# Defeating Complexity: New NMR Methods for Mixture Analysis

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**NMR Methodology Group**

The University of Manchester

61st Experimental Nuclear Magnetic Resonance Conference

Baltimore, Maryland

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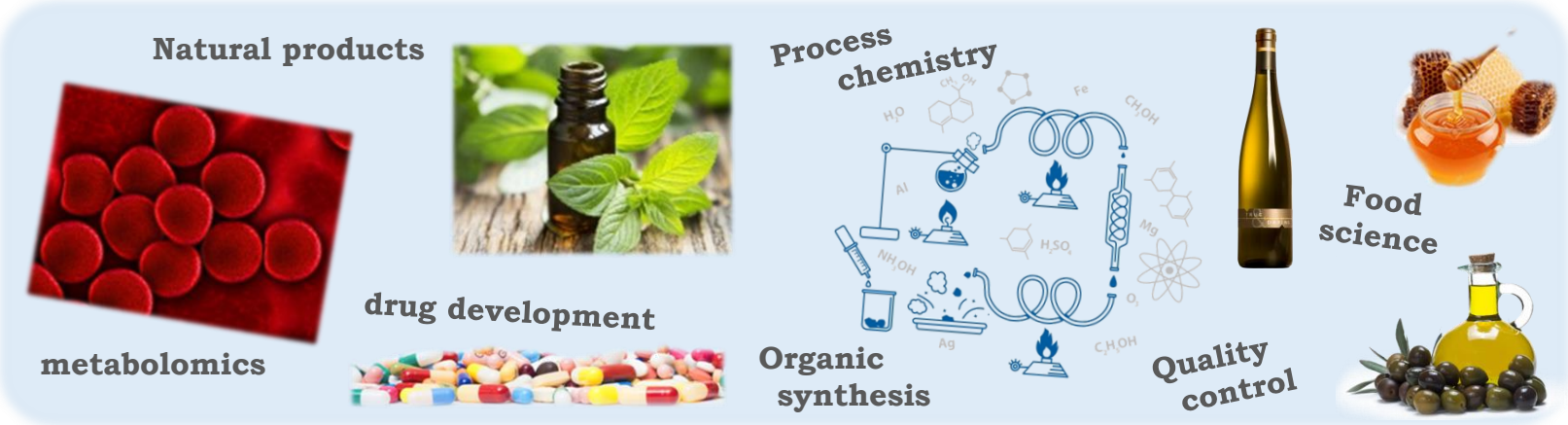
# The Importance of Mixture Analysis




## Qualitative analysis

*Mixture information*

Mixture fingerprint

Number of components

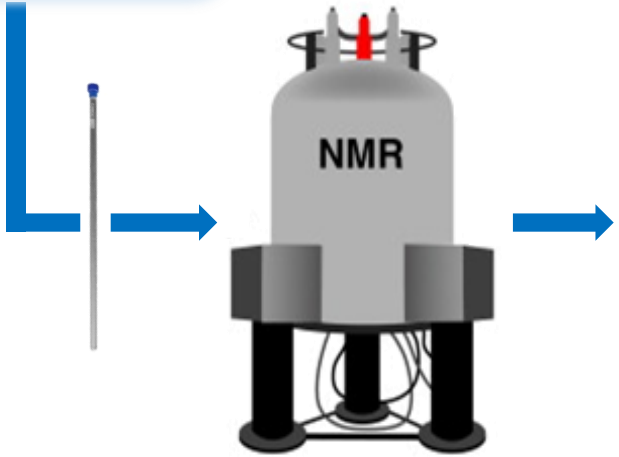
## Quantitative analysis

Individual components information

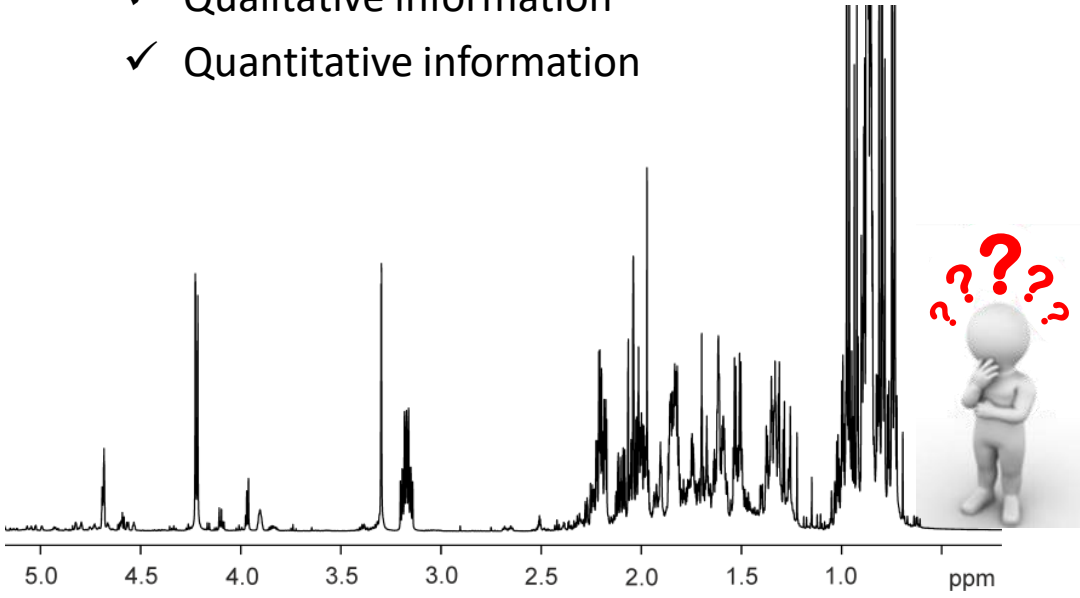
Chemical structure

Quantification

# NMR – A Powerful Tool for the Analysis of Mixtures


- ✓ Non-destructive
- ✓ Non-physical separation
- ✓ Qualitative information
- ✓ Quantitative information

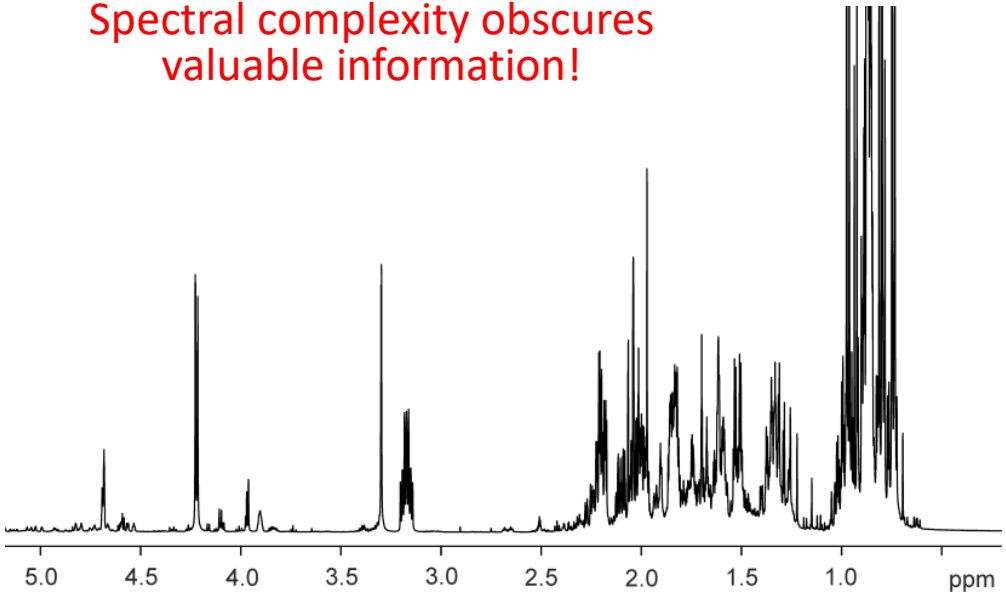


# 1D $^{1}$H NMR – Pros and Cons

- ✓ High sensitivity
- ✓ Ubiquity in Chemistry
- ✓ Structural information richness
- ✓ Mixture fingerprint

- X Signal overlap
- X Low spectral resolution
- X High spectral complexity

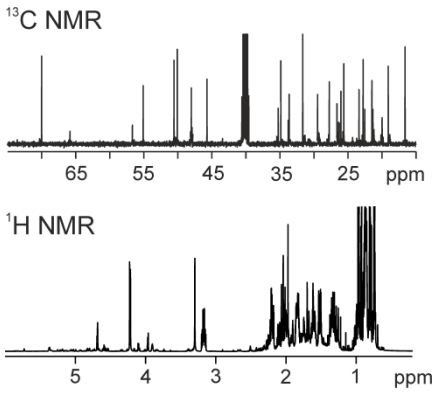
Spectral complexity obscures valuable information!



# Strategies for Alleviating Overlap

## Other nuclei

($^{13}$C, $^{19}$F, $^{31}$P)

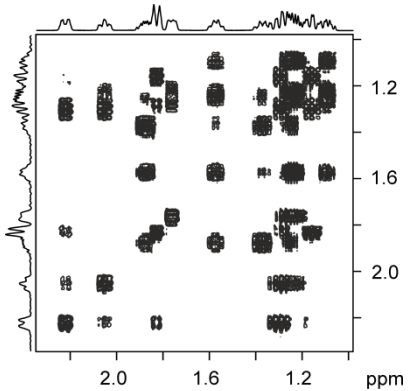



Low chemical abundance

Wide shift ranges

## 2D/3D NMR

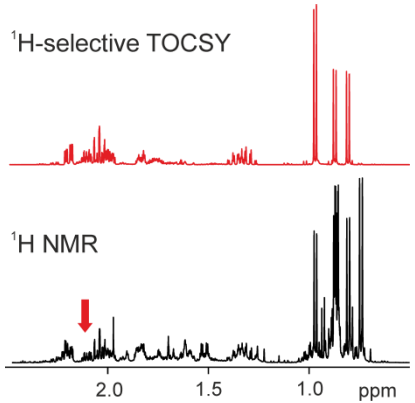
(COSY, HSQC-TOCSY)




Additional dimensions

## Virtual separation

(selective experiments, DOSY)

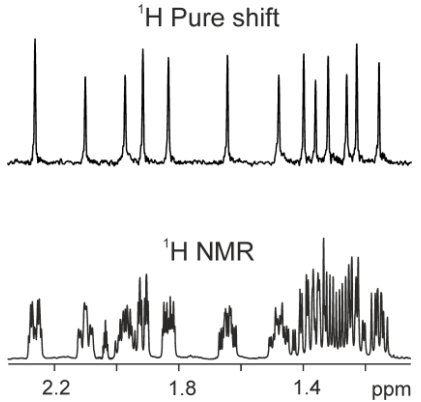



Simplify subspectra

Differential behaviour

## Pure shift NMR

(HOBS, ZS, PSYCHE, BIRD)




Effects of $J_{HH}$ suppressed

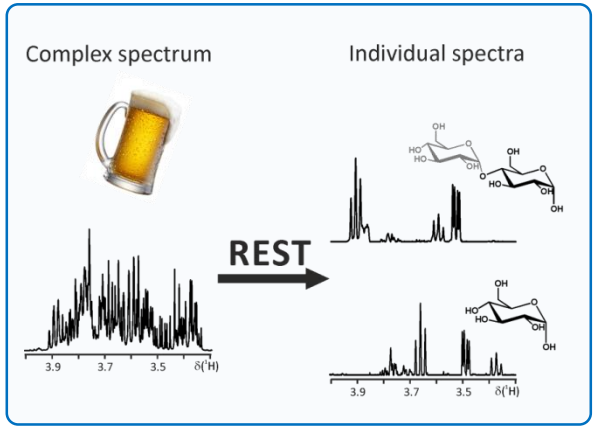
Single peak per signal

For complex mixtures we need more sophisticated methods!

# Novel NMR Methods for the Analysis of Complex Mixtures

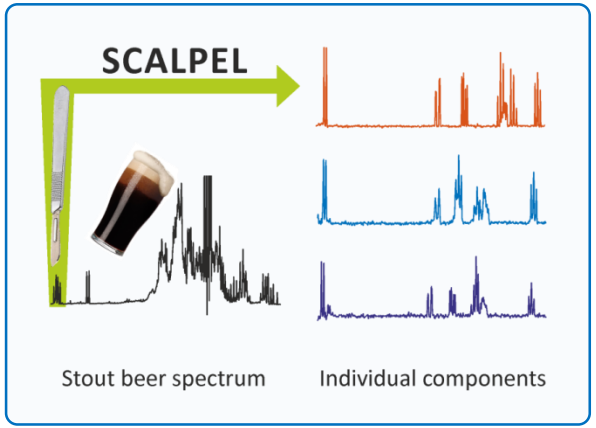
## REST

*Relaxation-Encoded Selective TOCSY*



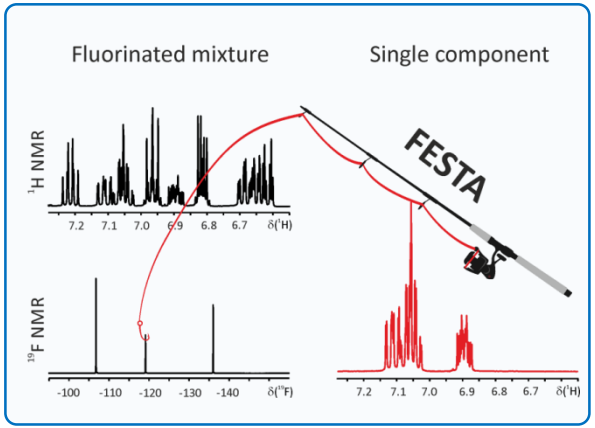

## SCALPEL

*Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components*




## FESTA

*Fluorine-Edited Selective TOCSY Acquisition*



# Novel NMR Methods for the Analysis of Complex Mixtures

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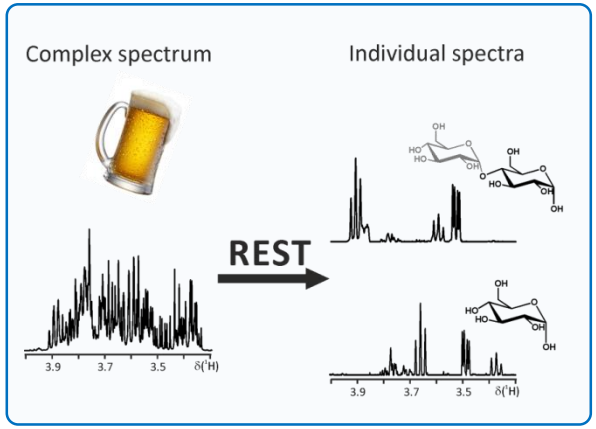



## SCALPEL

*Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components*

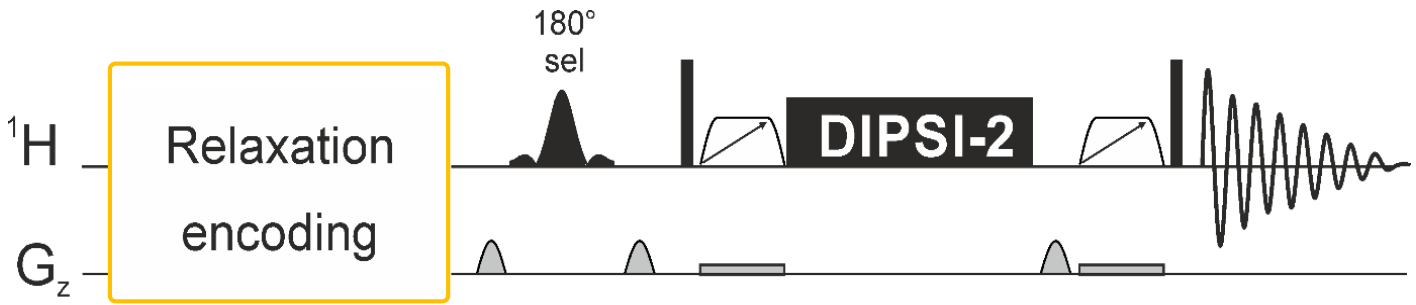



## FESTA

*Fluorine-Edited Selective TOCSY Acquisition*

# Relaxation-Encoded Selective TOCSY (REST) experiment

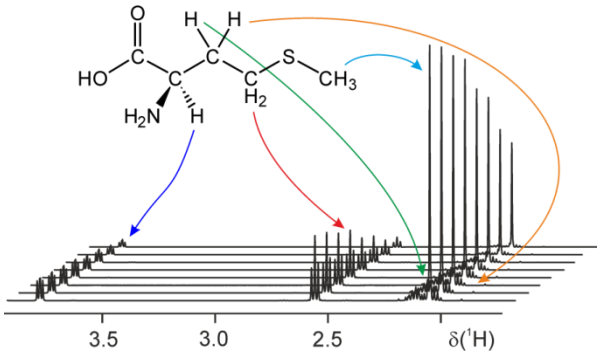
Virtual separation of the components of a mixture by exploiting differences in the relaxation behavior of spins

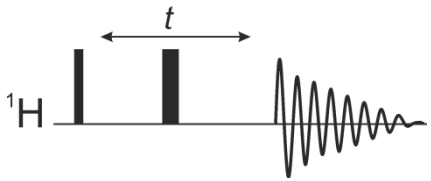



G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

# Relaxation NMR

## Relaxation array



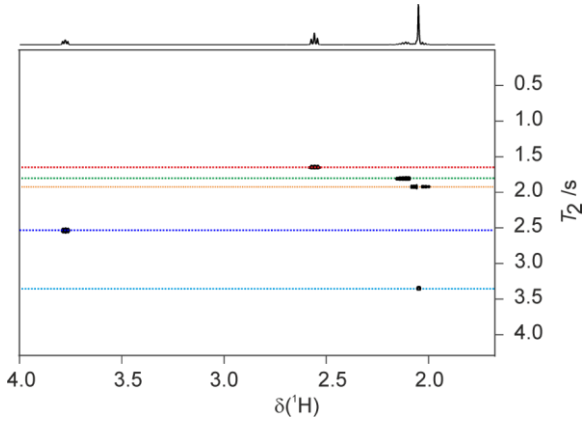




Prototype sequence

$$S(t) = S_0 e^{-\frac{t}{T_2}}$$

*Univariate analysis*

## Relaxation-Ordered SpectroscopY (ROSY)



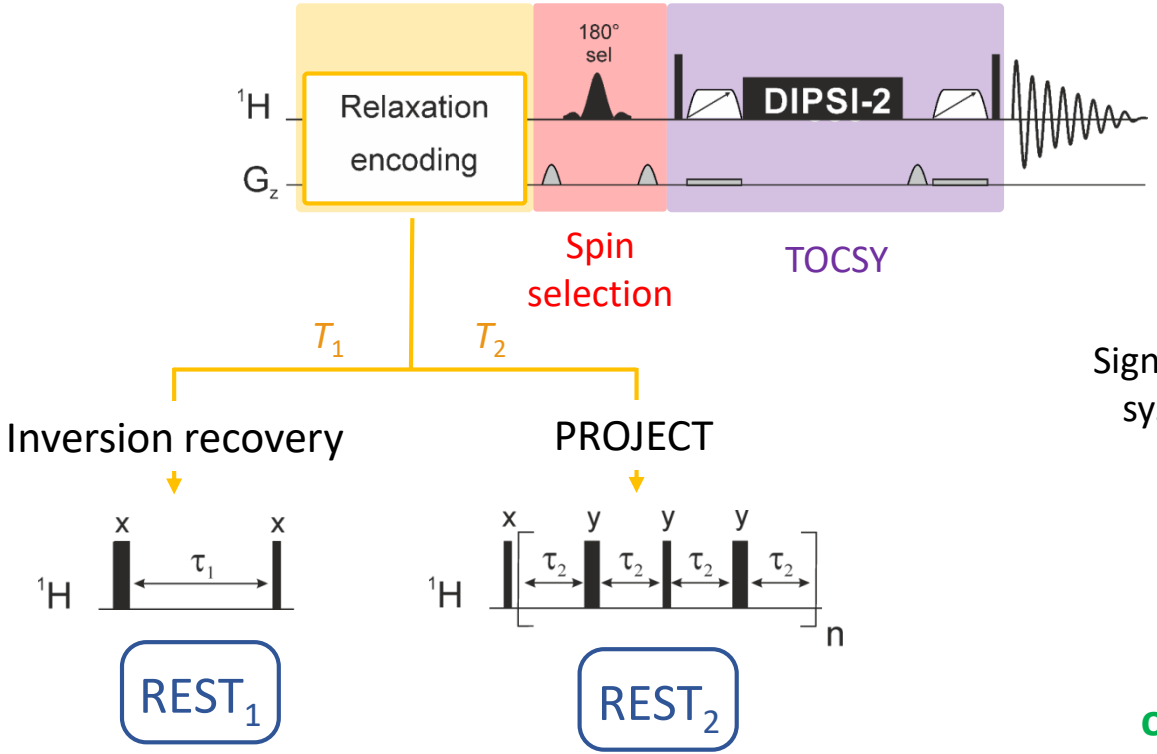

Signals from a given molecule:

- have different relaxation behaviour
- have different $T_1$ and/or $T_2$ values

**NO virtual separation of components**

(ROSY) Y. Nishiyama, M. H. Frey, S. Mukasa and H. Utsumi, *J. Magn. Reson.*, **2010**, *202*, 135.

# Relaxation-Encoded Selective TOCSY (REST) experiment



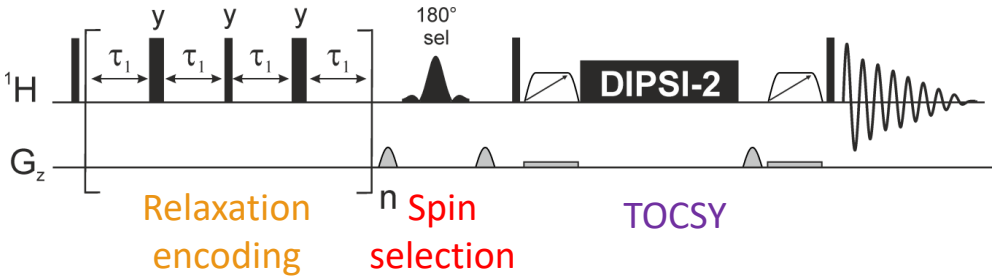

Signals from a given spin system appear at the same $T_1$ / $T_2$

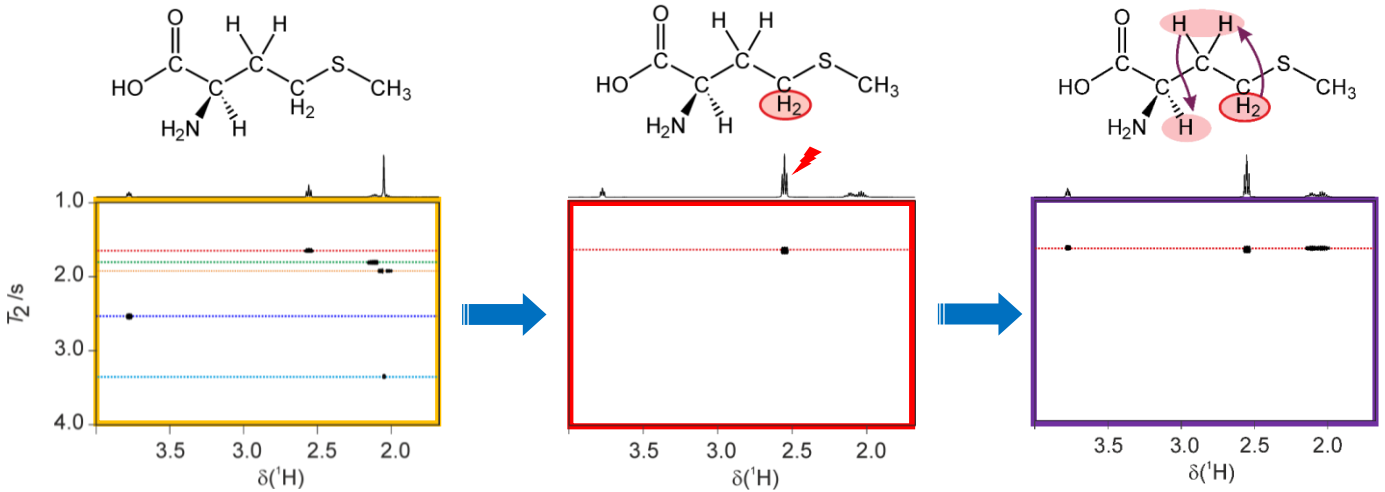
**Virtual separation of spin systems**

G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

# Relaxation-Encoded Selective TOCSY (REST) experiment

REST$_2$



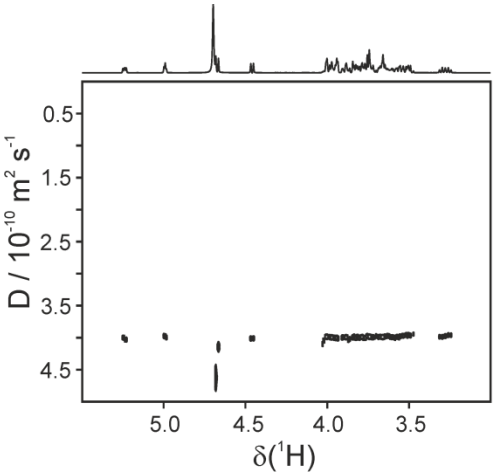




Signals from a given spin system appear at the same $T_2$

G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

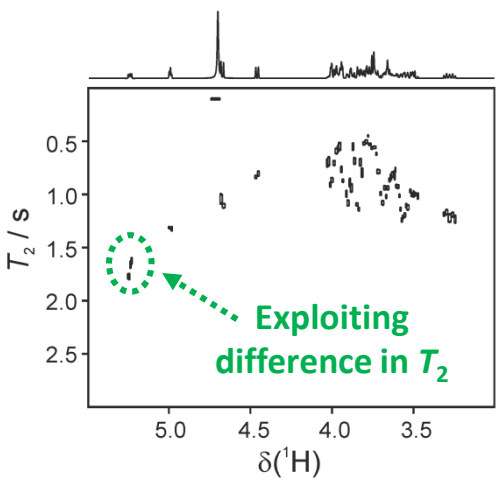
# REST & Sugars

## Oneshot DOSY



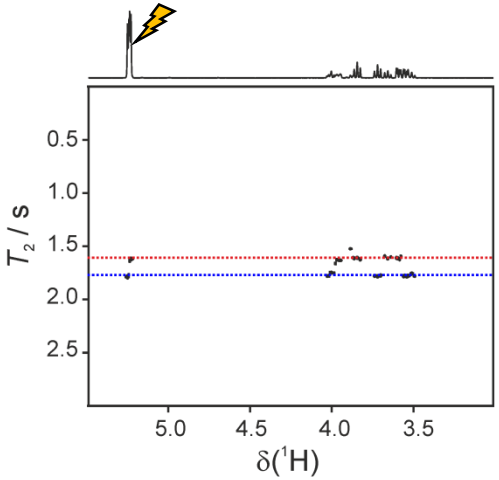

Sugar have almost identical *D*

## PROJECT ROSY

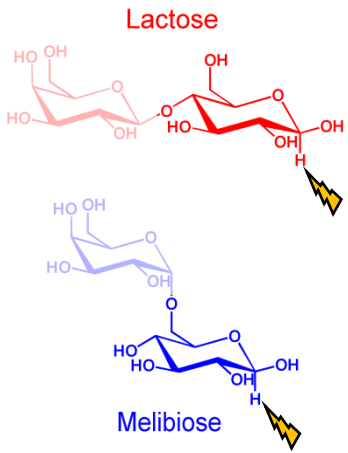



All sugar signals have different $T_2$

## $REST_2$ ROSY



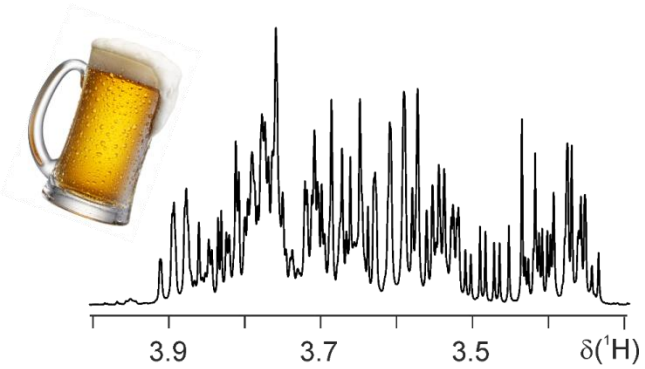

**Virtual separation of components**




(REST) G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

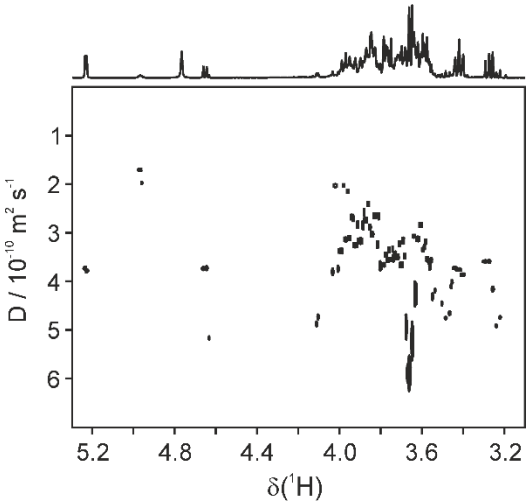
# REST & Beer

## $^1$H NMR



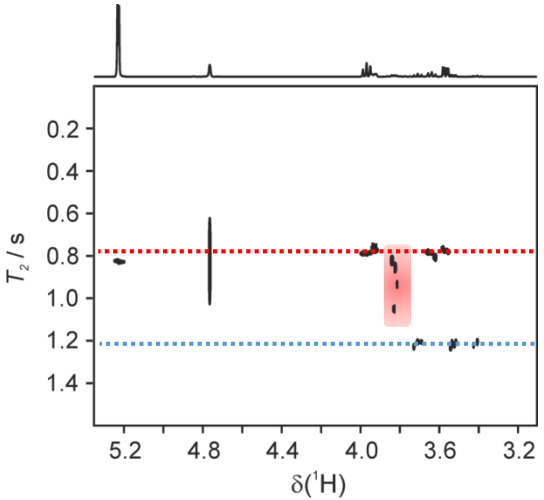

$^1$H NMR spectrum of beer
(Clausthaler classic premium lager)

## Oneshot DOSY




Partial signal overlap

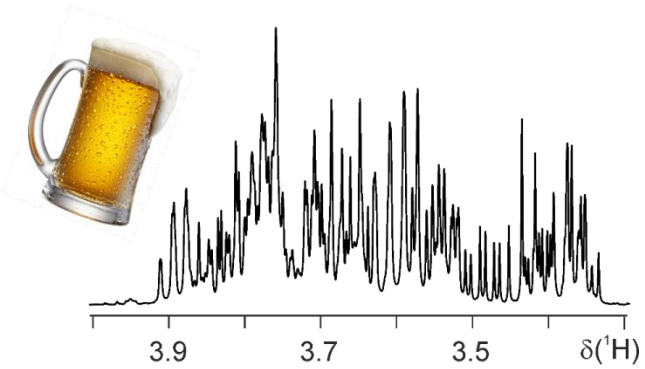
## REST$_2$ ROSY




G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

# REST & Beer

## Complex Mixture




$^1$H NMR spectrum of beer
(Clausthaler classic premium lager)

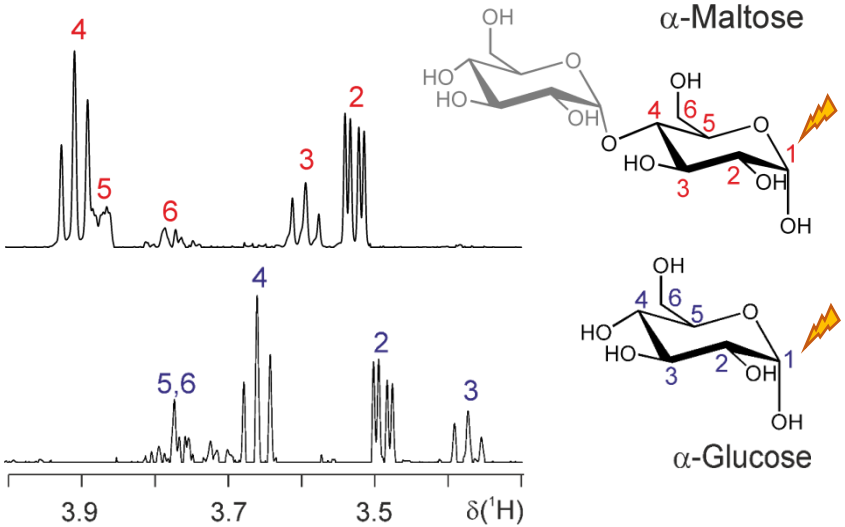
REST$_2$

OUTSCORE

Optimized Unmixing of True Spectra for COmponent Resolution

(***Multivariate analysis***)

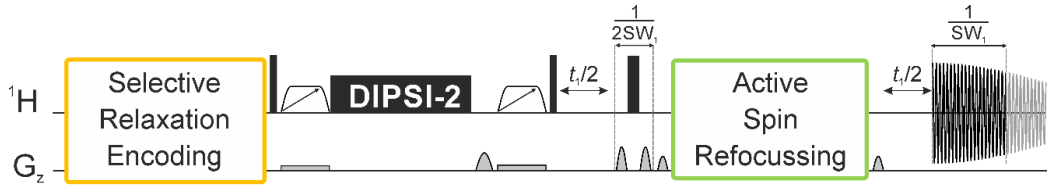
## Virtual separation of components



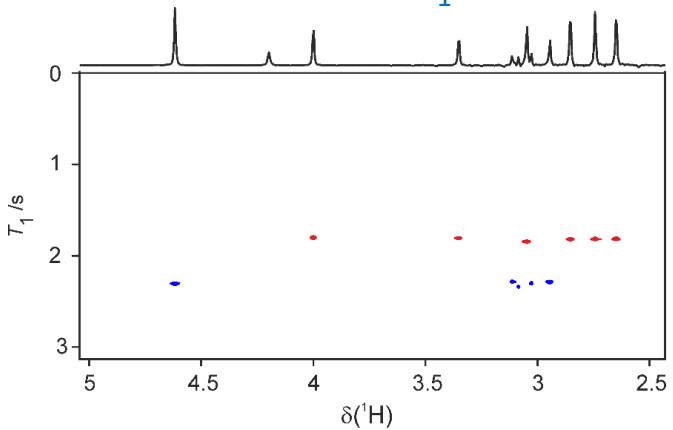

REST$_2$ OUTSCORE components for free α-glucose and for the α-glucose unit in α-maltose

(REST) G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

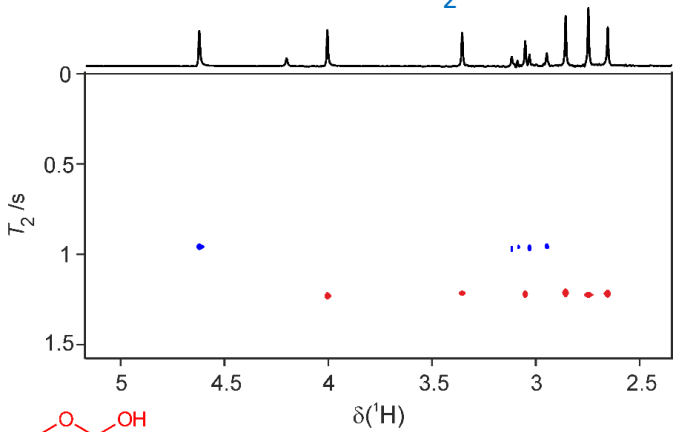
# Pure shift Relaxation-Encoded Selective TOCSY experiment

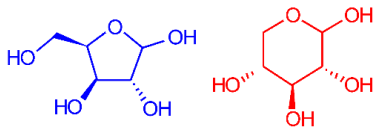



PS-REST$_1$




PS-REST$_2$




Xylose in $D_20$

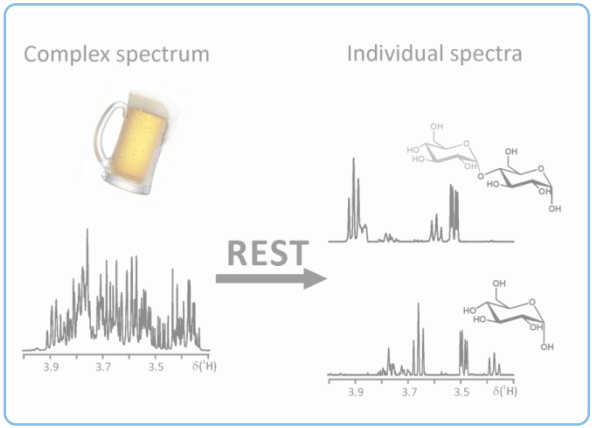
Marshall Smith

*Preliminary results*

# Novel NMR methods for the analysis of complex systems

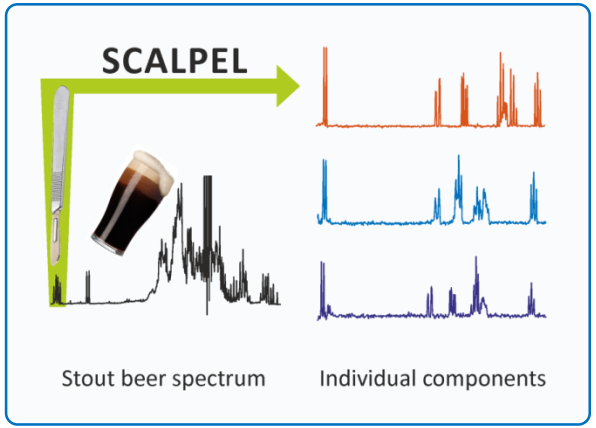
## REST

*Relaxation-Encoded Selective TOCSY*




## SCALPEL

*Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components*

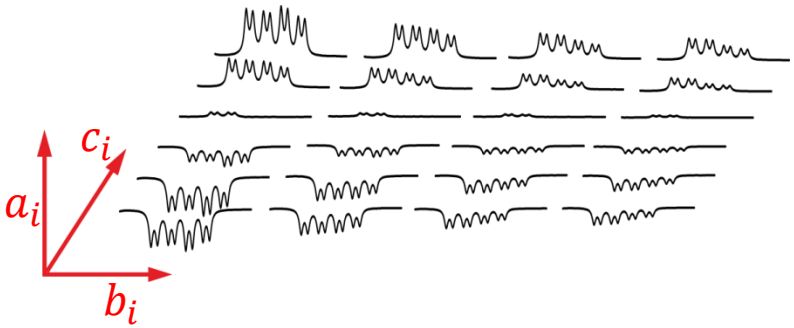



## FESTA

*Fluorine-Edited Selective TOCSY Acquisition*

# PARAllel FACtor (PARAFAC) tensor analysis

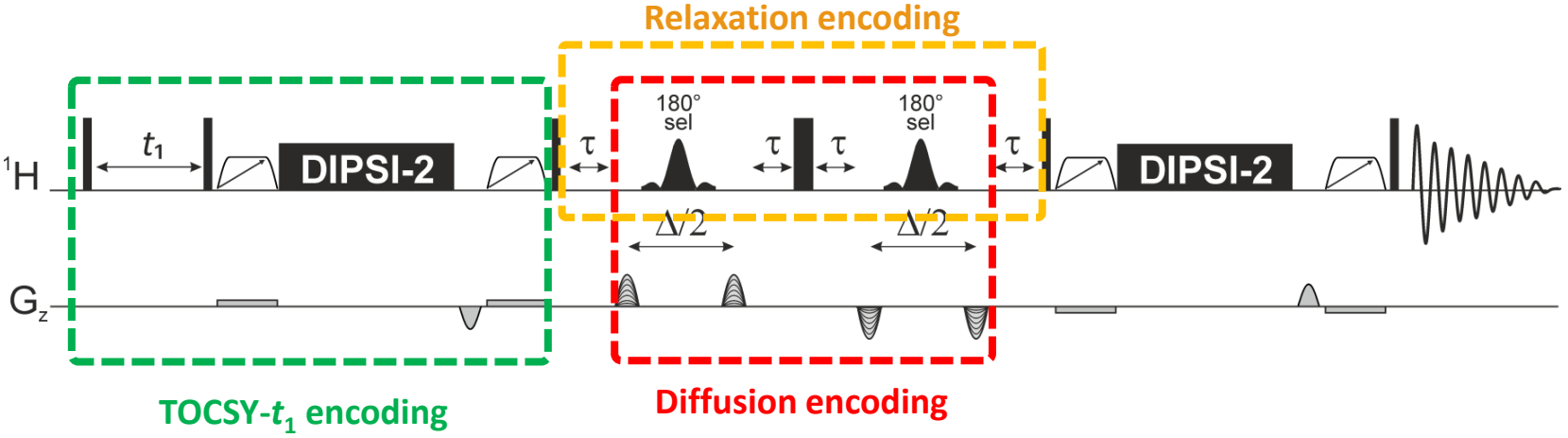
$$\text{Data} = \sum_{i=1}^{N} a_i \otimes b_i \otimes s_i + E$$




- Can distinguish components which are very similar
- Can distinguish several components
- Requires perfectly **orthogonal** dimensions ($a_i$, $b_i$, $s_i$)
- Requires 3D data
- Provides the three modes simultaneously
- Does not require data with high signal-to-noise ratio
- Requires only a few increments

R.Bro, *Chem. Int. Lab Sys.*, **1997**, *38*, 149.

# Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components (SCALPEL)




Data = $\sum_{i=1}^{N} a_i \otimes b_i \otimes s_i + E$

$s_i$ - spectrum of component $i$ as a function of frequency
$b_i$ - diffusion or relaxation
$a_i$ - TOCSY-$t_1$ (time) evolution

G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *J. Am. Chem. Soc.* **2019**, *141*, 5766.

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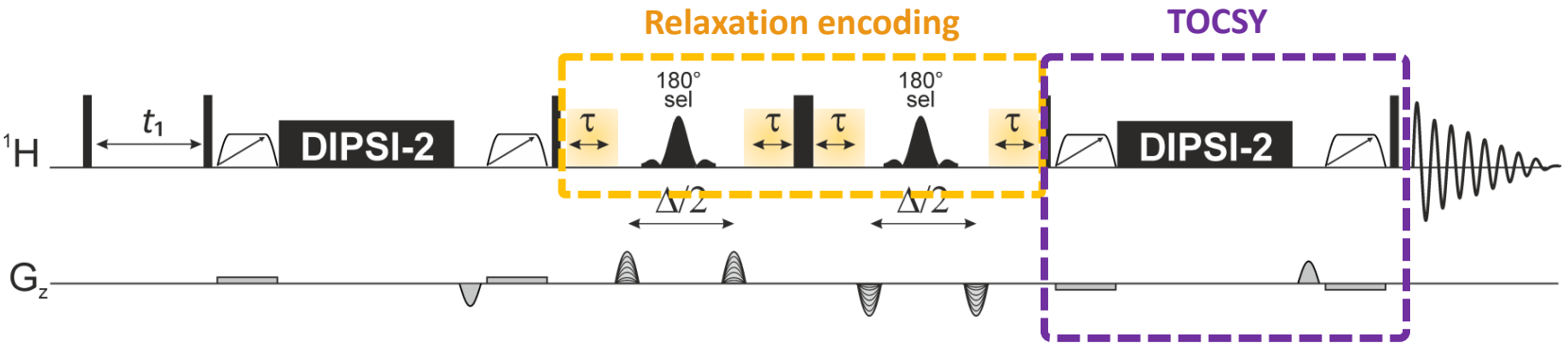


iREST$_2$

($T_2$ Relaxation internally-Encoded Selective TOCSY)

G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *J. Am. Chem. Soc.* **2019**, *141*, 5766.

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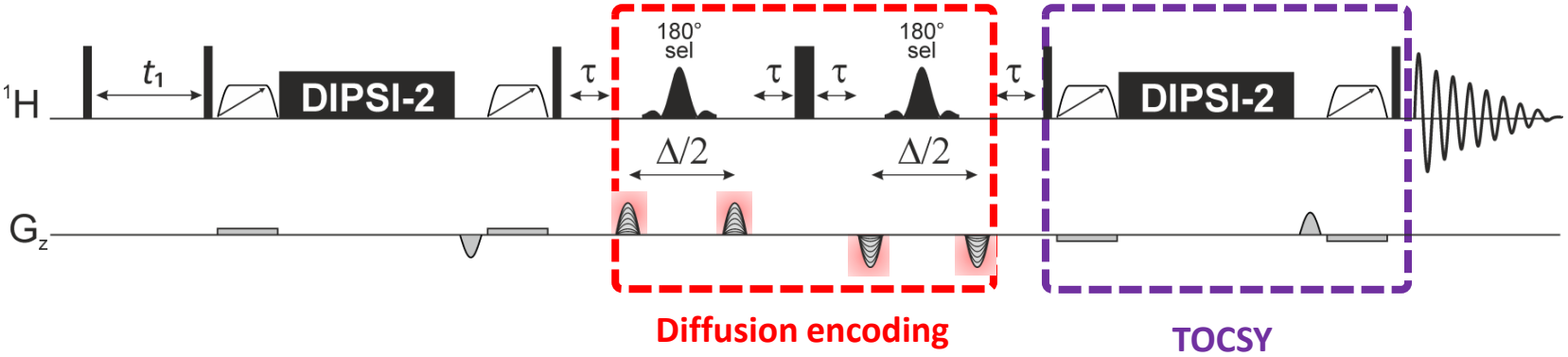
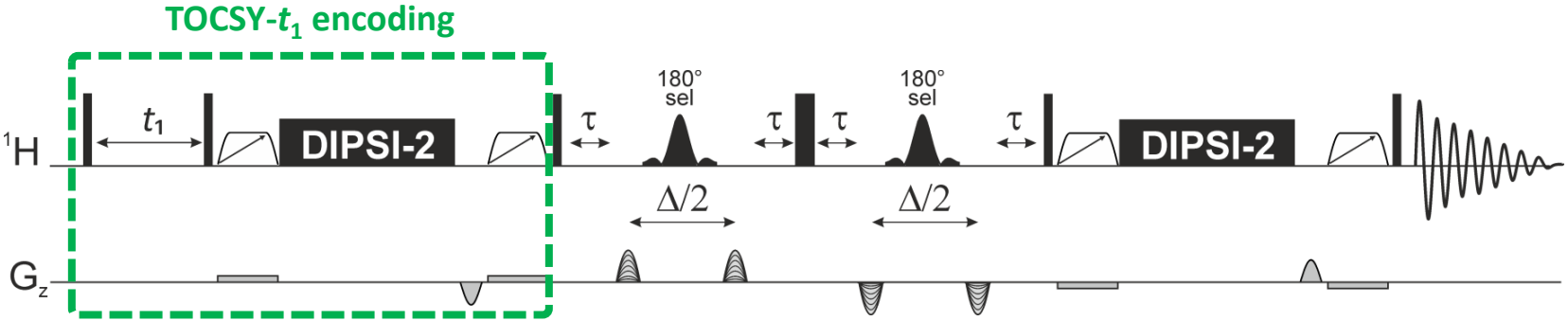
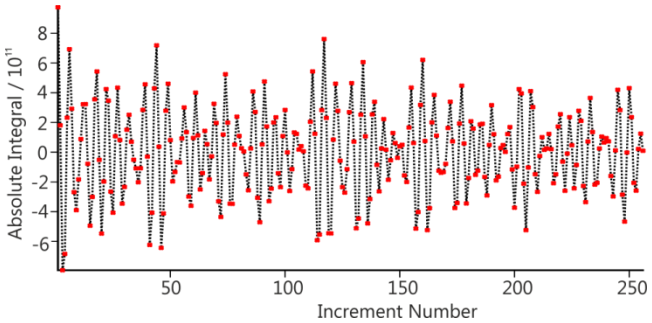



iDESTO
(Diffusion internally-Encoded Selective TOCSY)

G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *J. Am. Chem. Soc.* **2019**, *141*, 5766.

# Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components (SCALPEL)



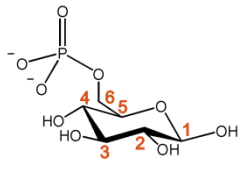



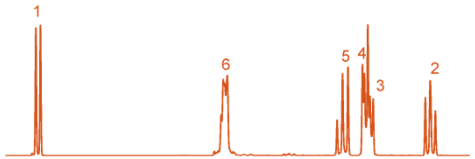

- TOCSY-$t_1$ encoding labelled spin systems based on their signal amplitude dependence on $t_1$.
- Signal amplitude is a sensitive function of the coupling constants in the spin system.
- This dimension is **not** Fourier transformed.

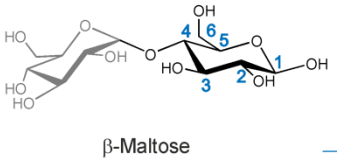
G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *J. Am. Chem. Soc.* **2019**, *141*, 5766.

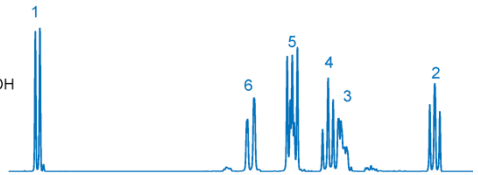
# SCALPEL & Sugars

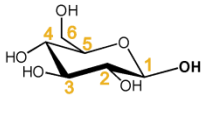
## Spectral mode

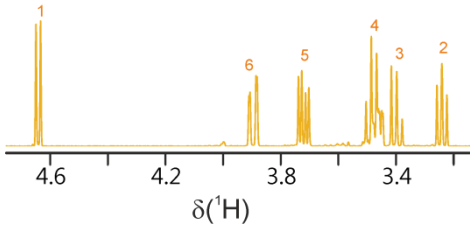


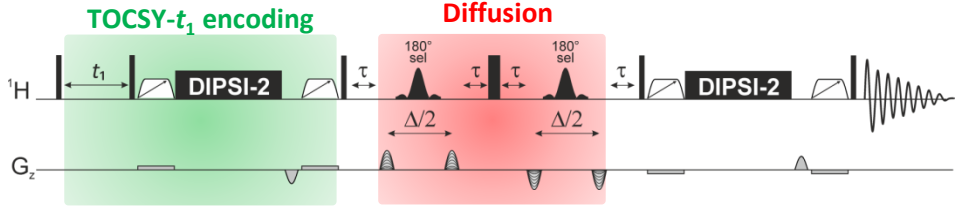



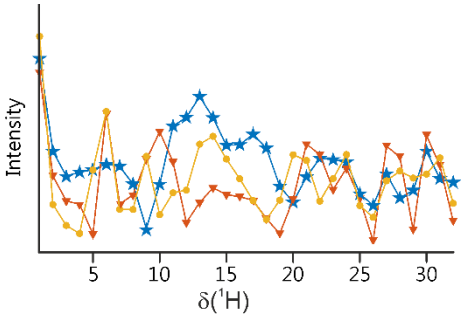



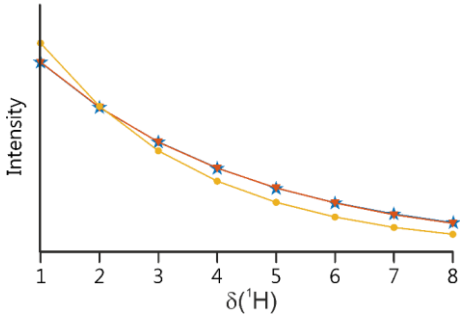




## TOCSY-$t_1$ evolution mode




## Diffusion mode

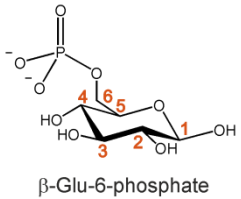


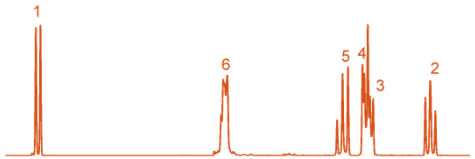

PARAFAC modes for SCALPEL experiment for a mixture of glucose, glucose 6-phosphate and maltose in $D_2O$

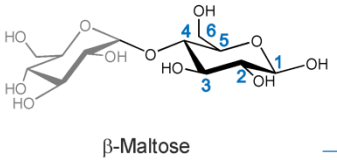
G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *J. Am. Chem. Soc.* **2019**, *141*, 5766.

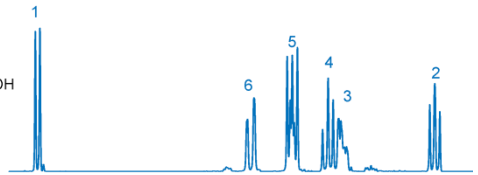
# SCALPEL & Sugars

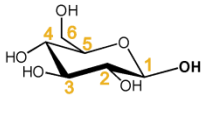
## Spectral mode

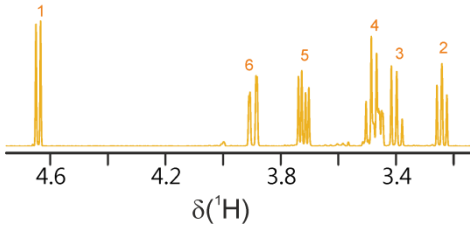


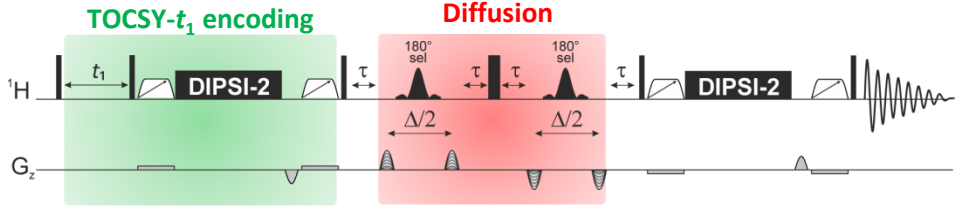



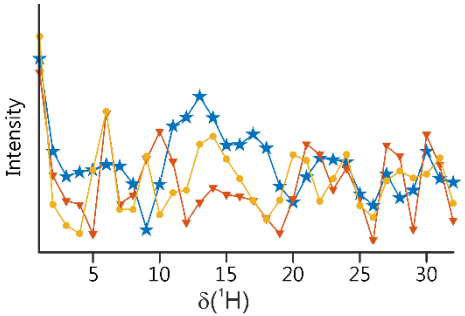



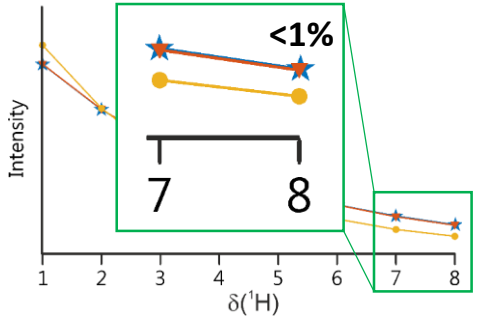




## TOCSY-$t_1$ evolution mode



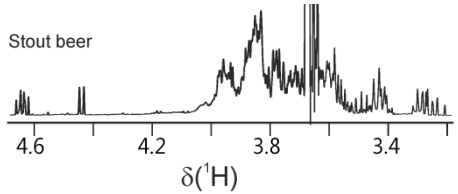

## Diffusion mode

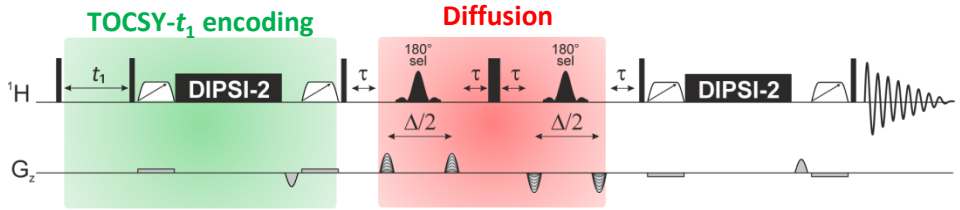



PARAFAC modes for SCALPEL experiment for a mixture of glucose, glucose 6-phosphate and maltose in $D_2O$

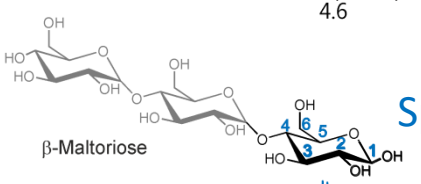
G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *J. Am. Chem. Soc.* **2019**, *141*, 5766.

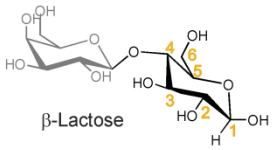
# SCALPEL & Beer

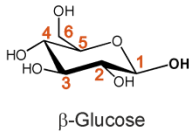


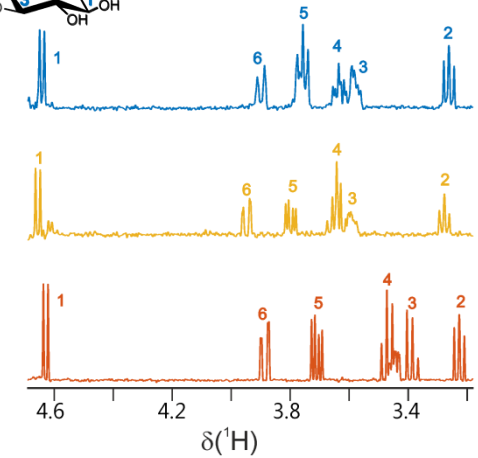




## Spectral mode

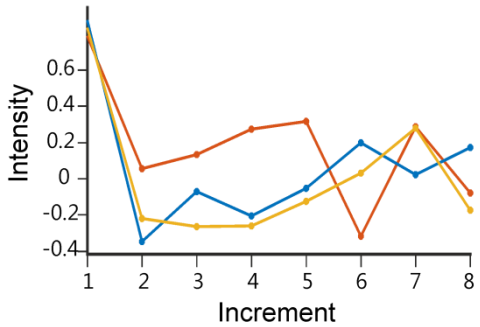


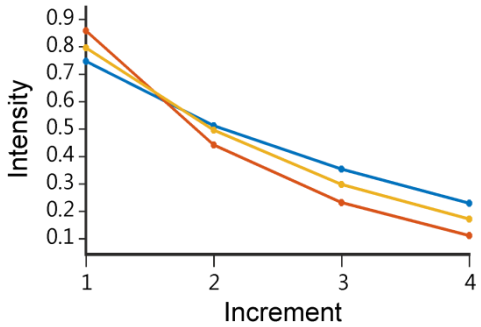




## TOCSY-$t_1$ evolution mode




## Diffusion mode

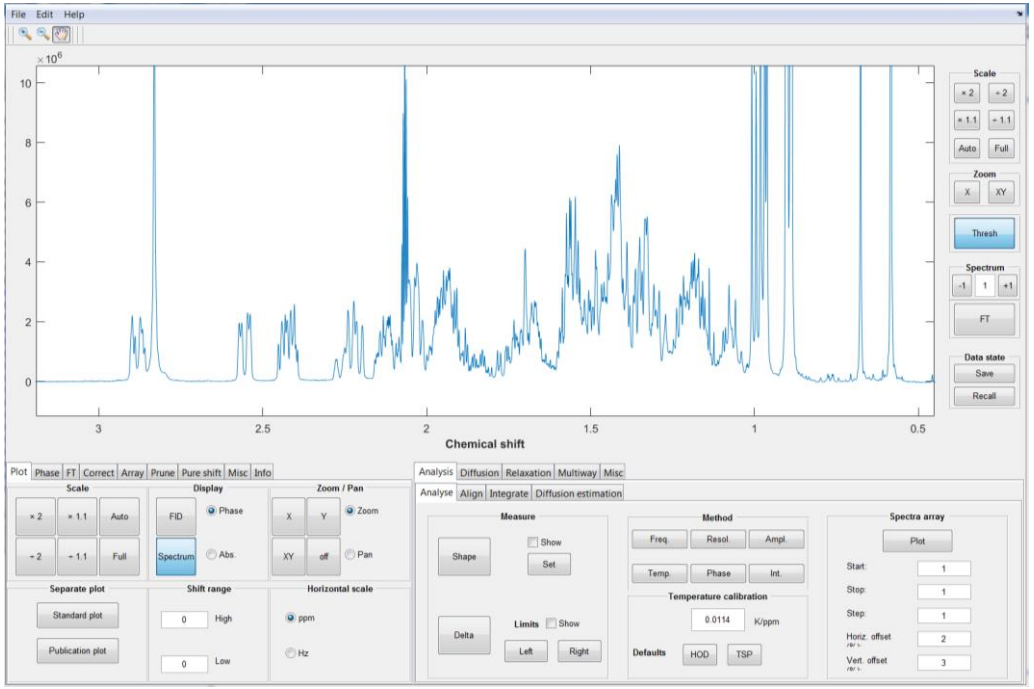



PARAFAC modes for SCALPEL experiment for a sample of stout beer (Mackeson) with 20% added $D_2O$

G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *J. Am. Chem. Soc.* **2019**, *141*, 5766.

# General NMR Analysis Toolbox (GNAT)

- For processing, visualising, and analysing NMR data
- Based on the DOSY Toolbox (*J. Magn. Reson.* **2009**, 200, 296)
- Free & open-source software
- User-friendly graphical interface
- MATLAB® language (free-standing compiled versions available)
- Univariate and multivariate analysis

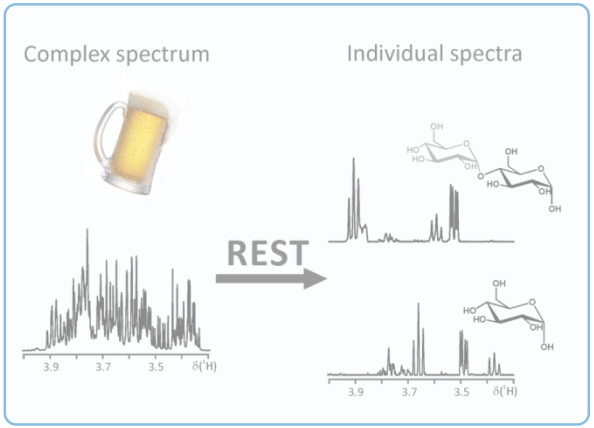



L. Castañar, G. Dal Poggetto, A. A. Colbourne, G. A. Morris, M. Nilsson, *Magn. Reson. Chem.* **2018**, *56*, 546.

# Novel NMR methods for the analysis of complex systems

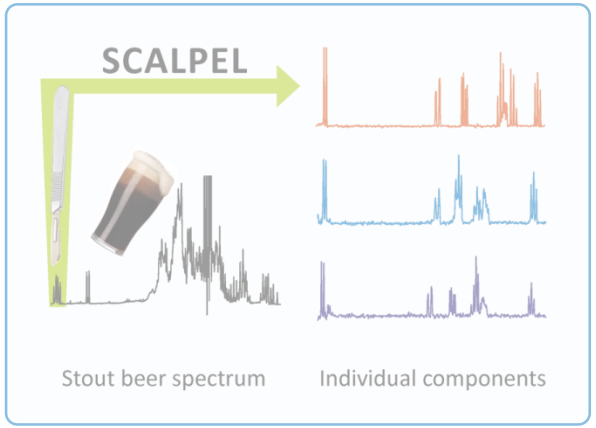
## REST

*Relaxation-Encoded Selective TOCSY*



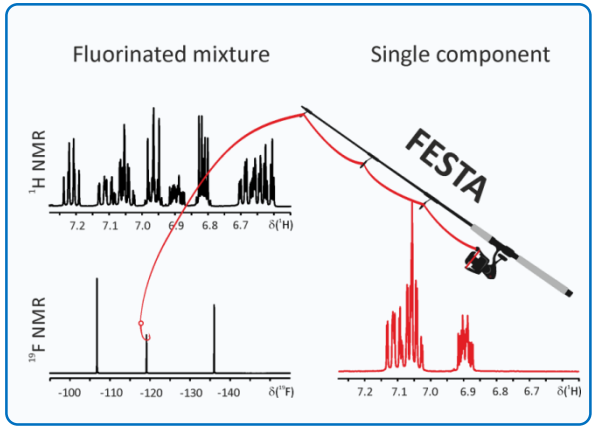

## SCALPEL

*Spectral Component Acquisition by Localized PARAFAC Extraction of Linear*



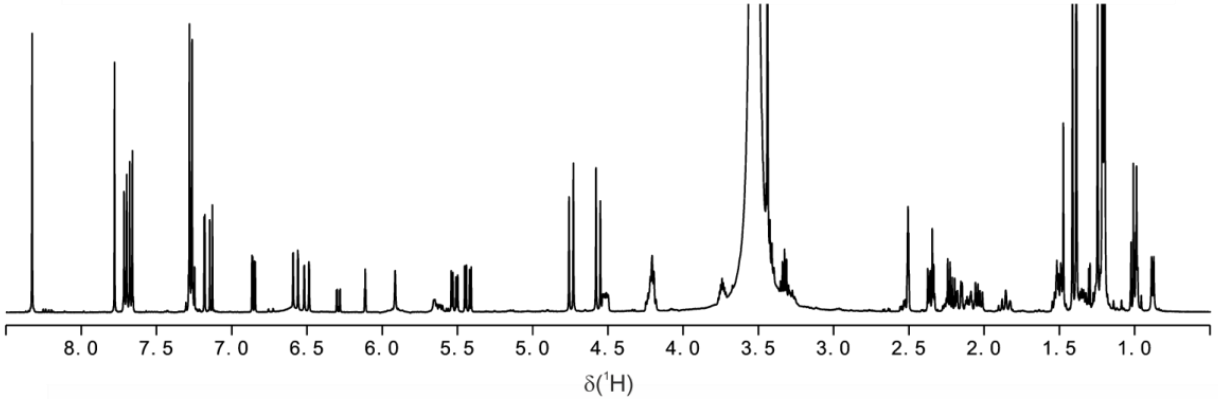

## FESTA

*Fluorine-Edited Selective TOCSY Acquisition*



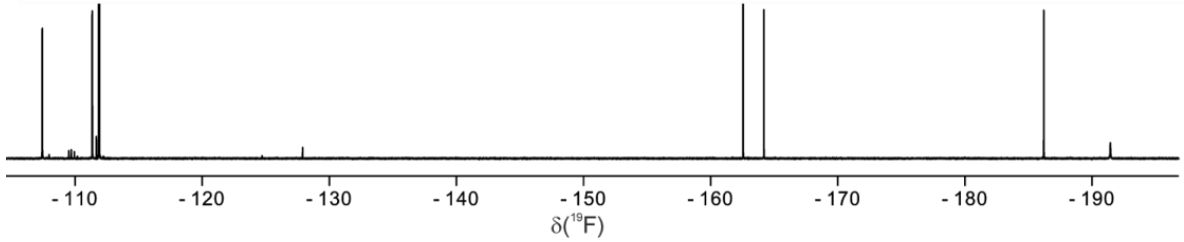
# Mixtures containing fluorinated species

$^{1}$H NMR



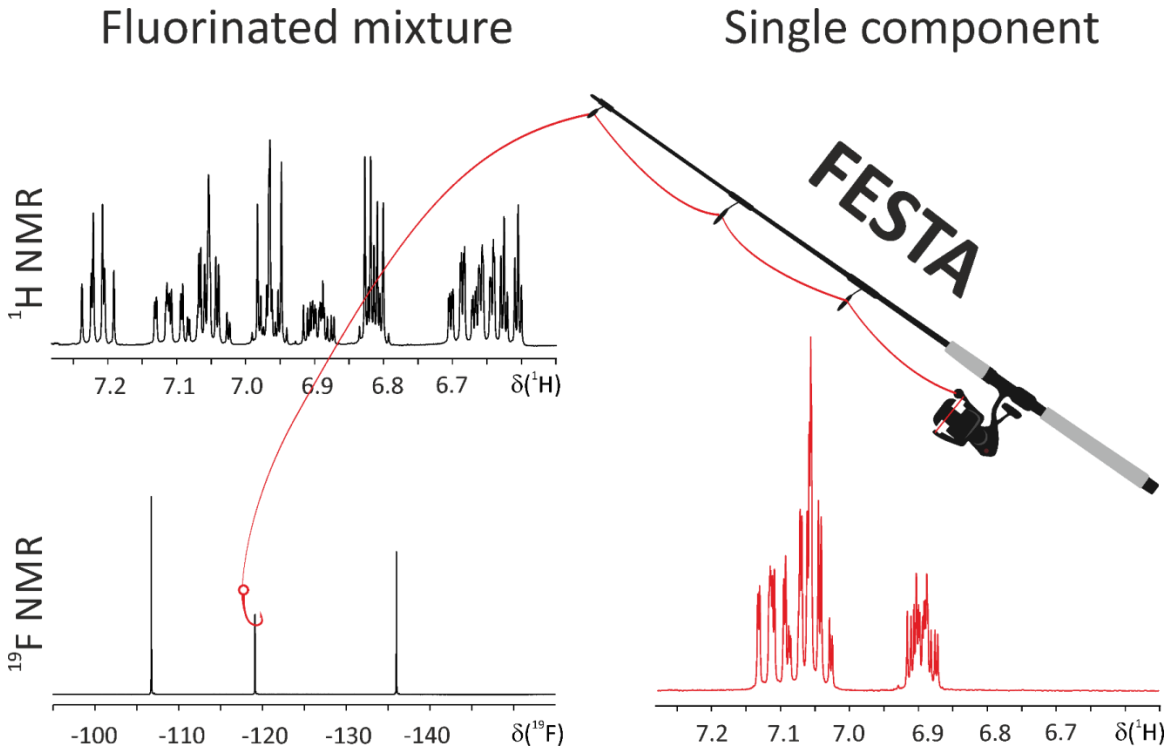

- ✗ Signal overlap
- ✗ Component information
- ✗ Structural information

$^{19}$F NMR




- ✓ Spectral resolution
- ✗ Component information
- ✗ Structural information

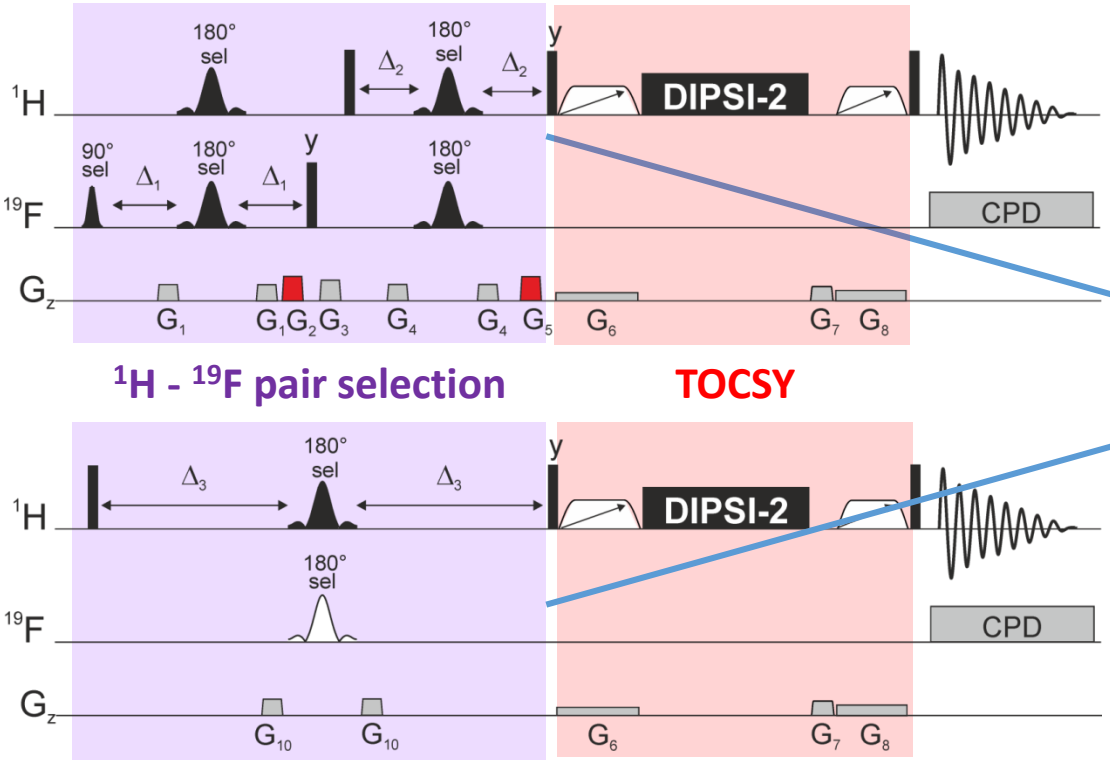
# Simplifying $^{1}$H NMR spectrum to extract structural information



# Fluorine-edited selective TOCSY Acquisition (FESTA)

SRI-FESTA

MODO-FESTA

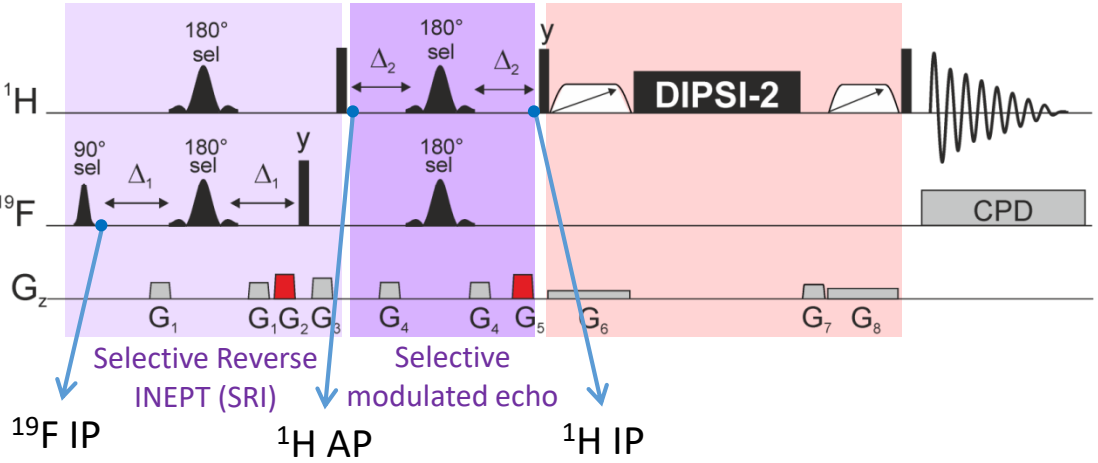



$^1$H magnetization in-phase (IP)

SRI: L. Castañar, P. Moutzouri, T. M. Barbosa, C. F. Tormena, R. Rittner, A. R. Phillips, S. R. Coombes, M. Nilsson, G. A. Morris, *Anal. Chem*. **2018**, *90,* 5445.
MODO: T. M. Barbosa, L. Castañar, P. Moutzouri, M. Nilsson, G. A. Morris, , R. Rittner C. F. Tormena,, *Anal. Chem*. **2020**, *92, 2224*.

# Fluorine-edited selective TOCSY Acquisition (FESTA)

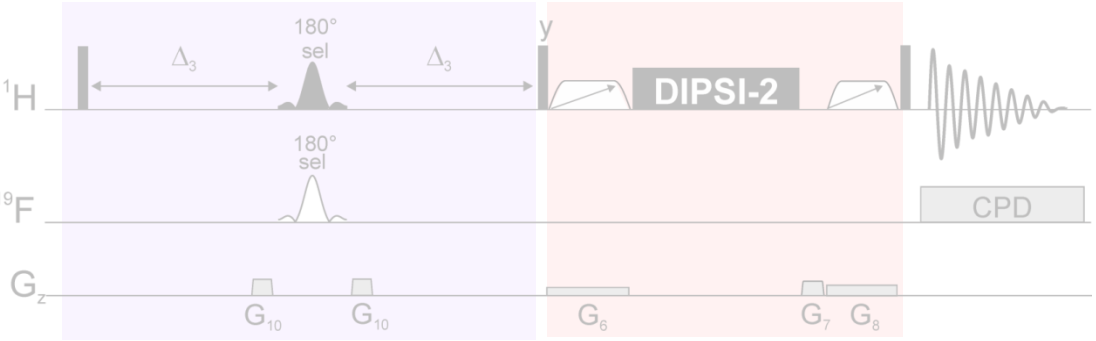
SRI-FESTA




$$\Delta_1 = \frac{1}{4J_{HF}n_H}$$

$$\Delta_2 = \frac{1}{4J_{HF}n_F}$$

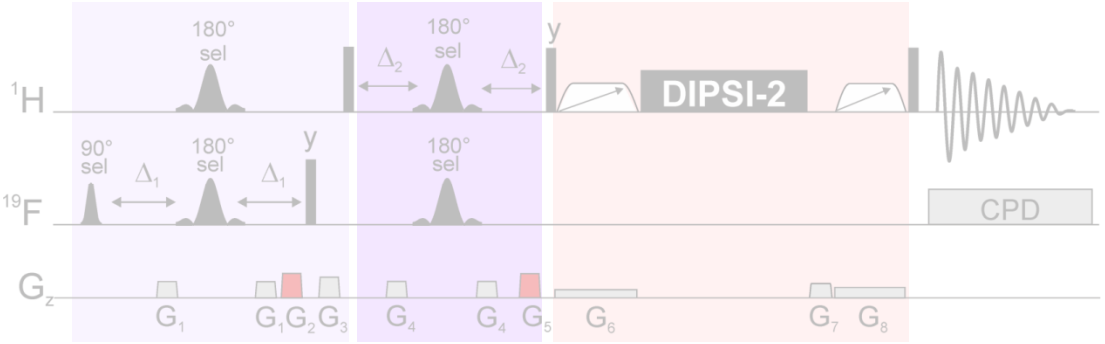
MODO-FESTA




SRI: L. Castañar, P. Moutzouri, T. M. Barbosa, C. F. Tormena, R. Rittner, A. R. Phillips, S. R. Coombes, M. Nilsson, G. A. Morris, *Anal. Chem*. **2018**, *90,* 5445.

# Fluorine-edited selective TOCSY Acquisition (FESTA)

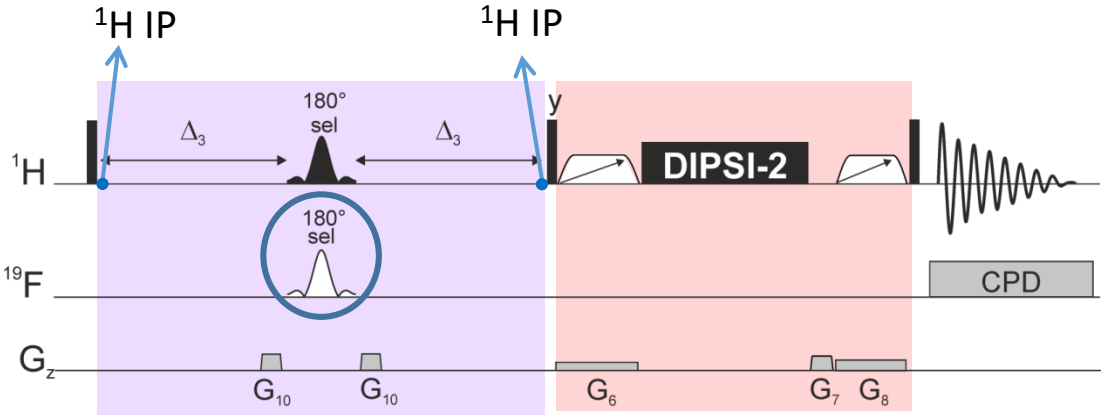
SRI-FESTA




$$\Delta_1 = \frac{1}{4J_{HF}n_H}$$

$$\Delta_2 = \frac{1}{4J_{HF}n_F}$$

MODO-FESTA



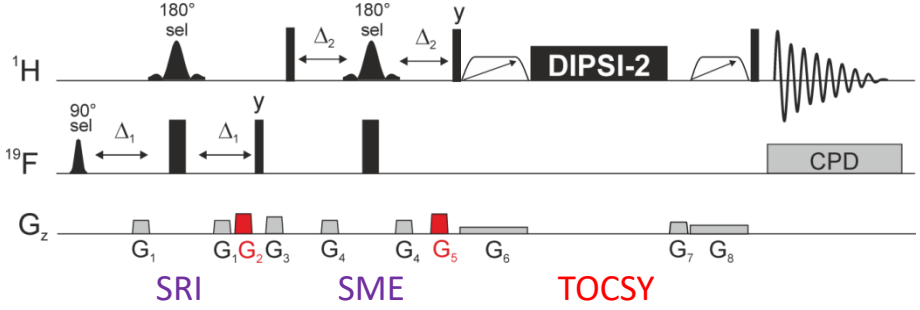

$$\Delta_3 = \frac{1}{2J_{HF}n_F}$$

$^{19}$F selective 180° pulse only applied in even-numbered scans

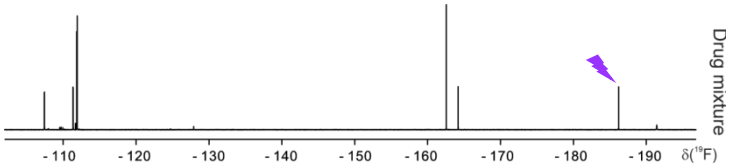
Selective MOdulated echO (MODO)

MODO: T. M. Barbosa, L. Castañar, P. Moutzouri, M. Nilsson, G. A. Morris, , R. Rittner C. F. Tormena,, *Anal. Chem*. **2020**, *92*, 2224.

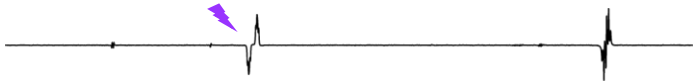
# How SRI-FESTA works




$^{19}$F

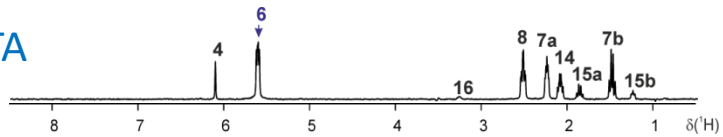



$^1$H SRI



$^1$H SRI-SME


$^1$H SRI-FESTA




**$^{19}$F** Spin selection

⬇ Heteronuclear transfer ($J_{HF}$)

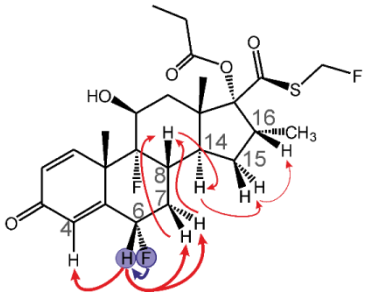
**$^1H_A$ $^1H_B$**

⬇ Spin selection

**$^1H_A$**

⬇ Homonuclear transfer ($J_{HH}$)

**$^1$H** → **$^1$H** → **$^1$H** →

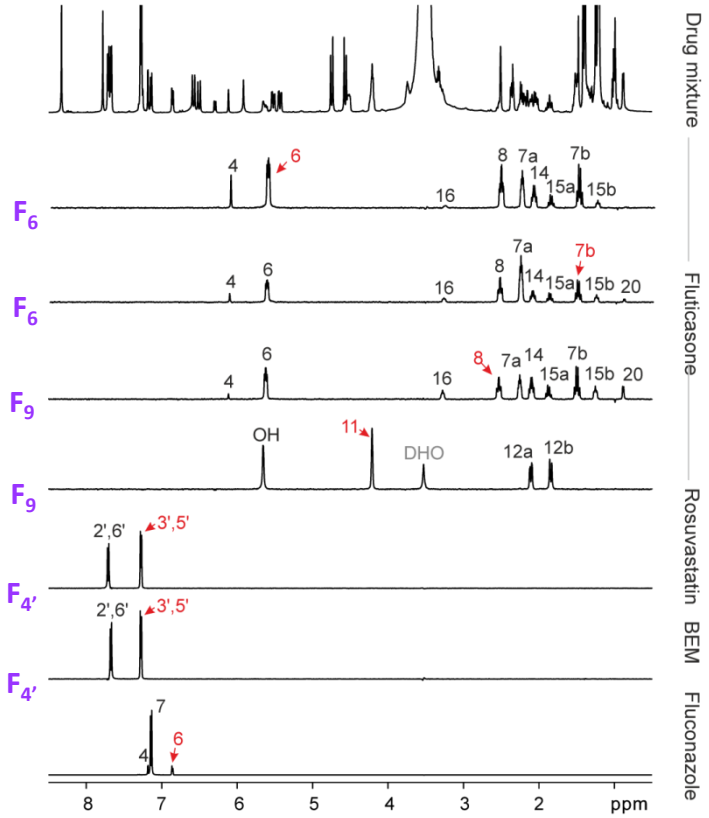


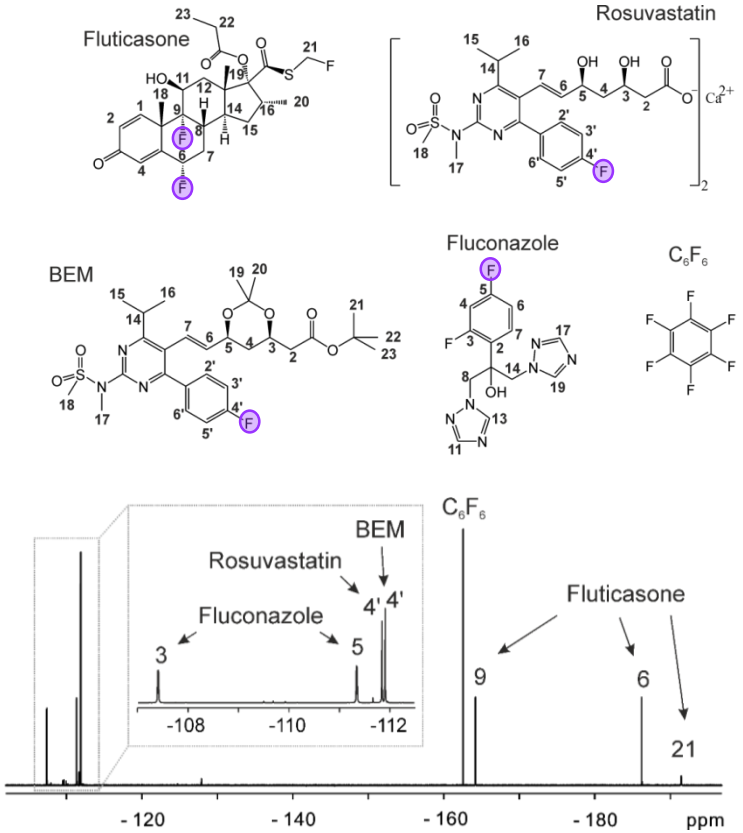

L. Castañar, P. Moutzouri, T. M. Barbosa, C. F. Tormena, R. Rittner, A. R. Phillips, S. R. Coombes, M. Nilsson, G. A. Morris, *Anal. Chem*. **2018**, *90,* 5445.

# FESTA & Drug mixture

$^1$H

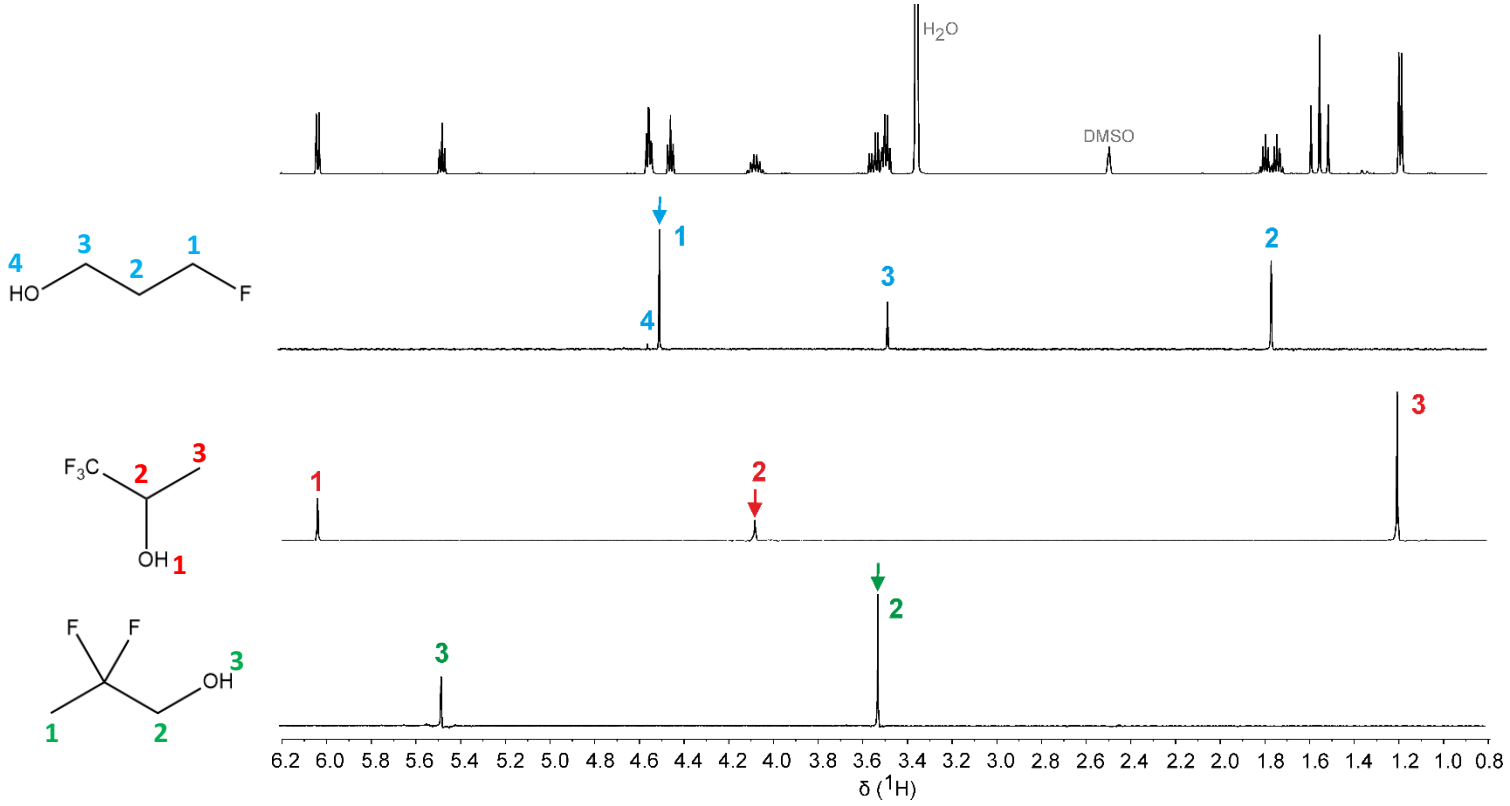
$^1$H FESTA




L. Castañar, P. Moutzouri, T. M. Barbosa, C. F. Tormena, R. Rittner, A. R. Phillips, S. R. Coombes, M. Nilsson, G. A. Morris, *Anal. Chem*. **2018**, *90,* 5445.

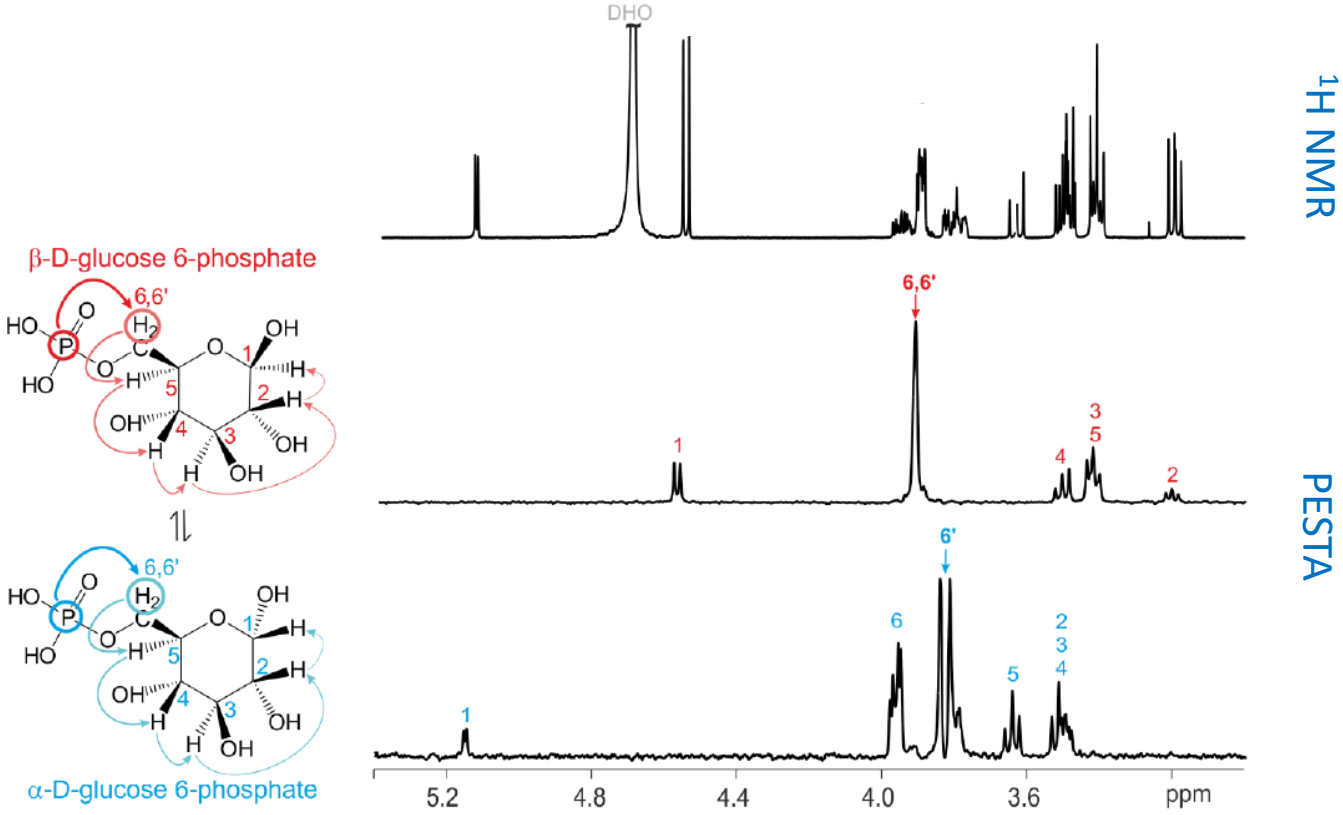
# Ultrahigh Resolution Pure Shift FESTA




*Preliminary results*

Coral Mycroft

# Phosporous-Edited Selective TOCSY Acquisition (PESTA)

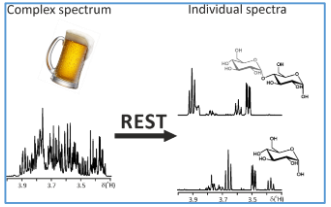


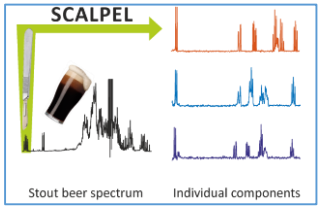

*Preliminary results*

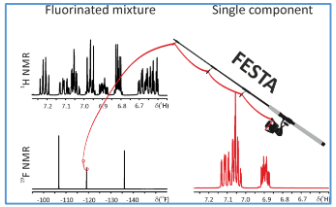
Carmen Tebar Soler

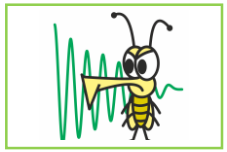
# Novel NMR methods and software for mixture analysis

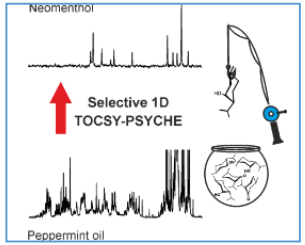
- REST – *Chem. Commun.* **2017**, *53*, 7461.
- SCALPEL – *J. Am. Chem. Soc.* **2019**, *141*, 5766.
- FESTA – *Anal. Chem.* **2018**, *90*, 5445.
  – *Anal. Chem.* **2020**, *92*, 2224.
- GNAT – *Mag. Reson. Chem.* **2018**, *56*, 546.
- MAGNATE – *Anal. Chem.* **2018**, *90*, 13695.
- Selective PSYCHE-TOCSY – *RSC Adv.* **2016**, *6*, 100063.
- PSYCHE-iDOSY – *Angew. Chem. Int. Ed.* **2016**, *55*, 15579.
- SAPPHIRE – *Chem. Commun.* **2017**, *53*, 10188.
- DISPEL – *Anal. Chem.*, **2017**, *89*, 11898.
  – *J. Magn. Reson.* **2018**, *295*, 6.

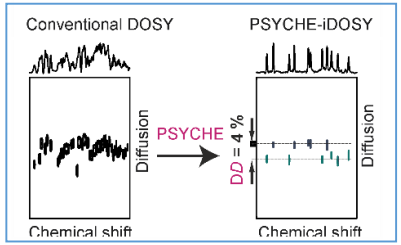


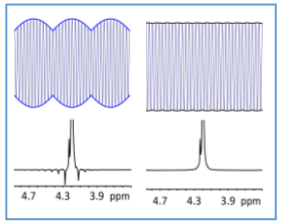



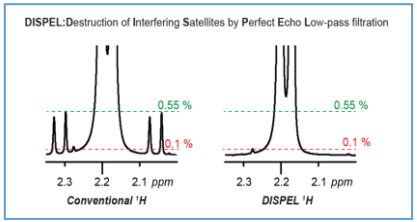





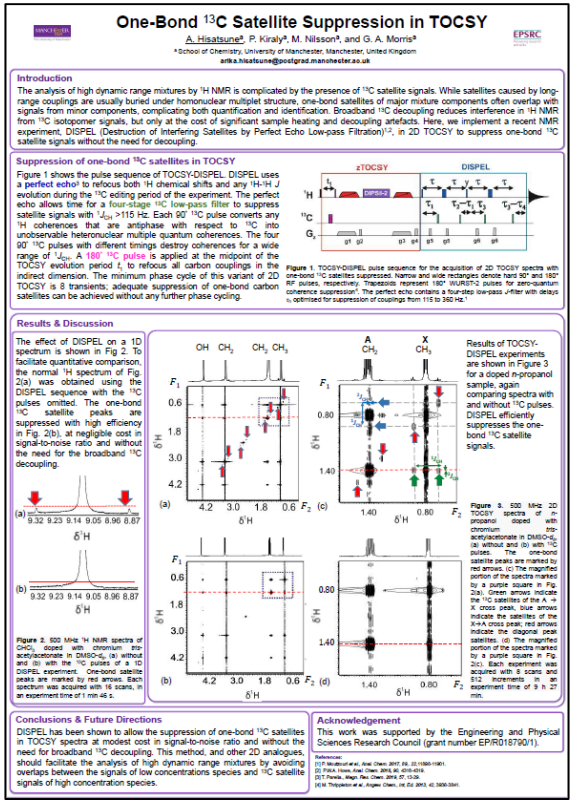



# One-Bond $^{13}C$ Satellite Suppression in TOCSY

## Poster 222

*Tuesday 10th*
*Thursday 12th*

*Key Ballroom 7-8*



One-Bond $^{13}C$ Satellite Suppression in TOCSY

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**Introduction**

The analysis of high dynamic range mixtures by $^1H$ NMR is complicated by the presence of $^{13}C$ satellite signals. While satellites caused by long-range couplings are usually buried under homonuclear multiplet structure, one-bond satellites of major mixture components often overlap with signals from minor components, complicating both quantification and identification. Broadband $^{13}C$ decoupling reduces interference in $^1H$ NMR from $^{13}C$ isotopomer signals, but only at the cost of significant sample heating and decoupling artefacts. Here, we implement a recent NMR experiment, DISPEL (Destruction of Interfering Satellites by Perfect Echo Low-pass Filtration)[1,2], in 2D TOCSY to suppress one-bond $^{13}C$ satellite signals without the need for decoupling.

**Suppression of one-bond $^{13}C$ satellites in TOCSY**

Figure 1 shows the pulse sequence of TOCSY-DISPEL. DISPEL uses a perfect echo[3] to refocus both $^1H$ chemical shifts and any $^1H$-$^1H$ $J$ evolution during the $^{13}C$ editing period of the experiment. The perfect echo allows time for a four-stage $^{13}C$ low-pass filter to suppress satellite signals with $^1J_{CH}$ >115 Hz. Each 90° $^{13}C$ pulse converts any $^1H$ coherences that are antiphase with respect to $^{13}C$ into unobservable heteronuclear multiple quantum coherences. The four 90° $^{13}C$ pulses with different timings destroy coherences for a wide range of $^1J_{CH}$. A 180° $^{13}C$ pulse is applied at the midpoint of the TOCSY evolution period $t_1$ to refocus all carbon couplings in the indirect dimension. The minimum phase cycle of this variant of 2D TOCSY is 8 transients; adequate suppression of one-bond carbon satellites can be achieved without any further phase cycling.




Figure 1. TOCSY-DISPEL pulse sequence for the acquisition of 2D TOCSY spectra with one-bond $^{13}C$ satellites suppressed. Narrow and wide rectangles denote hard 90° and 180° RF pulses, respectively. Trapezoids represent 180° WURST-2 pulses for zero-quantum coherence suppression[4]. The perfect echo contains a four-step low-pass $J$-filter with delays $\tau_1$ optimised for suppression of couplings from 115 to 360 Hz.[1]

**Results & Discussion**

The effect of DISPEL on a 1D spectrum is shown in Fig 2. To facilitate quantitative comparison, the normal $^1H$ spectrum of Fig. 2(a) was obtained using the DISPEL sequence with the $^{13}C$ pulses omitted. The one-bond $^{13}C$ satellite peaks are suppressed with high efficiency in Fig. 2(b), at negligible cost in signal-to-noise ratio and without the need for the broadband $^{13}C$ decoupling.




Figure 2. 600 MHz $^1H$ NMR spectra of $CrO_x$ doped with chromium tris-acetylacetonate in DMSO-$d_6$ (a) without and (b) with the $^{13}C$ pulses of a 1D DISPEL experiment. One-bond satellite peaks are marked by red arrows. Each spectrum was acquired with 16 scans, in an experiment time of 1 min 46 s.




Results of TOCSY-DISPEL experiments are shown in Figure 3 for a doped *n*-propanol sample, again comparing spectra with and without $^{13}C$ pulses. DISPEL efficiently suppresses the one-bond $^{13}C$ satellite signals.

Figure 3. 500 MHz 2D TOCSY spectra of *n*-propanol doped with chromium tris-acetylacetonate in DMSO-$d_6$ (a) without and (b) with $^{13}C$ pulses. The one-bond satellite peaks are marked by red arrows. (c) The magnified portion of the spectra marked by a purple square in Fig. 2(a). Green arrows indicate the $^{13}C$ satellites of the A → X cross peak, blue arrows indicate the satellites of the X→A cross peak, red arrows indicate the diagonal peak satellites. (d) The magnified portion of the spectra marked by a purple square in Fig. 2(c). Each experiment was acquired with 8 scans and 512 increments in an experiment time of 9 h 27 min.

**Conclusions & Future Directions**

DISPEL has been shown to allow the suppression of one-bond $^{13}C$ satellites in TOCSY spectra at modest cost in signal-to-noise ratio and without the need for broadband $^{13}C$ decoupling. This method, and other 2D analogues, should facilitate the analysis of high dynamic range mixtures by avoiding overlaps between the signals of low concentrations species and $^{13}C$ satellite signals of high concentration species.

**Acknowledgement**

This work was supported by the Engineering and Physical Sciences Research Council (grant number EP/R018790/1).

References:
[1] P. Modrzejewski et al., Anal. Chem. 2017, 89, 22, 11560-11561.
[2] P.M.A. Howe, Anal. Chem. 2015, 87, 4316-4319.
[3] T. Parella, Magn. Res. Chem. 2014, 57, 13-26.
[4] M. Tkáčiková et al., Angew. Chem., Int. Ed. 2003, 42, 3806-3841.

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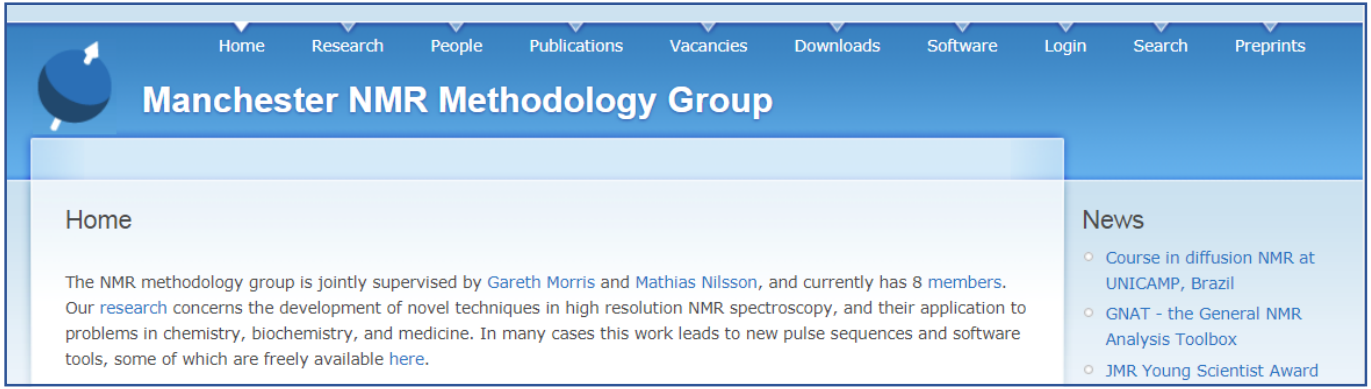
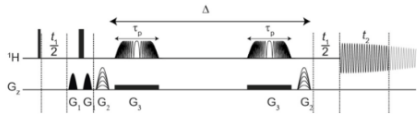



## Pulse Sequences

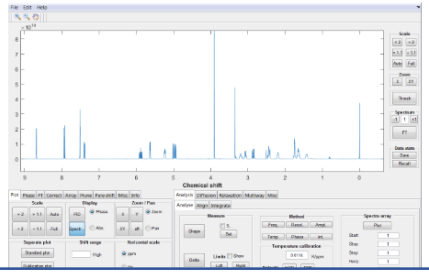
We are currently preparing many of our pulse sequences, parameter sets, example datasets and processing macros for the website. Some are available here but if you would like to use any of the other the sequences, as described in the publications section, please email us. The majority of sequences are available for Varian systems and we are gradually writing the Bruker variants.

The pulse sequences and any macros required for data conversion can be accessed from this part of the website.



## Software

Software produced in-house, including The GNAT (General NMR Analysis Toolbox), the legacy DOSY Toolbox, and diffusion estimation.



## Workshops and presentations

The slides from some of the workshops and presentations given by group members are available from this part of the website. There is a pure shift NMR package available for download as part of our 2017 workshop on pure shift NMR.

# Manchester NMR Methodology Group

## Collaborators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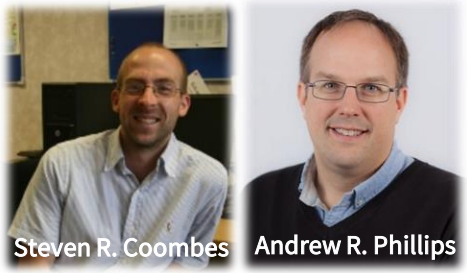
University of Campinas, Brazil

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Conselho Nacional de Desenvolvimento Científico e Tecnológico

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# Thank you very much for your attention!

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**NMR Methodology Group**
The University of Manchester

61st Experimental Nuclear Magnetic Resonance Conference
Baltimore, Maryland

10th March, 2020