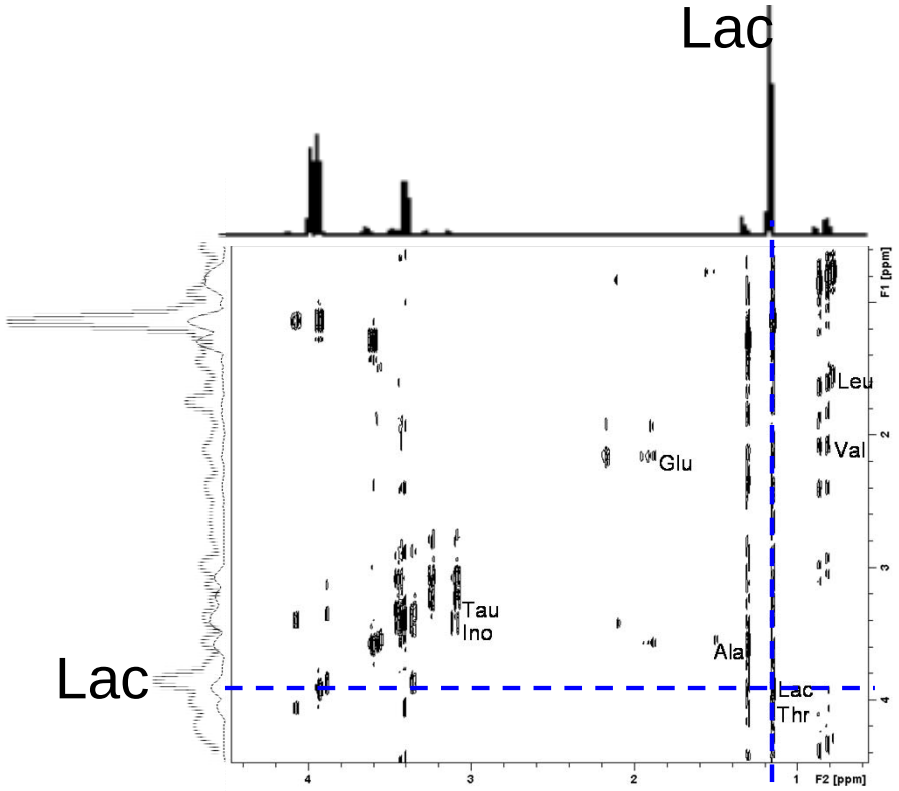
Better precision

A. Le Guennec, I. Tea, I. Antheaume, E. Martineau, B. Charrier, M. Pathan, S. Akoka, P. Giraudeau, *Anal. Chem.* 84 (2012) 10831

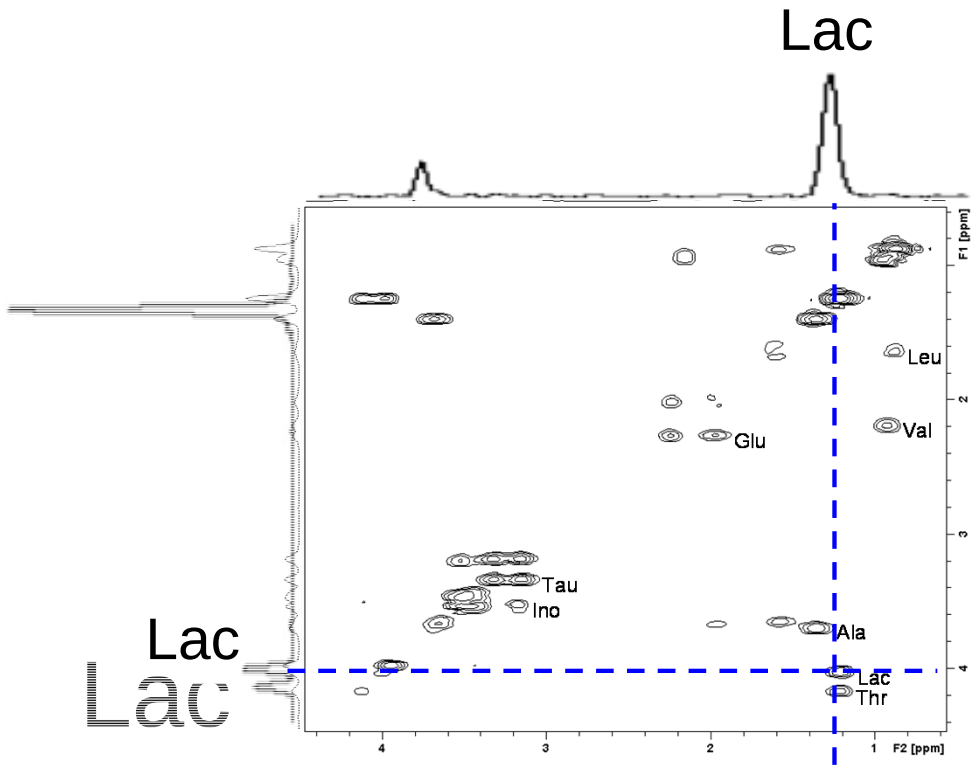
Applications to quantitative metabolomics

Why is long UF more repeatable ?

UF 2D NMR is more immune to hardware instabilities
This is particularly true in quantitative conditions



Conv. COSY, 34 min
NS = 1, N_1 = 64



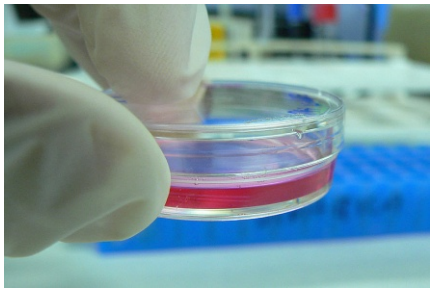
« Long ultrafast » COSY
34 min
NS = 64

M. Pathan, S. Akoka, I. Tea, B. Charrier, P. Giraudeau, *Analyst* 2011.

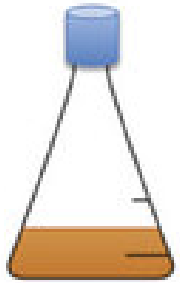
Applications to quantitative metabolomics

Application to biological samples

1. Growth of various breast cancer cell lines

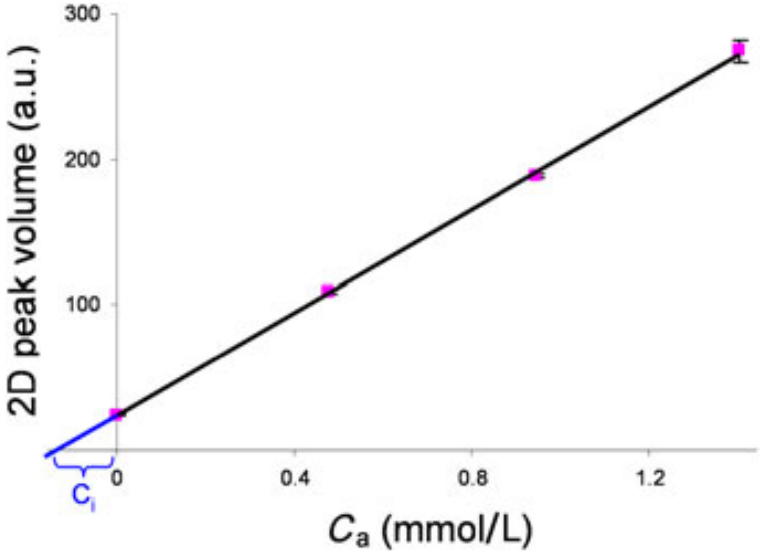


2. Optimized metabolite extraction



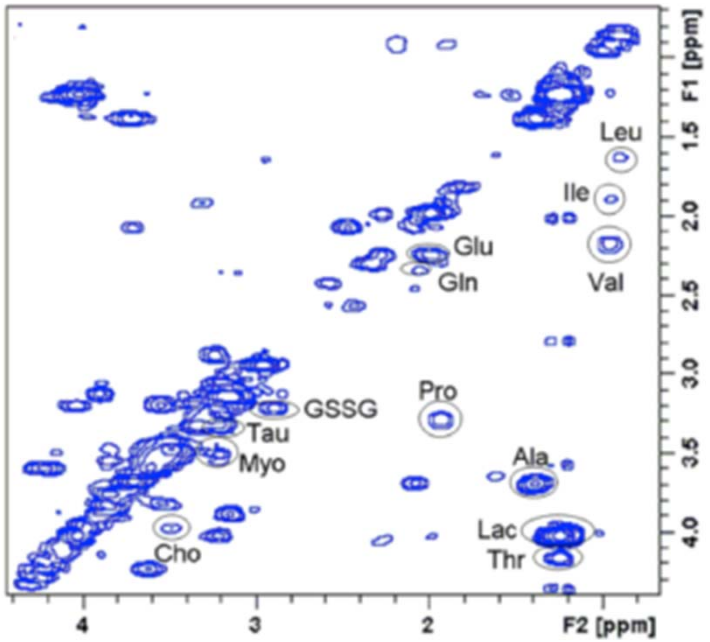
E. Martineau, I. Tea, P. Giraudeau, S. Akoka, *Anal. Bioanal. Chem.* 2011

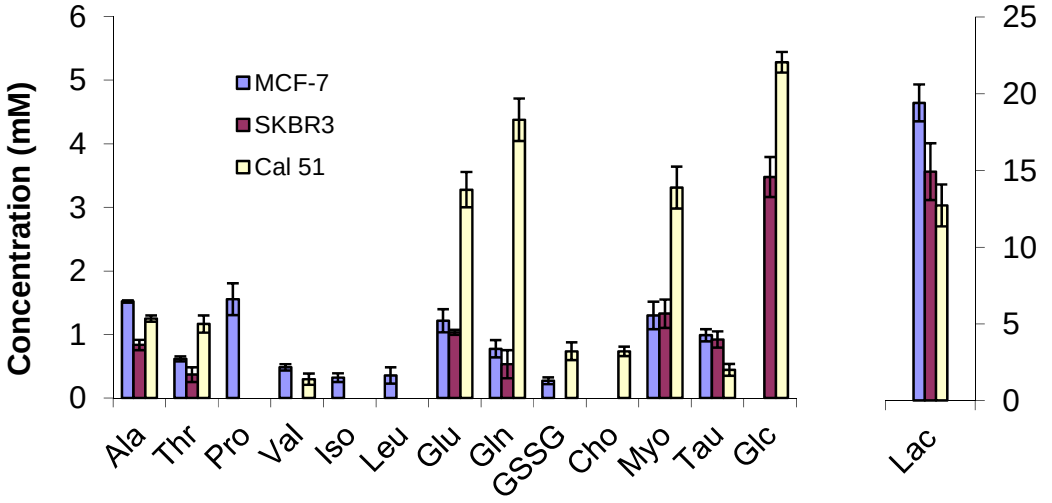
3. NMR quantification with standard addition procedure



Applications to quantitative metabolomics

Application to biological samples



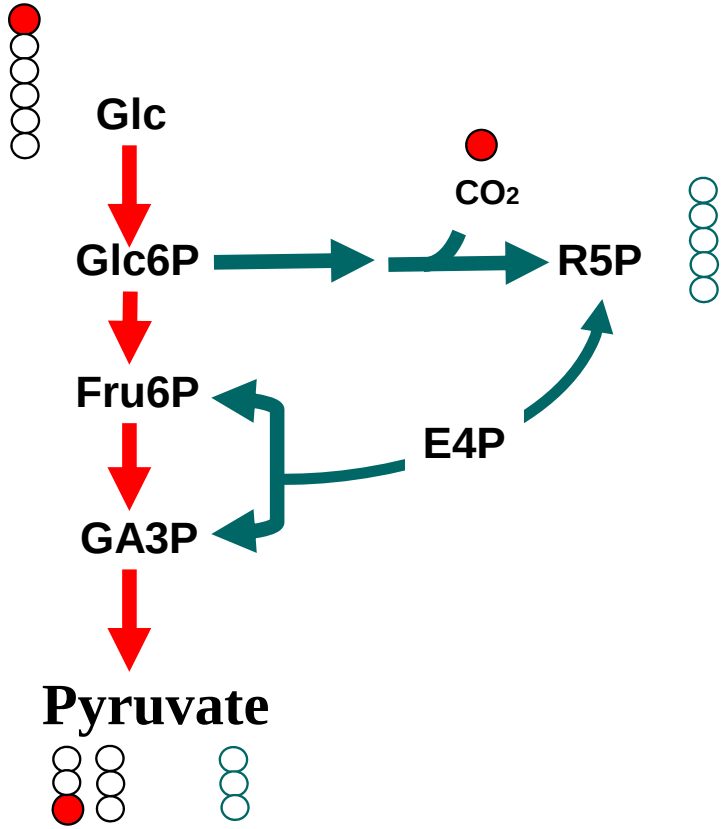


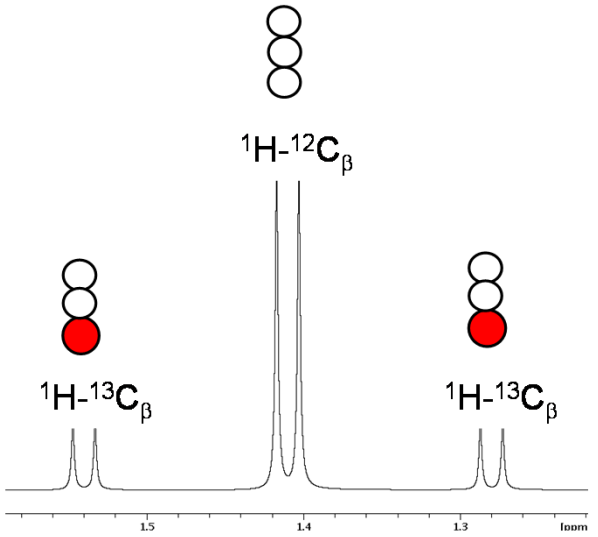
- Comparison of 3 breast cancer cell lines
- 20 min / spectrum
- Standard addition procedure

A. Le Guennec, I. Tea, I. Antheaume, E. Martineau, B. Charrier, M. Pathan, S. Akoka, P. Giraudeau, *Anal. Chem.* 84 (2012) 10831

Applications in fluxomics

Context : measurement of ^{13}C isotopic enrichments in fluxomics

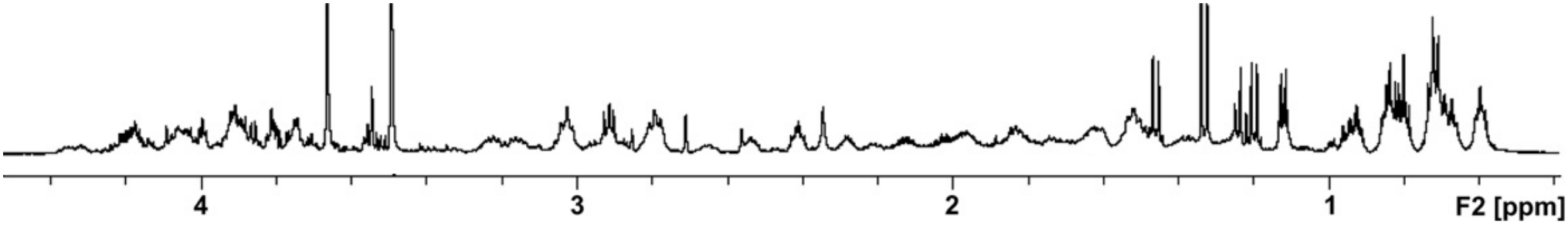




Simple cases: 1D ^{1}H NMR

Site-specific ^{13}C isotopic enrichment (IE) = $S^{satellites}/S^{total}$

Limitations of 1D NMR – biological mixtures

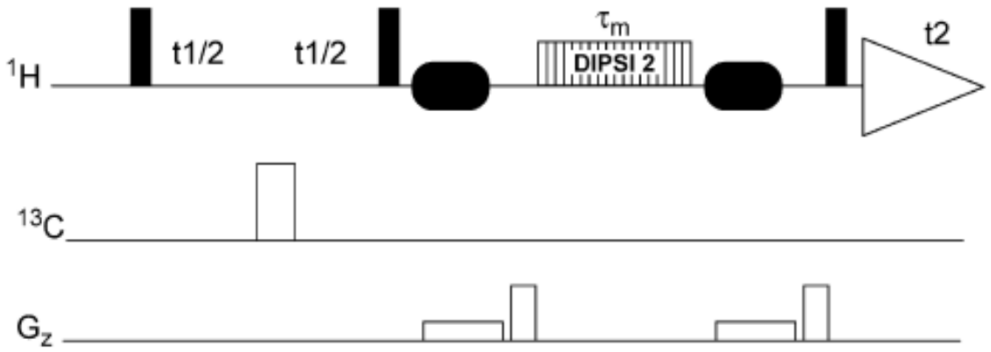


biomass hydrolysate from E. coli cells
grown on 20% [U-^{13}C]-glucose +80% [1-^{13}C]-glucose

Measurement of specific isotopic enrichments
IMPOSSIBLE

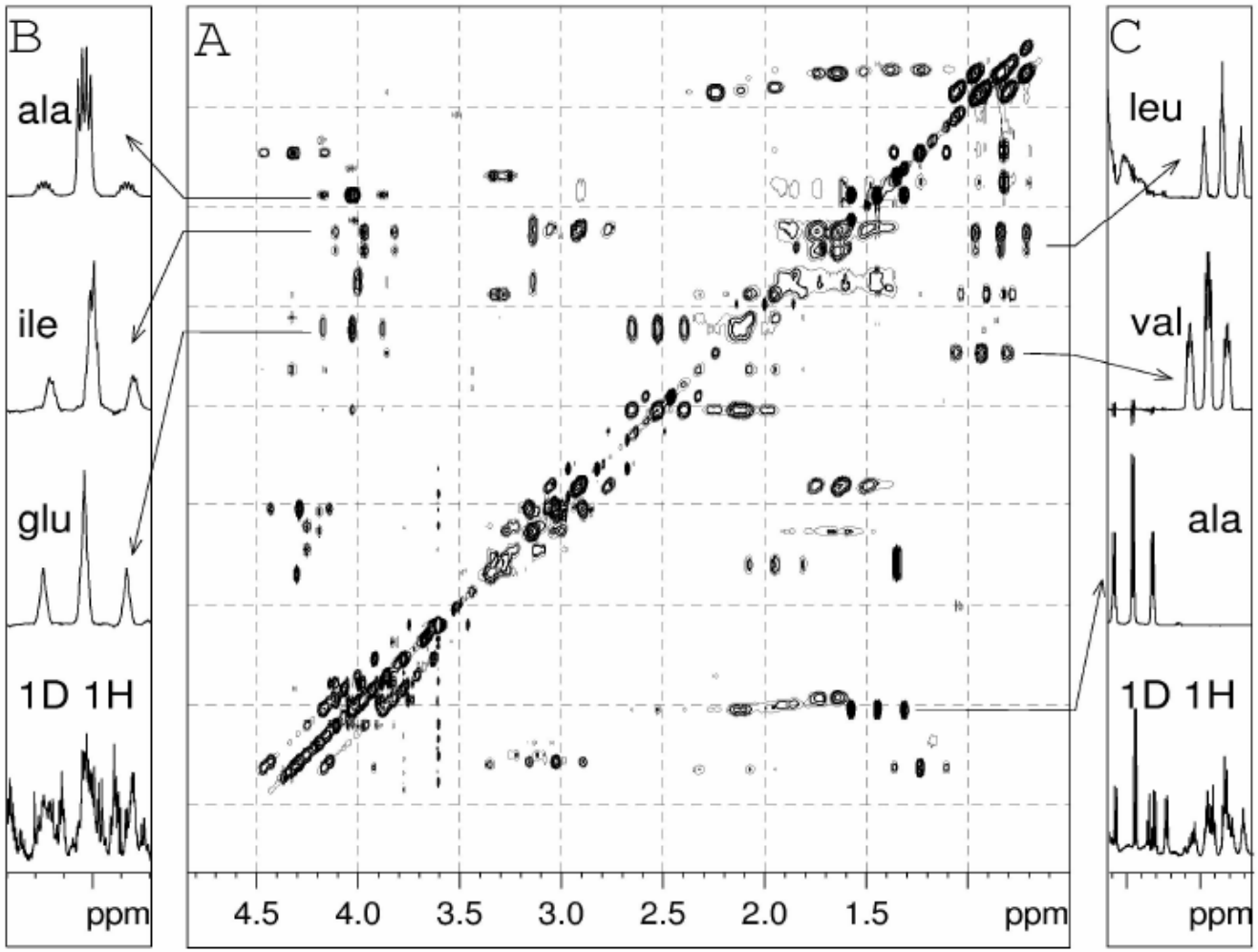
2D NMR

Applications in fluxomics



Conv. 2D ZQF TOCSY
^{13}C decoupling in F_1

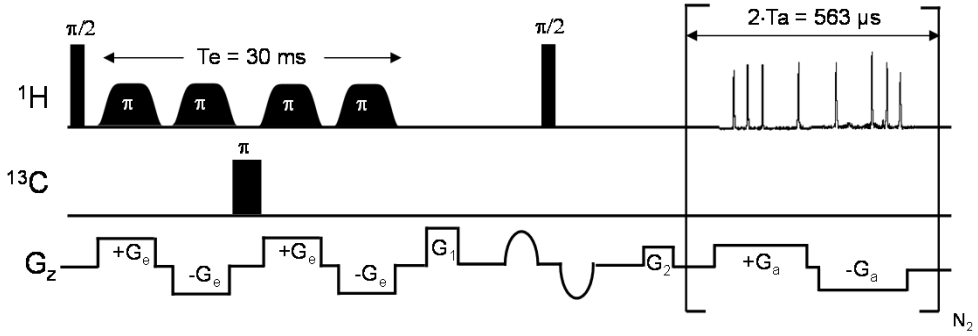
Acquisition time:
5-10h
in quantitative
conditions
($TR \geq 5 \cdot T_1^{max}$)

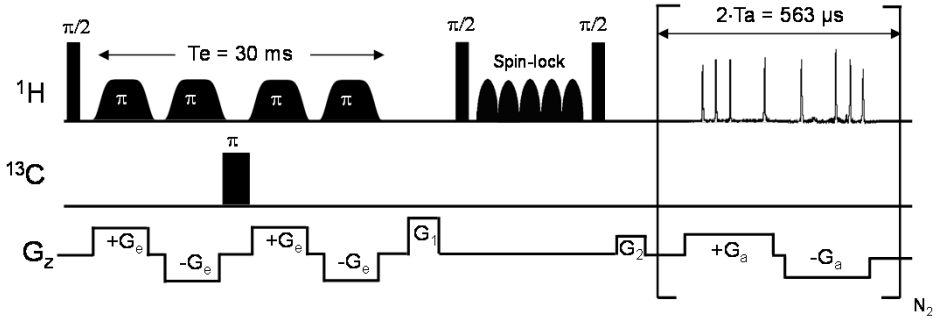


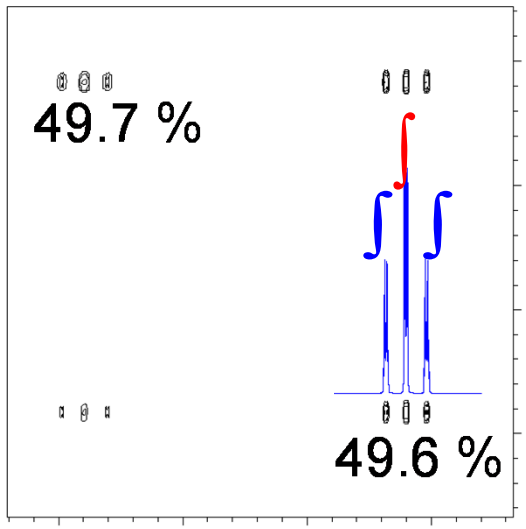
S. Massou *et* al, Metab. Eng. 2007

Applications in fluxomics

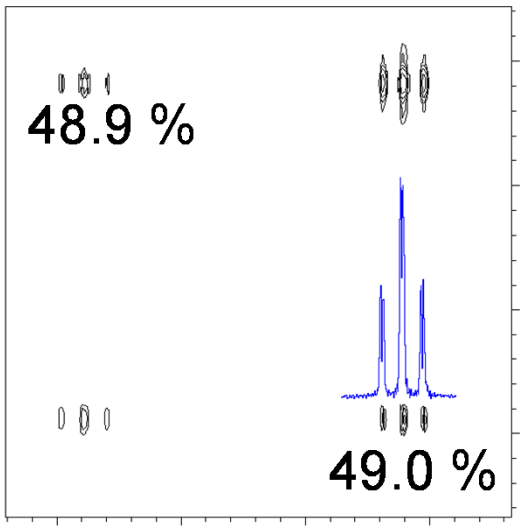
Ultrafast COSY and zTOCSY



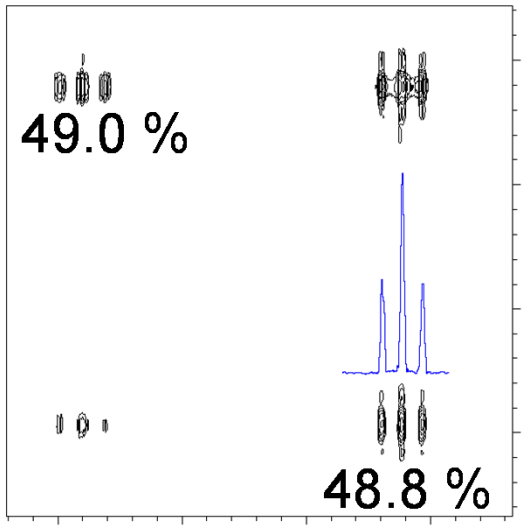




Conv. zTOCSY, 2.5 h



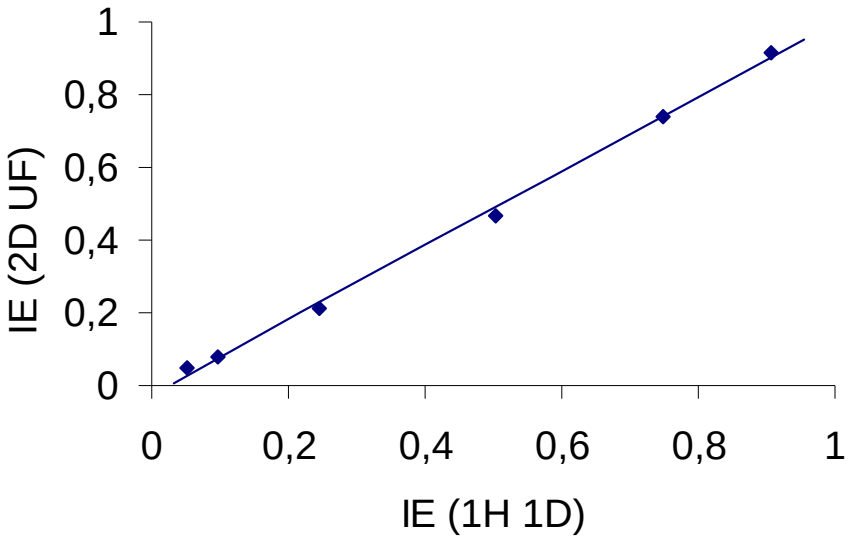
UF zTOCSY, 3 s



UF COSY, 3 s

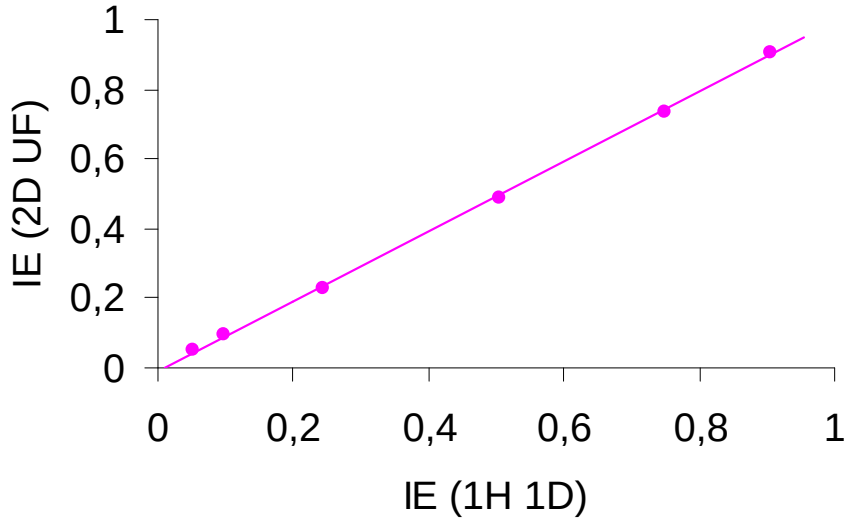
Mixture of variously labeled alanines, 400 MHz

Analytical performance – ^{13}C glucose samples



UF TOCSY

y = 1.018x + 0.023
R^2=0,998
Precision 3.7 %



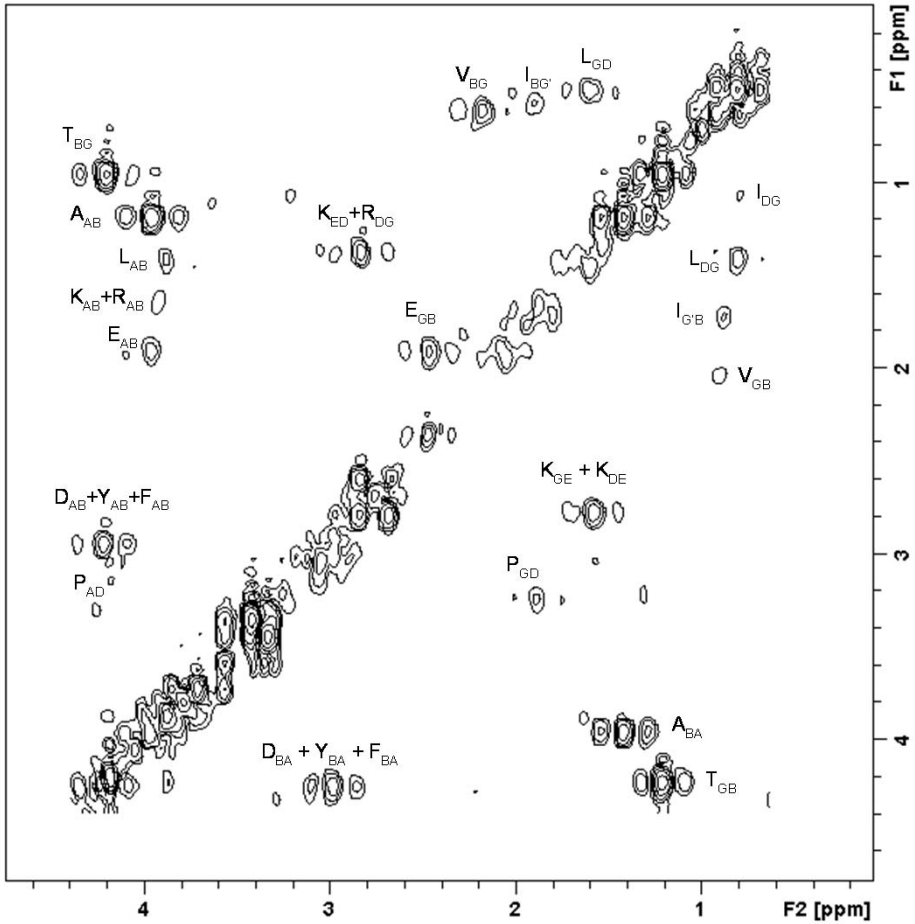
UF COSY

y = 1.000x + 0.008
R^2=0,999
Precision 3.0 %

Application to a biological sample

COSY UF

Ta = 40 s



Biomass hydrolyzate (*E. coli*)
Grown on
50 % [U-13C]-glucose
and 50 % *n.a.* glucose

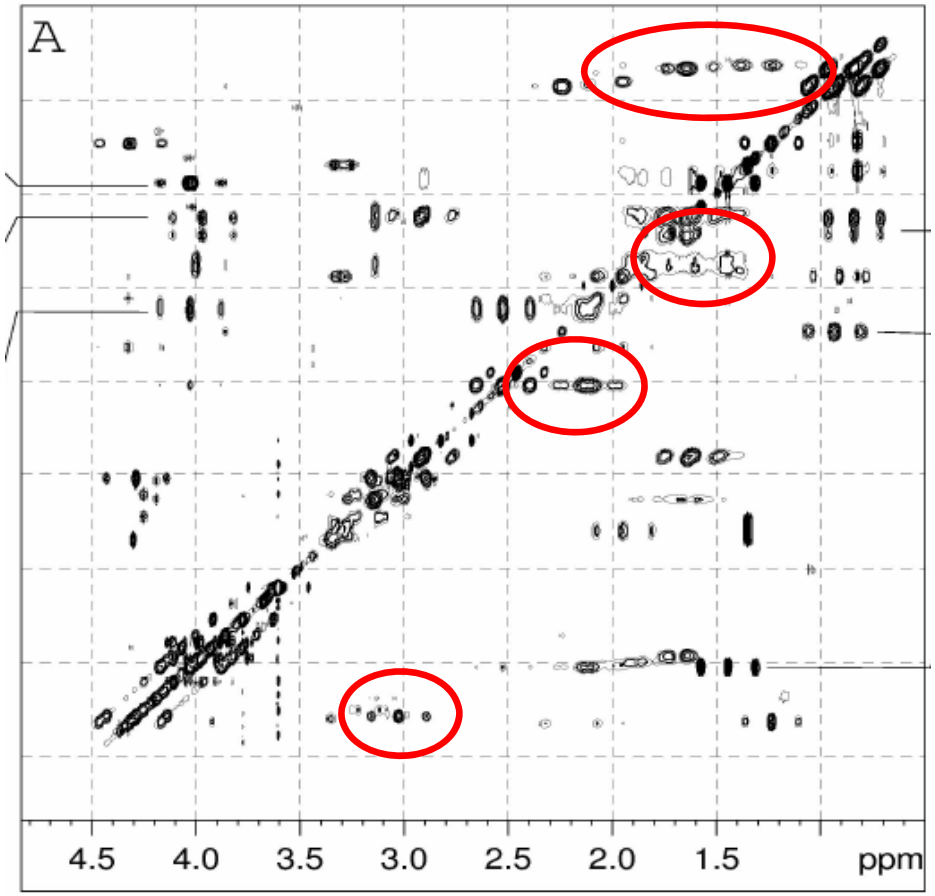
Isotopic enrichments measured from 2D spectra

	TOCSY Conv.	TOCSY UF	COSY UF
A_{AB}	44.5	45.7	41.2
A_{BA}	46.8	51.3	50.7
E_{AB}	47.9	44.7	45.2
L_{AB}	48.3	43.8	43.4
L_{DG}	47.9	42.7	45.1
P_{AD}	47.0	43.1	42.0
T_{BG}	26.9	23.0	25.8
T_{GB}	24.5	23.3	24.7
$V_{GG'}$	47.9	42.3	47.6

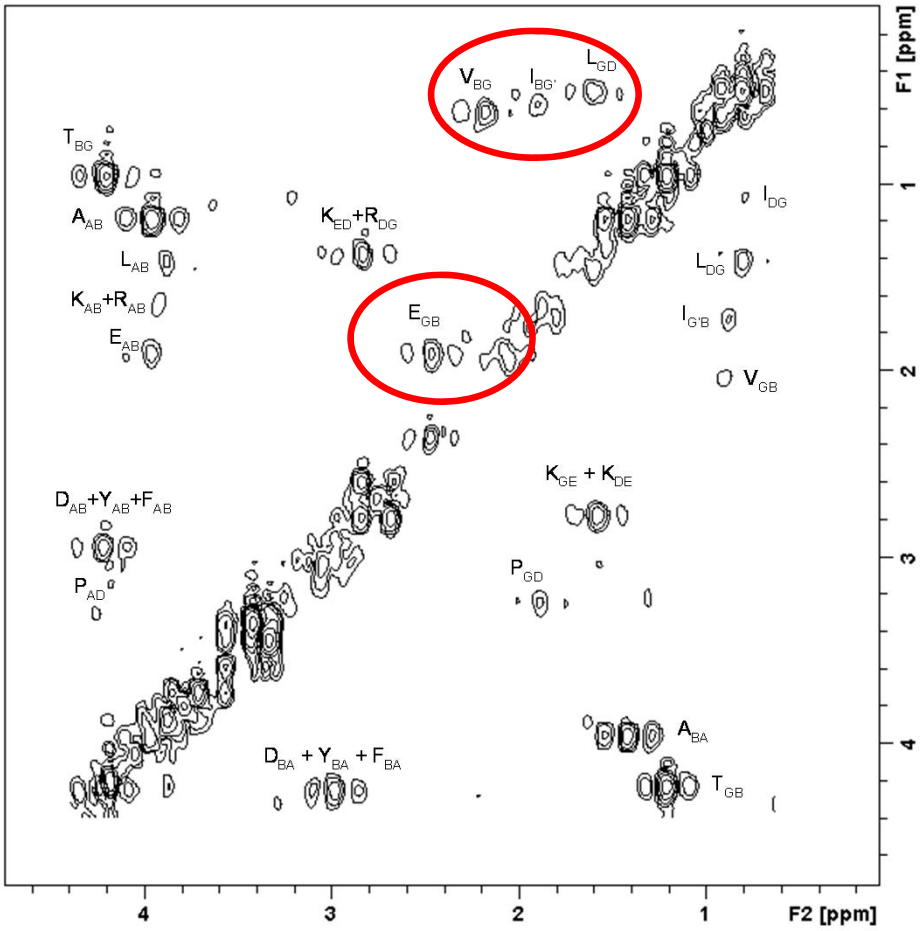
P. Giraudeau, S. Massou, Y. Robin, E. Cahoreau, J.C. Portais, S. Akoka *Anal. Chem.* 2011.

Limitations of 2D NMR

Conventional 2D

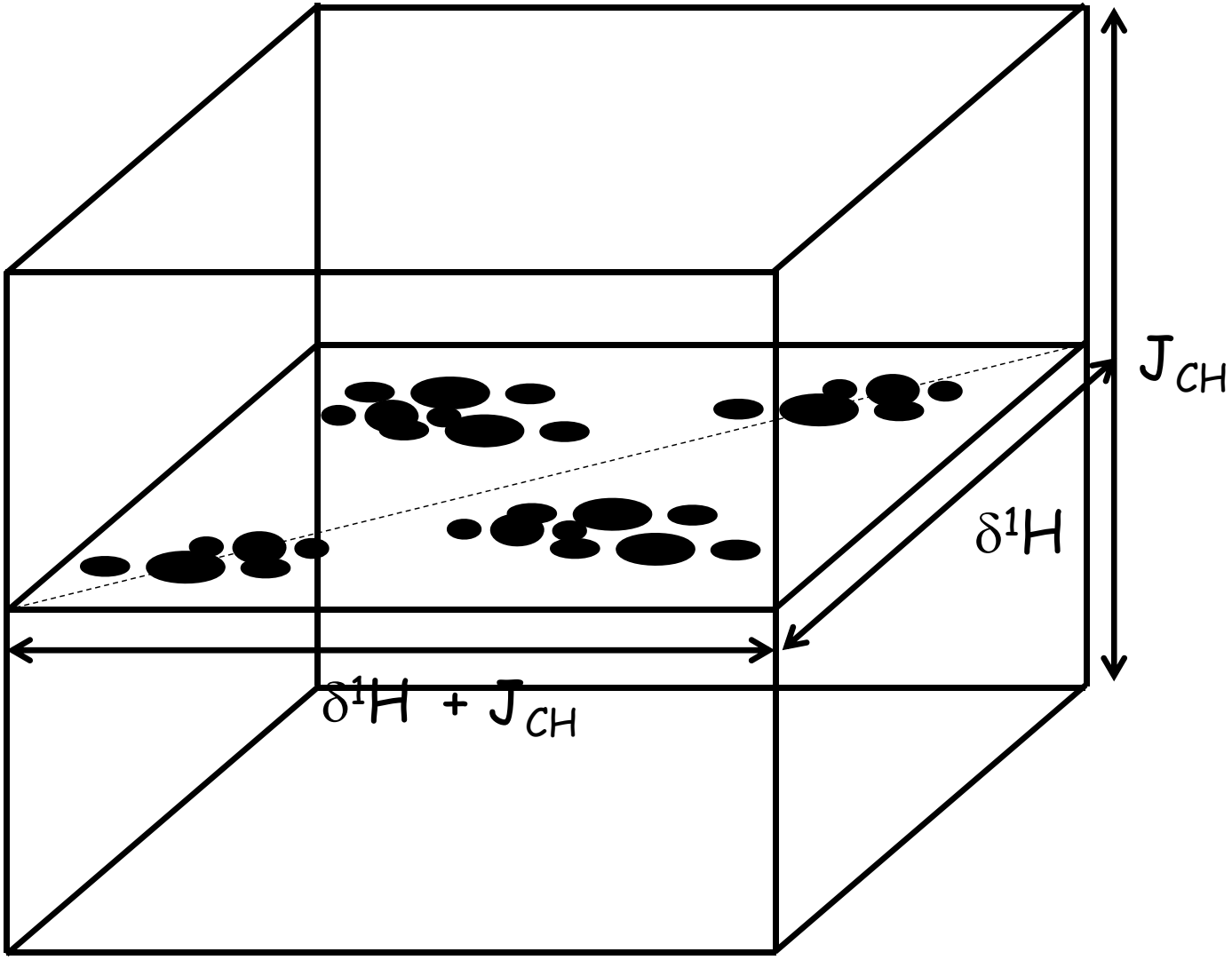


2D UF



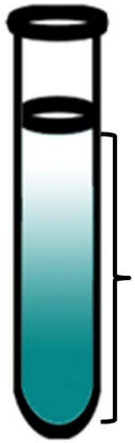
Peak overlaps still prevent accurate quantification

3D NMR

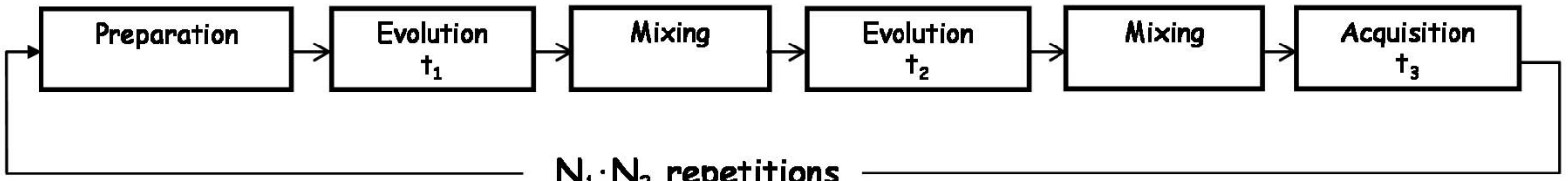


Applications in fluxomics

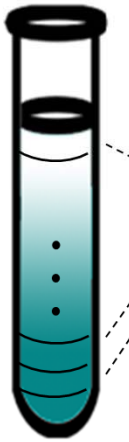
3D NMR



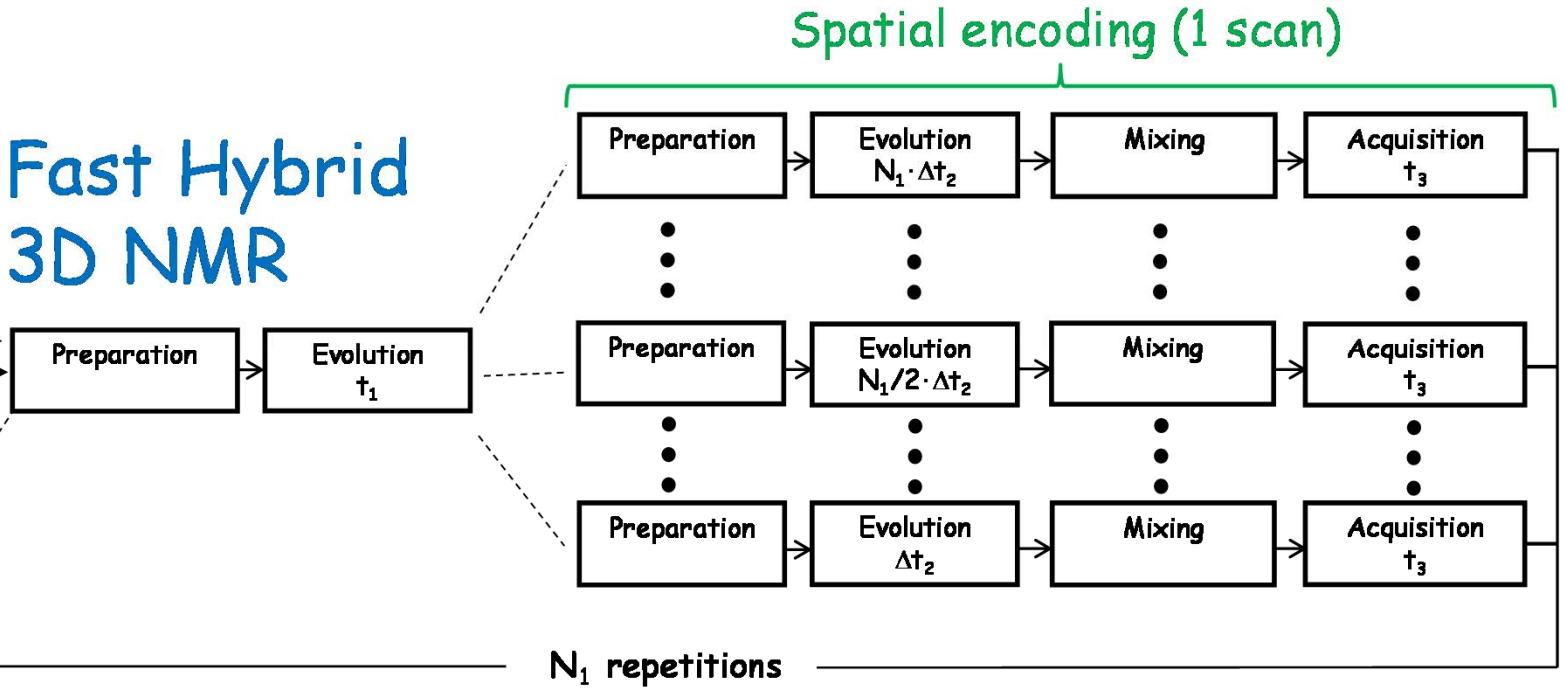
Conventional 3D NMR



$$T_a = TR \cdot Ns \cdot N_1 \cdot N_2$$



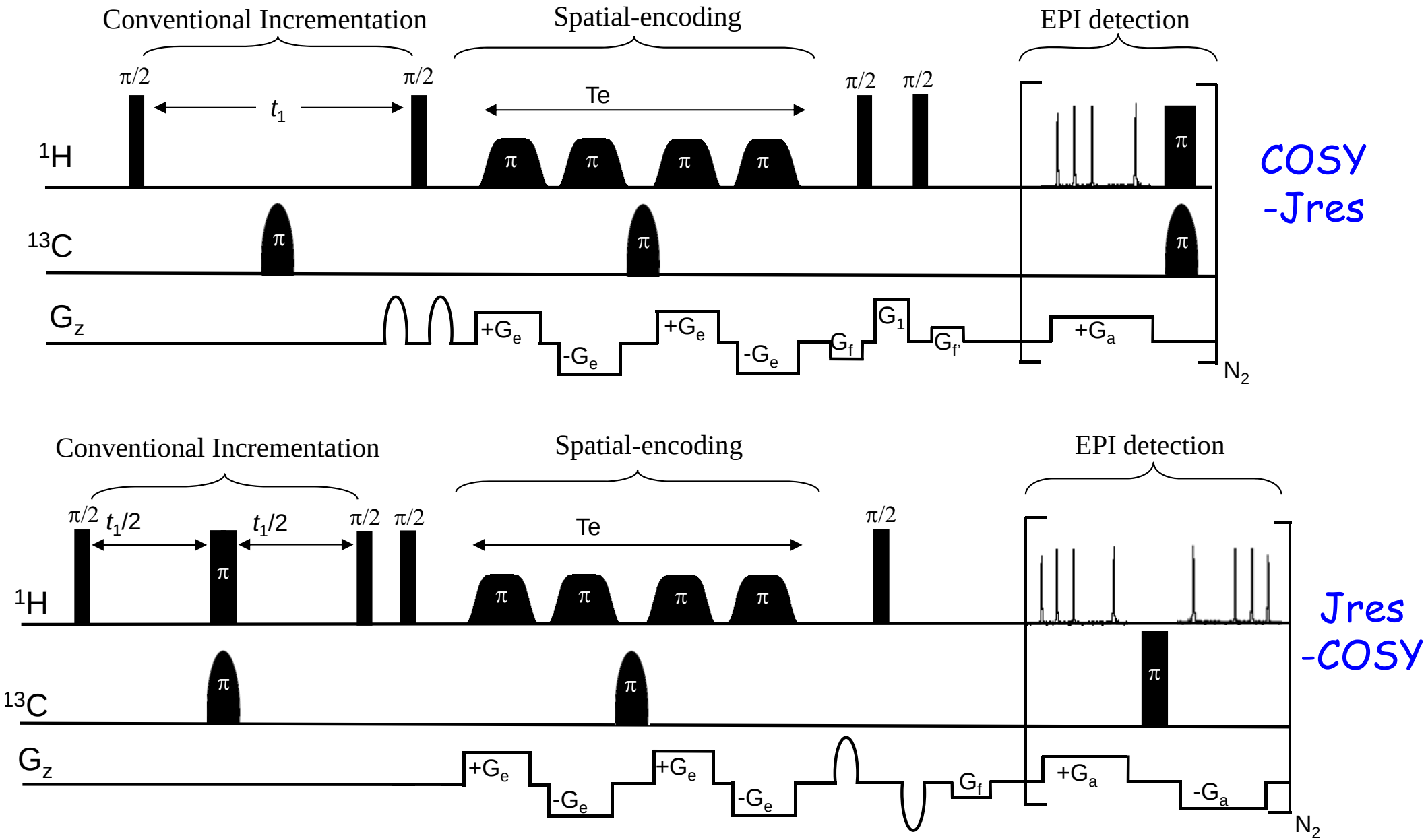
Fast Hybrid 3D NMR



$$T_a = TR \cdot Ns \cdot N_1$$

Applications in fluxomics

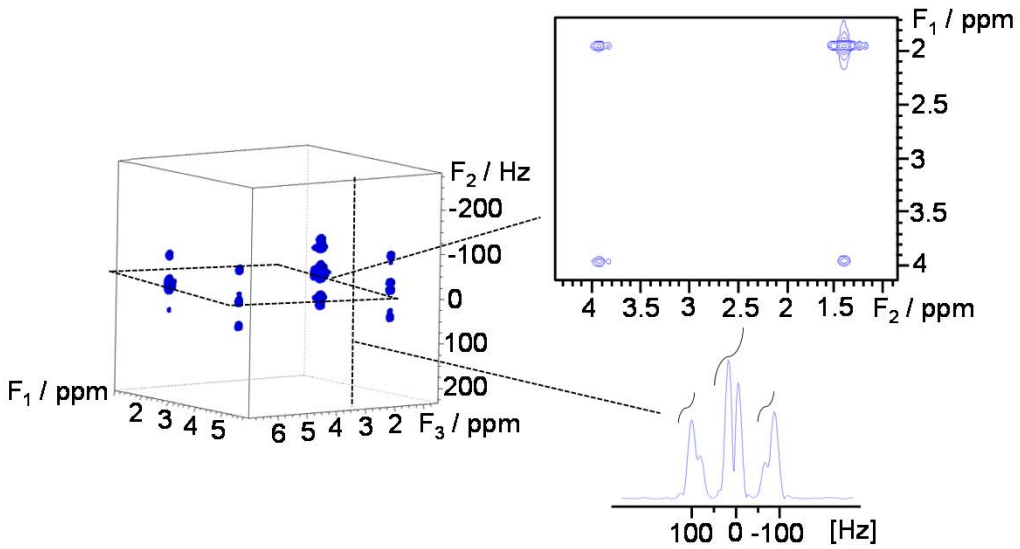
Fast-Hybrid 3D NMR



P. Giraudeau, E. Cahoreau, S. Massou, M. Pathan, J.-C. Portais, S. Akoka, *ChemPhysChem* 2012

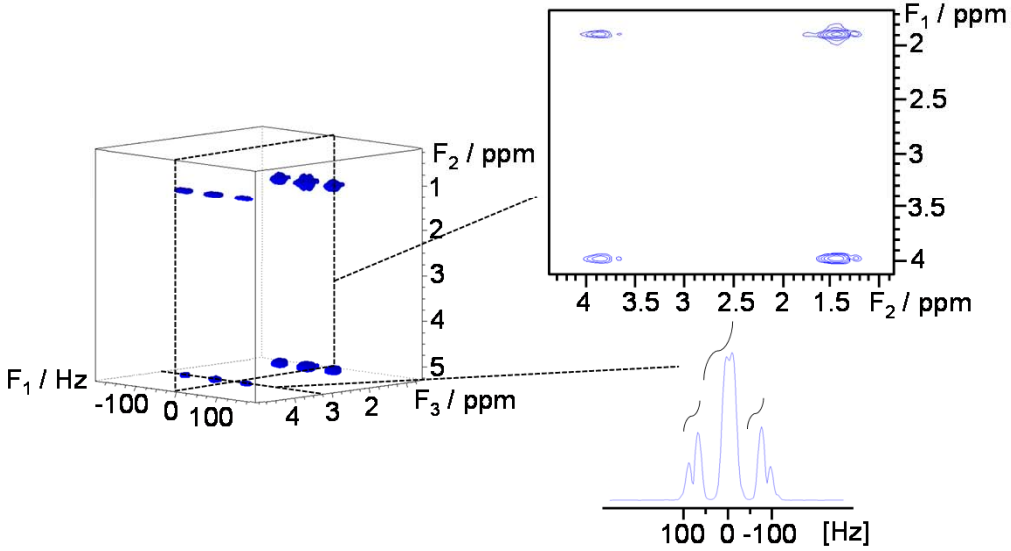
Applications in fluxomics

Fast-Hybrid 3D NMR (12 min, labeled alanine sample)



COSY-Jres

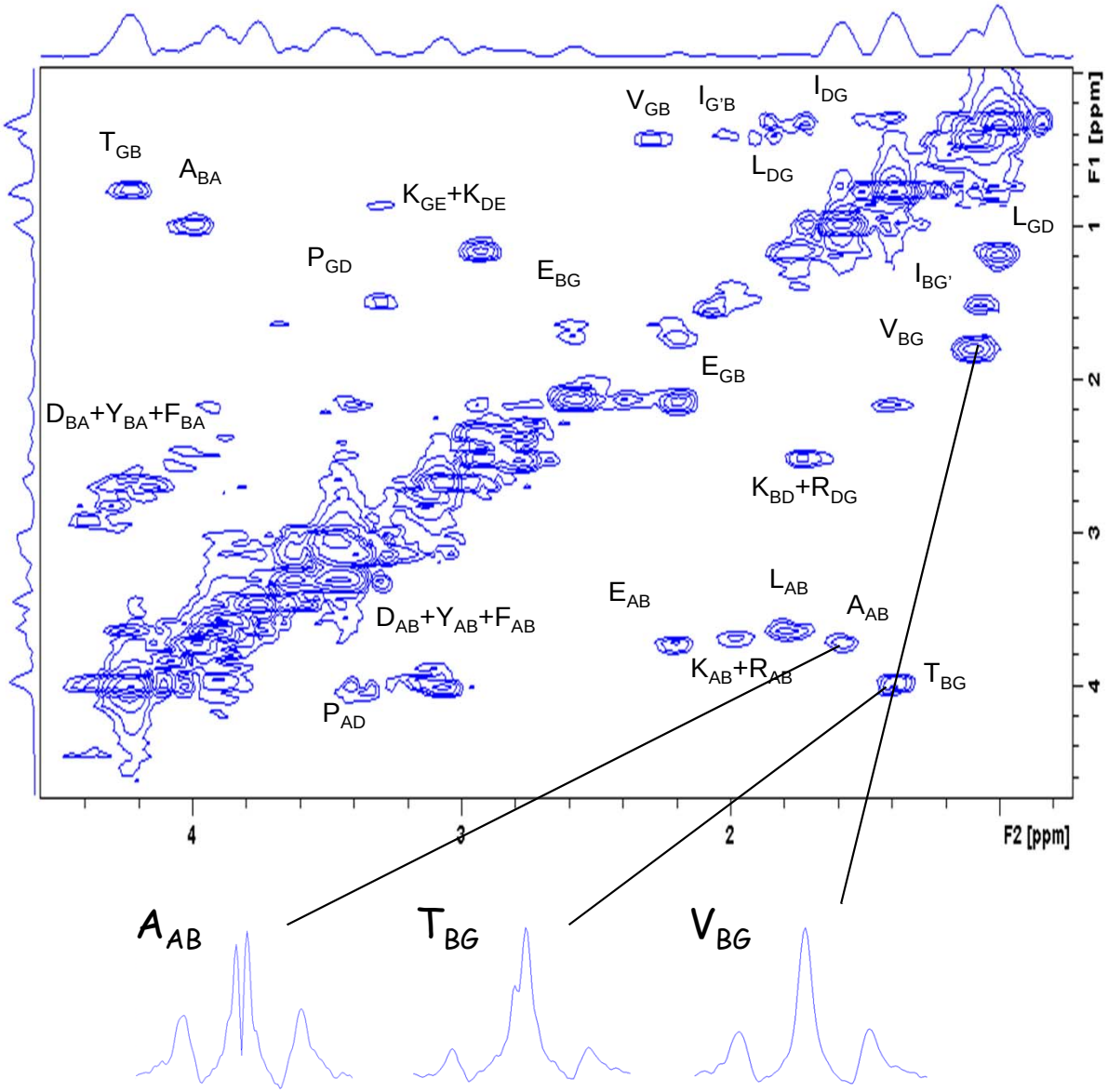
Jres-COSY



P. Giraudeau, E. Cahoreau, S. Massou, M. Pathan, J.-C. Portais, S. Akoka, *ChemPhysChem* 2012

Applications in fluxomics

Fast-Hybrid 3D NMR: application to a biological sample (12 min)



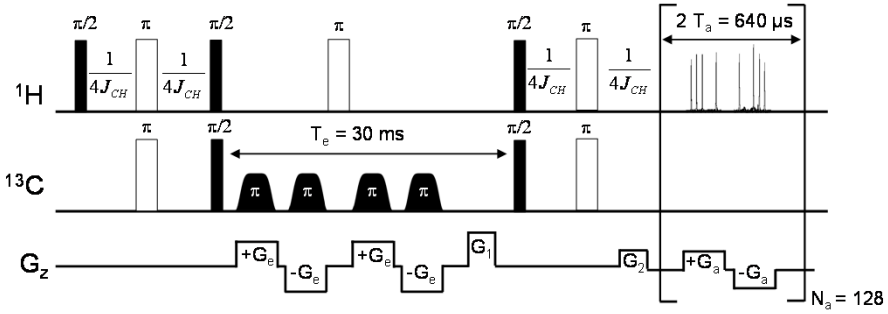
Site-specific IEs

peak	2D UFCOSY	3D COSY-Jres
A_{AB}	41,2	44,7
A_{BA}	50,7	47,1
E_{AB}	45,2	47,9
E_{BG}	-	48,6
E_{GB}	37,6	49,9
$I_{G'B}$	44,8	46,9
L_{AB}	43,4	48,5
L_{DG}	45,1	47,9
P_{AD}	42	47,2
T_{BG}	25,8	27,1
T_{GB}	24,7	24,9
V_{BG}	47,6	48,0
V_{GB}	-	46,8
P_{GD}	-	41,4

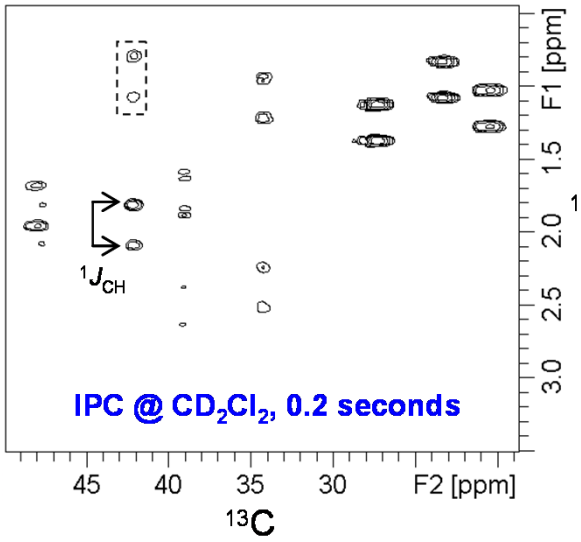
R. Boisseau, B. Charrier, S. Massou, J.-C. Portais, S. Akoka, Submitted for publication

Other recent analytical applications

Measurement of Residual Dipolar Couplings (RDCs) in oriented media

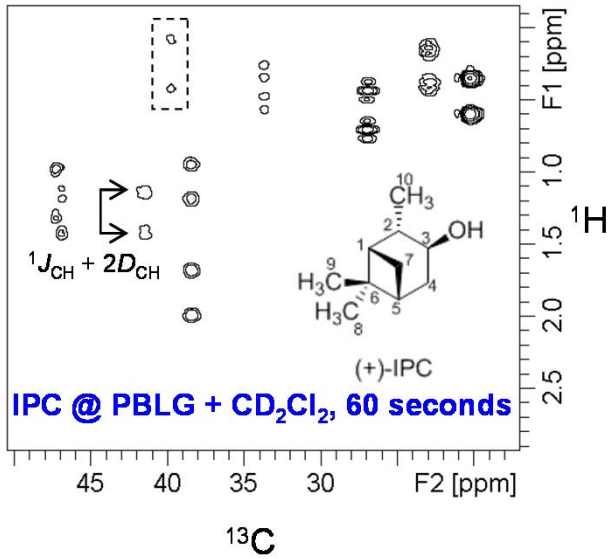


F_2-coupled UF HSQC



Residual Dipolar Couplings (RDCs)

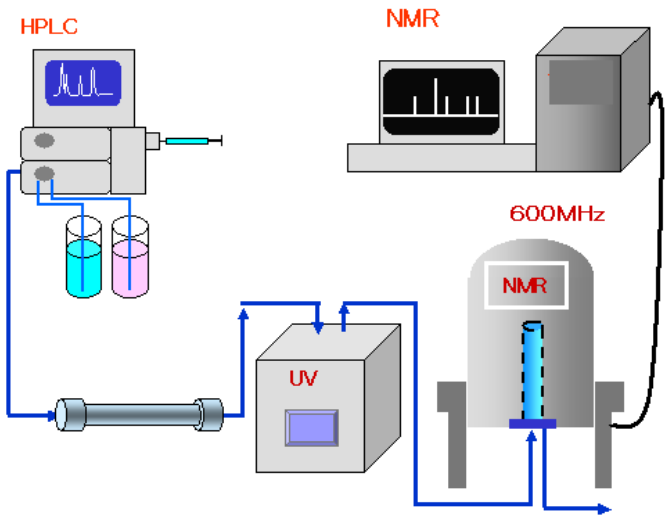
Faster structural analysis



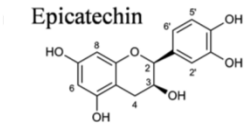
P. Giraudeau, T. Montag, B. Charrier, C.M. Thiele, *Magn. Reson. Chem.* 2012.

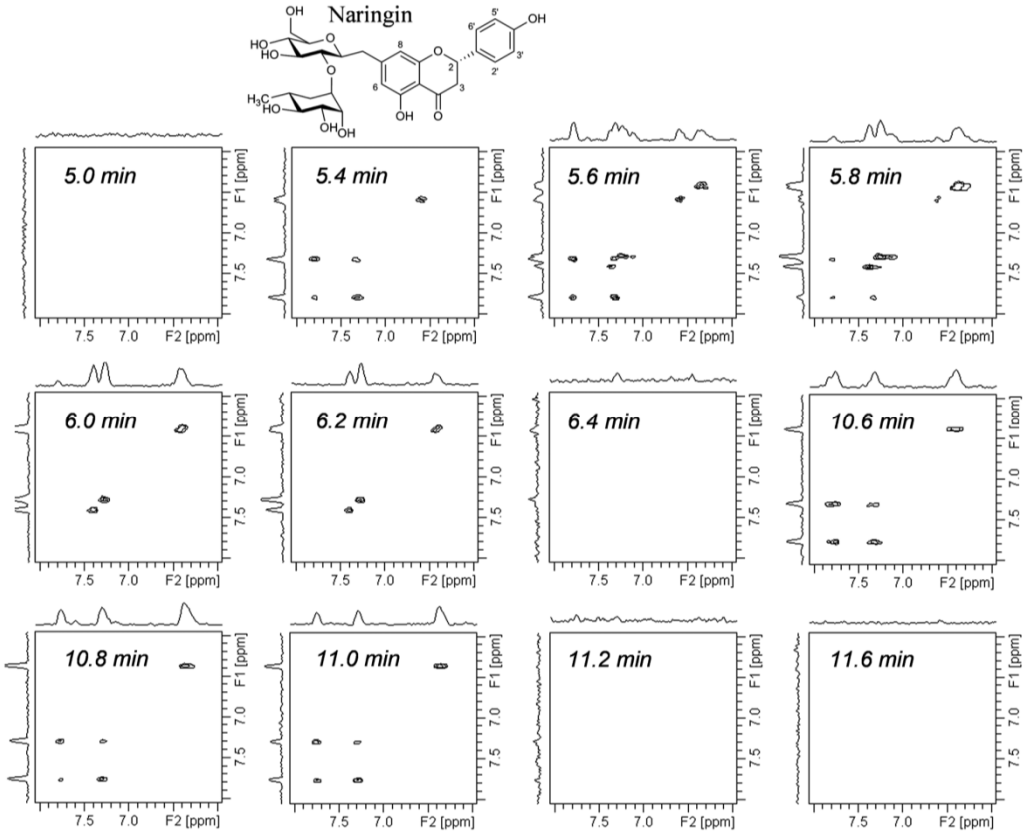
Other recent analytical applications

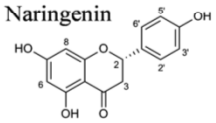
Coupling with on-line HPLC



Real-time separation of natural products



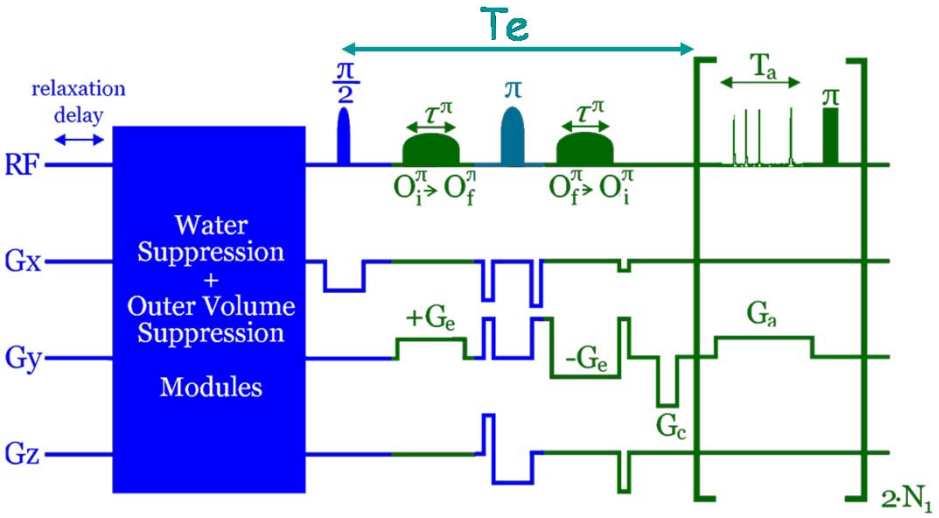




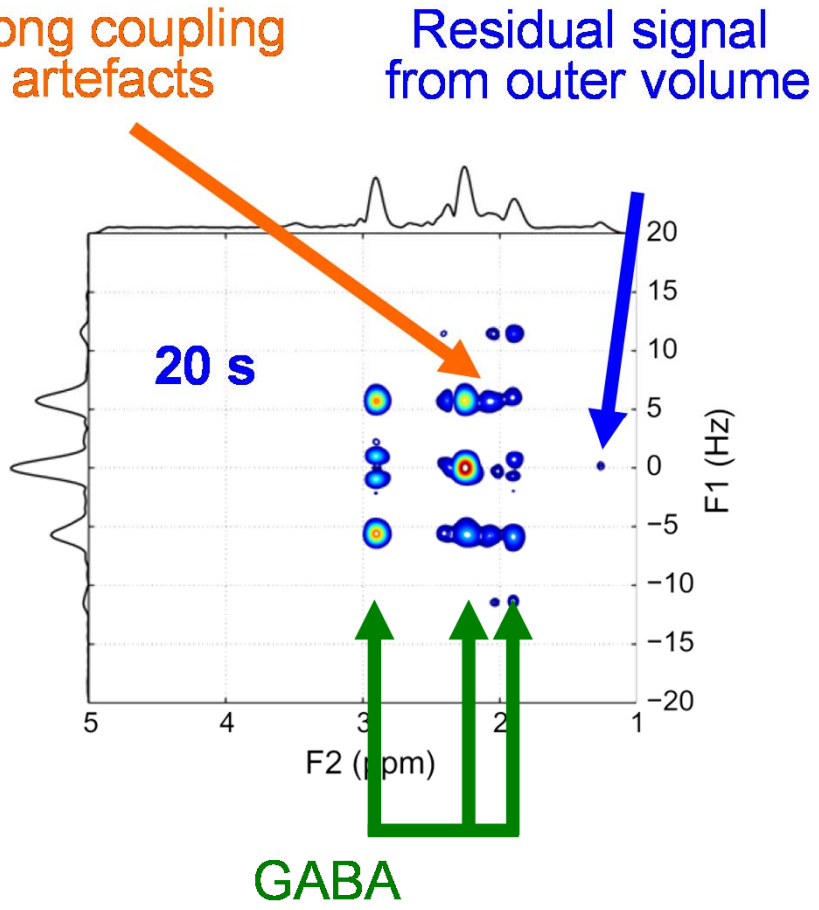
L.H.K. Queiroz Jr., D.P.K. Queiroz, L. Dhooghe, A.G. Ferreira, P. Giraudeau, *Analyst* 137 (2012) 2357

Other recent analytical applications

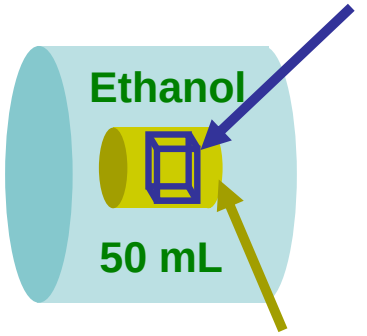
Towards ultrafast *in vivo* spectroscopy



PRESS + UF Jres



Voxel 5mm.5mm.5mm



GABA 10% @ H_2O
1.5 mL

T. Roussel, P. Giraudeau, H. Ratiney, S. Akoka, S. Cavassila, *J. Magn. Reson.* 2012

Conclusion & Perspectives

- Improved analytical performance of ultrafast experiments
- Immunity to spectrometer temporal instabilities
- High potential for quantitative analysis
- Application to samples of increasing complexity
- Next steps : *in vivo* and hyperpolarization

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Illa Tea

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Collaborators

INSA Toulouse, France
Stéphane Massou
Jean-Charles Portais
Edern Cahoreau

Univ. Lyon, France
Tangi Roussel
Hélène Ratiney
Sophie Cavassila

Tech. Univ. Darmstadt, Germany
Christina Thiele
Tobias Montag

Univ. Barcelona, Spain
Teodor Parella
Laura Castañar

Weizmann Institute of Science, Israel
Lucio Frydman

Univ. Sao Carlos, Brazil
Antonio Gilberto Ferreira

Univ. Goias, Brazil
Luiz Keng Queiroz Jr.

Univ. Geneva, Switzerland
Damien Jeannerat
Mohammadali Foroozandeh