New NMR tools for getting the most out of complex spectra

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NMR Methodology Group

The University of Manchester

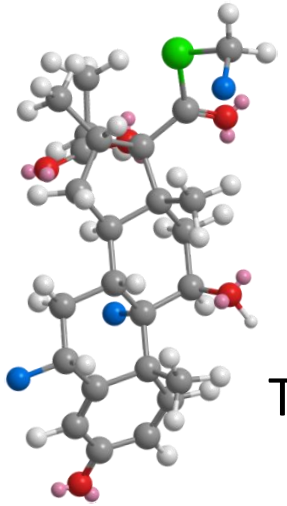
Small Molecule NMR Conference

Porto, Portugal

September 25th 2019

The importance of chemical structure

Structure – Function relations



Chemical properties

Biological functions

Physical properties

Technological applications

…

NMR

Economic/social implications

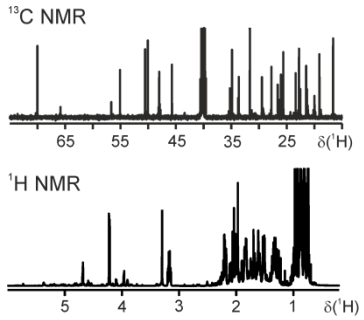
Chemical synthesis

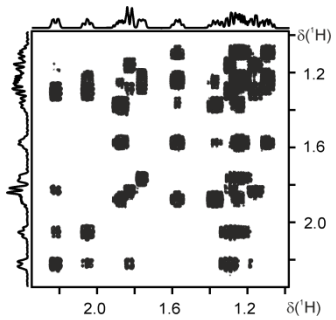
Drug discovery

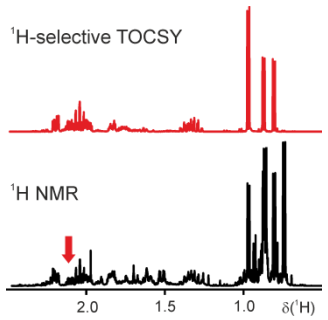
Materials science

Fuel development

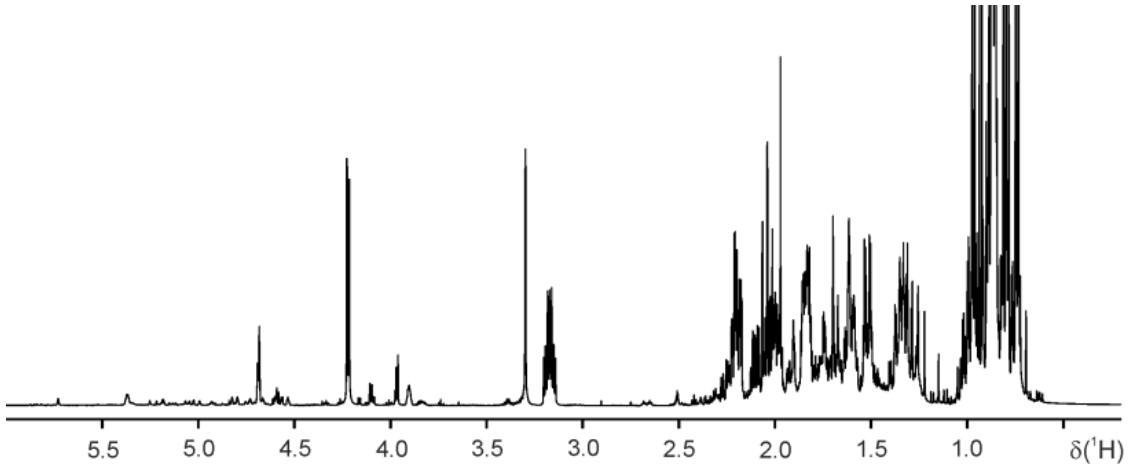
…







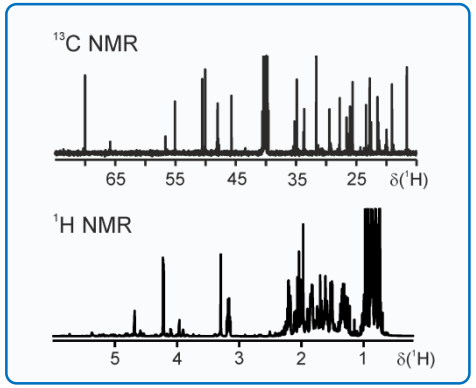
1D ^{1}H NMR



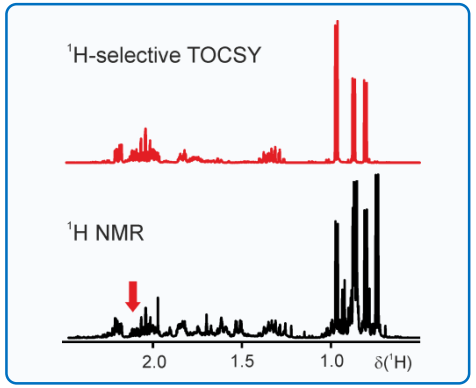
- ✓ High sensitivity
- ✓ The most abundant nuclei
- ✓ Structural information
- ✗ Signal overlap
- ✗ Low resolution spectra

Strategies for alleviating overlap

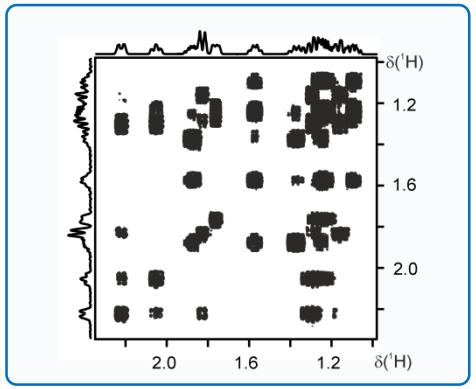
Other nuclei



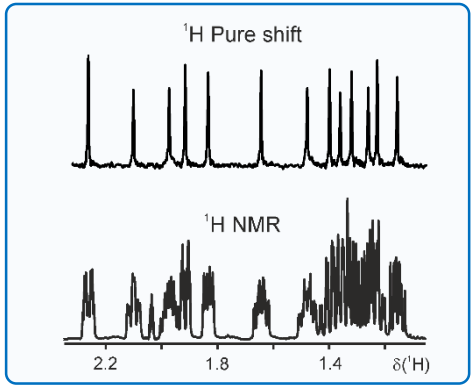
Virtual separation



nD NMR



Pure shift NMR

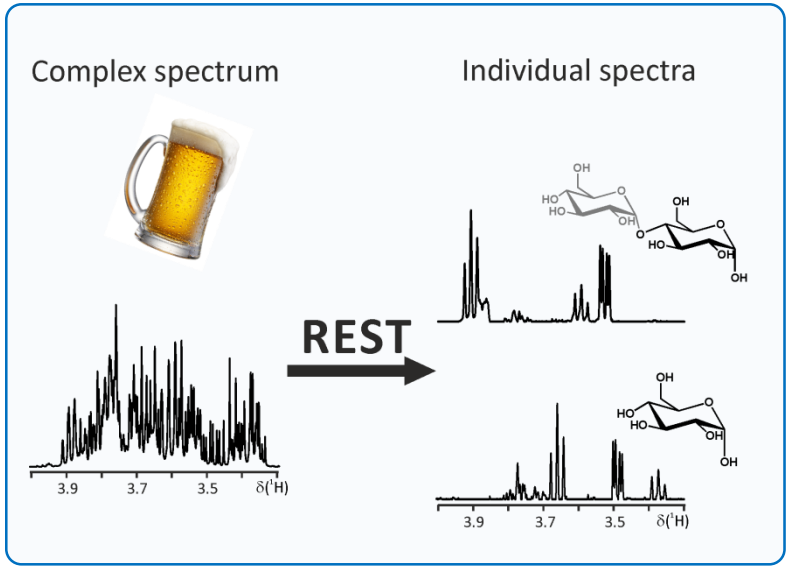


Spectral complexity obscures structural information:
we need better experimental methods!

Novel NMR methods for the analysis of complex systems

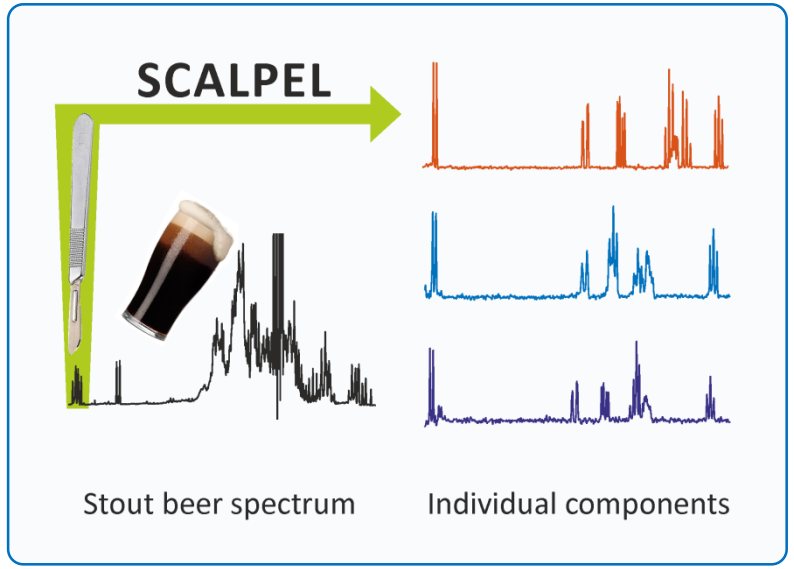
REST

Relaxation-Encoded Selective TOCSY



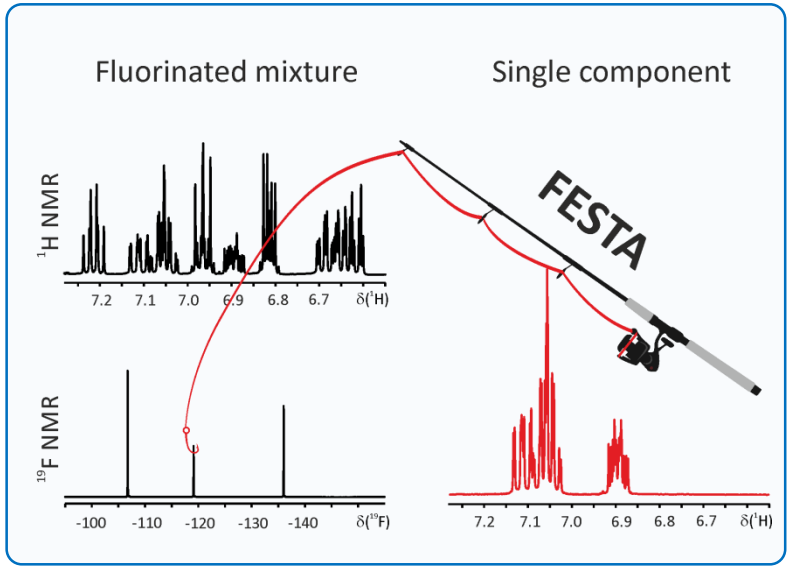
SCALPEL

Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components



FESTA

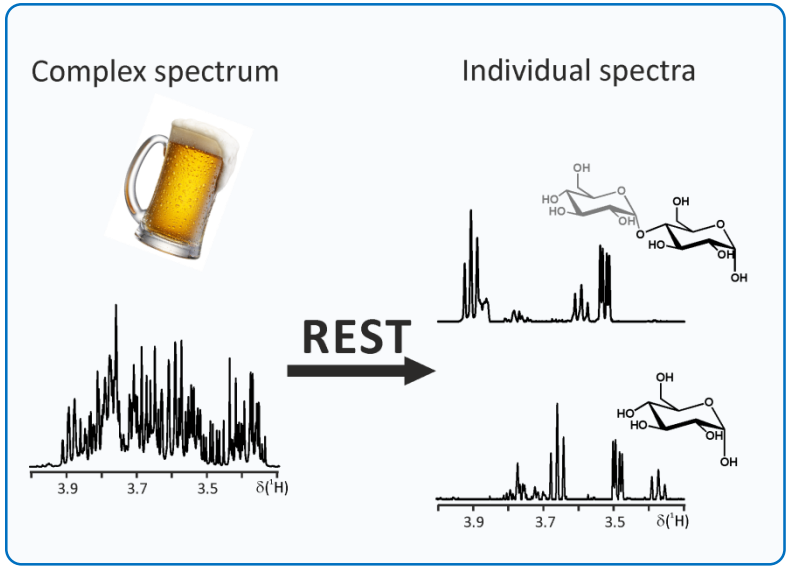
Fluorine-Edited Selective TOCSY Acquisition



Novel NMR methods for the analysis of complex systems

REST

Relaxation-Encoded Selective TOCSY



SCALPEL

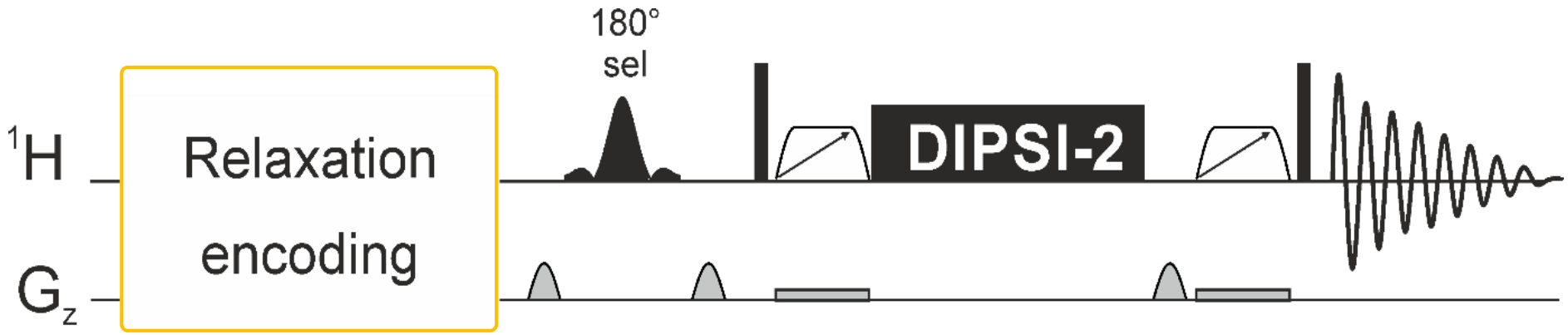
Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components

FESTA

Fluorine-Edited Selective TOCSY Acquisition

Relaxation-Encoded Selective TOCSY (REST) experiment

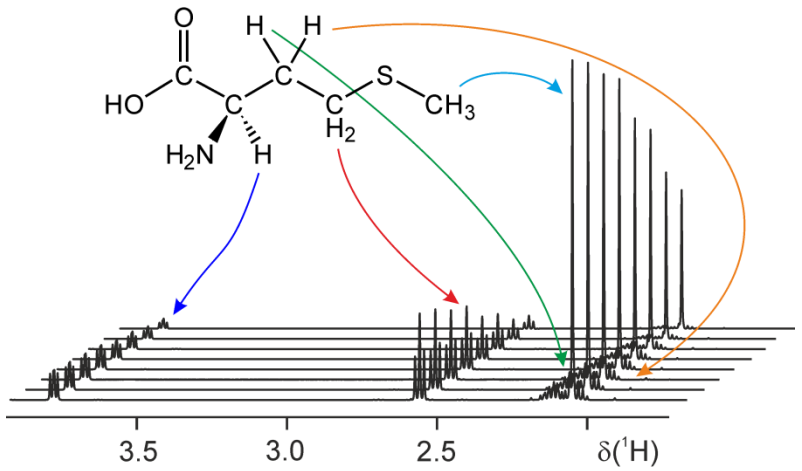
Virtual separation of the components of a mixture by exploiting differences in the relaxation behavior of spins

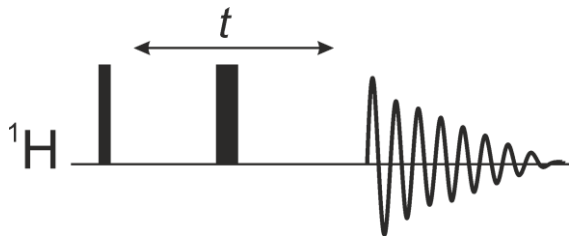


G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

Relaxation NMR

Relaxation array



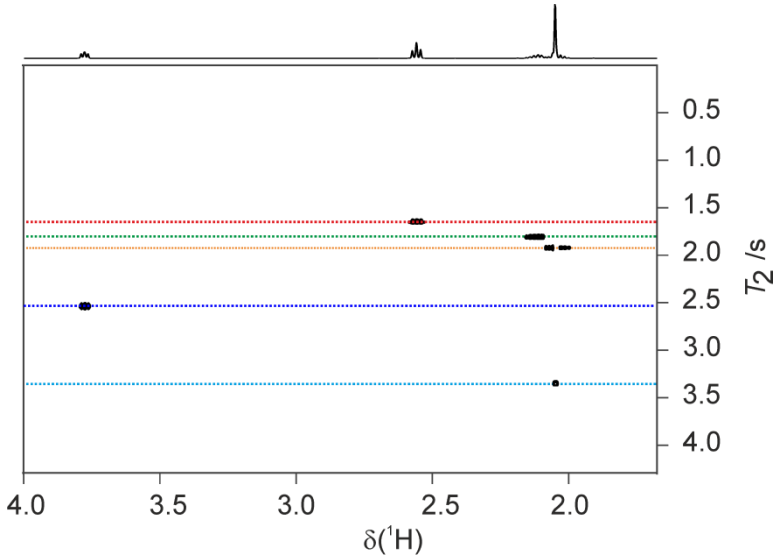


Prototype sequence

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

Univariate analysis

Relaxation-Ordered SpectroscopY (ROSY)



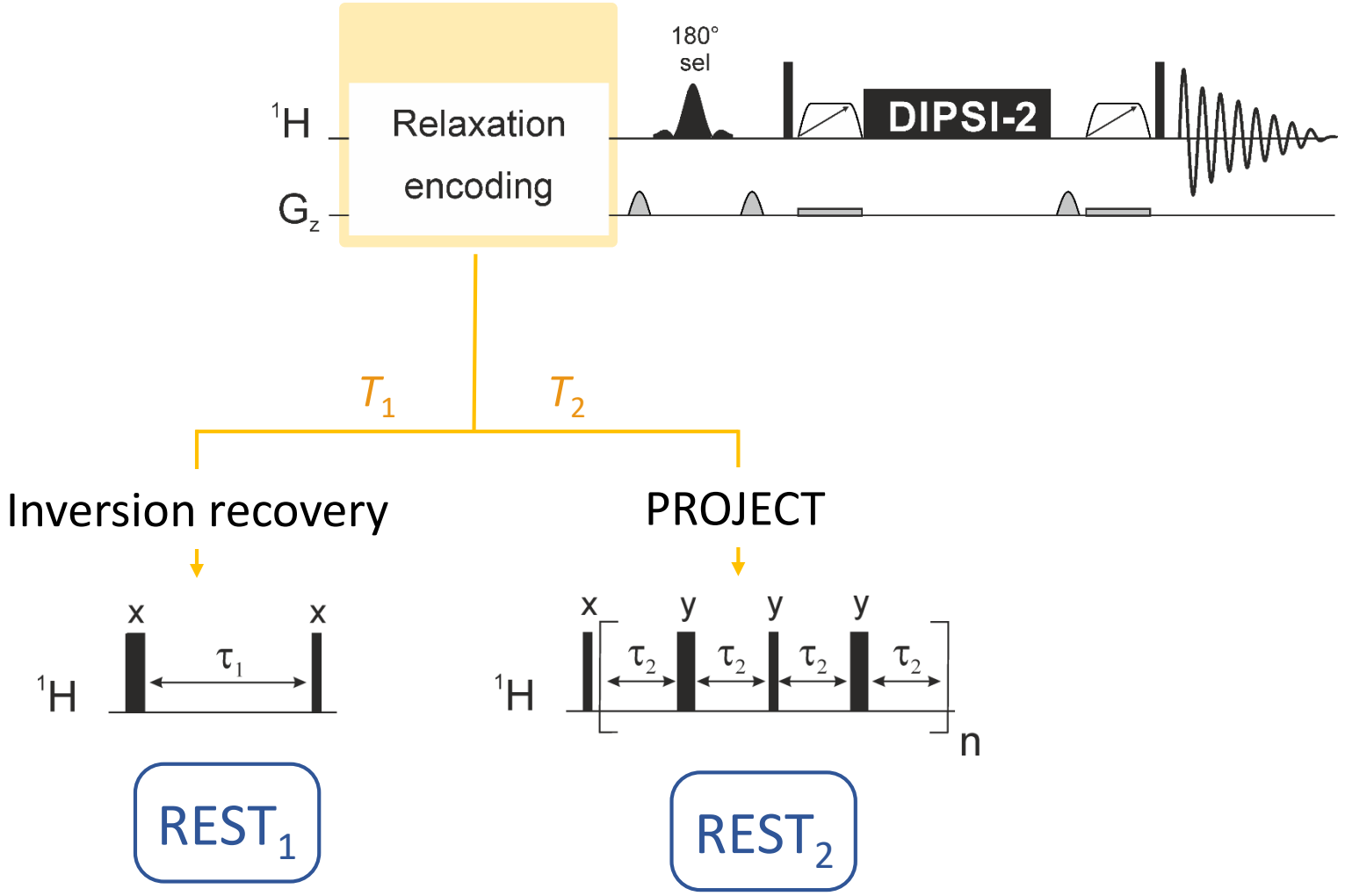
Signals from a given molecule:

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

NO virtual separation of components

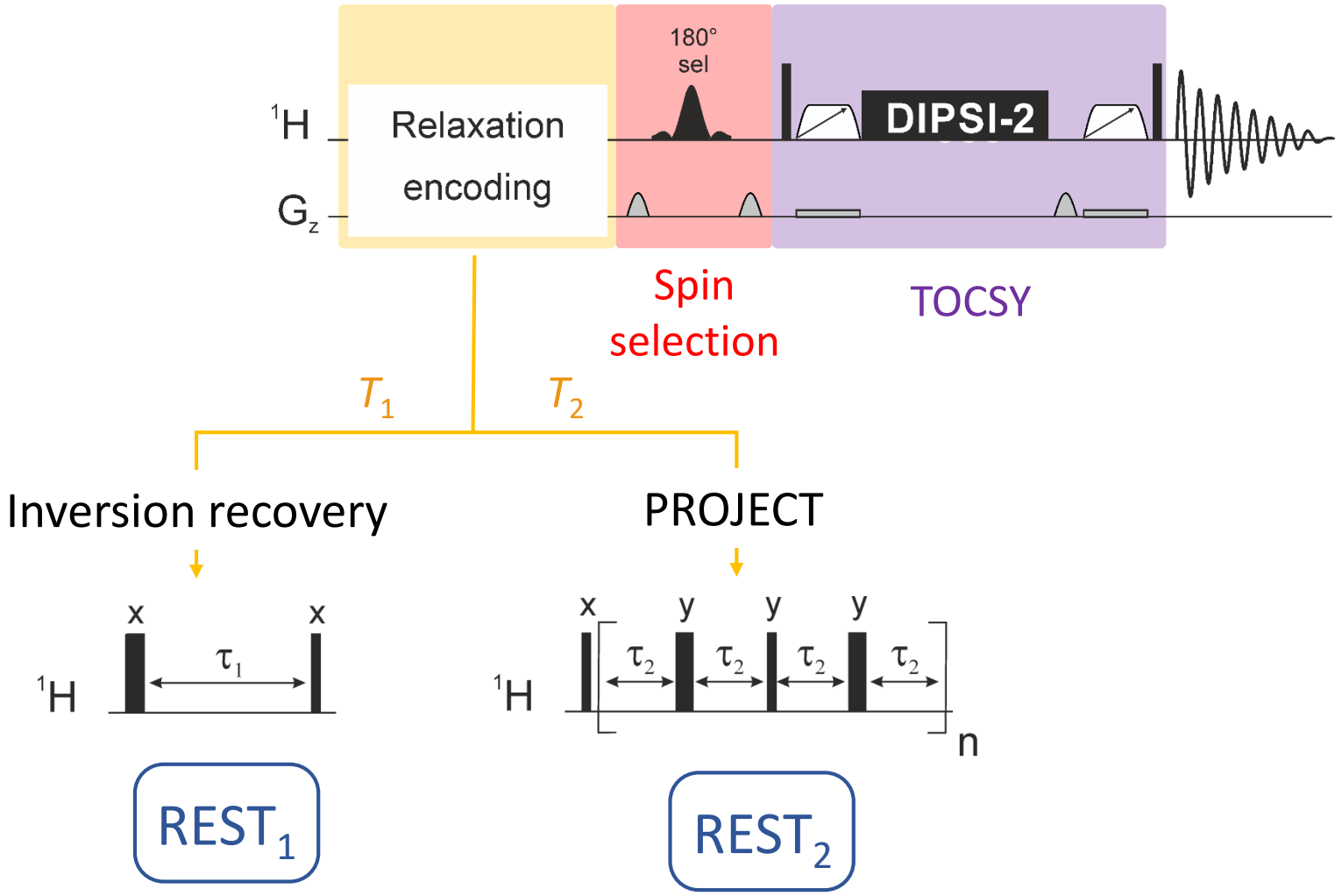
(ROSY) Y. Nishiyama, M. H. Frey, S. Mukasa and H. Utsumi, *J. Magn. Reson.*, **2010**, *202*, 135.

Relaxation-Encoded Selective TOCSY (REST) experiment



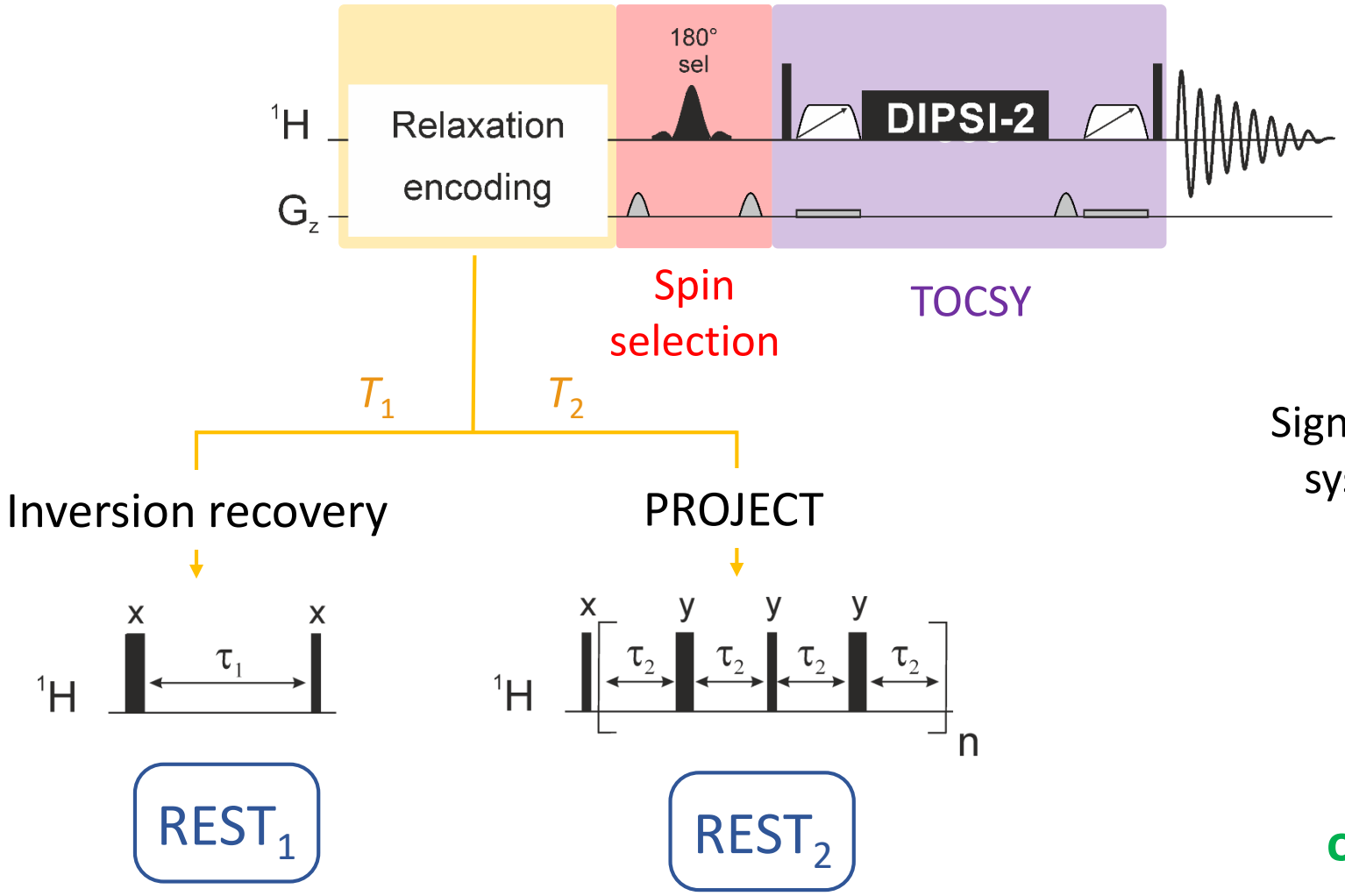
(Inversion recovery) R. L. Vold, J. S. Waugh, M. P. Klein and D. E. Phelps, *J. Chem. Phys*., **1968**, *48*, 3831.
(PROJECT) J. A. Aguilar, M. Nilsson, G. Bodenhausen and G. A. Morris, *Chem. Commun*., **2012**, *48*, 811.

Relaxation-Encoded Selective TOCSY (REST) experiment



(Selective TOCSY) M. J. Thrippleton and J. Keeler, *Angew. Chem., Int. Ed., 2003, 42,* 39.

Relaxation-Encoded Selective TOCSY (REST) experiment



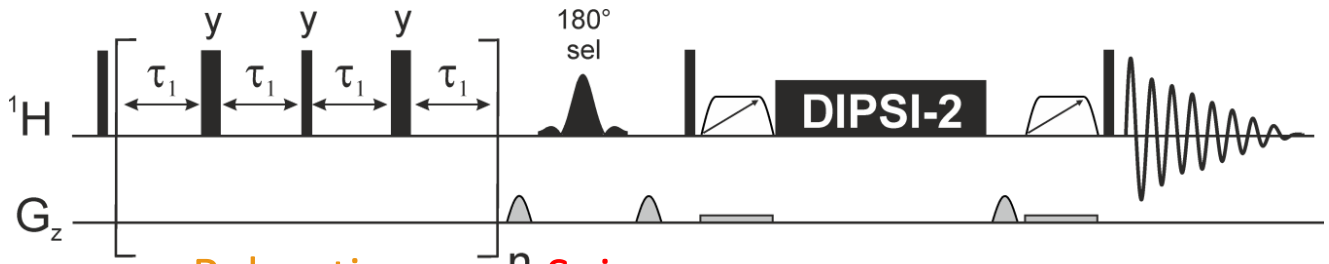
Signals from a given spin system appear at the same T_1 / T_2

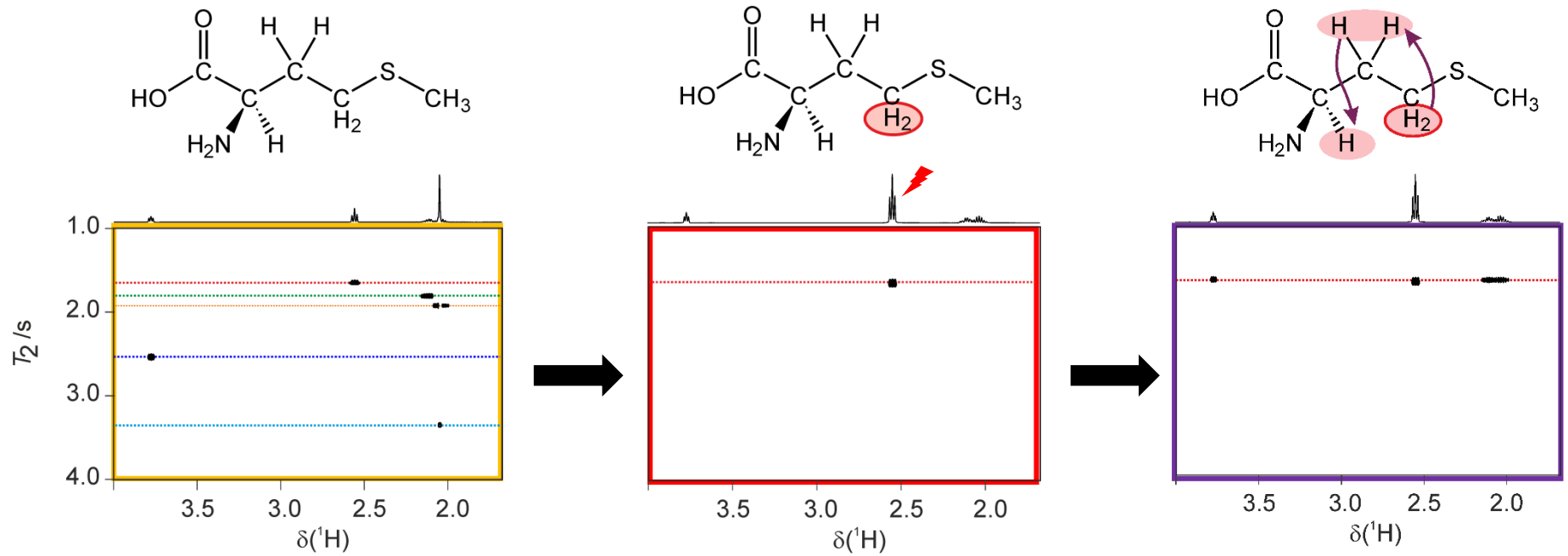
Virtual separation of spin systems

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$_2$



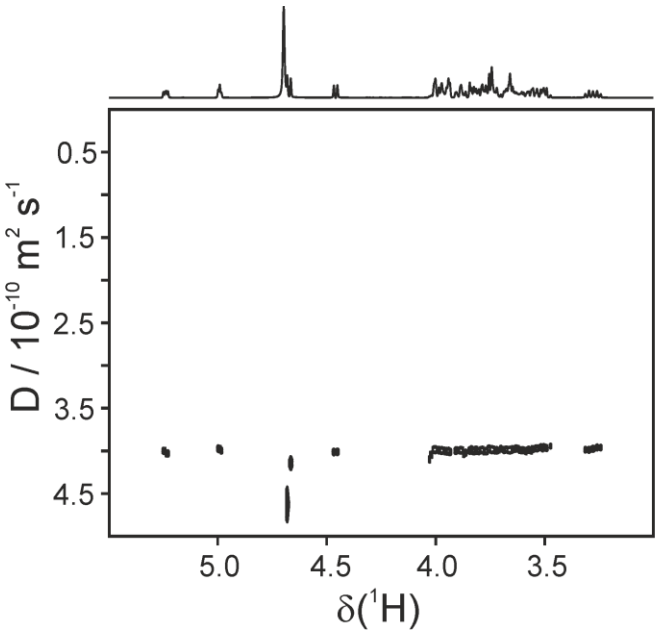


Signals from a given spin system appear at the same T_2

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

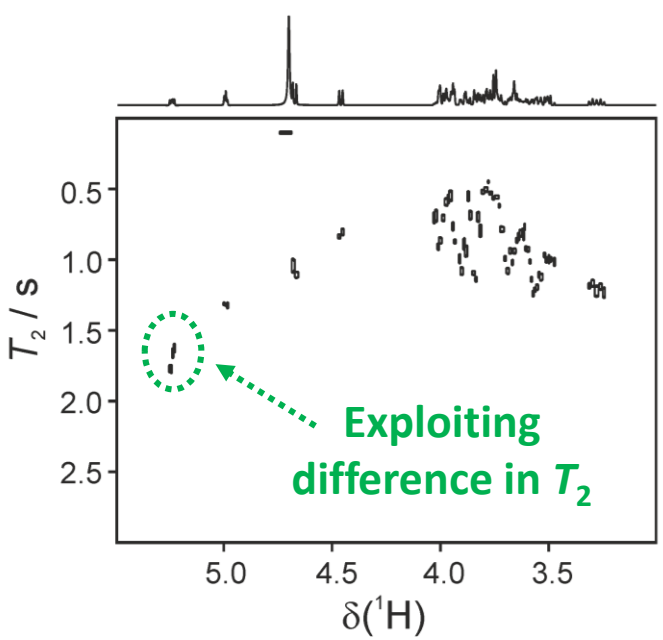
REST & Sugars

Oneshot DOSY



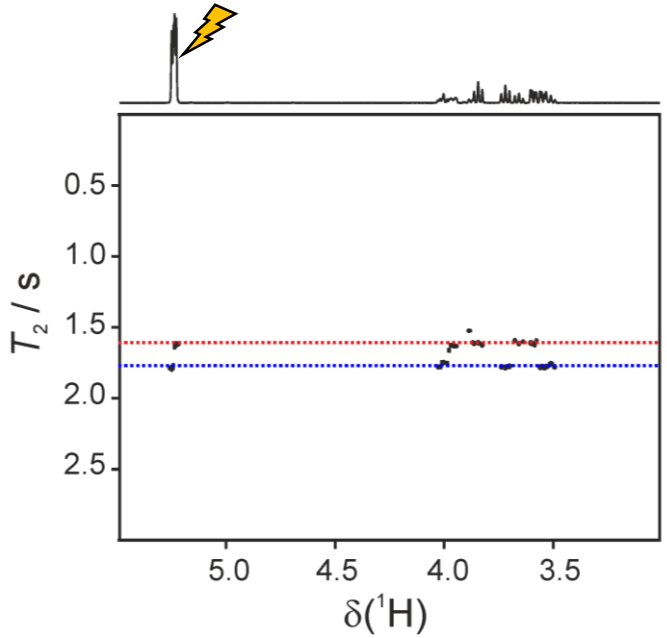
Sugar have almost identical *D*

PROJECT ROSY

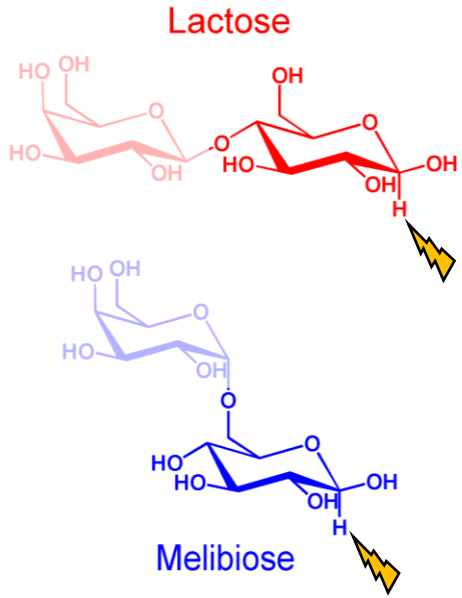


All sugar signals have different T_2

REST$_2$ ROSY



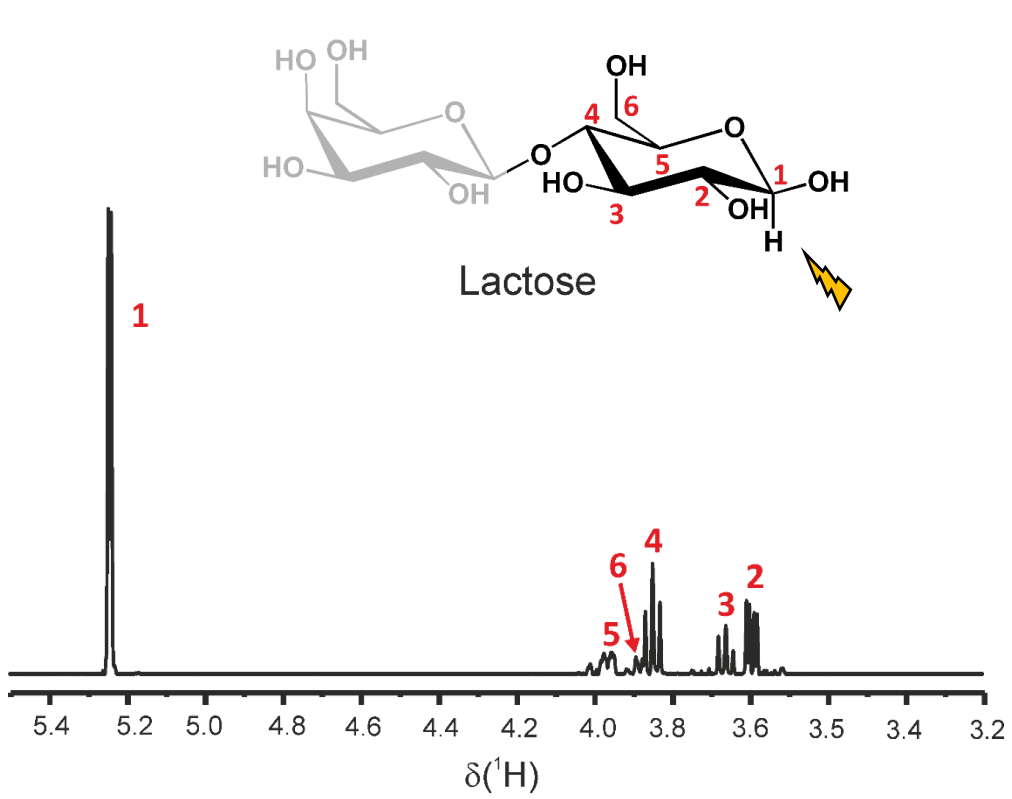
Virtual separation of components

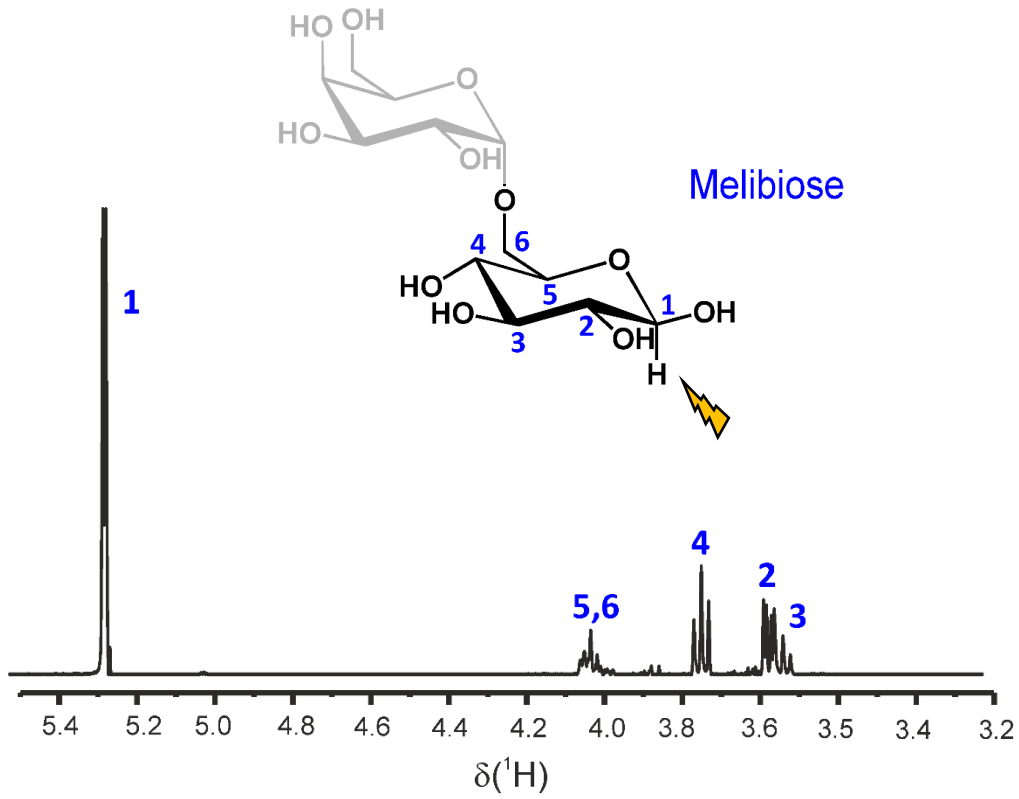


(Oneshot DOSY) M. D. Pelta, G. A. Morris, M. J. Stchedroff, S.J. Hammond, *Magn. Reson. Chem*. **2002**, *40*, S147.
(REST) G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

REST & Sugars

Virtual separation of components



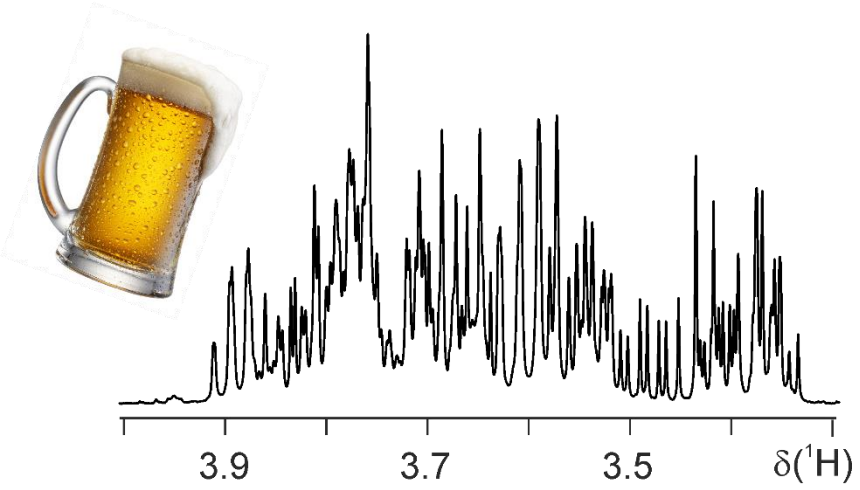


$REST_2$ SCORE components (multivariate analysis) for the disaccharide sample in D_2O, fitting for 2 components using non-negativity constraint

(Speedy Component Resolution, SCORE) M. Nilsson and G. A. Morris, *Anal. Chem.*, **2008**, *80*, 3777.
(REST) G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

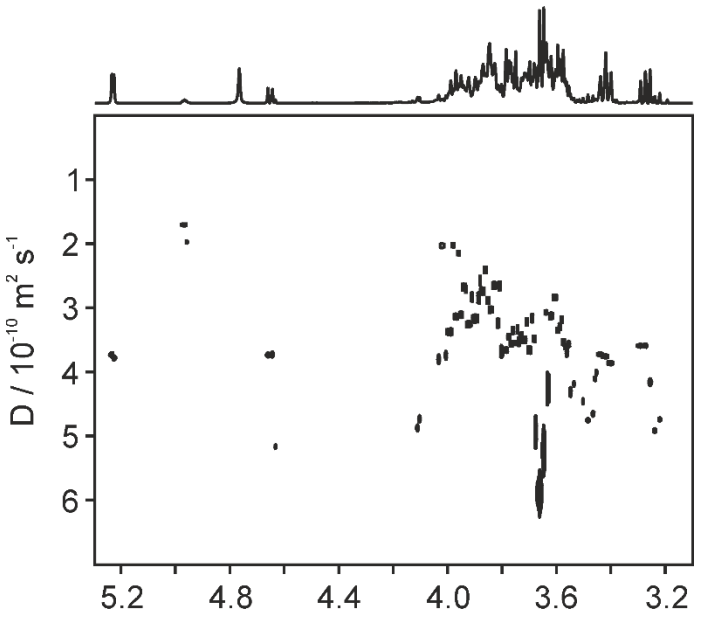
REST & Beer

^{1}H NMR



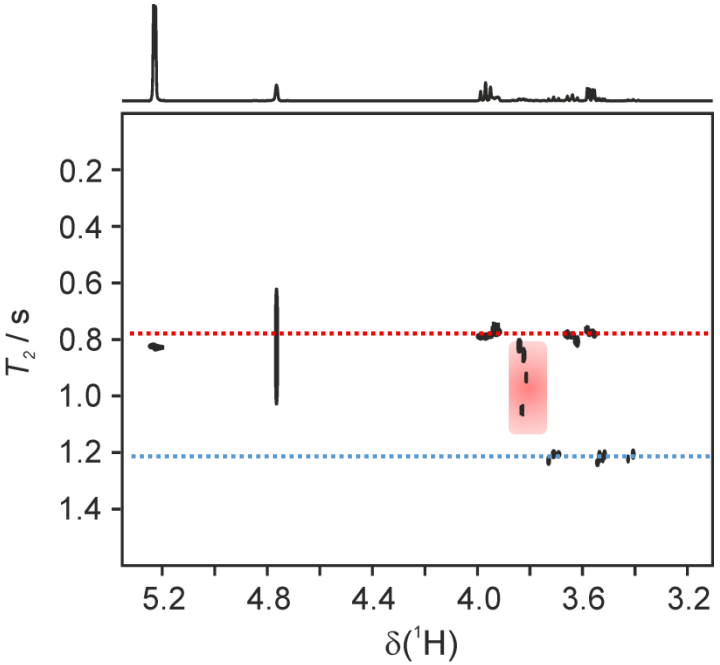
^{1}H NMR spectrum of beer
(Clausthaler classic premium lager)

Oneshot DOSY



Partial signal overlap

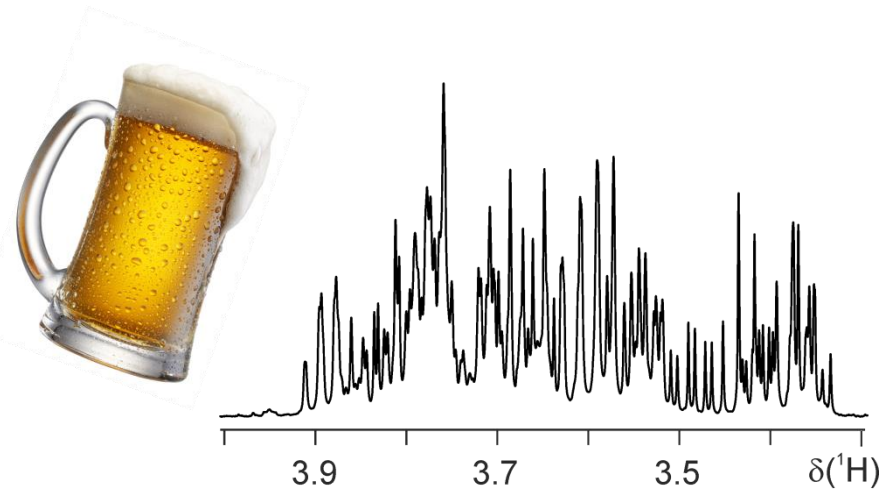
REST$_2$ ROSY



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)

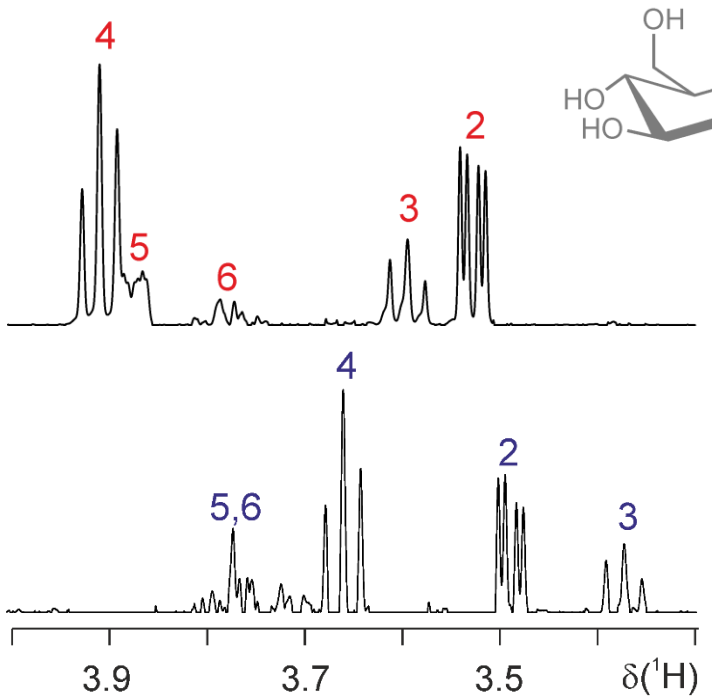
REST$_2$

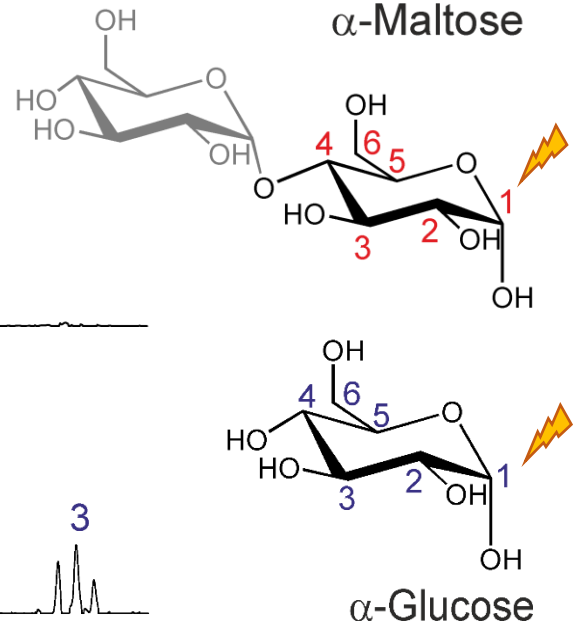
OUTSCORE

Optimized Unmixing of True Spectra for COmponent Resolution

(Multivariate *analysis*)

Virtual separation of components





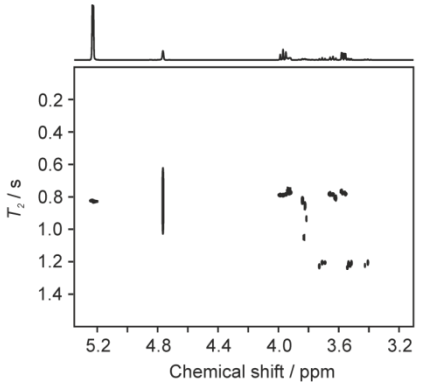
REST$_2$ OUTSCORE components for free α-glucose and for the α-glucose unit in α-maltose

(OUTSCORE) A. A. Colbourne, S. Meier, G. A. Morris and M. Nilsson, *Chem. Commun.*, **2013,** *49*, 10510.
(REST) G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *Chem. Commun.* **2017**, *53*, 7461.

Summary

REST allows the extraction of component subspectra from mixtures using isotropic mixing to label whole spin systems with the relaxation times (T_1 or T_2) of individual spins.

Univariate analysis (ROSY)

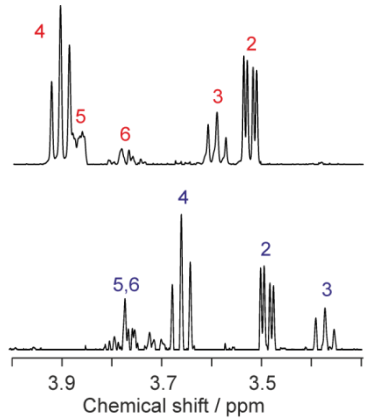


$$S(t) = S_0 e^{-\frac{t}{T_2}}$$

✓ Small differences in T_1/T_2 (>5%)

✘ Partially overlapped signals

Multivariate analysis (SCORE/OUTSCORE)



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

a_i - spectra
b_i - relaxation
E = error

✓ Uses all data at once

✓ Partially overlapped signals

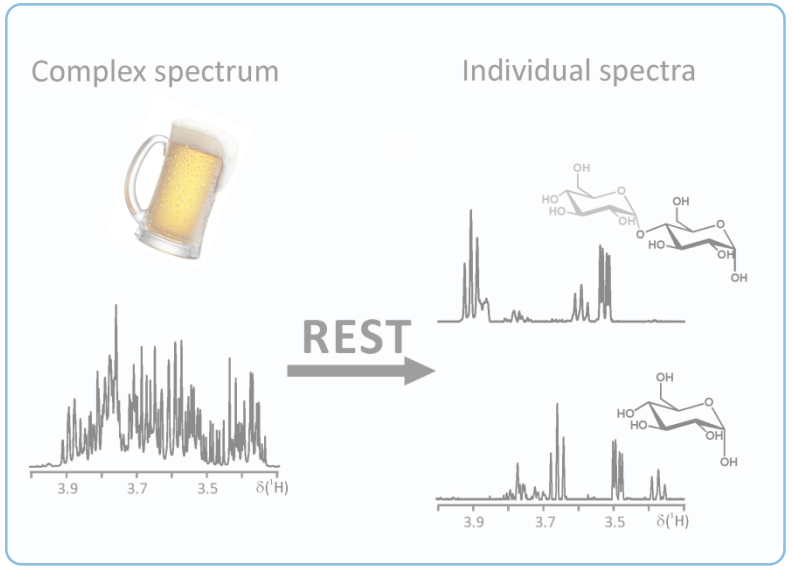
✘ Small number of components (2 - 5)

✘ Significant differences in T_1/T_2 (>10%)

Novel NMR methods for the analysis of complex systems

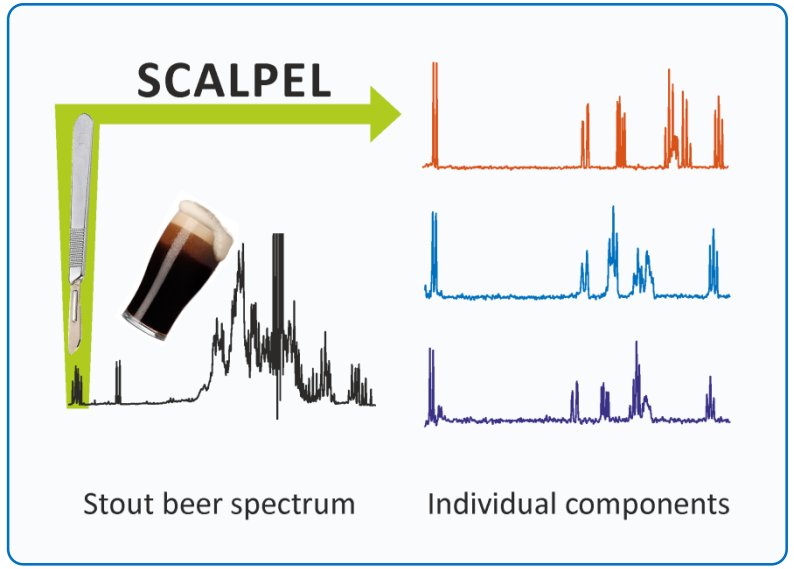
REST

Relaxation-Encoded Selective TOCSY



SCALPEL

Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components

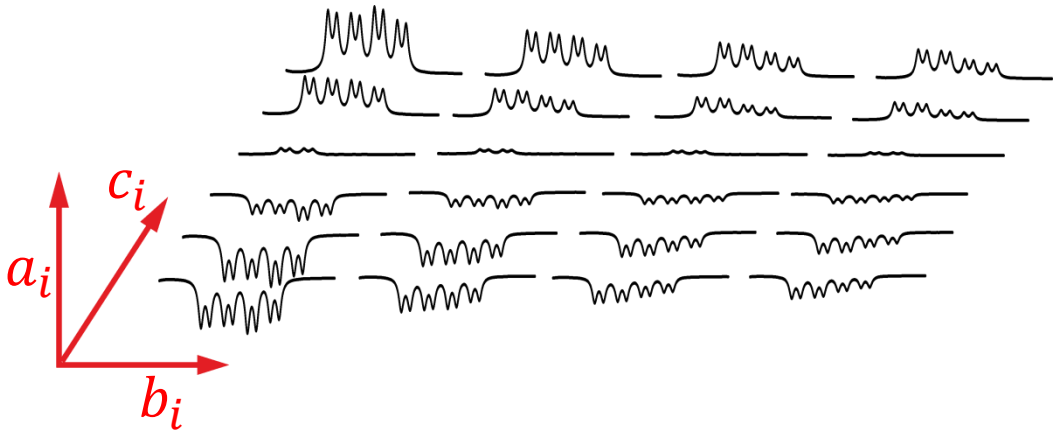


FESTA

Fluorine-Edited Selective TOCSY Acquisition

PARAllel FACtor (PARAFAC) tensor analysis

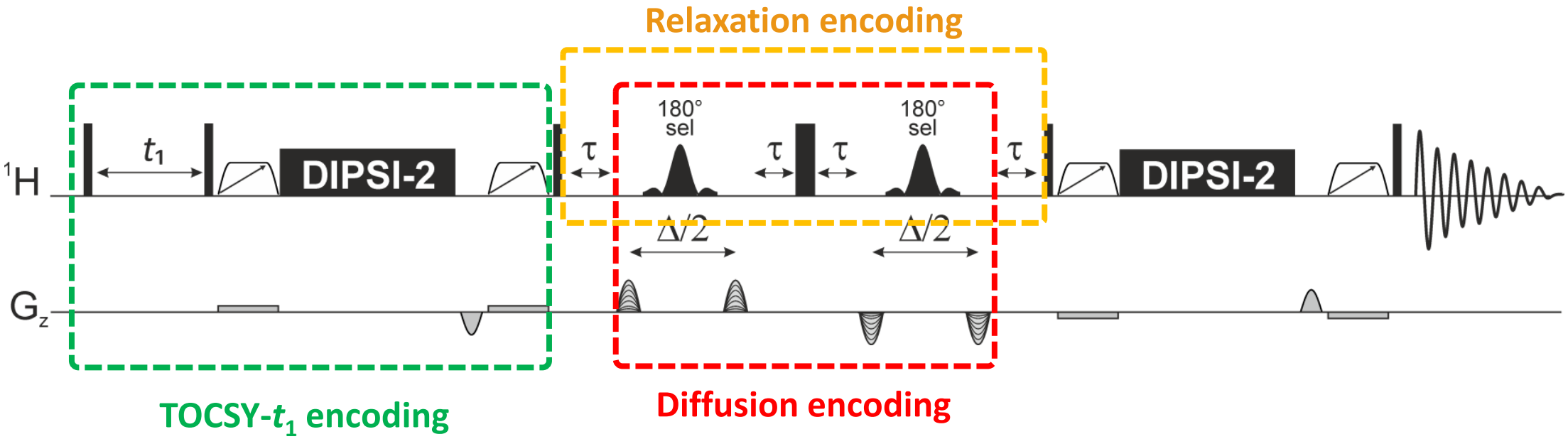
Data = $\sum_{i=1}^{N} a_i \otimes b_i \otimes s_i + E$



- 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)

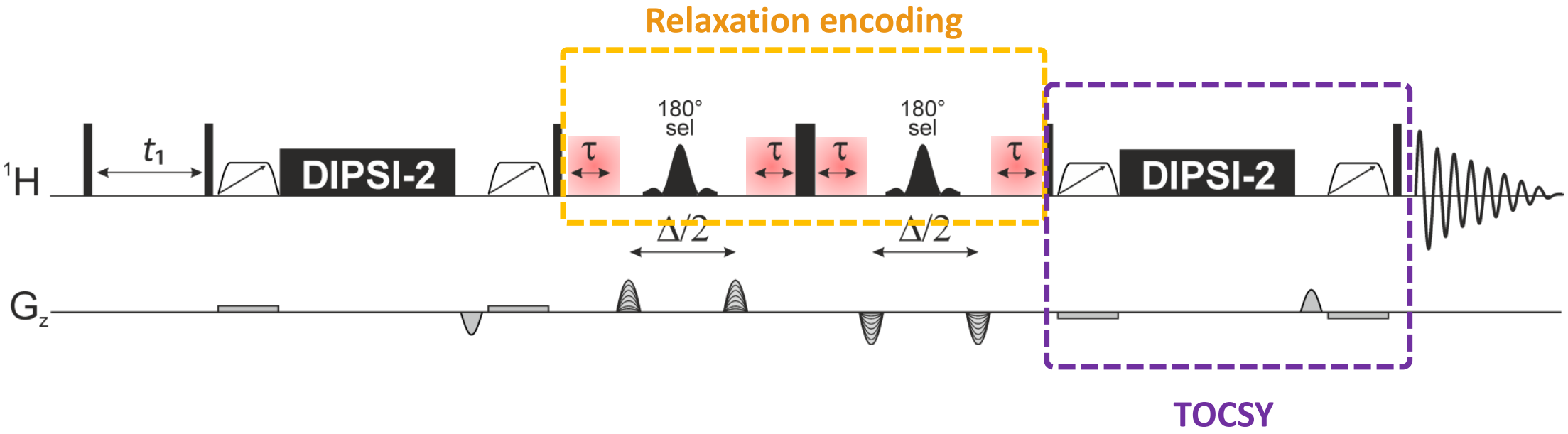


Data = $\sum_{i=1}^{N} a_i \otimes b_i \otimes s_i + E$

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.

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

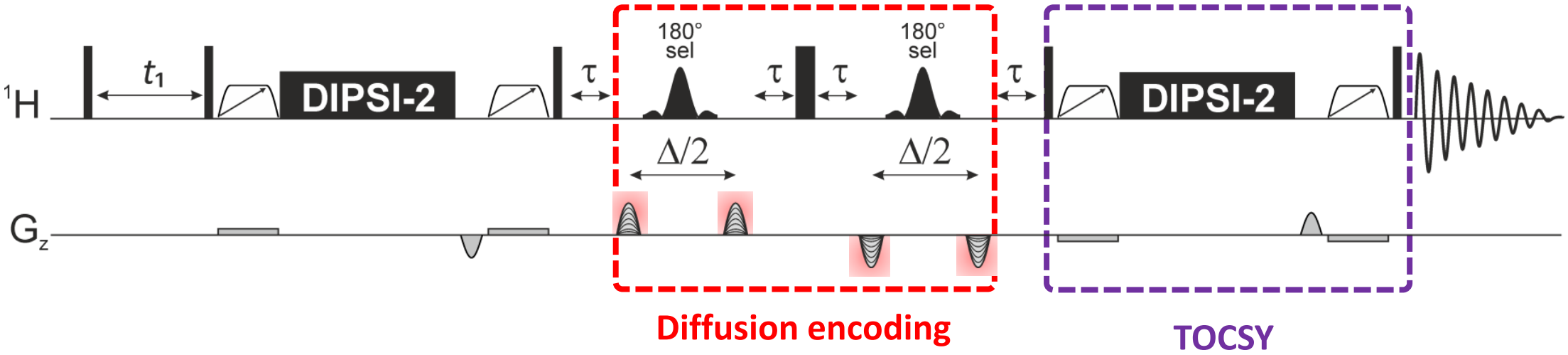


iREST$_2$

(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.

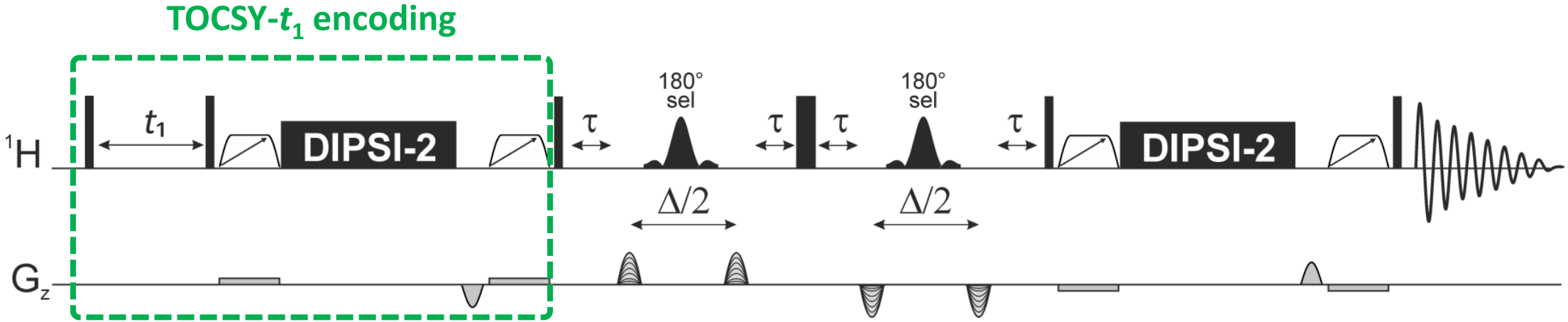
Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components (SCALPEL)

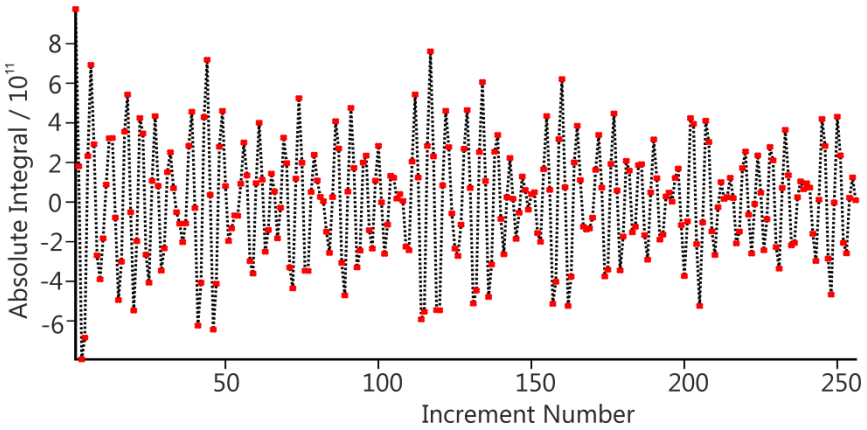


iDESTO
(Diffusion internally-Encoded Selective TOCSY)

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)



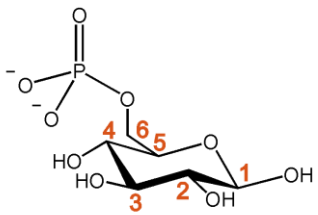


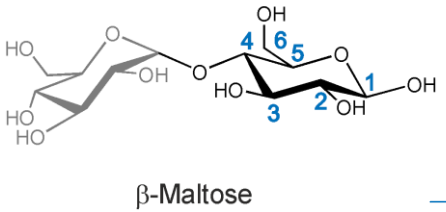
- TOCSY-t_1 encoding labelled spin systems based on their signal amplitude dependence on t_1.
- Signal amplitude is a sensitive function of the coupling constants in the spin system.
- This dimension is **not** Fourier transformed.

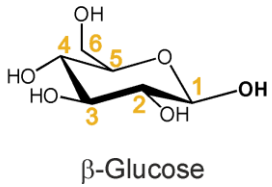
G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *J. Am. Chem. Soc.* **2019**, *141*, 5766.

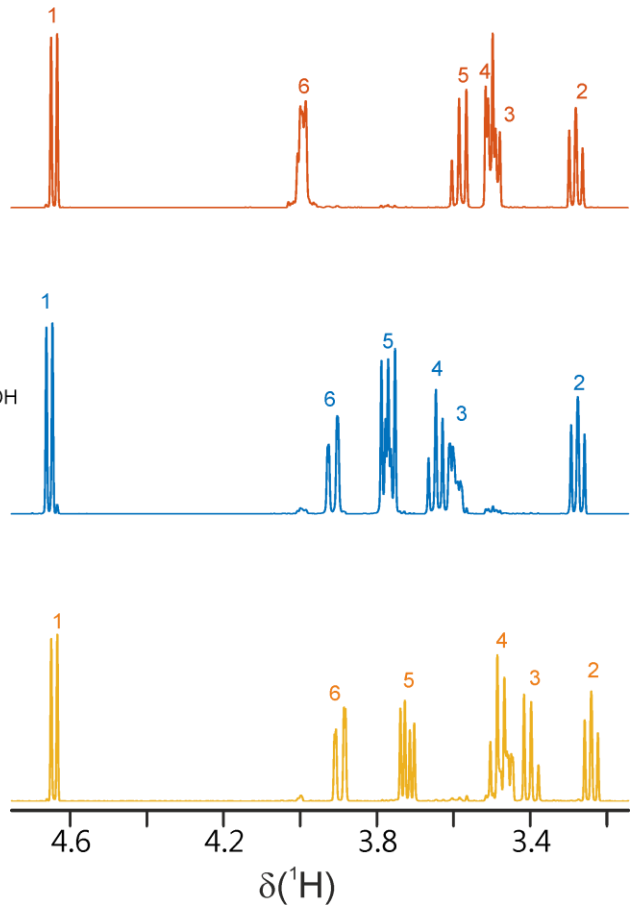
SCALPEL & Sugars

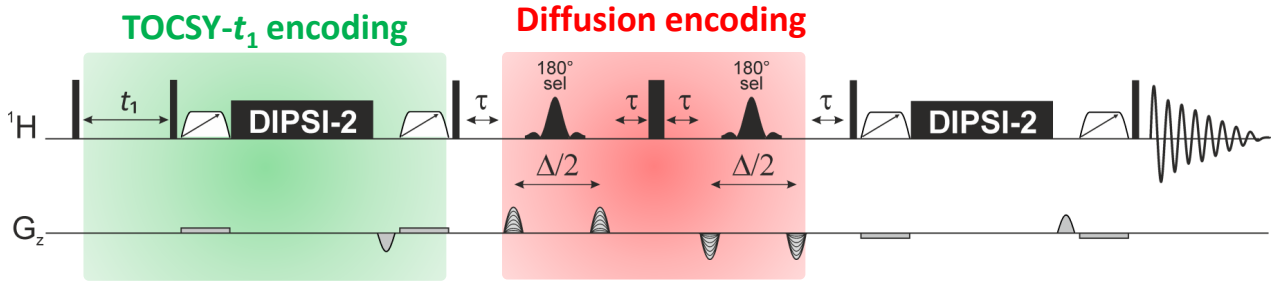
Spectral mode



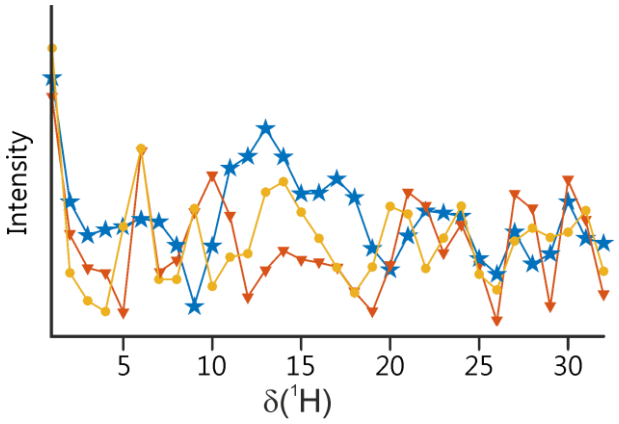




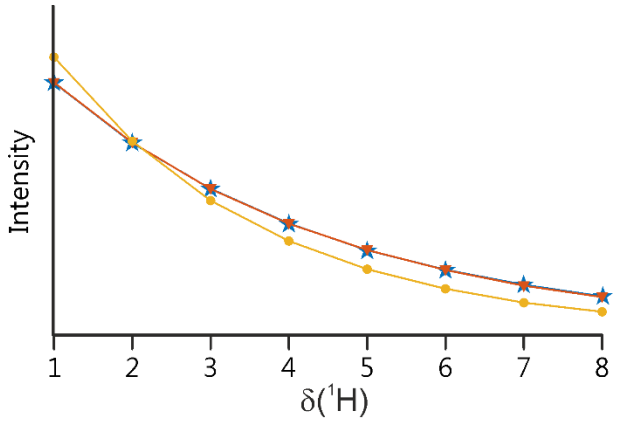




TOCSY-t_1 evolution mode



Diffusion mode

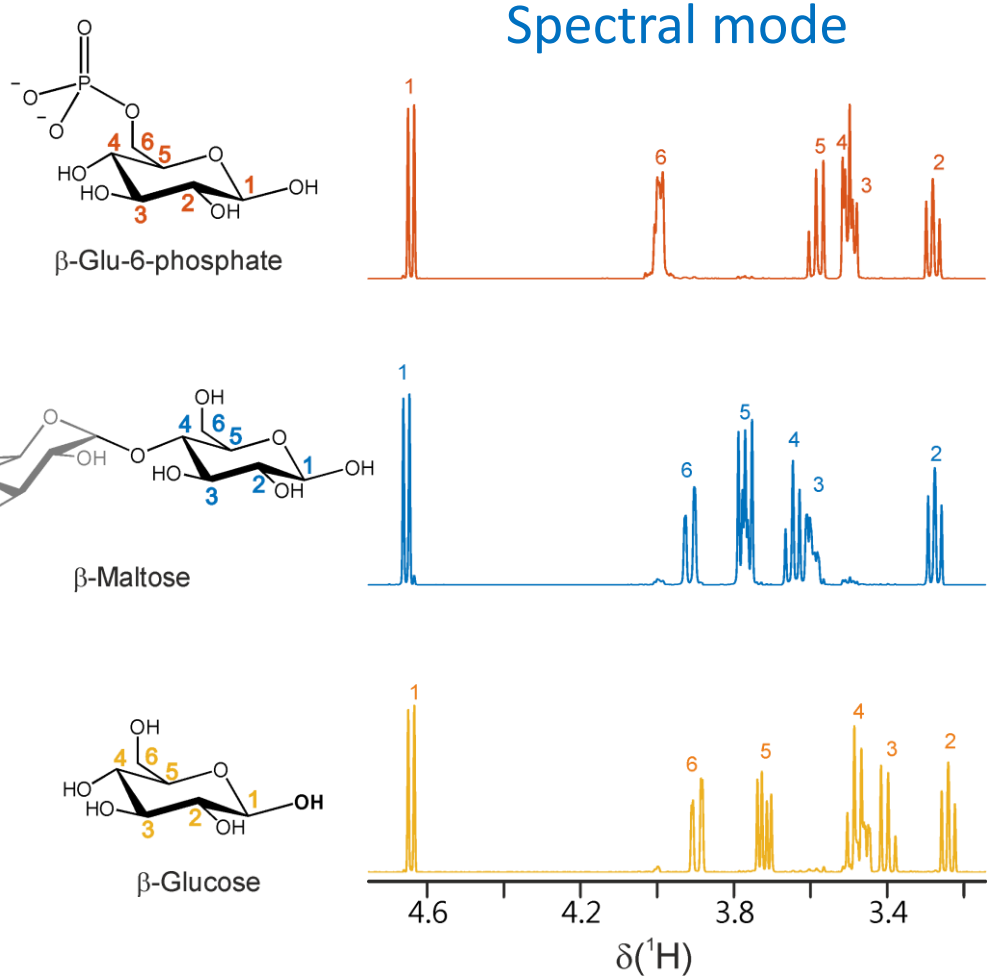


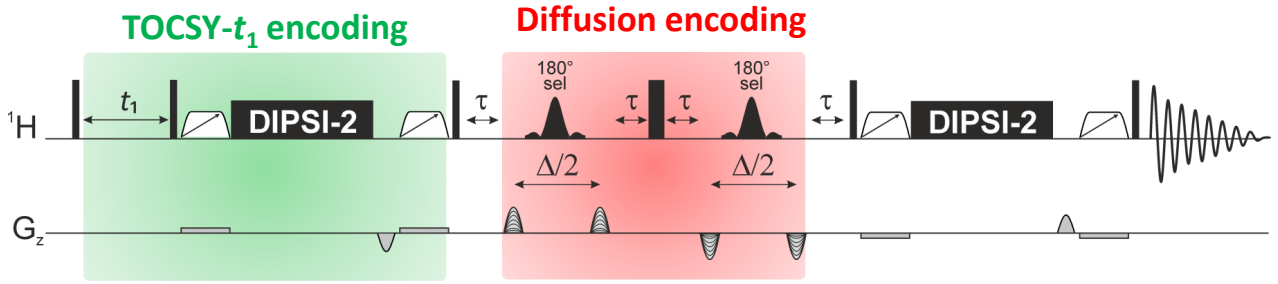
PARAFAC modes for SCALPEL experiment for a mixture of glucose, glucose 6-phosphate and maltose in D_2O

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

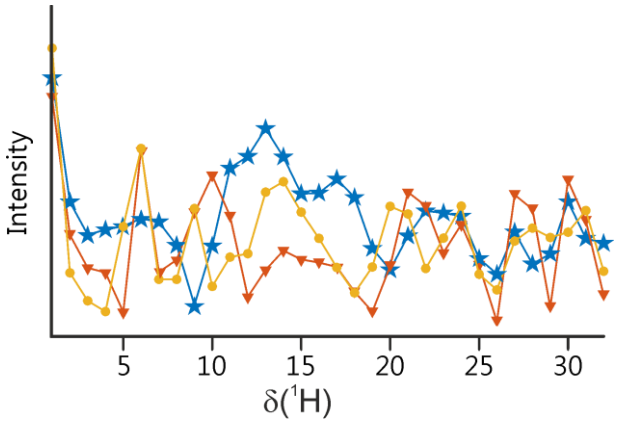
SCALPEL & Sugars

Spectral mode

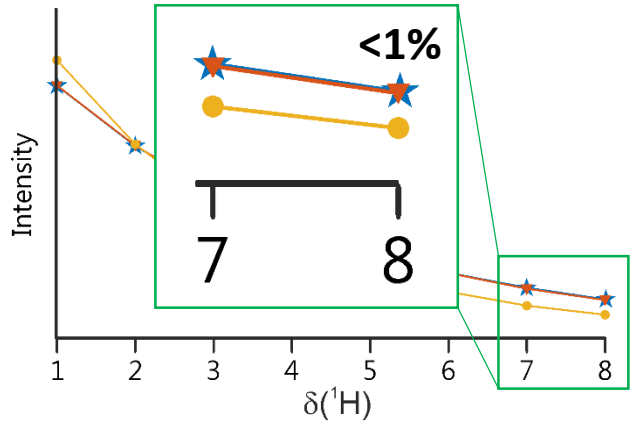




TOCSY-t_1 evolution mode



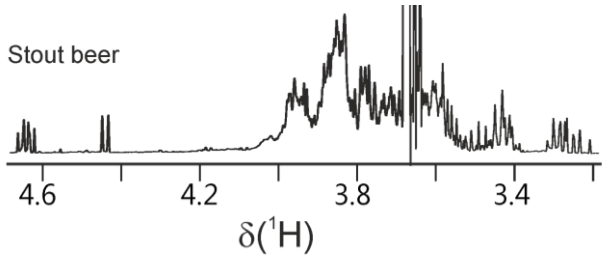
Diffusion mode

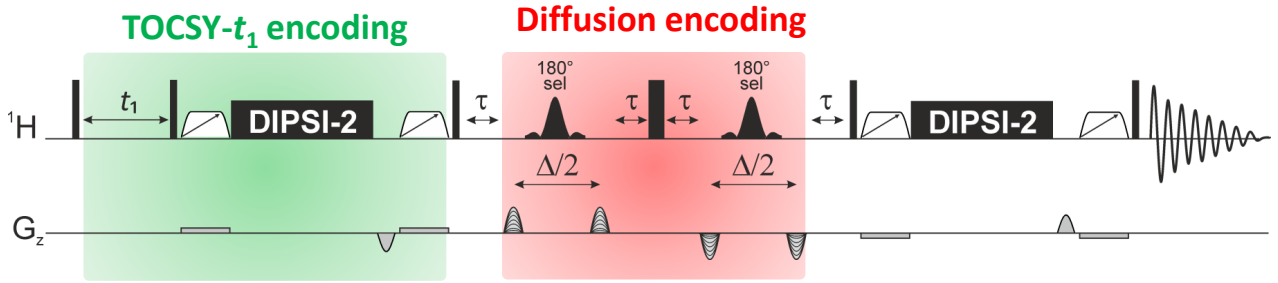


PARAFAC modes for SCALPEL experiment for a mixture of glucose, glucose 6-phosphate and maltose in D_2O

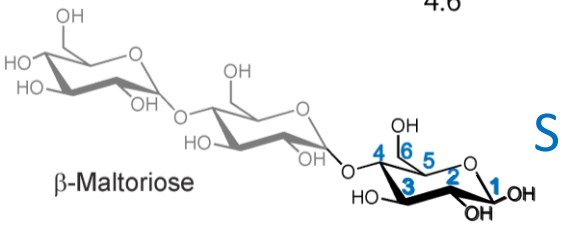
G. Dal Poggetto, L. Castañar, R. W. Adams, G. A. Morris, M. Nilsson, *J. Am. Chem. Soc.* **2019**, *141*, 5766.

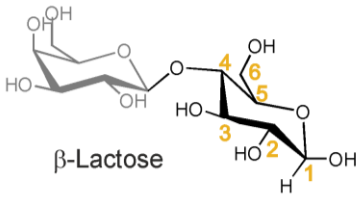
SCALPEL & Beer

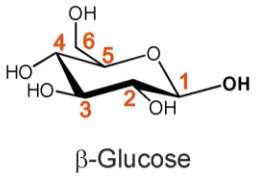


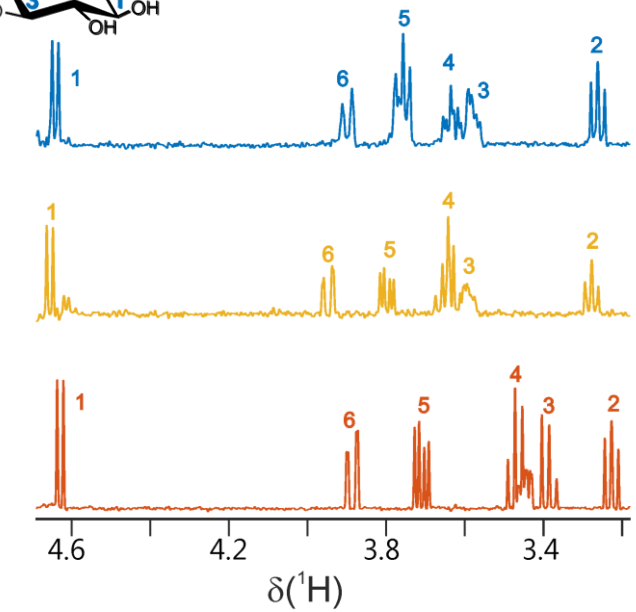


Spectral mode

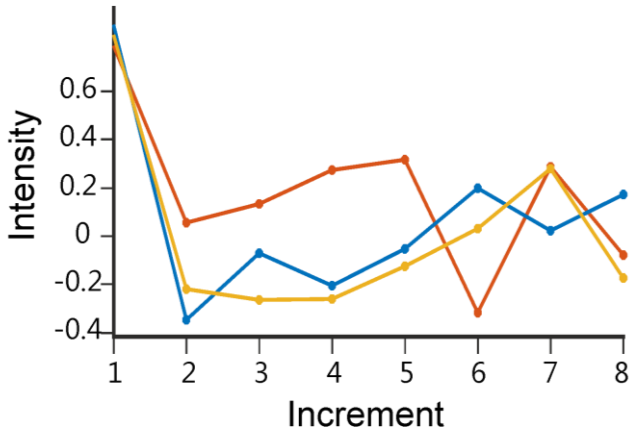




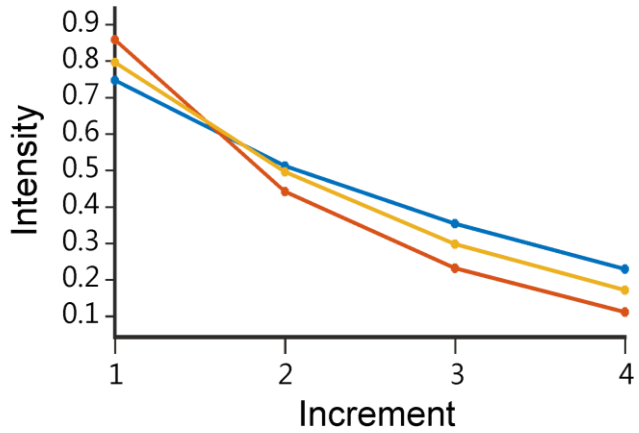




TOCSY-t_1 evolution mode



Diffusion mode

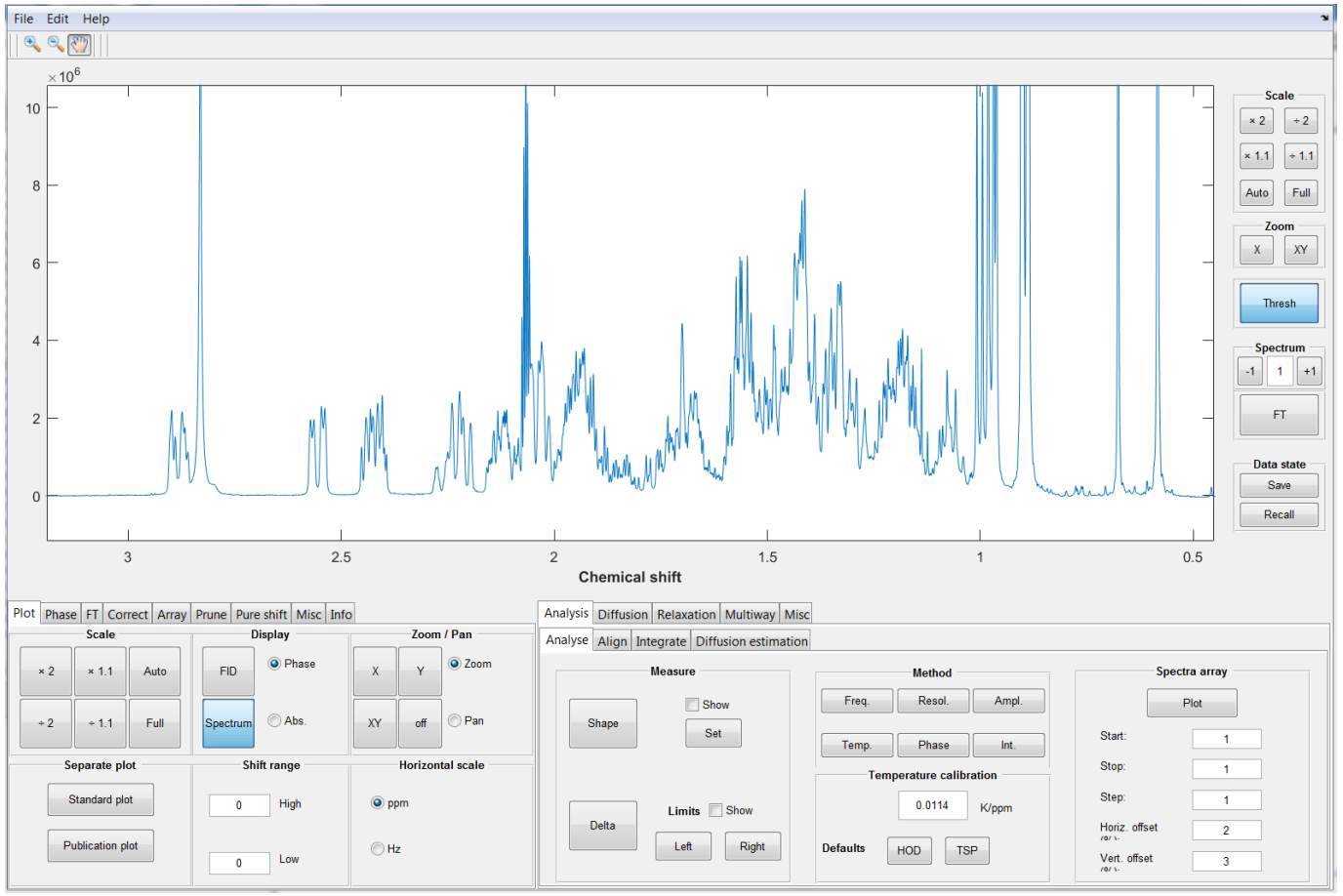


PARAFAC modes for SCALPEL experiment for a sample of stout beer (Mackeson) with 20% added D_2O

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

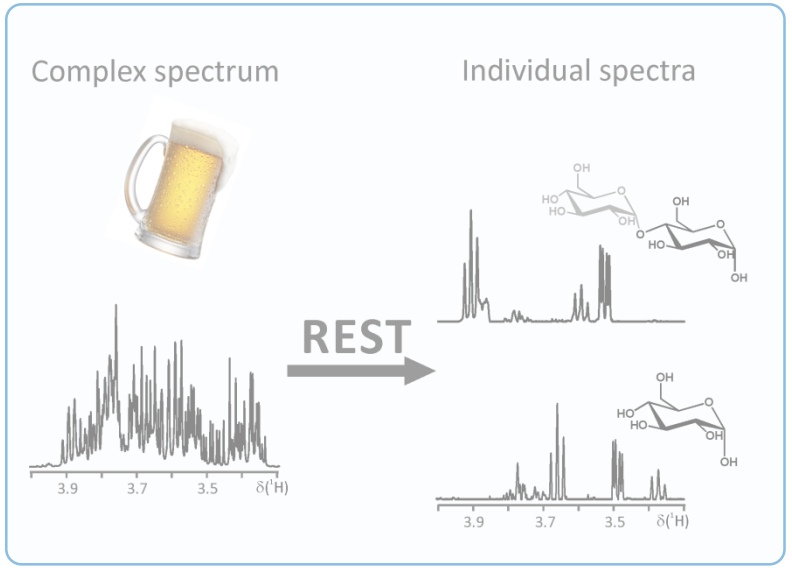


L. Castañar, G. Dal Poggetto, A. A. Colbourne, G. A. Morris, M. Nilsson, *Magn. Reson. Chem.* **2018**, *56*, 546.

Novel NMR methods for the analysis of complex systems

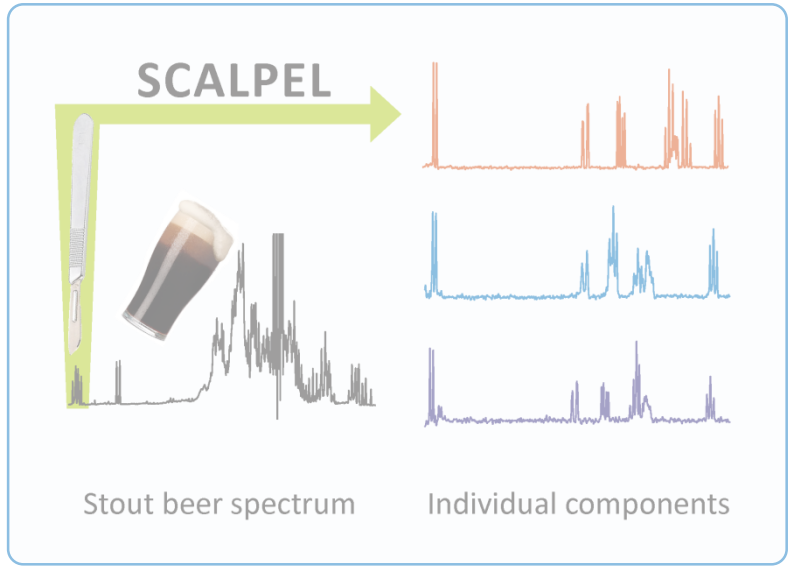
REST

Relaxation-Encoded Selective TOCSY



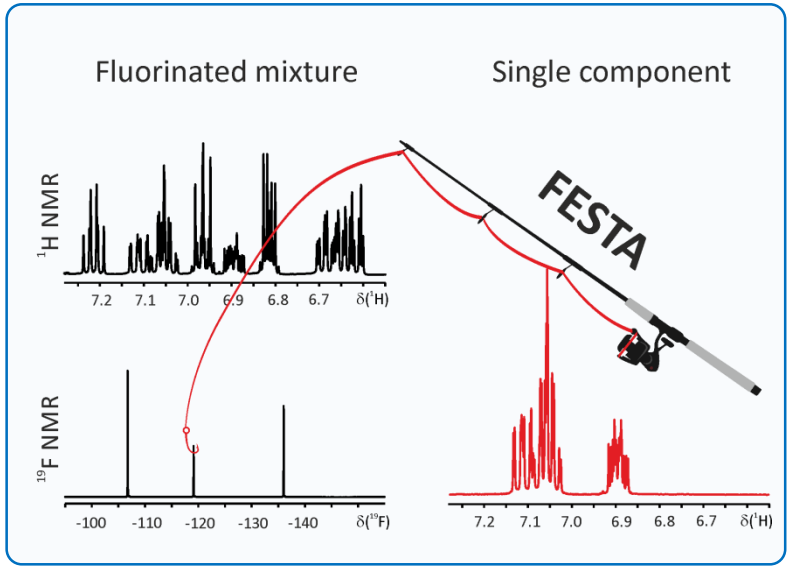
SCALPEL

Spectral Component Acquisition by Localized PARAFAC Extraction of Linear

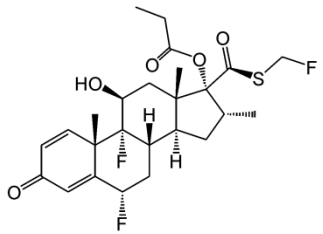


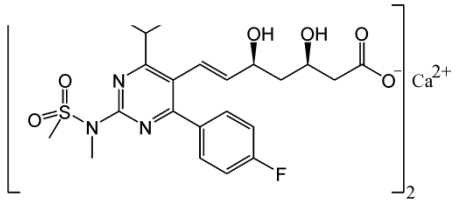
FESTA

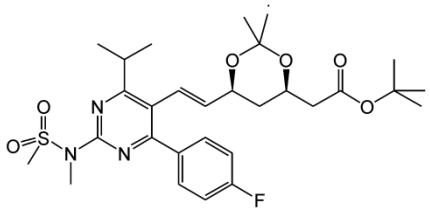
Fluorine-Edited Selective TOCSY Acquisition

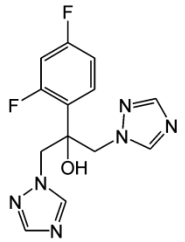


Mixtures containing fluorinated species

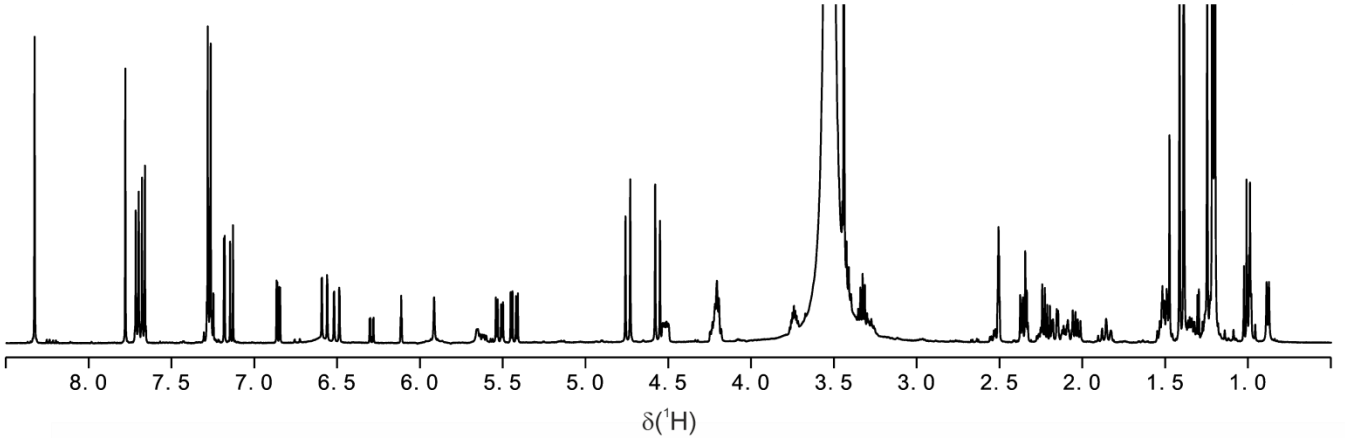






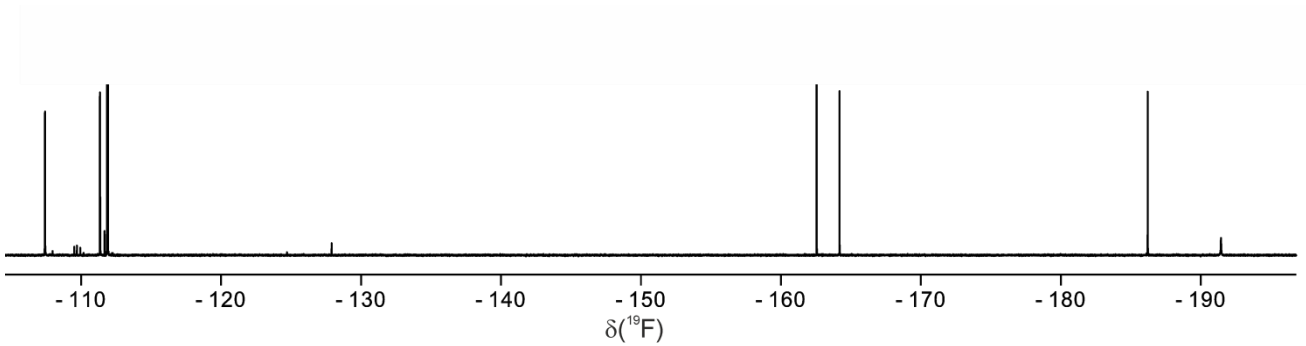


^{1}H NMR



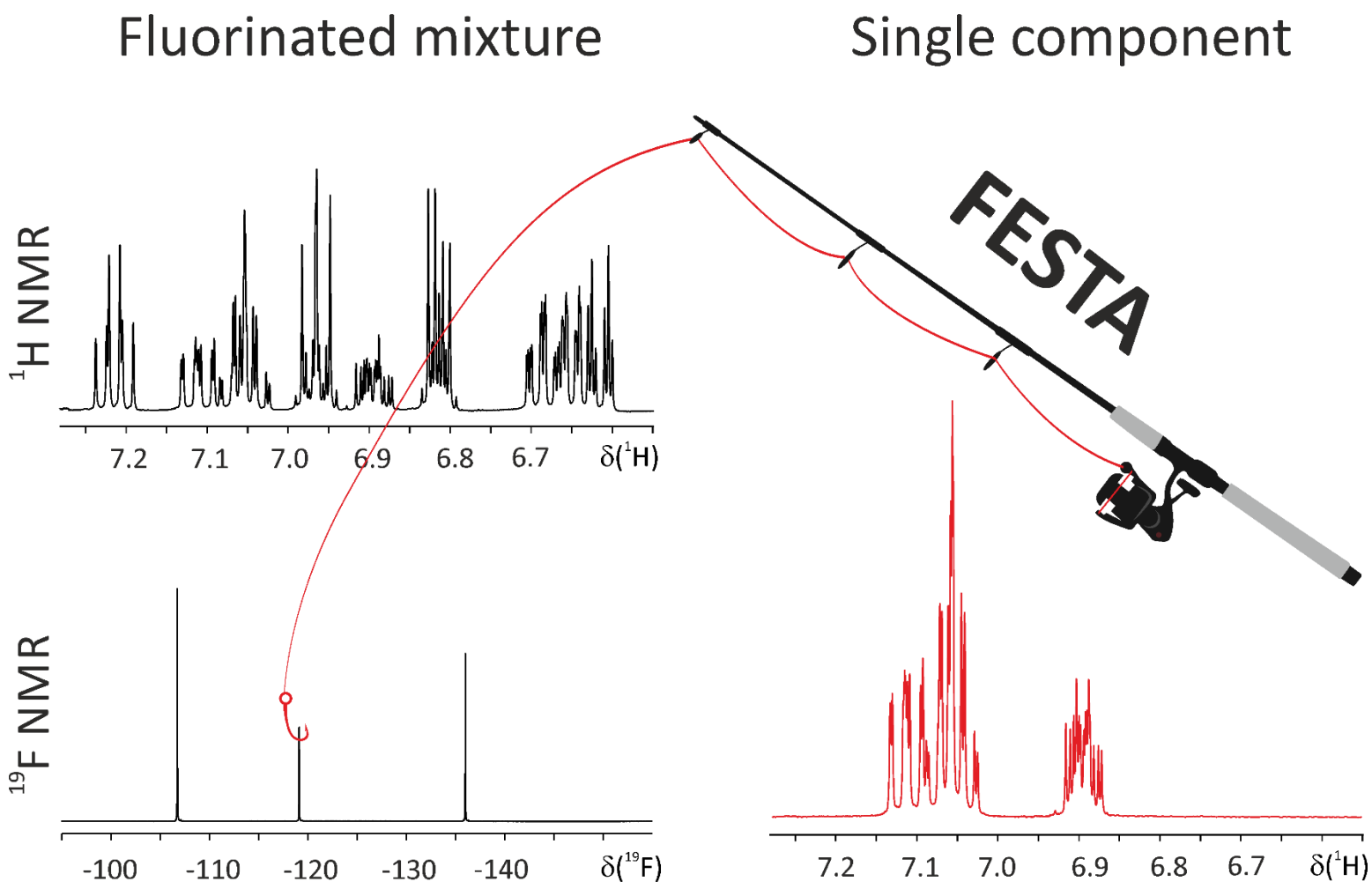
- ✗ Signal overlap
- ✗ Component information
- ✗ Structural information

^{19}F NMR



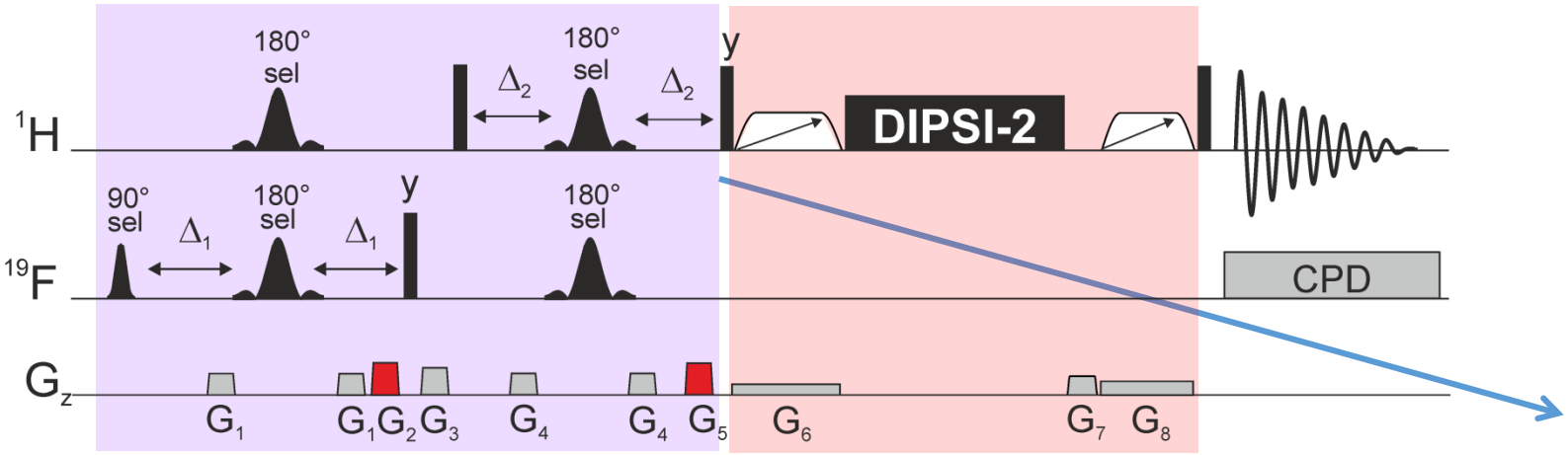
- ✓ Spectral resolution
- ✗ Component information
- ✗ Structural information

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



Fluorine-edited selective TOCSY Acquisition (FESTA)

SRI-FESTA

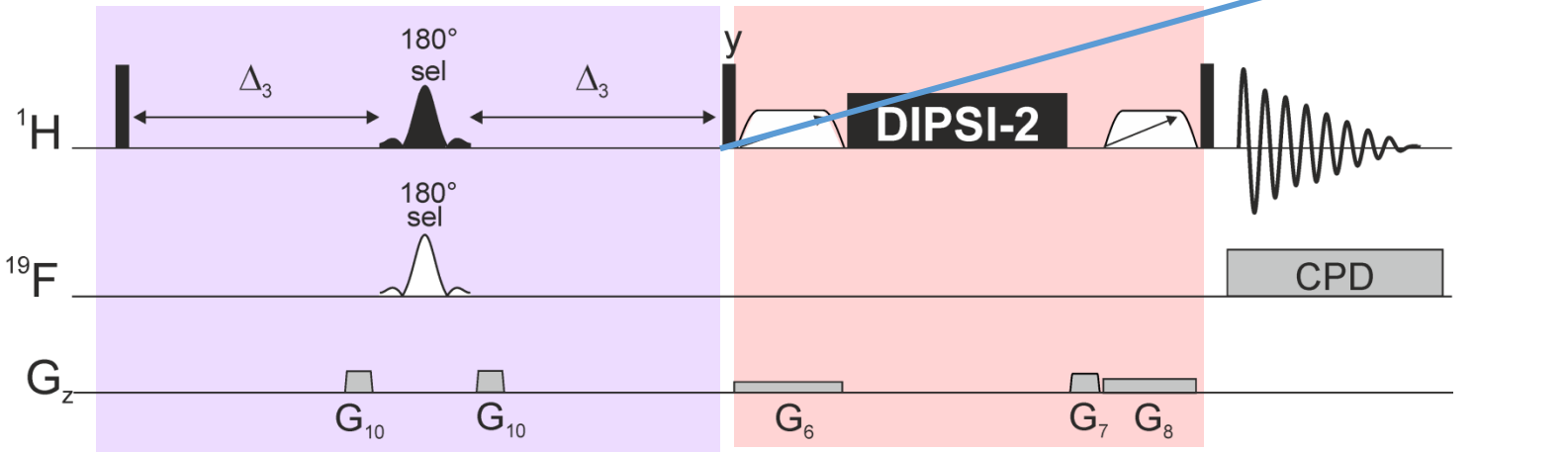


^{1}H magnetization in-phase

^{1}H - ^{19}F pair selection

TOCSY

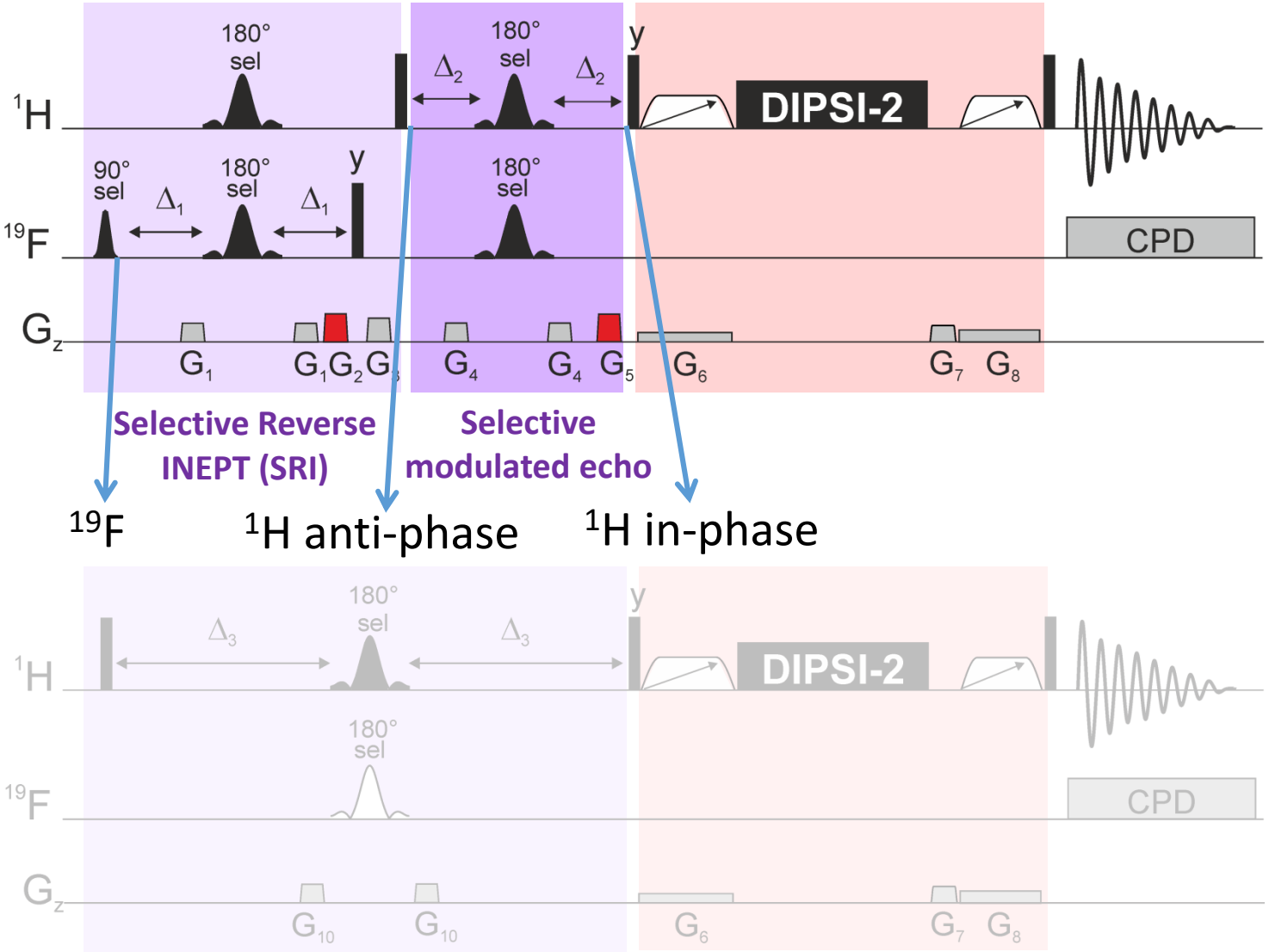
MODO-FESTA



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. / MODO: *unpublished*

Fluorine-edited selective TOCSY Acquisition (FESTA)

SRI-FESTA



MODO-FESTA

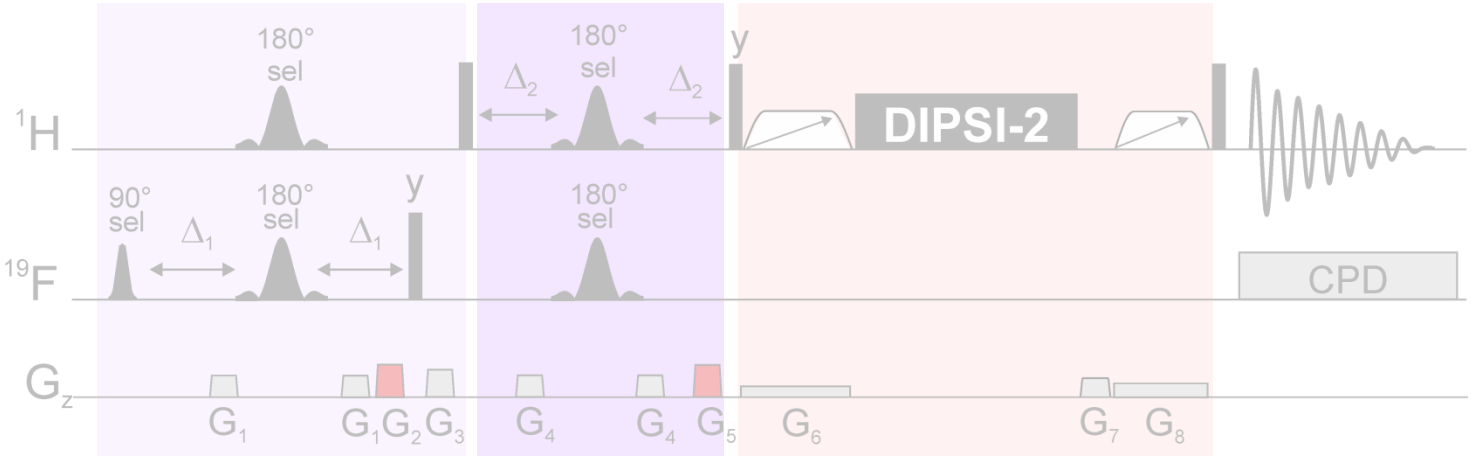
$$\Delta_1 = \frac{1}{4J_{HF}n_H}$$

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

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)

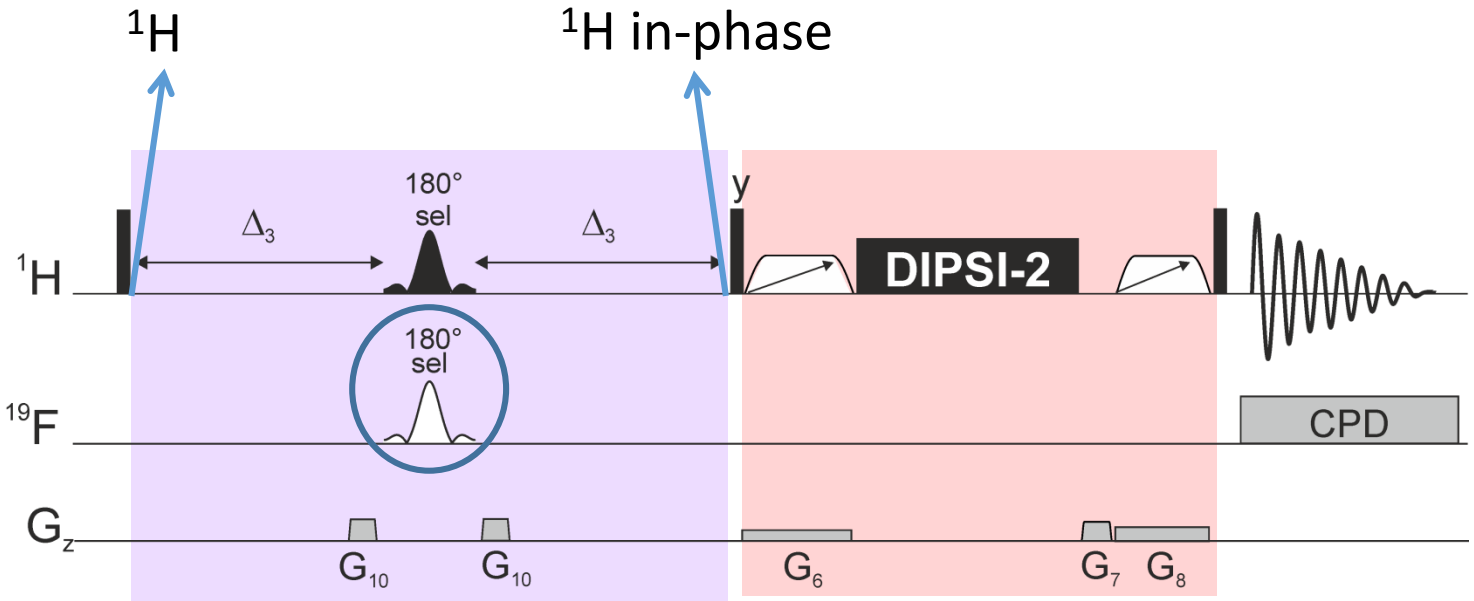
SRI-FESTA



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

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

MODO-FESTA



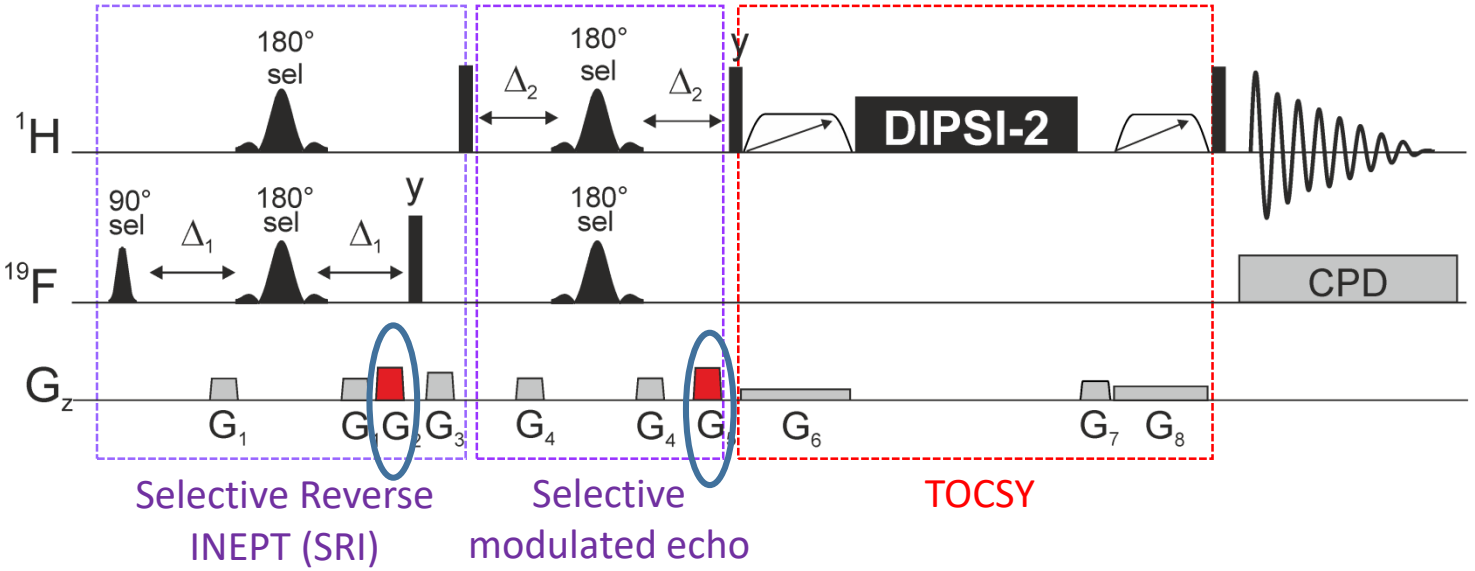
Selective MOdulated echO (MODO)

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

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

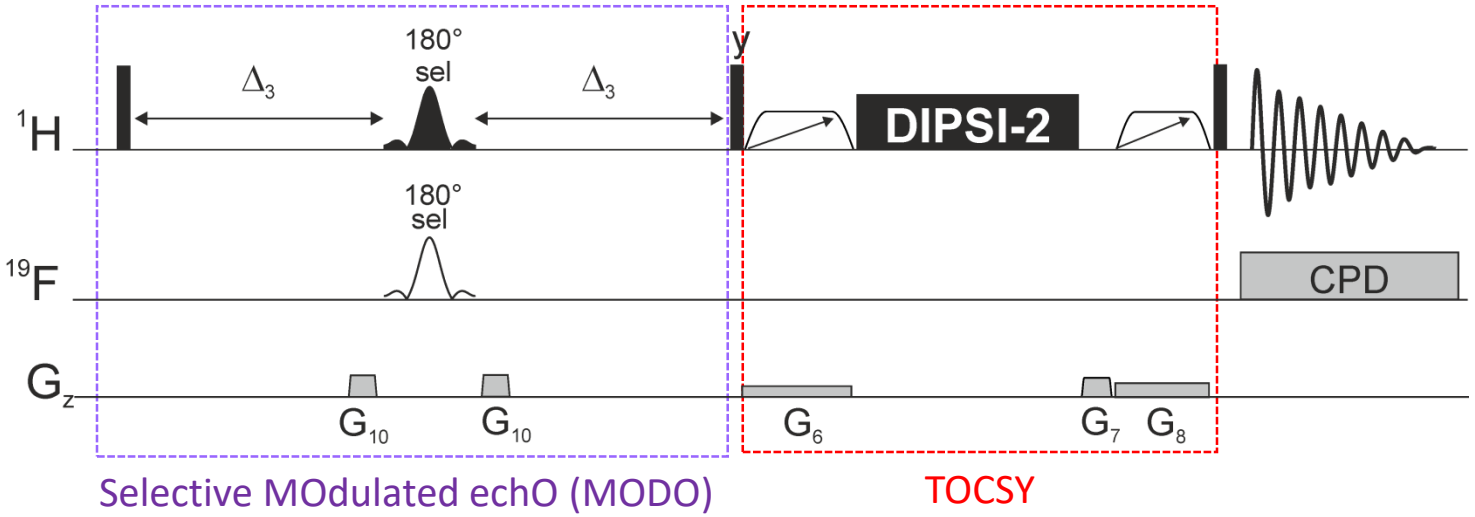
Fluorine-edited selective TOCSY Acquisition (FESTA)

SRI-FESTA



Heteronuclear coherence transfer pathway can be enforced

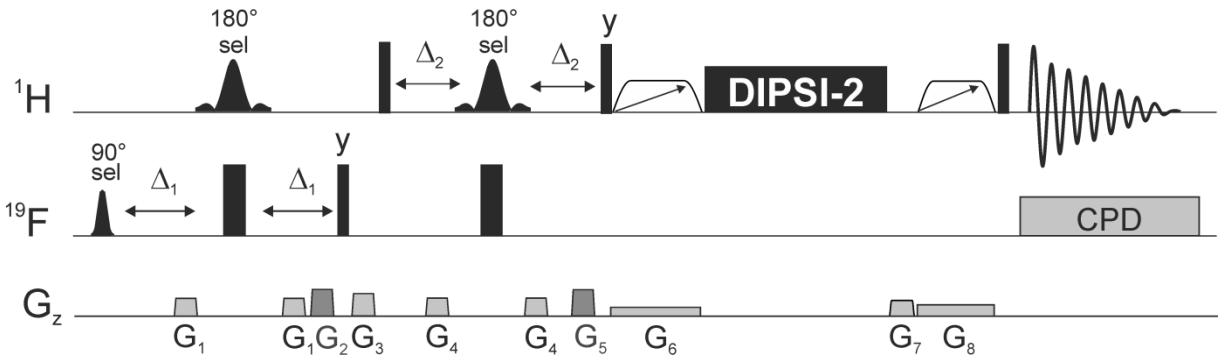
MODO-FESTA



Heteronuclear coherence selection relies on phase cycling only

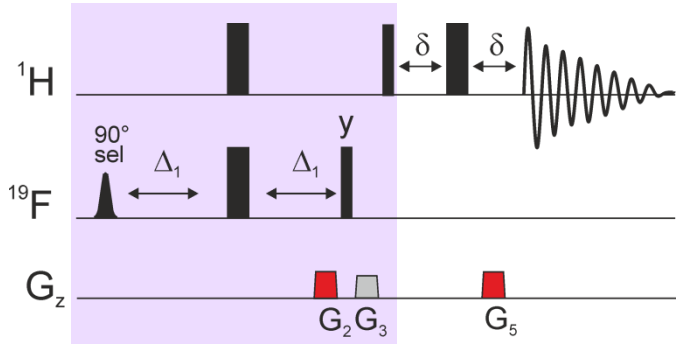
Higher sensitivity

How SRI-FESTA works



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.

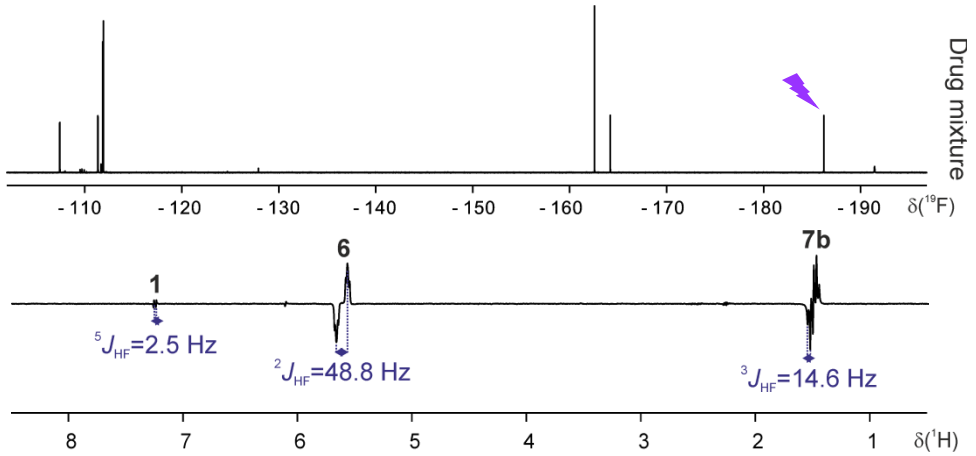
How SRI-FESTA works



Selective reverse INEPT (SRI)

^{19}F

^{1}H SRI

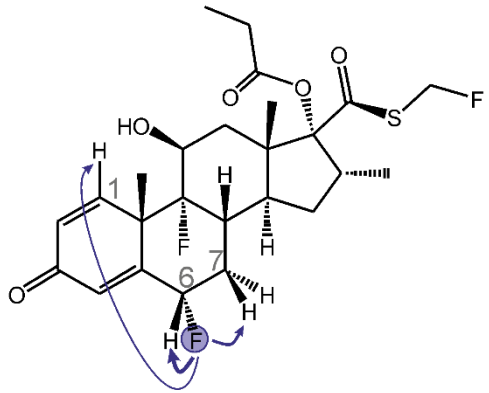


^{19}F Spin selection

↓

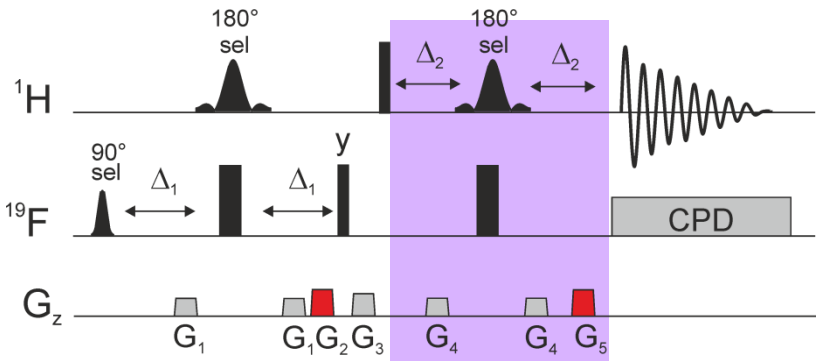
Heteronuclear transfer (J_{HF})

^1H_A ^1H_B



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.

How SRI-FESTA works

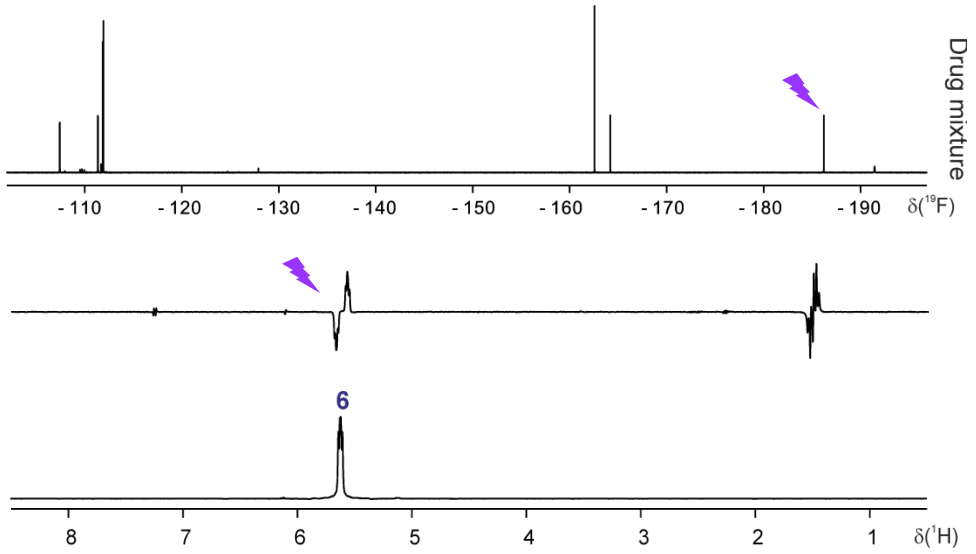


Selective modulated echo (SME)

^{19}F

^{1}H SRI

^{1}H SRI-SME



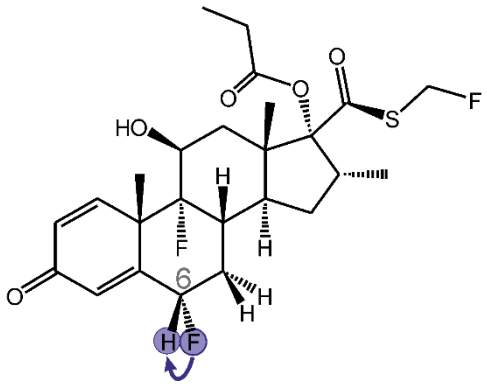
^{19}F Spin selection

↓ Heteronuclear transfer (J_{HF})

^1H_A ^1H_B

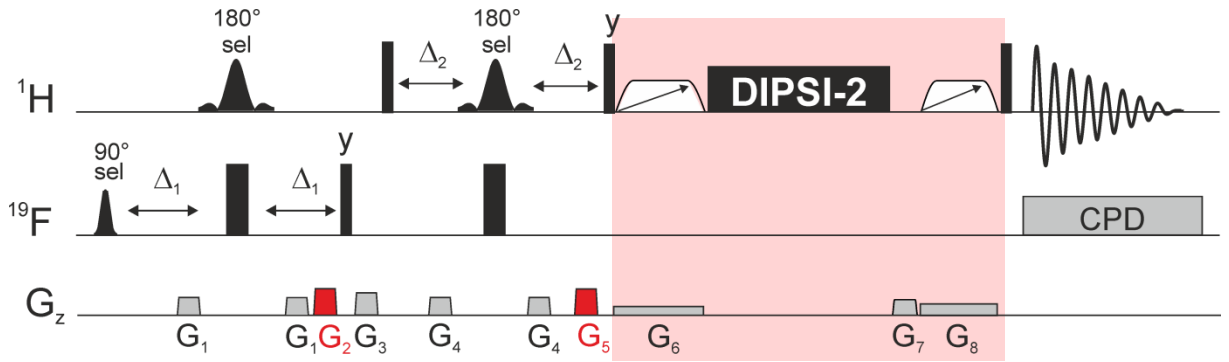
↓ Spin selection

^1H_A



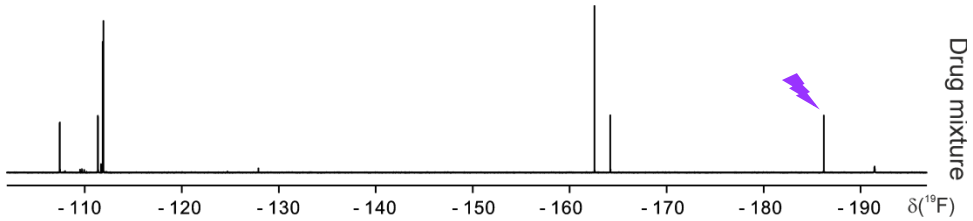
L. Castañar, P. Moutzouri, T. M. Barbosa, C. F. Tormena, R. Rittner, A. R. Phillips, S. R. Coombes, M. Nilsson, G. A. Morris, *Anal. Chem*. **2018**, *90,* 5445.

How SRI-FESTA works

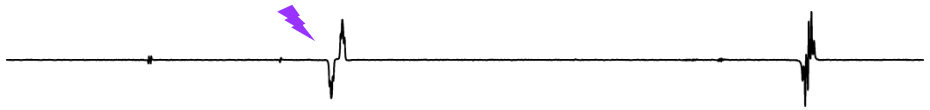


TOCSY

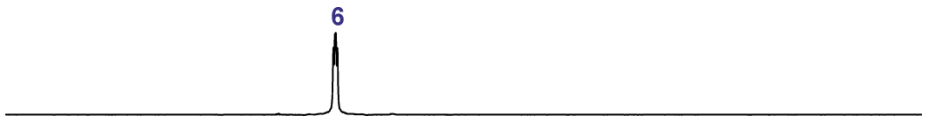
^{19}F



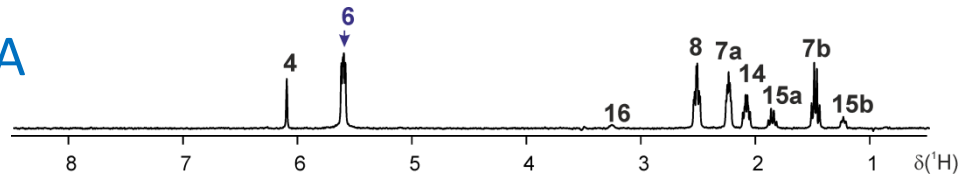
^{1}H SRI



^{1}H SRI-SME



^{1}H SRI-FESTA



^{19}F Spin selection

↓ Heteronuclear transfer (J_{HF})

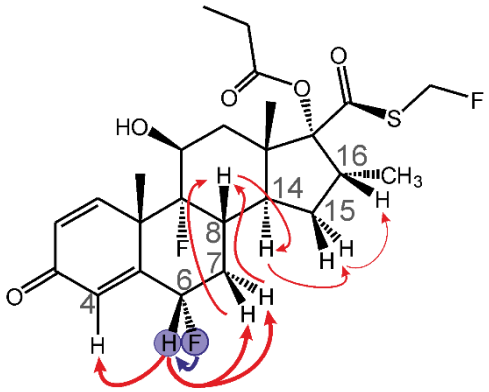
^1H_A ^1H_B

↓ Spin selection

^1H_A

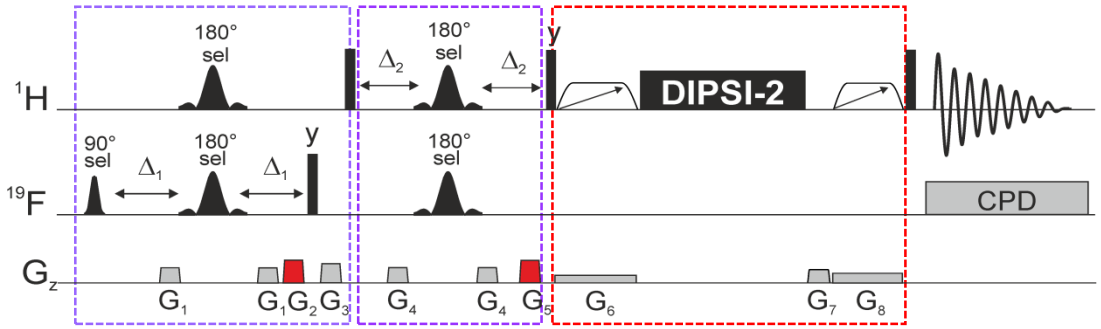
↓ Homonuclear transfer (J_{HH})

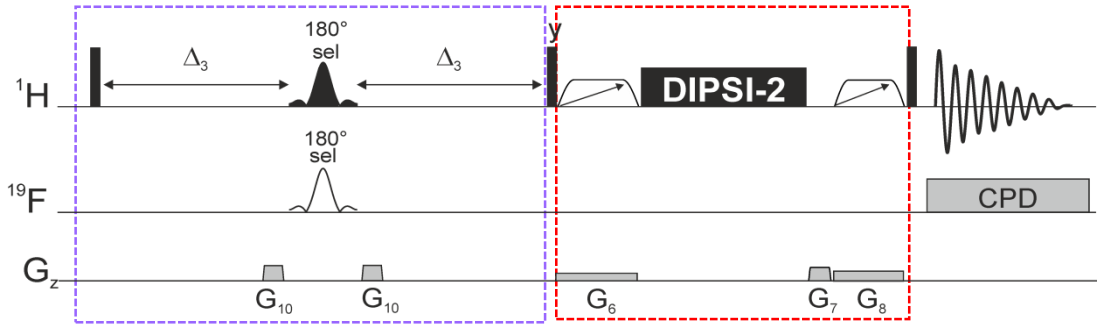
^{1}H → **^{1}H** → **^{1}H** →

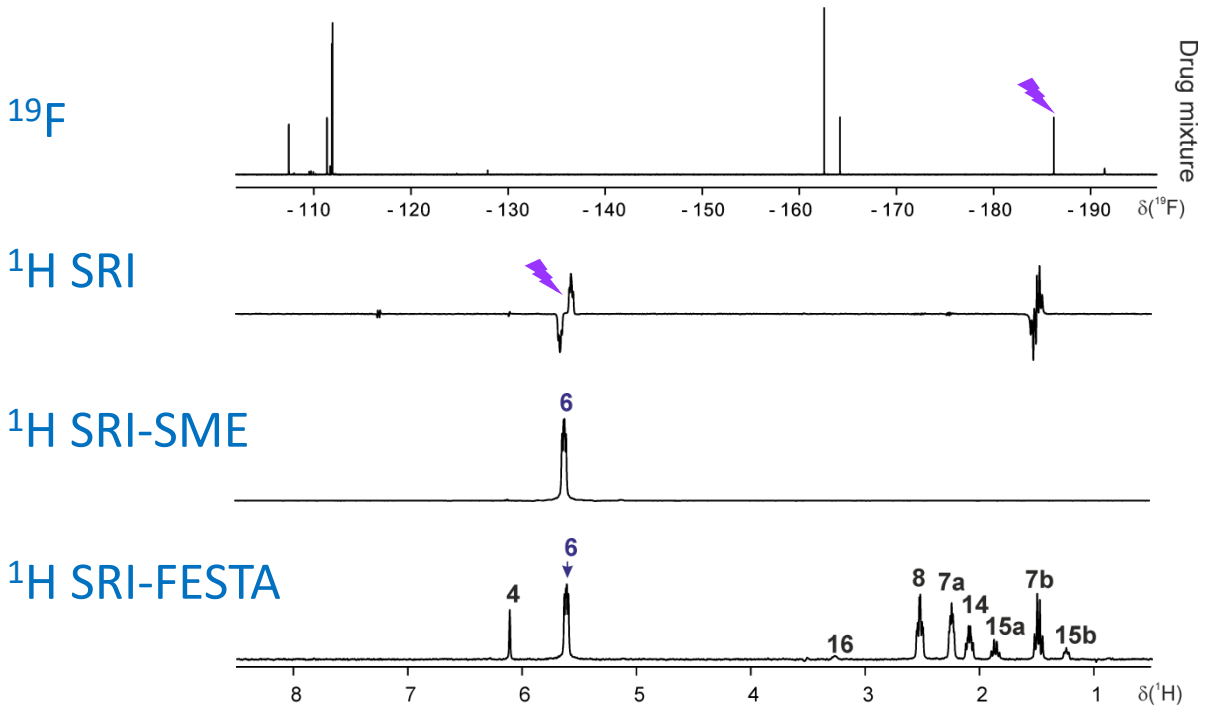


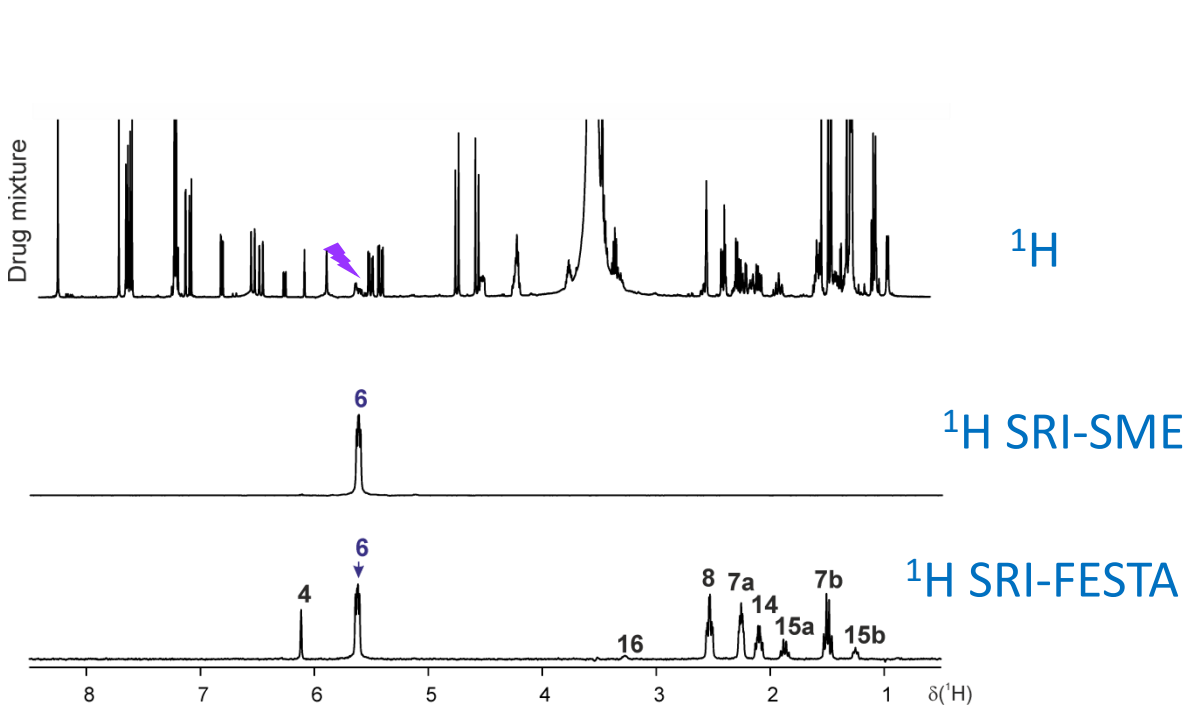
L. Castañar, P. Moutzouri, T. M. Barbosa, C. F. Tormena, R. Rittner, A. R. Phillips, S. R. Coombes, M. Nilsson, G. A. Morris, *Anal. Chem*. **2018**, *90,* 5445.

How SRI- and MODO-FESTA work







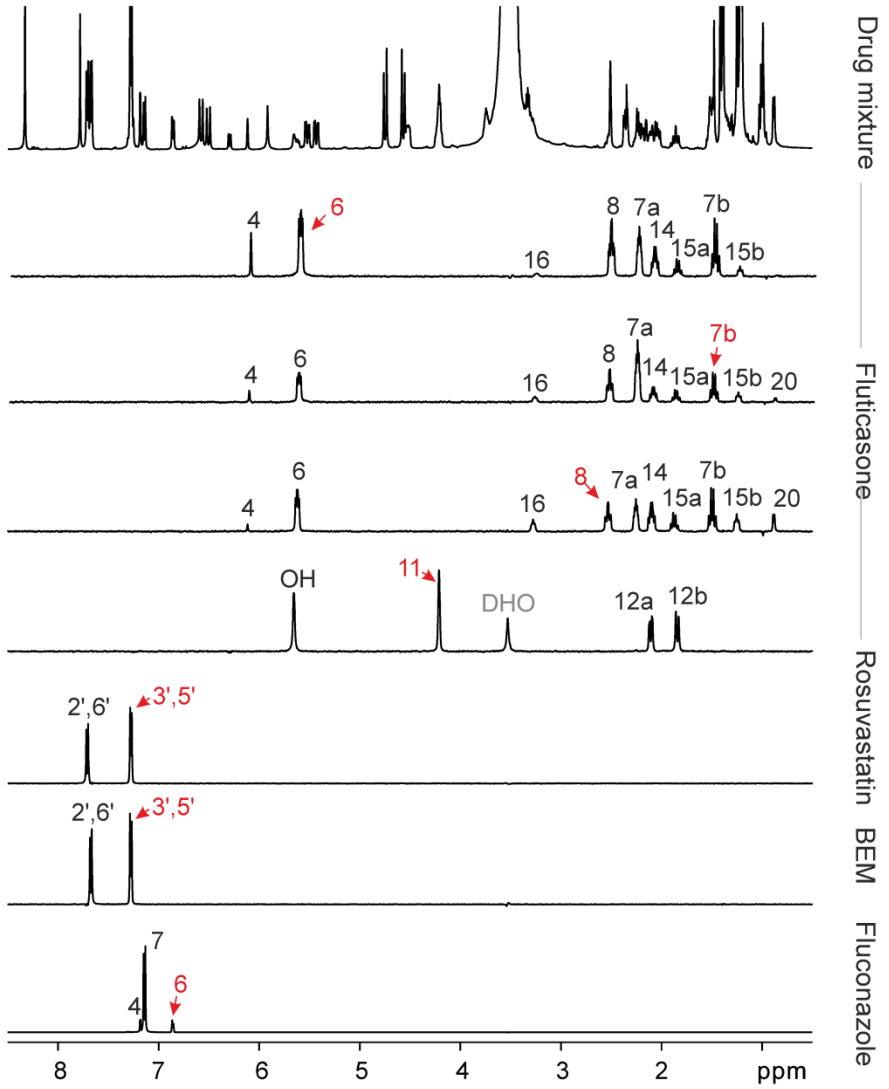


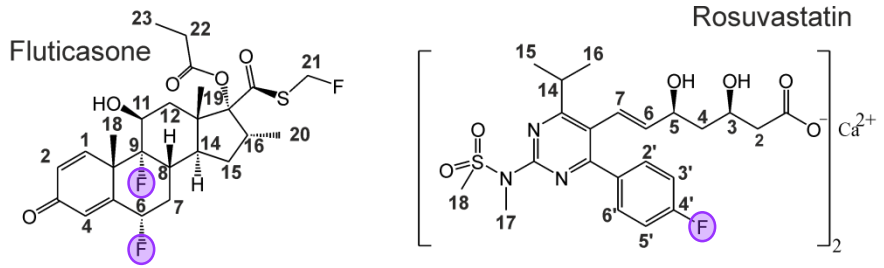
SRI: L. Castañar, P. Moutzouri, T. M. Barbosa, C. F. Tormena, R. Rittner, A. R. Phillips, S. R. Coombes, M. Nilsson, G. A. Morris, *Anal. Chem*. **2018**, *90,* 5445. / MODO: *unpublished*

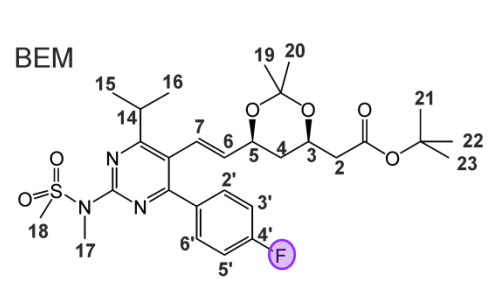
FESTA & Drug mixture

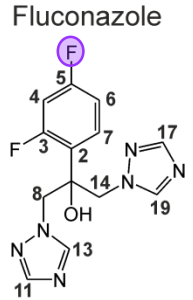
^{1}H

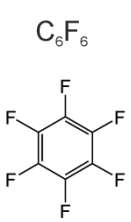
^{1}H FESTA

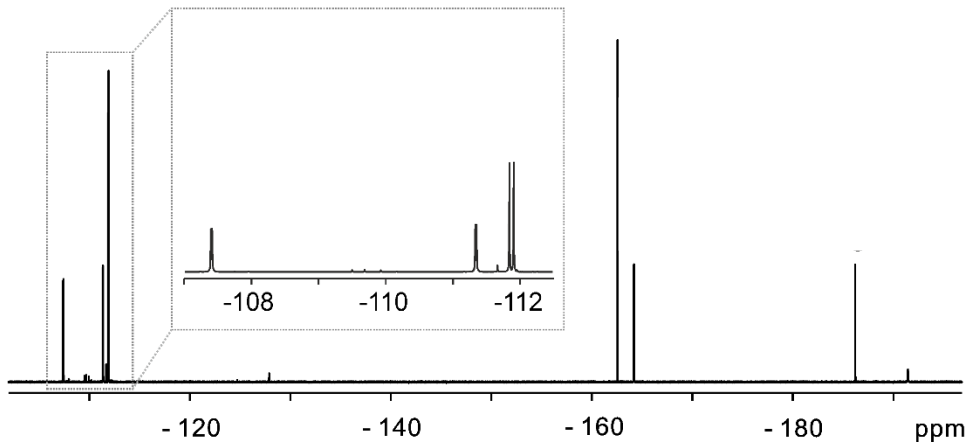












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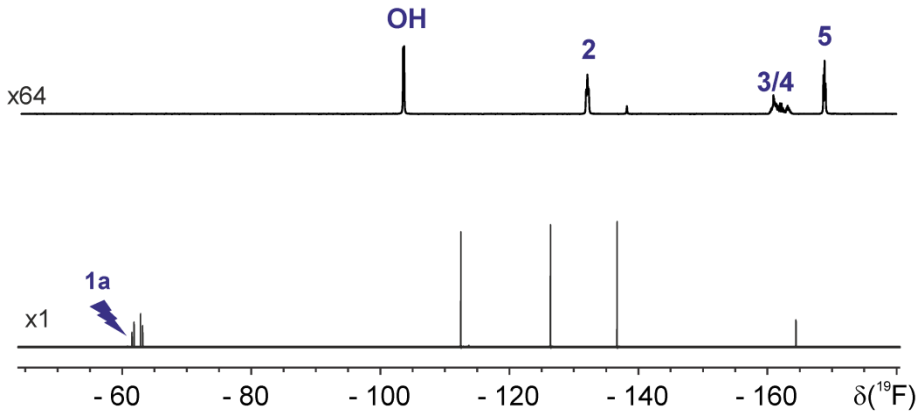
FESTA methods: Comparison

SRI-FESTA

SNR

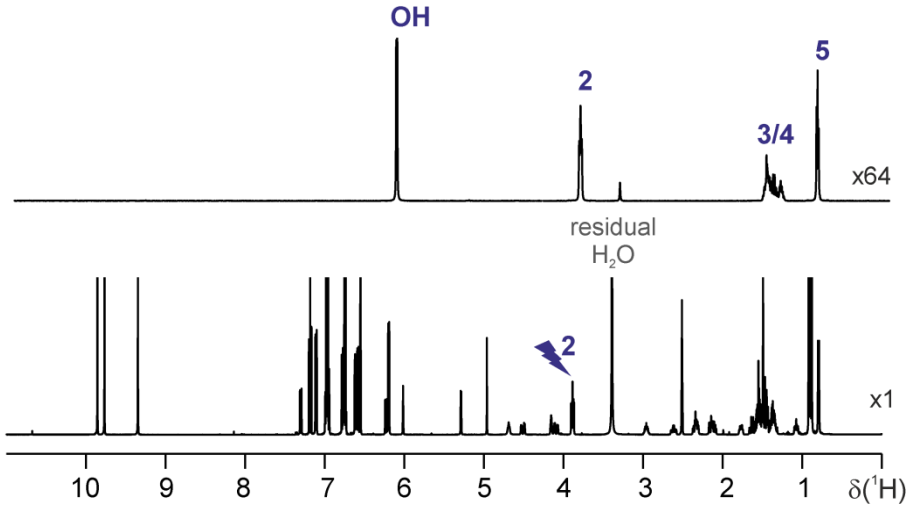
360

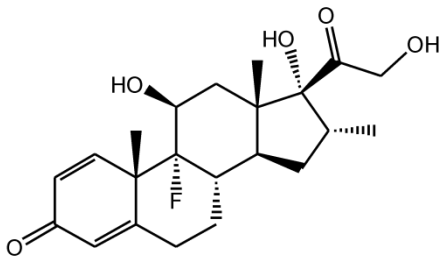


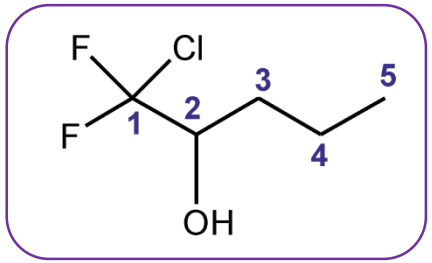
MODO-FESTA

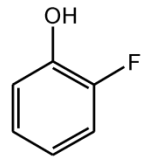
SNR

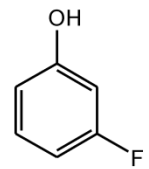
810

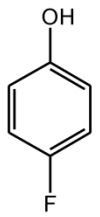












FESTA methods: Comparison

SRI-FESTA

MODO-FESTA

^{1}H sel 180

SNR 400

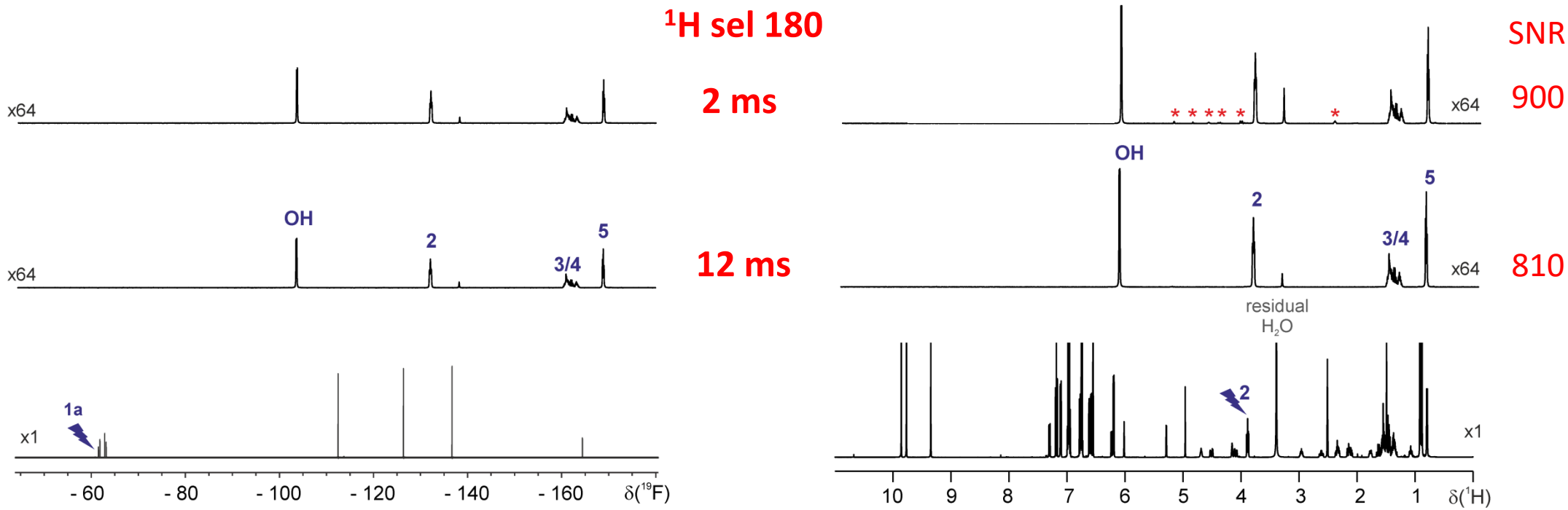
2 ms

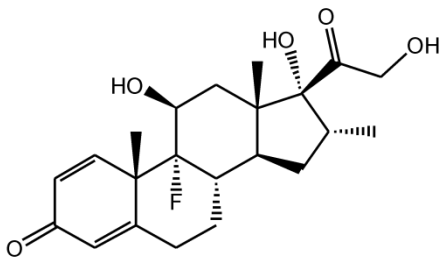
SNR 900

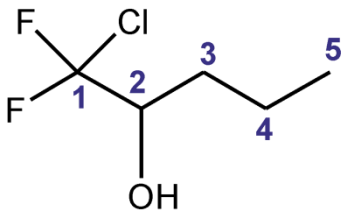
SNR 360

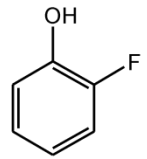
12 ms

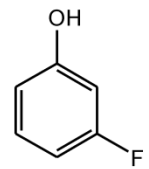
SNR 810

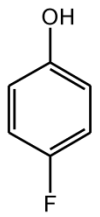






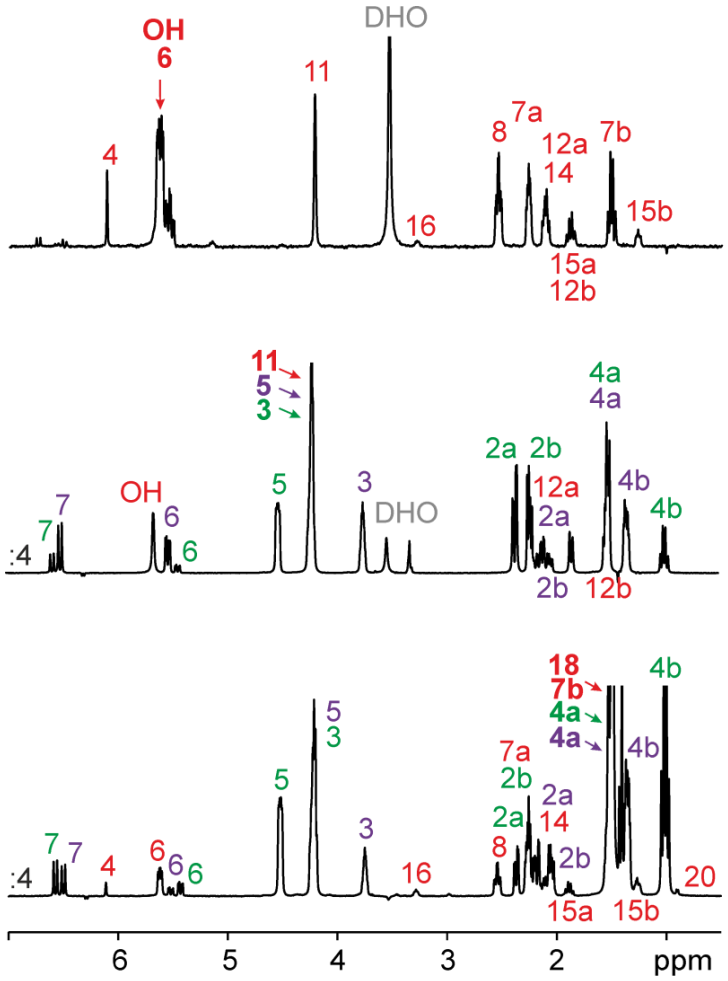




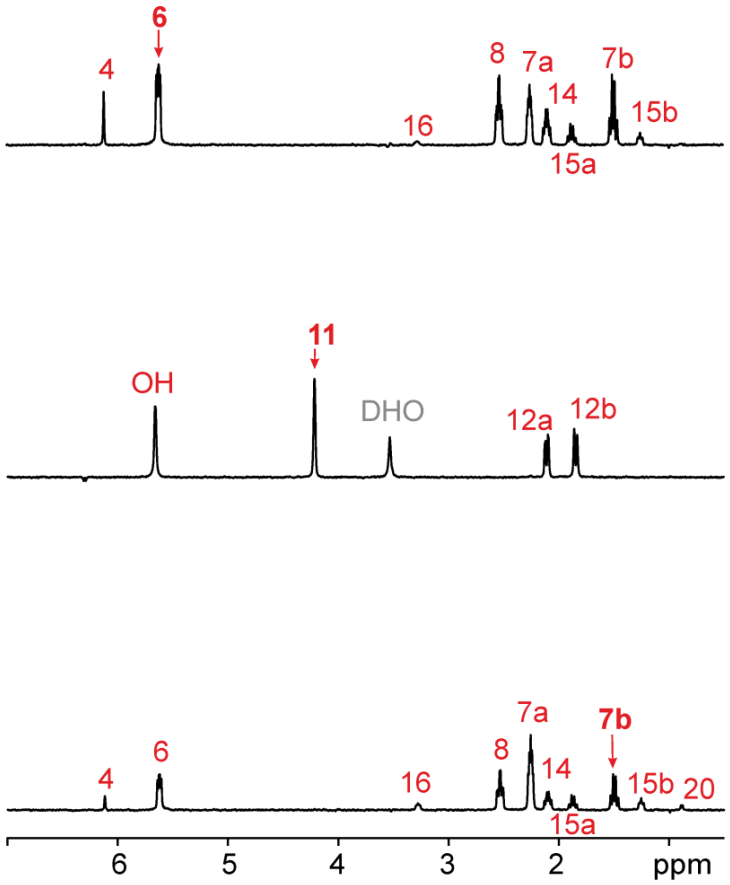


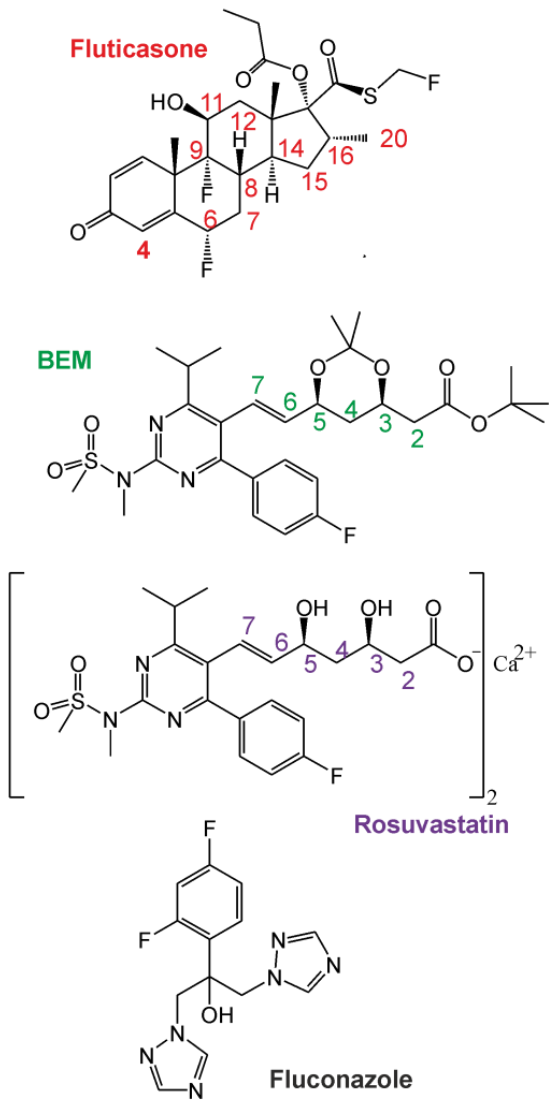
FESTA & Drug mixture

Selective TOCSY



SRI-FESTA





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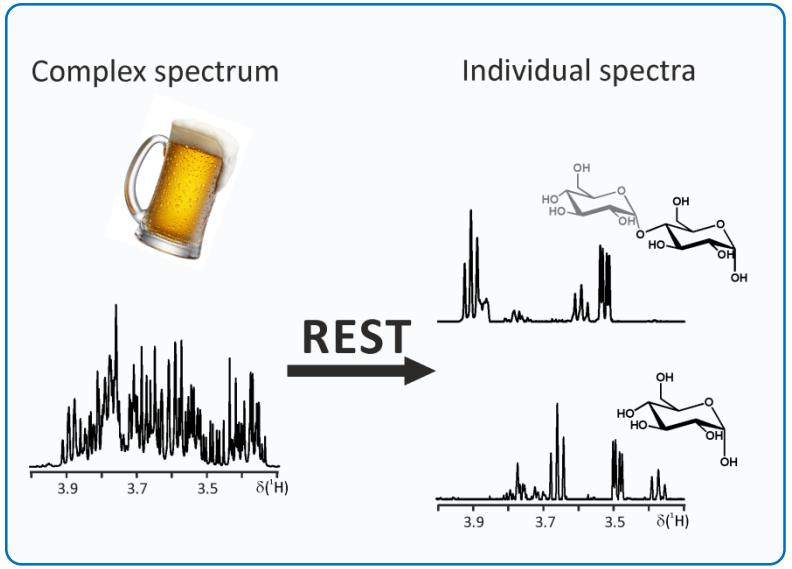
Novel NMR methods for the analysis of complex systems

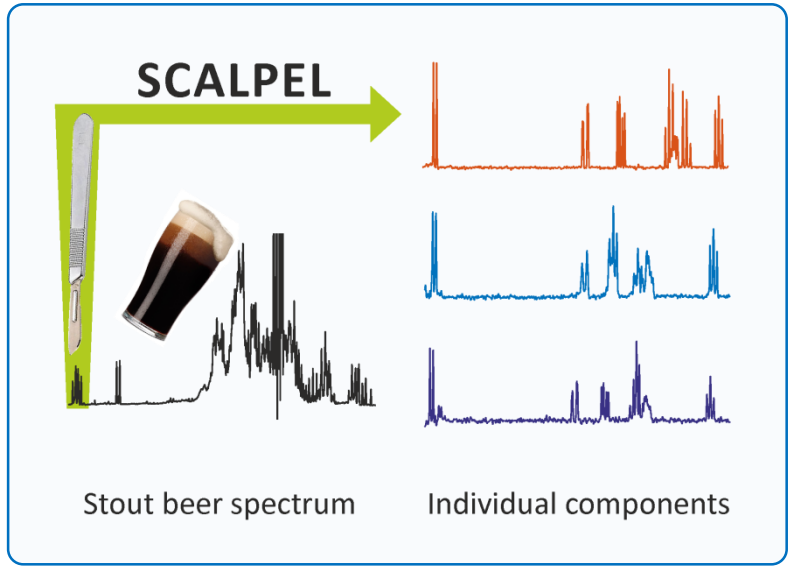
REST

Relaxation-Encoded Selective TOCSY



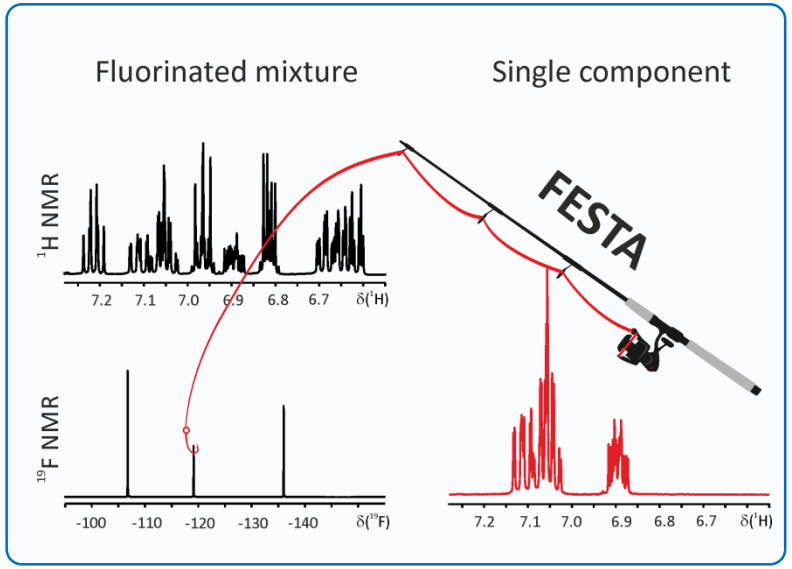
SCALPEL

Spectral Component Acquisition by Localized PARAFAC Extraction of Linear component



FESTA

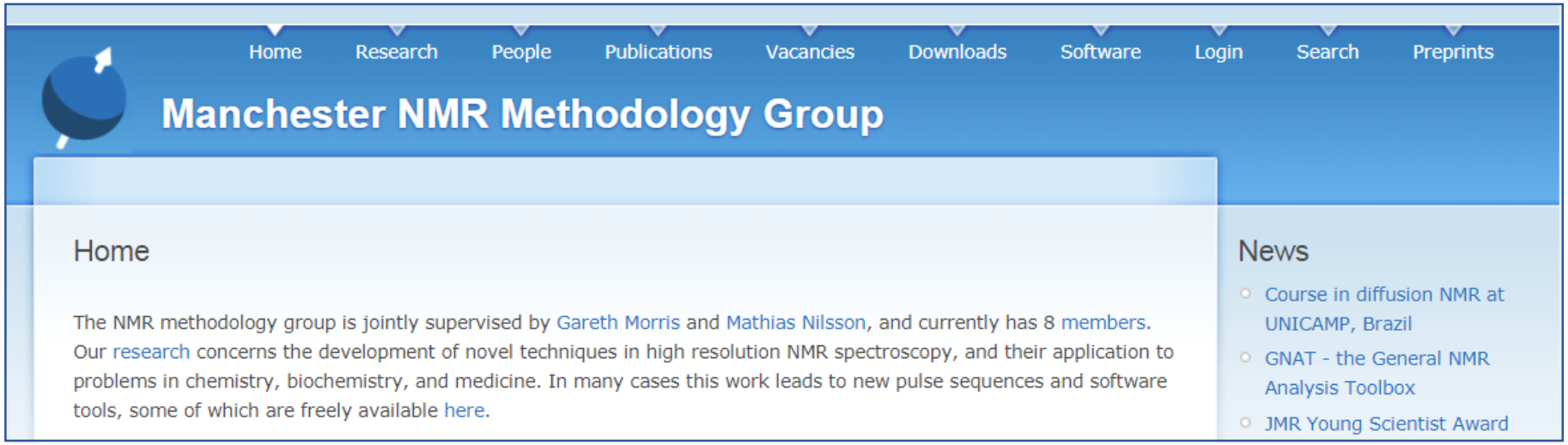
Fluorine-Edited Selective TOCSY Acquisition



Manchester NMR Methodology Group

@mancNMR

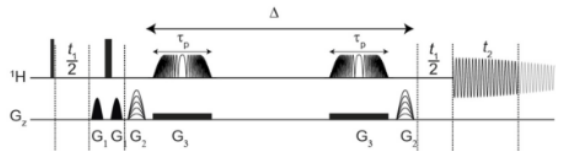
https://nmr.chemistry.manchester.ac.uk



Pulse Sequences

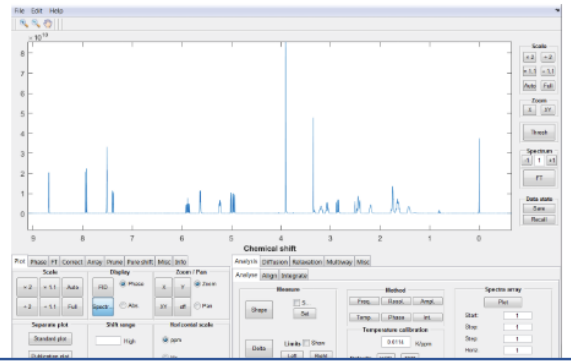
We are currently preparing many of our pulse sequences, parameter sets, example datasets and processing macros for the website. Some are available here but if you would like to use any of the other the sequences, as described in the publications section, please email us. The majority of sequences are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

Acknowledgments

Manchester NMR Methodology Group

Prof Gareth A. Morris

Prof Mathias Nilsson

Dr Ralph W. Adams

Dr Guilherme Dal Poggetto (Univ. of Campinas)

Dr Pinelopi Moutzouri (EPFL)

Dr Mohammadali Foroozandeh (Oxford Univ.)

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The University of Manchester

CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico

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CIÊNCIA SEM FRONTEIRAS

Thank you very much for your attention!

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NMR Methodology Group
The University of Manchester

Small Molecule NMR Conference
Porto, Portugal
September 25th 2019