Reducing experimental time using **M**ultiple **F**id Acquisition

Pau Nolis, Kumar Motiram-Corral, Miriam Pérez-Trujillo, Teodor Parella

Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona

Universitat Autònoma de Barcelona

Introduction

Speeding-up NMR molecular analysis is an important research field which has been continuously advancing since NMR early days. The relevant benefits are clear and evident:

- ✓ Reduce analysis timer per sample => reduce analysis cost.
- ✓ Gain Spectrometer time to analyze new samples => improve spectrometer efficiency

Multiple FID Acquisition (MFA) strategy consists in the design of a pulse sequence experiment accommodating N acquisition windows, each registering different relevant structural information. This strategy is faster than perform a traditional sequential acquisition of N separated experiments. Mainly, time savings come from scaping long d1 recovery delay. Several design strategies are possible:

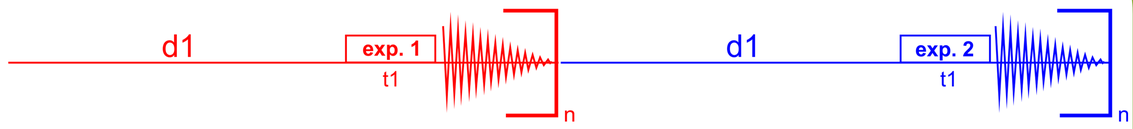
Orthogonal CTP Acquisition (Coherence Transfer Pathways) was firstly described in the COCONOSY experiment[1], were COSY and NOESY experiments where combined in one (COSY was acquired during NOESY mixing time).

Afterglow Acquisition, that is the application of a mixing sequence after the FID, transforming signal to new information which is then acquired again. Typical mixings are COSY, RELAY and TOCSY transfers. Applications have been demonstrated successfully in the obtention of up to 4 experiments in a single 1, in both, homonuclear and heteronuclear experiments[2].

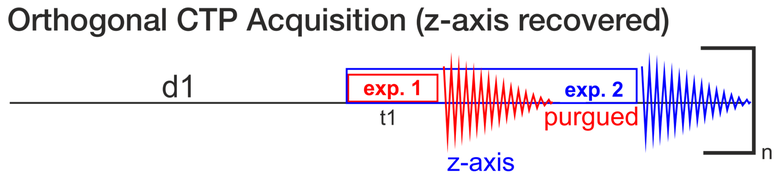
NOAH (NMR Ordered Acquisition with 1H-detection)[3]. This strategy nest independent experiments ordered from low to higher sensitive and waits for a single recovery delay. Table below summarize its properties in a two

Orthogonal CTP	Afterglow	NOAH
Exp1 embedded in Exp2	Exp1 embedded in Exp2	Exp1 and Exp2 are nested
Exp1 and Exp2 shares d1 and t1	Exp1 and Exp2 shares d1 and t1	Exp1 and Exp2 have a single common d1 but independent t1
Orthogonal CTP aquired	Continuous CTP acquired	Independent CTP experiments
2nd Magnetization stored in z-axis during 1st FID	2nd Magnetization comes from acquired 1st FID	2nd Magnetization starts being generated after 1st FID
Diffusion dependency in PFG selection	T2 dependency	Signal Saturation dependency (recovery between experiments)
Standard FIDS allowed	Truncated FIDS (moderate resolution)	Long FIDS recommended, acting as recovery delay (lowering saturation)
Typically 2 FID recorded	Up to 4 FIDS are plausible	NOAH-5 experiments are published

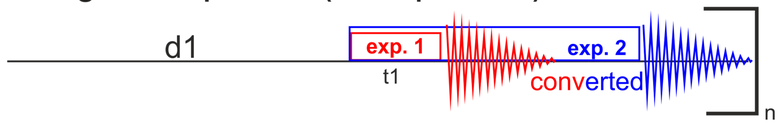
Sequential Standard Acquisition



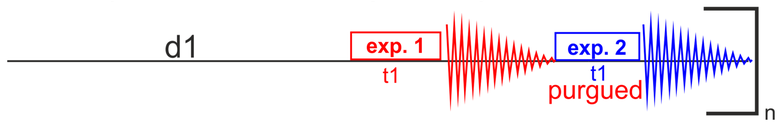
Orthogonal CTP Acquisition (z-axis recovered)

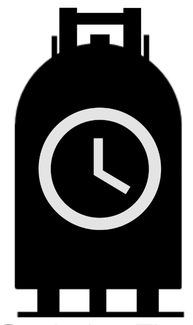


Afterglow Acquisition (T2-dependant)



NOAH (NMR Ordered Acquisition)





It is important to remark, that such designs are not incompatible one each other and can be combined between them. For instance, a triple COSY/TOCSY/TOCSY experiments can be designed in many different ways[4]. Another example is a zz-filter NOAH-2BS[5] design where first experiment HMBC stores Z-magnetization to be used for the second HSQC therefore minimizing saturation losses on it.

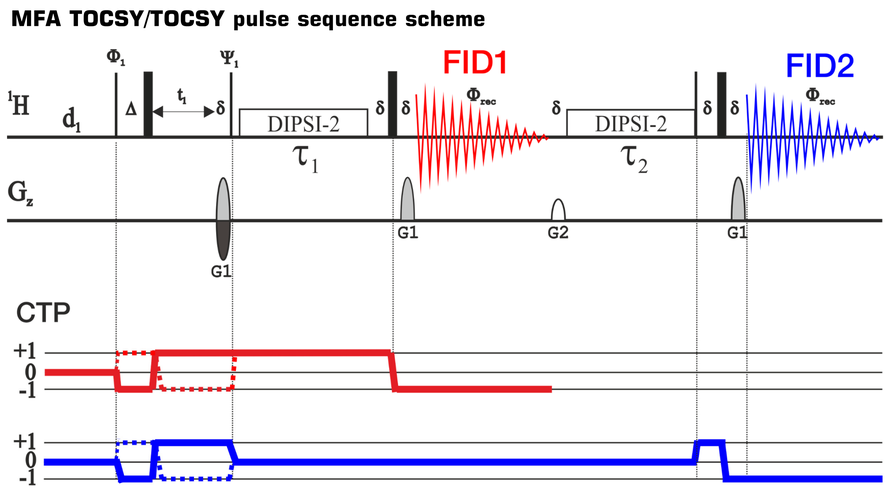
In this poster is presented the design of the Interleaved (orthogonal) CTP acquisition of Equivalent Transfer Pathways in Sensitivity Enhanced (SE) version of TOCSY and HSQC. Due to poster space requirements only two of the possible applications are shown[6].

Interleaved Dual NMR Acquisition of Equivalent Transfer Pathways in TOCSY

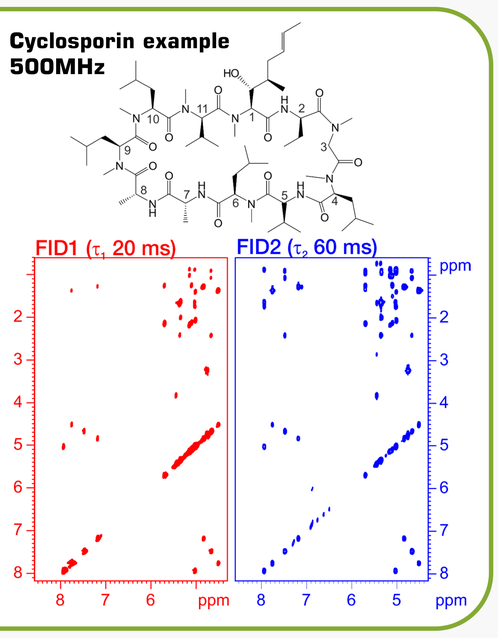
MFA TOCSY/TOCSY experiment where two equivalent CTPs components are acquired in interleaved mode. Sequence incorporates gradient-enhanced CTP selection based on the well-known echo/anti-echo acquisition and processing protocols to provide pure absorption line shapes.

FID1 is designed to observe exclusively transverse I_x components generated during the τ_1 mixing time, whereas the other I_z component remains non-observable. After that, the purging G2 gradient is applied to remove any residual transverse magnetization, and a subsequent 90° pulse flips the unexploited I_z component to the transverse plane to be acquired during a second acquisition period FID2 inserted after another TOCSY transfer. This method allowed the single-shot dual acquisition of two different TOCSY experiments sharing the same variable t_1 period but recorded with two different mixing times (τ_1 and τ_2, respectively). It is important to comment that a slight reduction in sensitivity is observed in FID2 due to diffusion. Nevertheless, the benefits of sensitivity per time unit are demonstrated to be favorable using MFA compared to sequential acquisition.

MFA TOCSY/TOCSY pulse sequence scheme



Cyclosporin example
500MHz



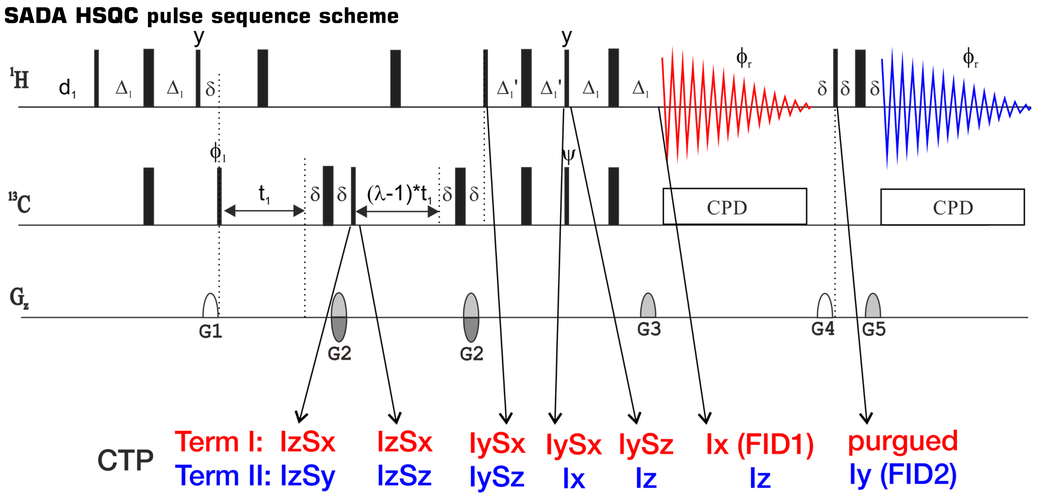
For experimental details and settings, please see reference [4]

Spectral Aliasing in Dually Acquired HSQC (SADA-HSQC)

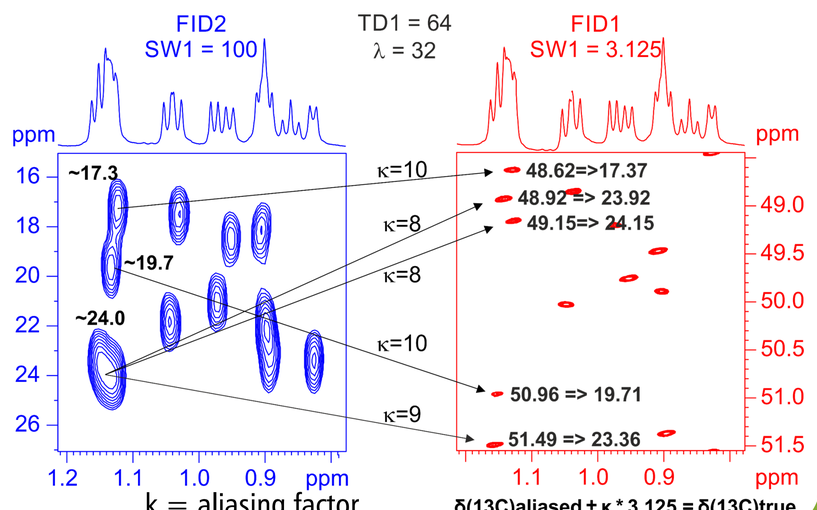
In SADA-HSQC experiment two equivalent CTPs components are acquired in interleaved mode, allowing to obtain two HSQC with different spectral widths simultaneously. The key point is the differential t1 evolution for each orthogonal magnetization component. While term I and term II evolves equally during t1. There is an extra period (λ-1)·t1 where term I continues to evolve, while term II is stored as zz-magnetization. Thus, the SADA-HSQC experiment yields a 2D HSQC spectrum with its full spectral width ($SW1^{normal}$) in FID 2 whereas an aliased HSQC spectrum showing a reduced $SW1^{aliased}$ according to the λ factor is obtained in FID1:

$$SW_{aliased} = SW_{normal}/\lambda$$

SADA HSQC pulse sequence scheme



Cyclosporin example (methyl region)
600MHz



For experimental details and settings, please see reference [6]

References

[1] C.A.G. Haasnoot, F.J.M. van de Ven, C.W. Hilbers. T. *J. Magn. Reson.*, 1984, *56*, 343-349.
[2] (a) P. Nolis, M. Pérez-Trujillo, T.Parella. *Angew. Chem. Int. Ed.* 2007, *46(39)*, 7495-7497. (b) K. Motiram-Corral, M. Pérez-Trujillo, P. Nolis, T. Parella. *Chem. Comm.* 2018, *54*, 13507-13510.
[3] E. Kupce, T. Claridge, *Angewandte Claridge*. 2017, *56(39)*, 11779-11783.
[4] P. Nolis, T. Parella, *Magn. Reason. Chem.* 2019, 57(4), S85-S94.
[5] E. Kupce, T. Claridge, *Chem.Com.* 2018, *54*, 7139-7142.
[6] P. Nolis, K. Motiram-Corral, M. Pérez-Trujillo, T. Parella. *J. Magn. Res.*, 2019, *300*, 1-7.

Summary

- MFA offers a time-efficient strategy, recording equivalent pathways in a dual interleaved mode.
- Acquiring two TOCSY with different mixing time in a single experiment is shown.
- Acquiring conventional and aliased HSQC spectra in a single experiment is demonstrated.

Financial support from the MINECO (CTQ2015-64436-P) is gratefully acknowledged.