

Divide and rule!

New NMR methods

for the analysis of mixtures

Laura Castañar Acedo

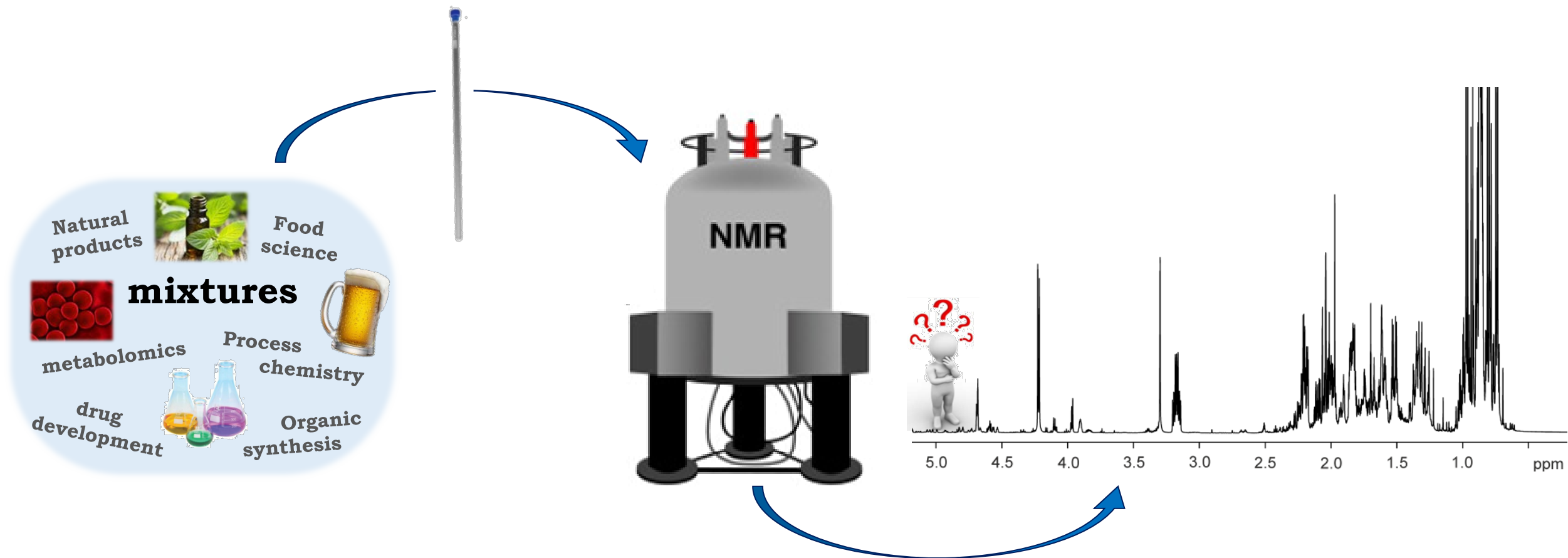
NMR Methodology group

The University of Manchester

European Magnetic Resonance Meeting

Nantes, France

July 4th 2018



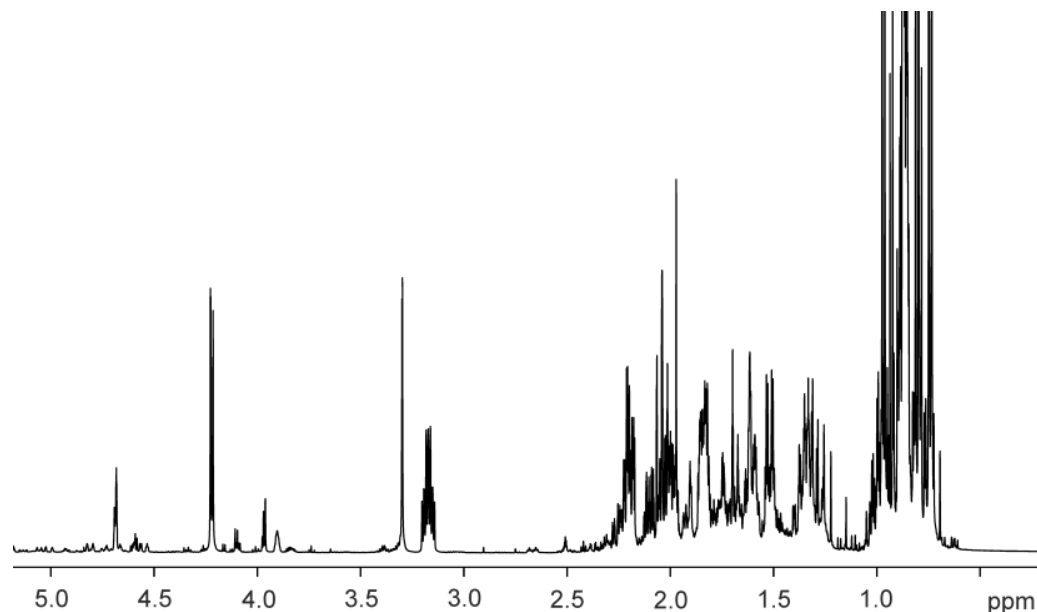
✓ Non-destructive

✓ Structural information

✓ Non-physical separation

✓ Individual species information

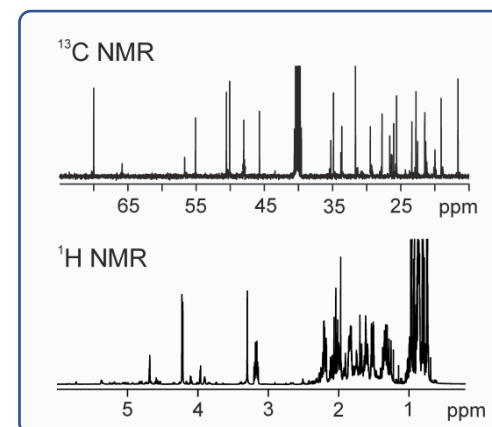
1D ^1H NMR & Mixture analysis



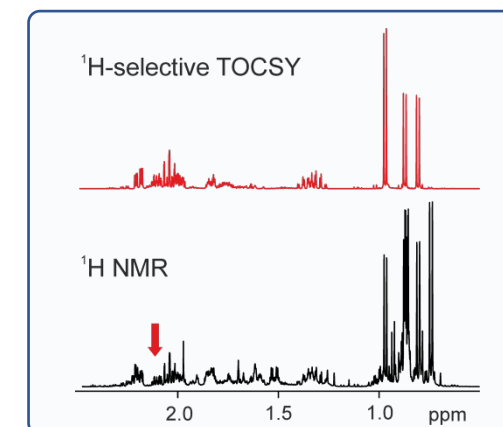
- ✓ The most abundant nuclei
- ✓ High sensitivity
- ✓ Structural information
- ✗ Signal overlap
- ✗ Low resolution spectra

Classical strategies for alleviating overlap

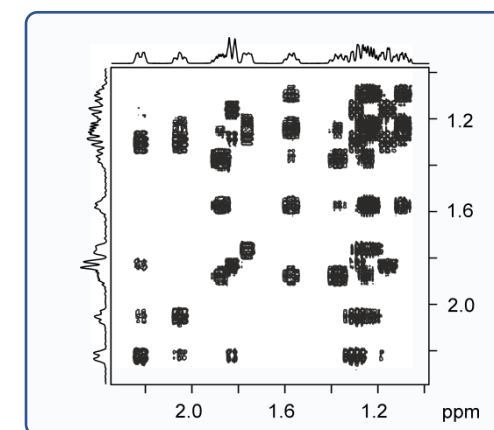
Other nuclei



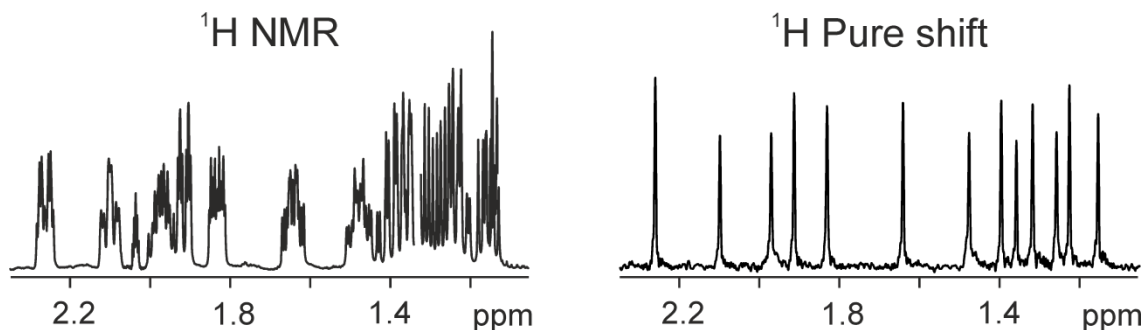
Spectral editing



nD NMR



Recent strategies for alleviating overlap: Pure shift NMR



Peak positions determined solely by chemical shifts

- ✓ Reduced spectral complexity
- ✓ Enhanced signal resolution
- ✓ Easier spectral analysis

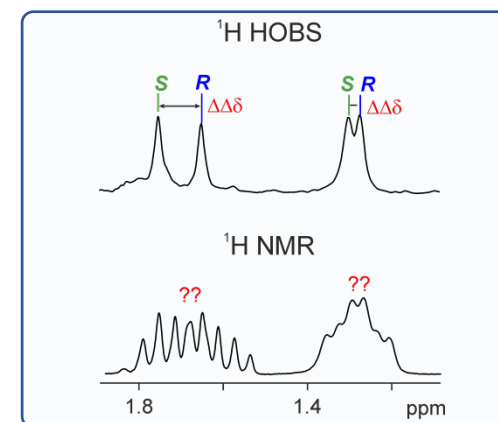
eMagRes **2014**, 3, 295

Magn. Reson. Chem. **2015**, 53, 399

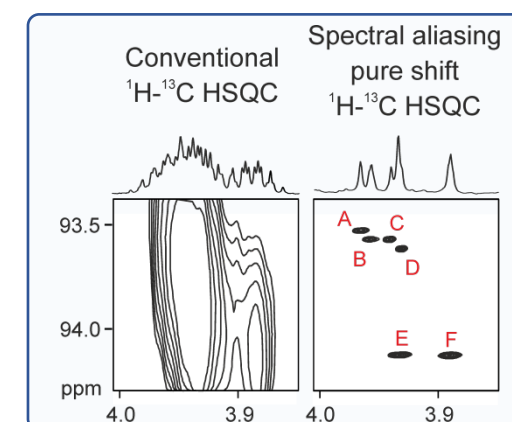
Prog. Nucl. Mag. Res. Sp. **2015**, 86, 1

Magn. Reson. Chem. **2017**, 55, 47

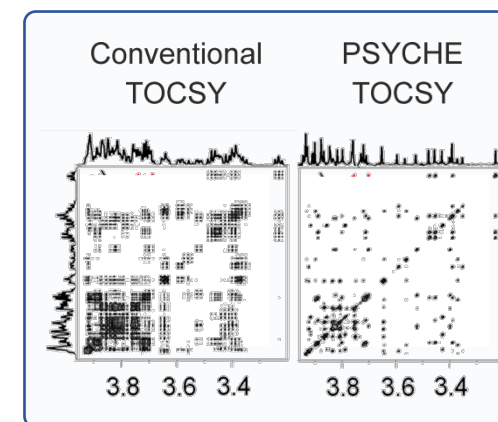
Pure shift NMR of mixtures



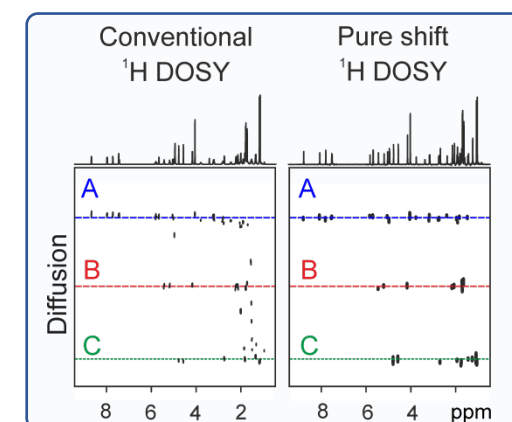
ChemPhysChem **2014**, 15, 854
Chem. Commun. **2014**, 50, 2512
Chem. Commun. **2015**, 51, 13492



Chem. Commun. **2014**, 50, 10214
Chem. Eur. J. **2015**, 21, 7682
Chem. Commun. **2016**, 52, 6142

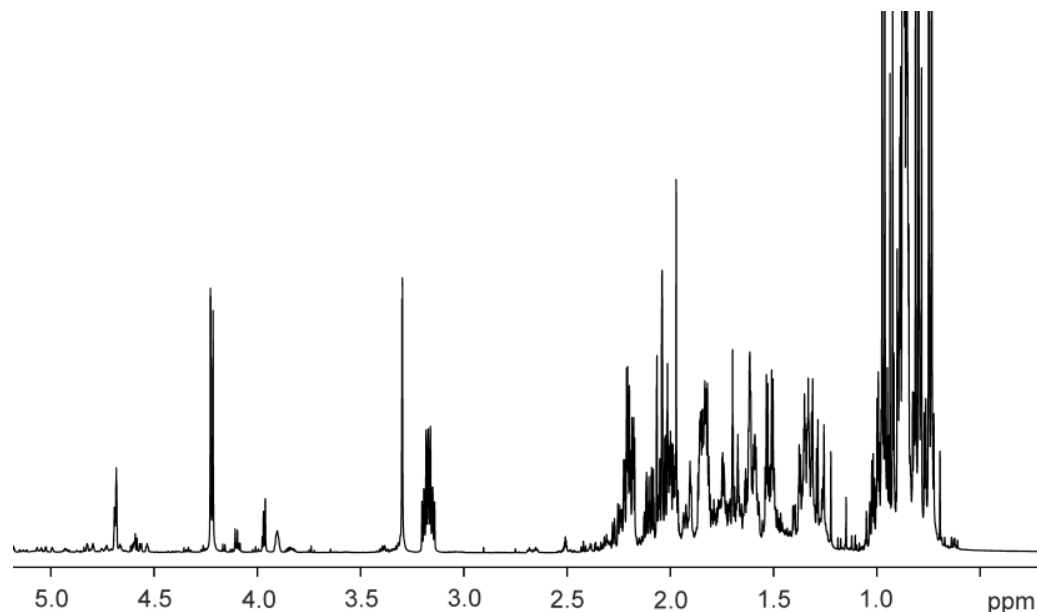


ChemPhysChem **2016**, 17, 2304
Magn. Reson. Chem. **2016**, 54, 308



Chem. Commun. **2007**, 933
Chem. Eur. J. **2014**, 20, 11171
Chem. Comm. **2014**, 50, 4073

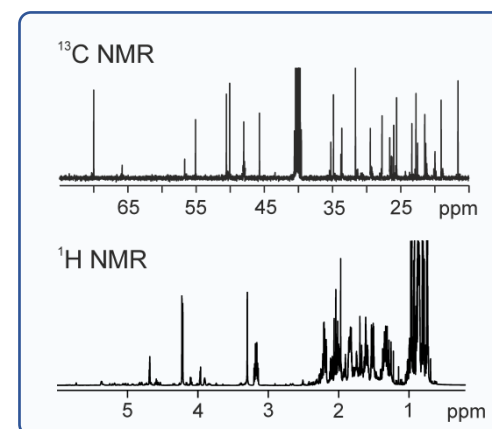
1D ^1H NMR & Mixture analysis



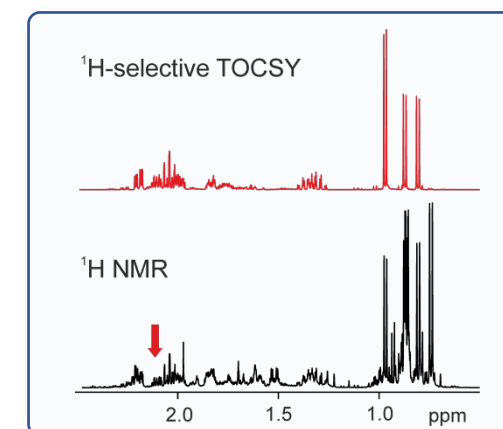
- ✓ The most abundant nuclei
- ✓ High sensitivity
- ✓ Structural information
- ✗ Signal overlap
- ✗ Low resolution spectra

Strategies for alleviating overlap

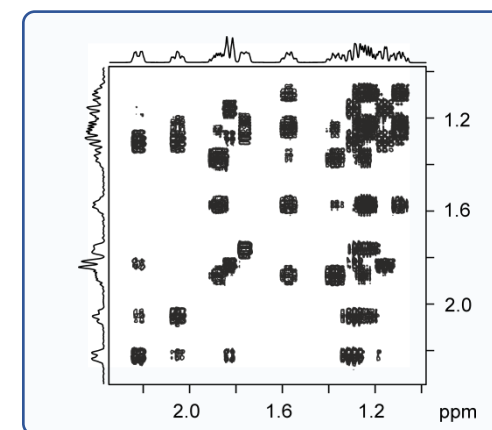
Other nuclei



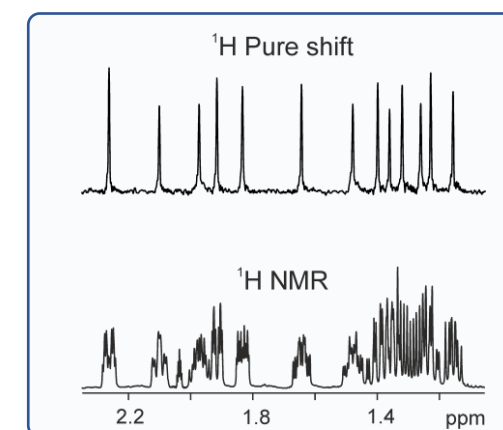
Spectral editing



nD NMR

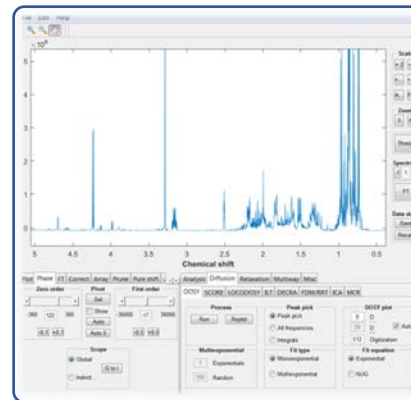


Pure shift NMR



New NMR software

GNAT

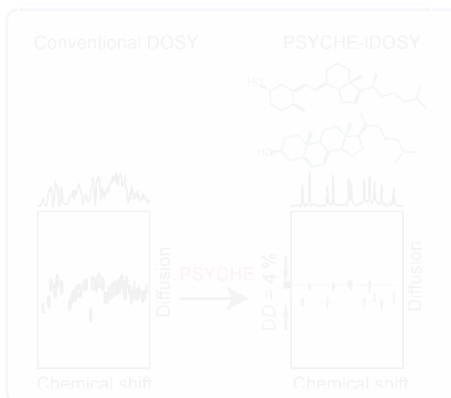


MAGNATE

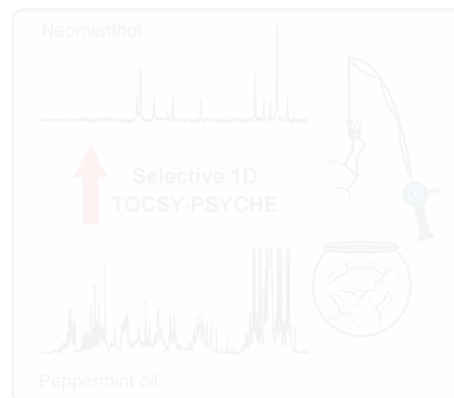


New NMR methods

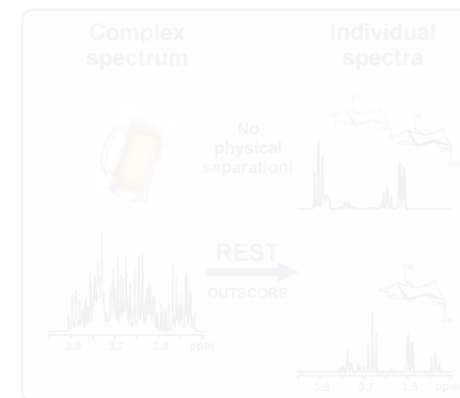
PSYCHE-iDOSY



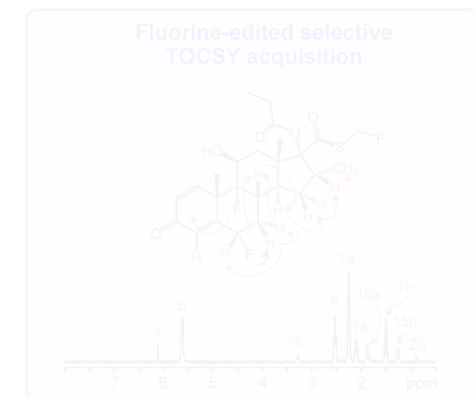
Selective 1D TOCSY-PSYCHE



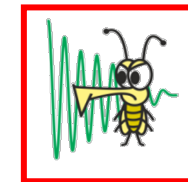
REST



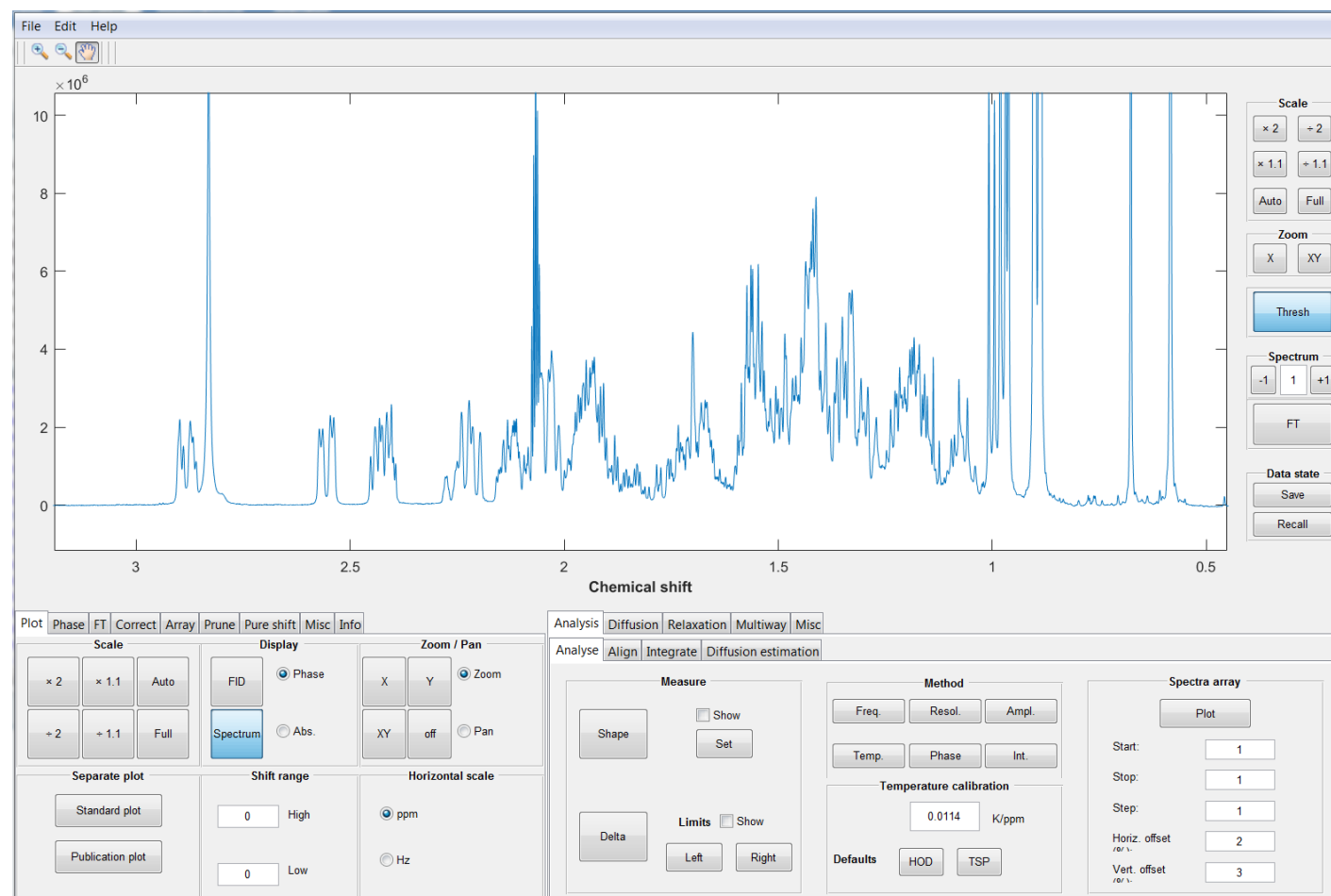
FESTA



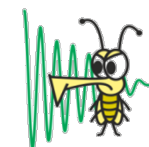
General NMR Analysis Toolbox (GNAT)



- 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)



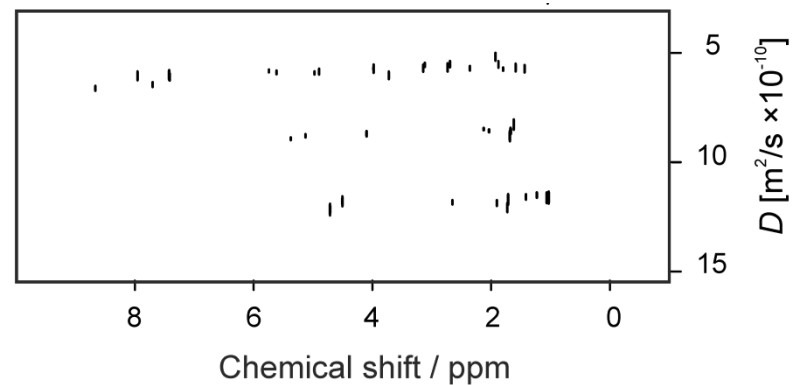
Main window of the graphical interface of the GNAT



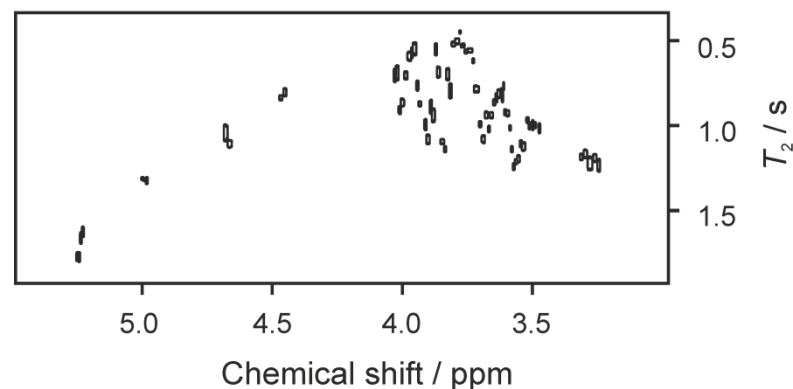
GNAT & Mixture analysis

Univariate methods

Diffusion (DOSY)



Relaxation (ROSY)



Diffusion related parameters

Calculate diffusion parameters by pulse sequence type

Monopolar Bipolar

Δ : Δ' : δ :

γ : τ :

dosyconstant:

	T/m
1	0.0681
2	0.1208
3	0.1566
4	0.1856
5	0.2107
6	0.2331
7	0.2536
8	0.2725

Relaxation related parameters

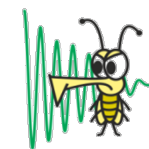
Convert counter to delays

Loop duration (s):

For CPMG => 2*tau+p2
For PROJECT => 4*tau+2*p2+p1

Revert to original values

Delays (vclist)		Counter (vclist)	
	sec		count
1	0.0089	1	4
2	0.0533	2	24
3	0.1066	3	48
4	0.2132	4	96
5	0.2843	5	128
6	0.4265	6	192
7	0.5686	7	256
8	0.7108	8	320



GNAT & Mixture analysis

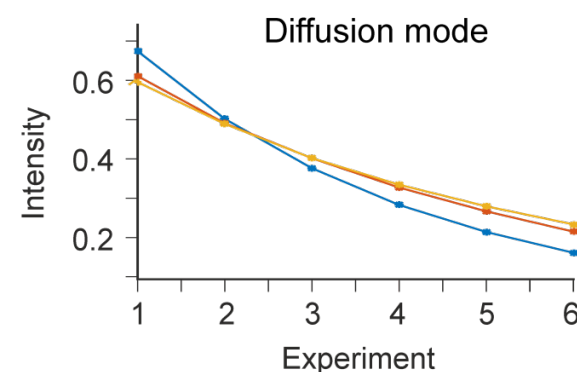
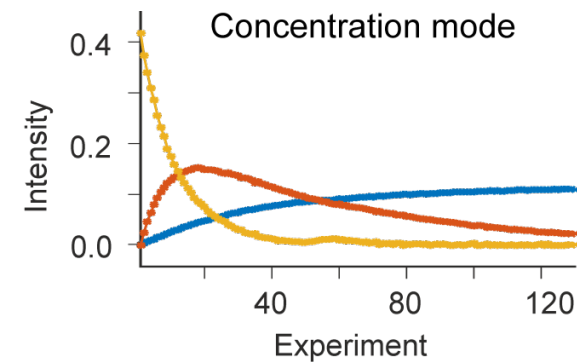
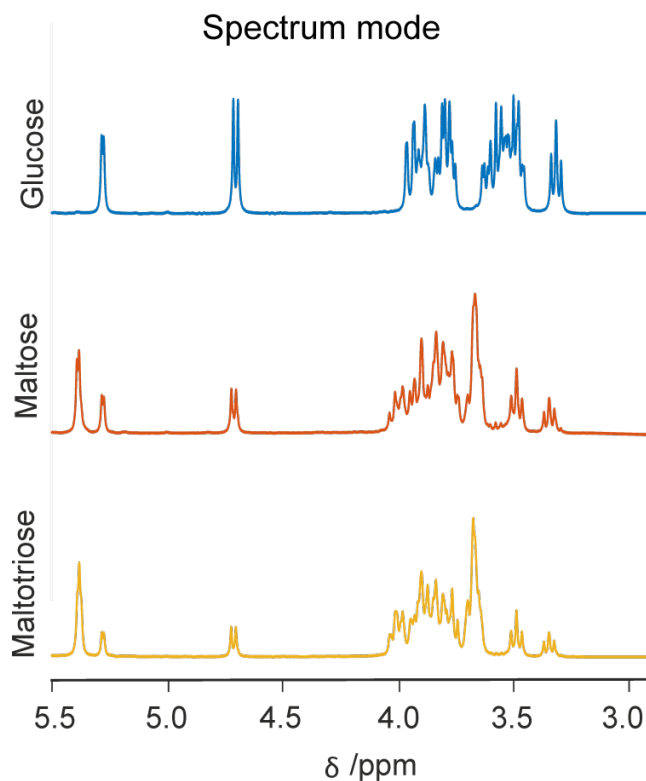
Multivariate methods

SCORE/RSCORE

OUTSCORE

LOCODOSY

DECRA



FDM/RRT

ICA

MRC

PARAFAC

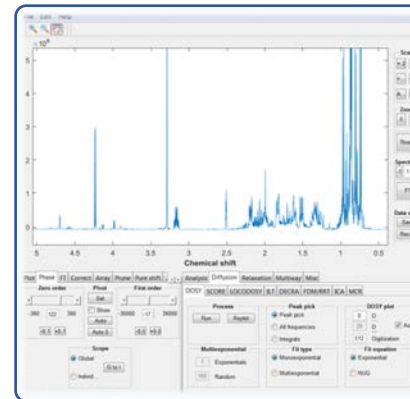
Slicing

PARAFAC analysis of the hydrolysis of maltotriose. Diffusion NMR experiments were performed continuously over the course of the reaction.

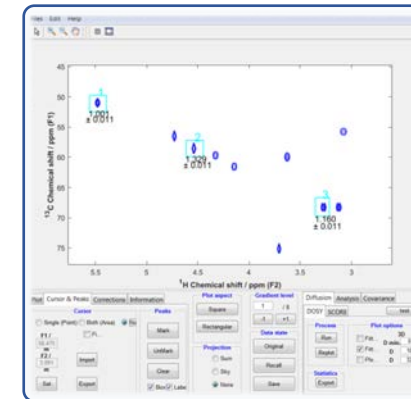
Anal. Chem. **2009**, *81*, 8119.

New NMR software

GNAT



MAGNATE

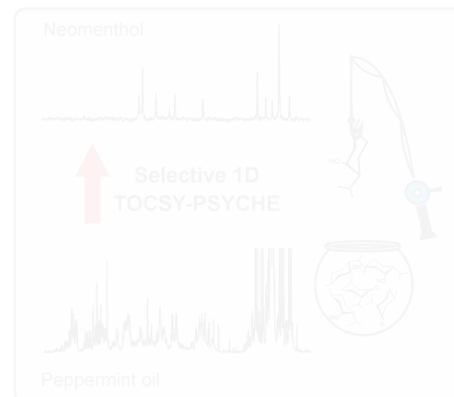


New NMR methods

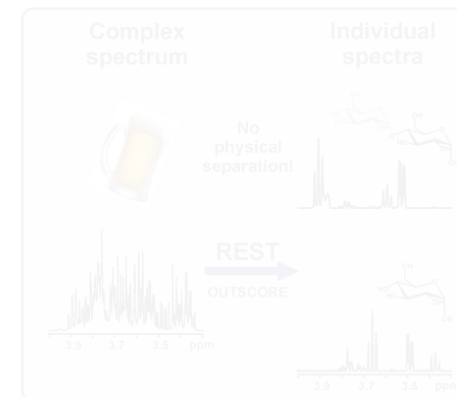
PSYCHE-iDOSY



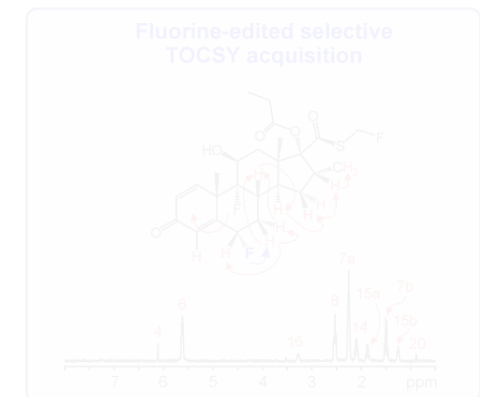
Selective 1D TOCSY-PSYCHE



REST

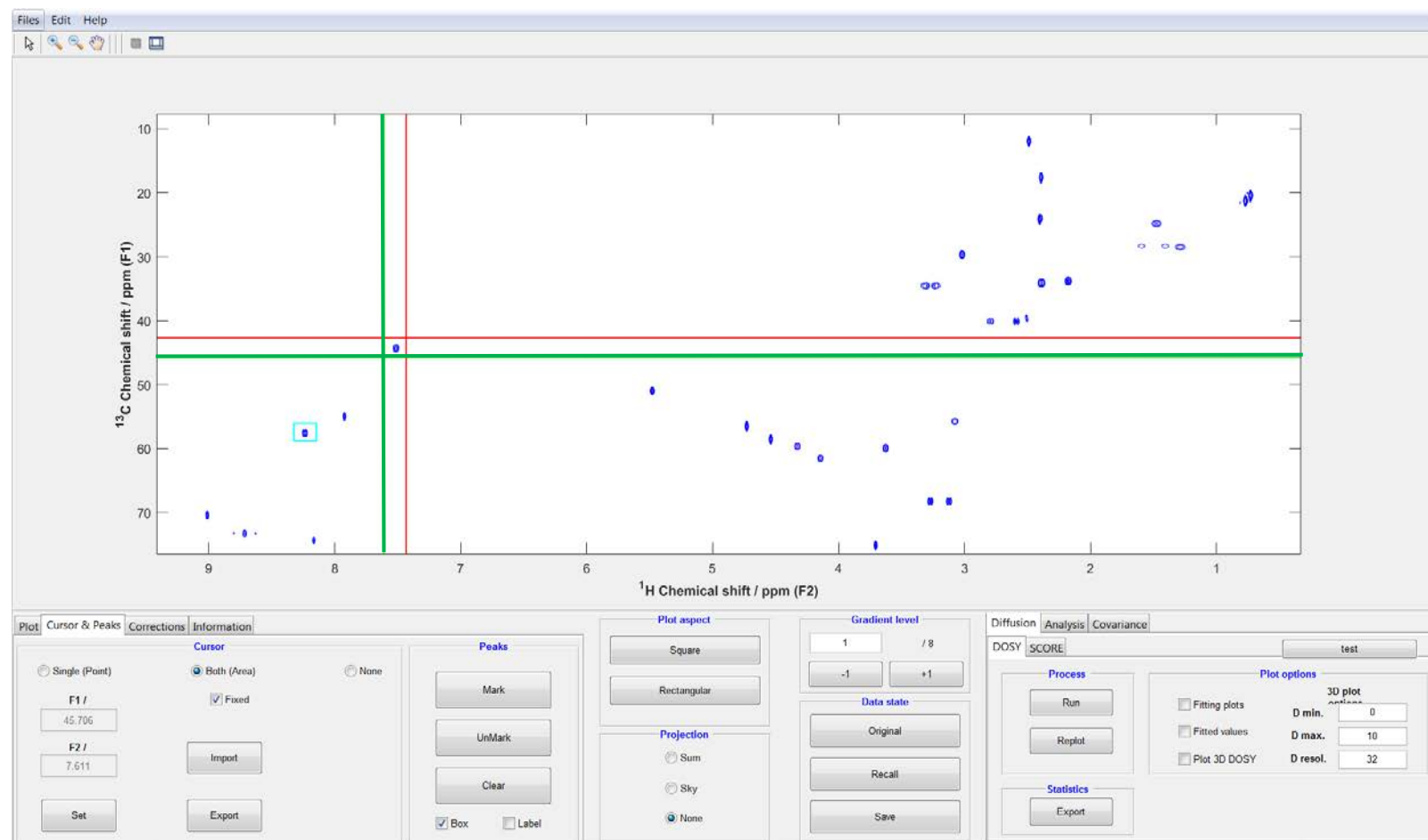


FESTA



Multidimensional Analysis for the GNAT Environment (MAGNATE)

- For processing, visualising, and analysing 3D diffusion NMR data
- Free & open-source software
- User-friendly graphical interface
- MATLAB[®] language (free-standing compiled versions available)

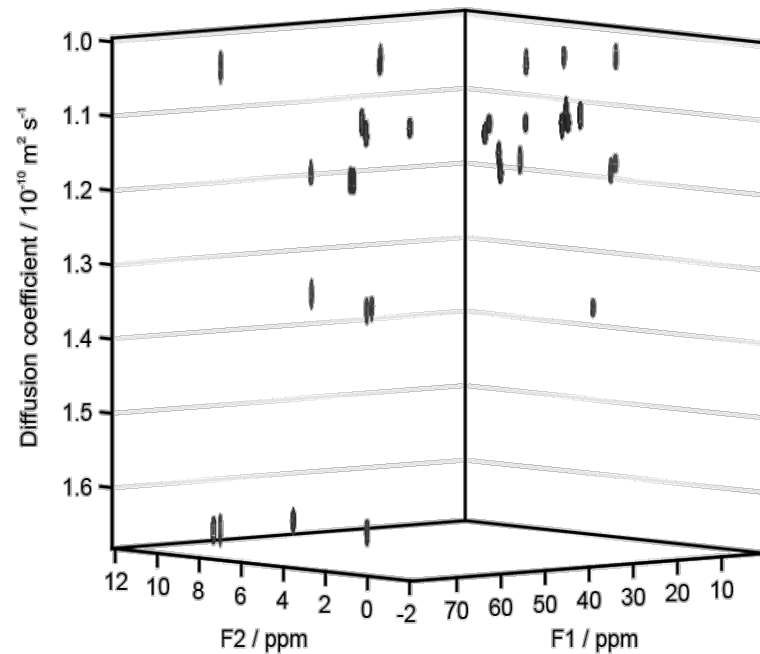


Main window of the graphical interface of the MAGNATE

MAGNATE & Mixture analysis

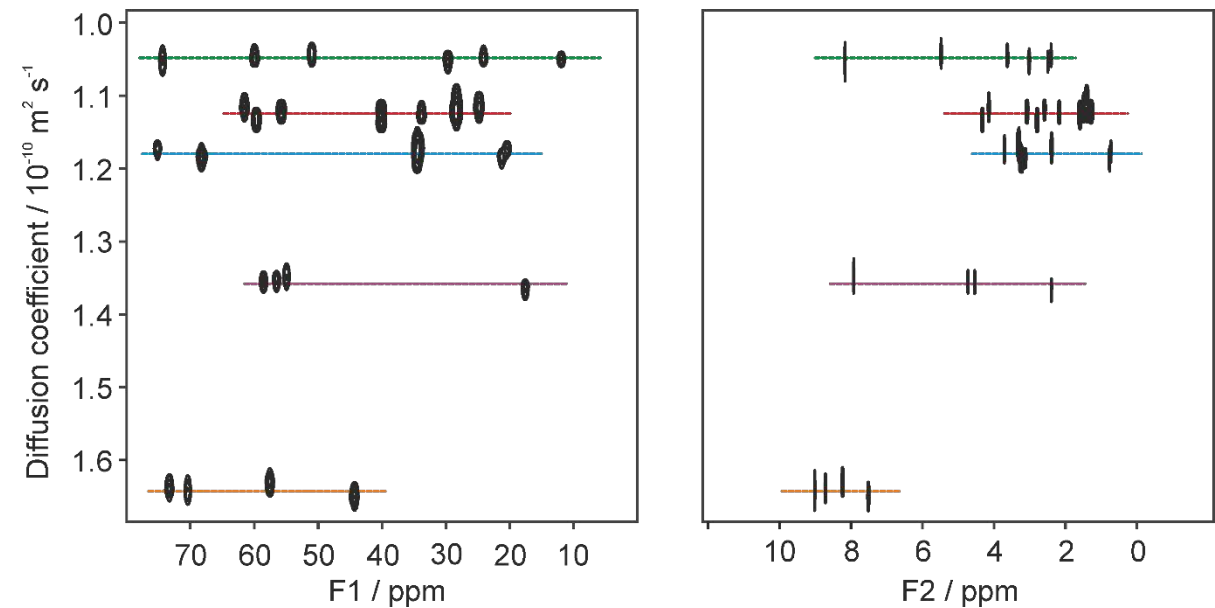
Univariate methods

3D DOSY



3D DOSY plot from a Oneshot-HSQC experiment on a mixture of B vitamins

2D Projections

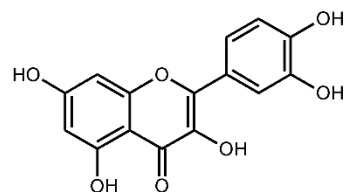


2D projection along ^1H dimension

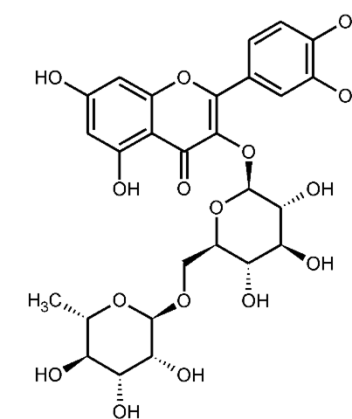
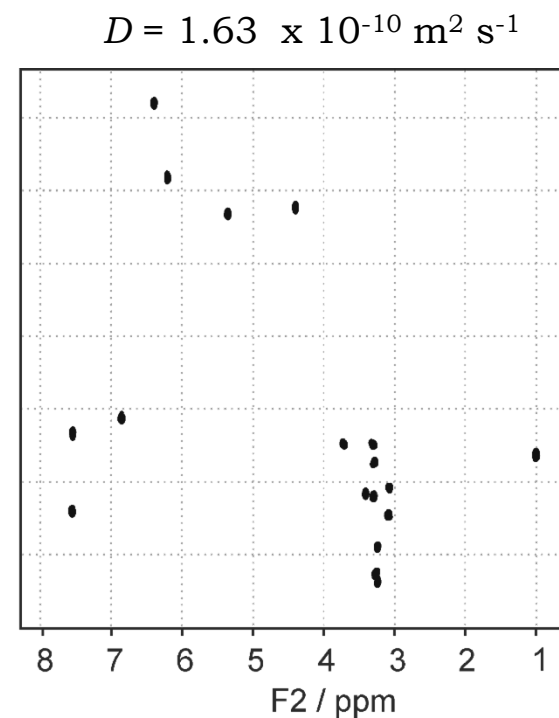
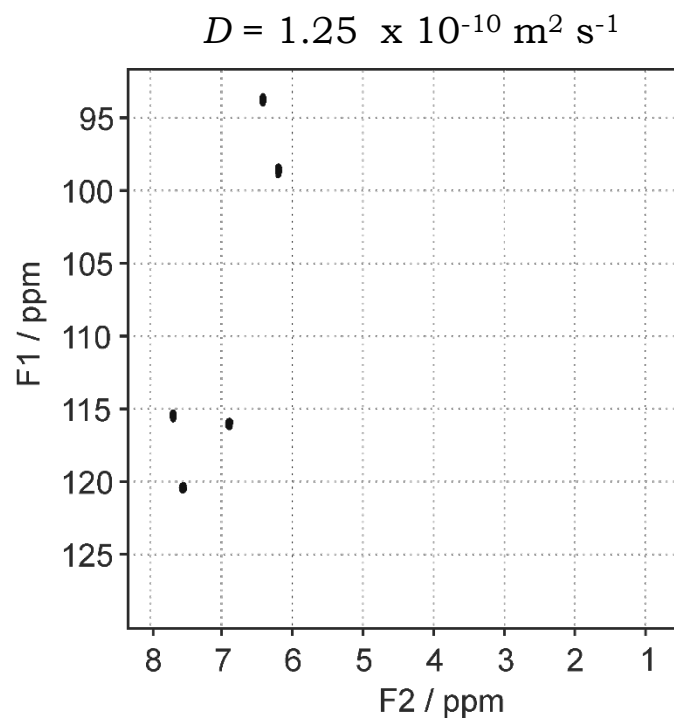
2D projection along ^{13}C dimension

MAGNATE & Mixture analysis

Multivariate methods



Quercetin



Rutin

2D HSQC spectra obtained from OUTSCORE analysis of the Oneshot-HSQC data for the components of a mixture of flavonoids

Poster 359

A new analysis tool for 3D diffusion NMR experiments



Guilherme Dal Poggetto



A new analysis tool for 3D diffusion NMR experiments

G. Dal Poggetto, L. Castañar, M. Foroozandeh, P. Kiraly, R. W. Adams, G. A. Morris and M. Nilsson

School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK. Email: guilherme.dalpoggetto@manchester.ac.uk

Introduction

In diffusion-ordered spectroscopy (DOSY), one of the most commonly used NMR experiments in the analysis of mixtures, the amplitude or integral of each signal in an array of 1D spectra is fitted to an appropriate variant of the Stejskal-Tanner equation,² and displayed as a Gaussian peak in a second dimension which represents diffusion coefficient. DOSY is commonly used to identify signals from different components in a mixture. However, signal overlap can obscure diffusion information and complicate spectral interpretation. A useful way to minimise signal overlap in the spectral dimension is to add a diffusion dimension to a 2D (or higher dimensionality) NMR experiment, generating a 3D (or higher) DOSY spectrum.³ This greatly reduces the probability of overlap, since cross-peaks are spread out over a plane rather than along a single axis. Here we introduce a new Matlab® based toolbox, MAGNATE (“Multidimensional Analysis for the GNAT Environment”), which complements the recently introduced GNAT (“General NMR Analysis Toolbox”,⁴ the former DOSYToolbox⁵), for analysing 3D diffusion NMR data. Within this new environment (Figure 1) it is possible for the first time to analyse 3D diffusion data (e.g. 3D DOSY-HSQC, as shown in the examples below) using both univariate and multivariate methods in a user-friendly graphical interface (GUI). The Matlab code and test data are available at <https://nmr.chemistry.manchester.ac.uk/>.

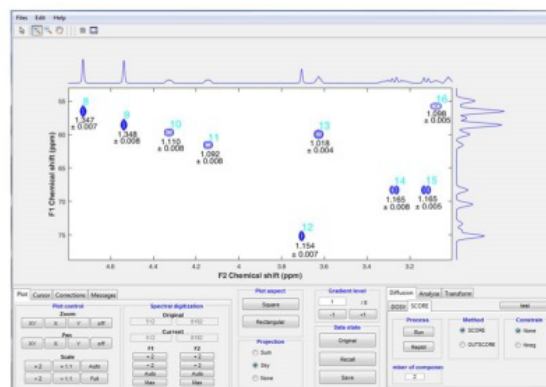


Figure 1. Main window of the MAGNATE GUI: The spectrum shows a small region of the least attenuated 2D spectrum of an F_1 -folded 3D Oneshot-HSQC experiment on vitamin B mixture in DMSO- d_6 . It illustrates one of the available display modes, in which each peak is numbered (in light blue) and labelled (in black) with its diffusion coefficient and estimated

Results and Discussion

Taking advantage of the user-friendly interface of MAGNATE, it is possible to manipulate the 3D DOSY spectrum generated by univariate processing, as shown in Figure 3. Several different plotting options are available, among them the extraction of 2D or integrals over a defined diffusion range (Figures 4 and 5), which gives individual spectra of the mixture components without the need for physical separation. Alternatively, it is possible to use multivariate methods (SCORE⁷ or OUTSCORE⁸ algorithms) to extract the spectra of the individual spectral components, in a quick and semi-automatic way.

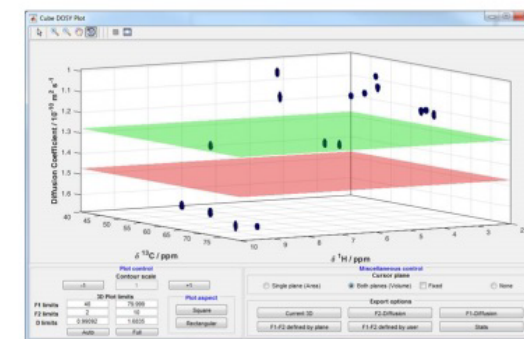


Figure 3. 3D DOSY plot GUI. The GUI is opened when univariate DOSY is used for processing and the box “Plot 3D DOSY” is selected, and this window is controlled independently of the main window.

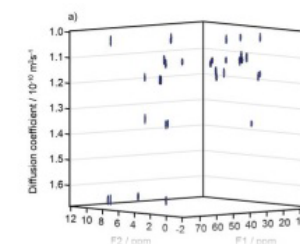
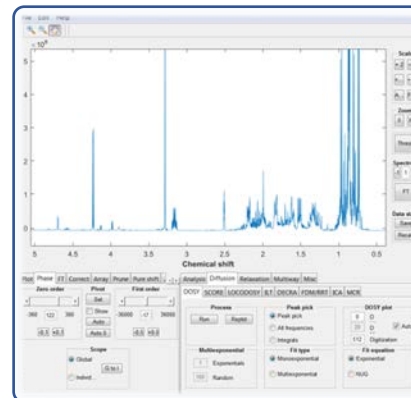


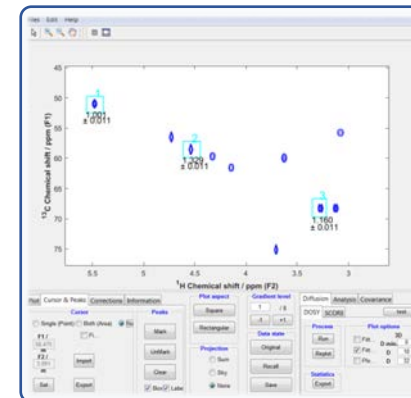
Figure 4. a) Pseudo-3D DOSY plot of the Oneshot-HSQC experiment of a mixture of B vitamins, in DMSO- d_6 , b) 2D projection along F_2 , and c) 2D projection along F_1 . F_1 and F_2 are the ^{13}C and ^1H dimensions, respectively. Dashed lines are introduced for aiding visualization and indicate the individual diffusion coefficients of: biotin B7 (red), pyridoxine hydrochloride B6

New NMR software

GNAT

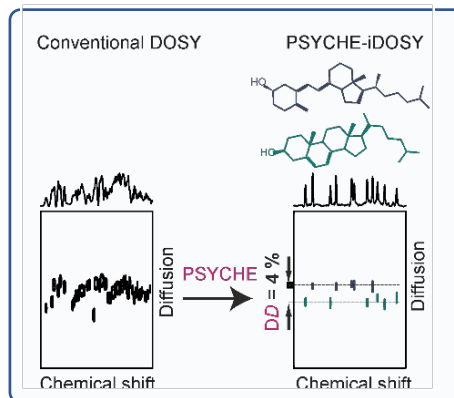
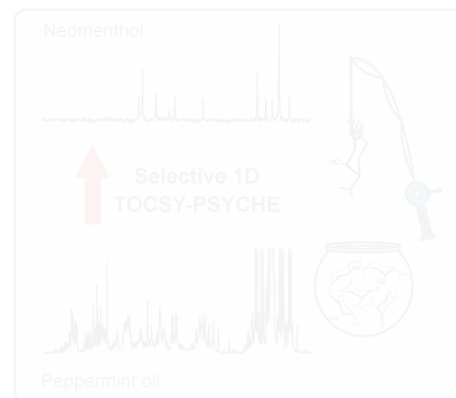


MAGNATE

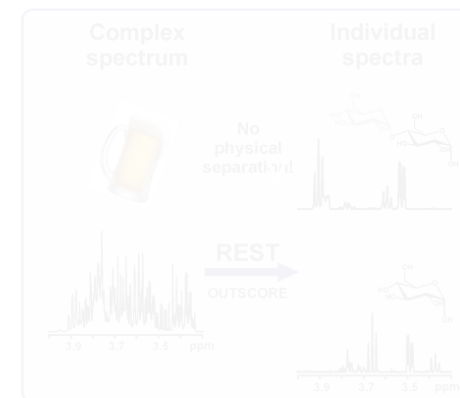


New NMR methods

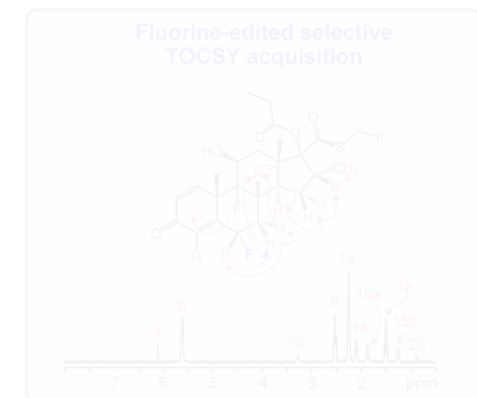
PSYCHE-iDOSY

Selective 1D
TOCSY-PSYCHE

REST

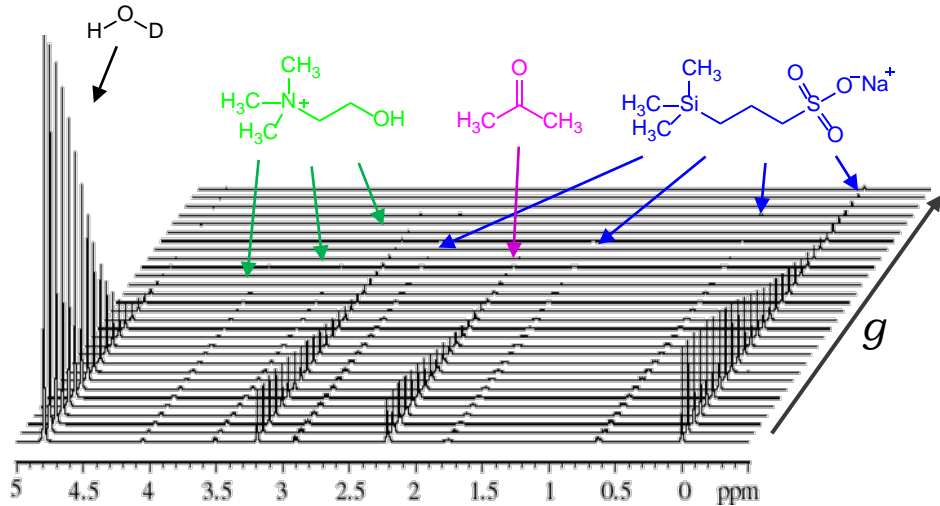


FESTA



Diffusion NMR

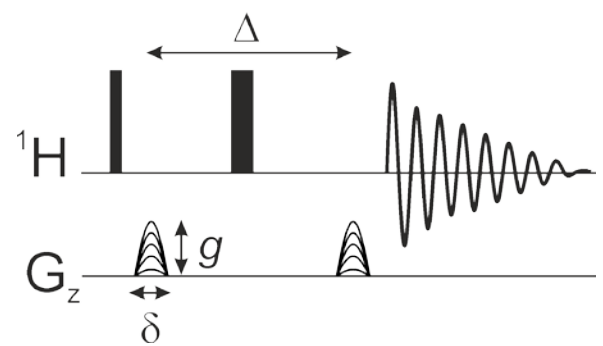
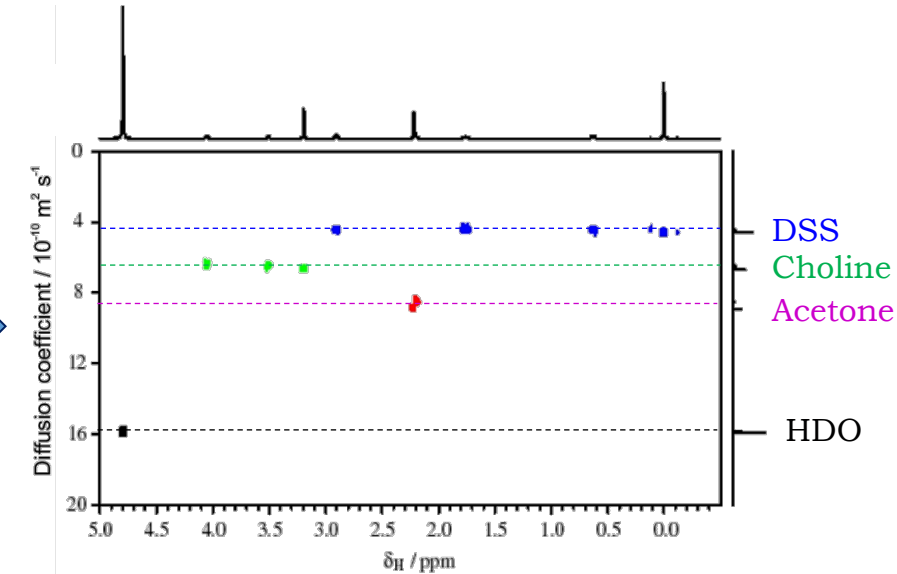
Diffusion array



$$S(g) = S_0 e^{-D\gamma^2 \delta^2 g^2 \Delta'}$$

Stejskal-Tanner

Diffusion-Ordered Spectroscopy (DOSY)

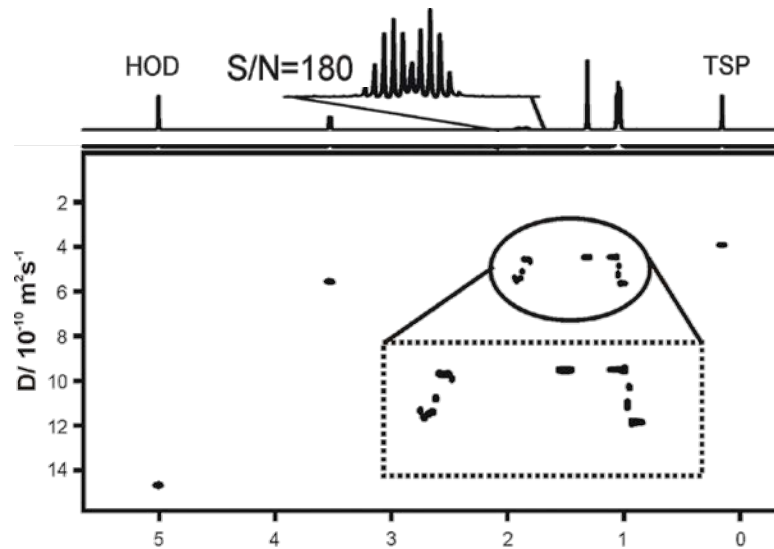


Prototype sequence

All signals from a given molecule:
 - have same diffusion behaviour
 - signals overlap **X** - should appear at the same *D*

Diffusion NMR & Pure shift NMR

Conventional DOSY

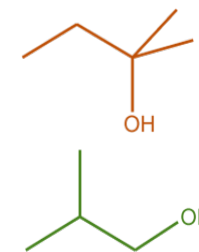
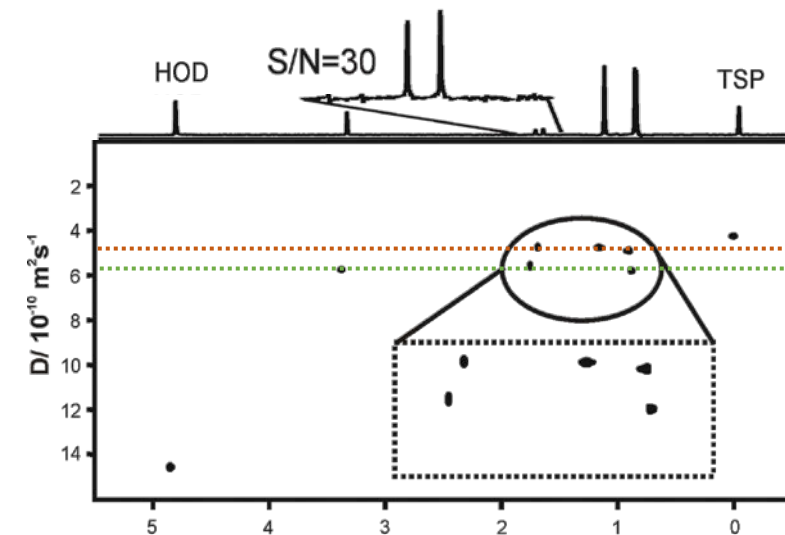
Interferogram
Zangger-Sterk

Chem. Commun. 2007, 933

Real time
Zangger-Sterk

Chem. Eur. J. 2014, 20, 11171

Pure shift DOSY



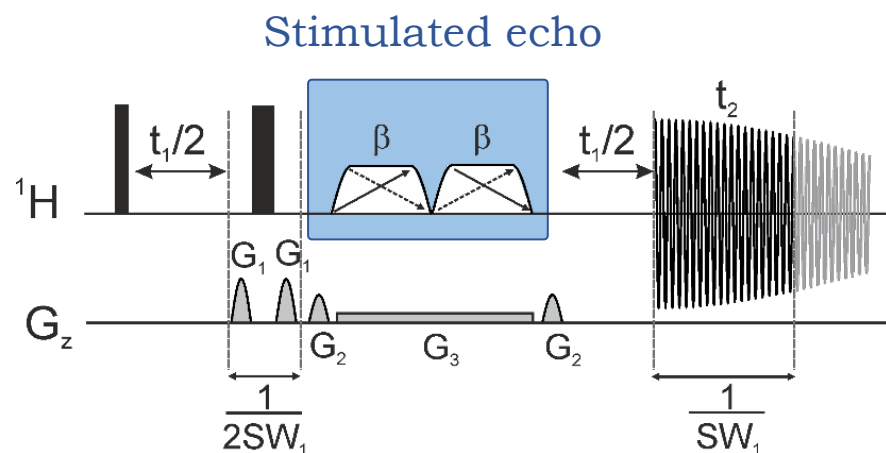
- ✗ Signals overlap
- ✗ Misleading peaks

- ✓ Higher signal resolution
- ✓ Accurate D measurements

Low SNR
PFG spatial variation

PSYCHE-iDOSY experiment

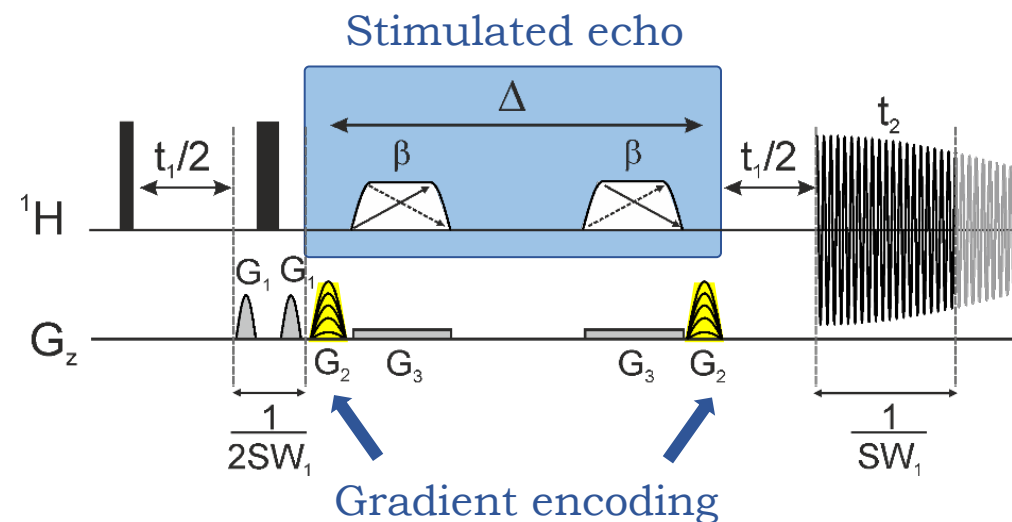
PSYCHE



PSYCHE benefits

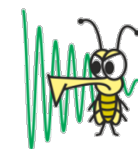
Higher sensitivity
Less sensitive to spatial
non-uniformity of PFG

PSYCHE-iDOSY



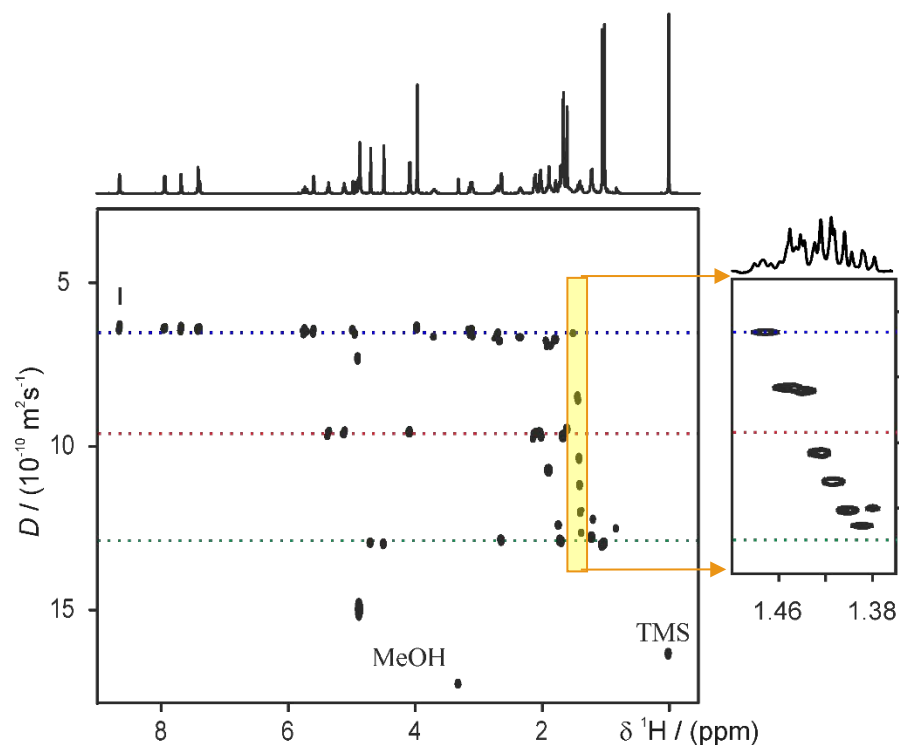
PSYCHE element

Inverts active spins
Provides internal diffusion
encoding (iDOSY)



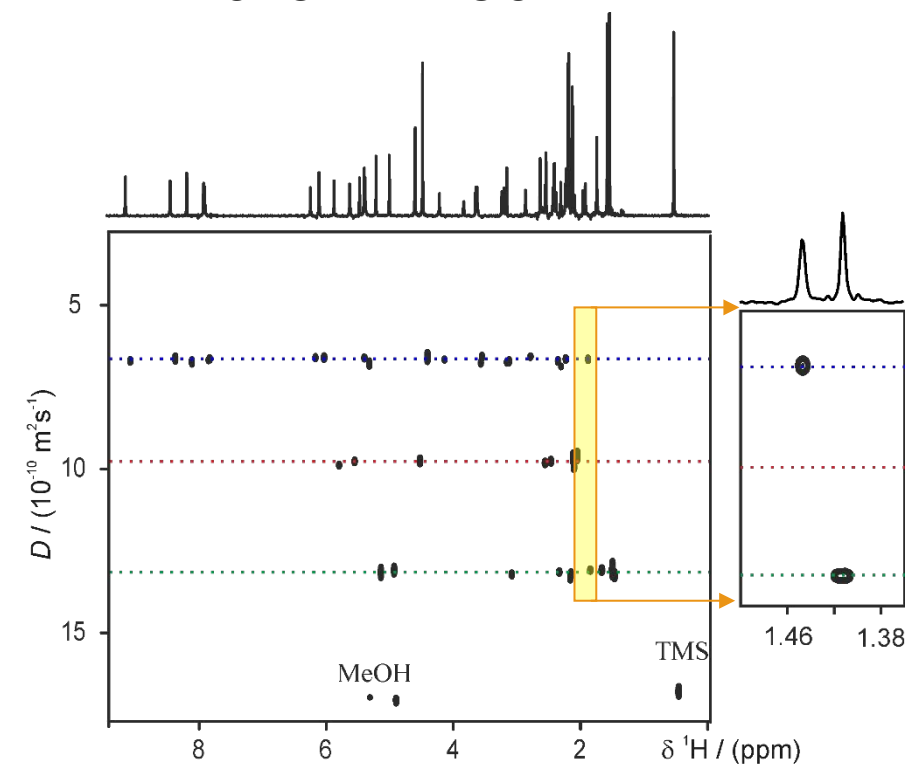
Pure shift DOSY & Mixtures

Oneshot DOSY

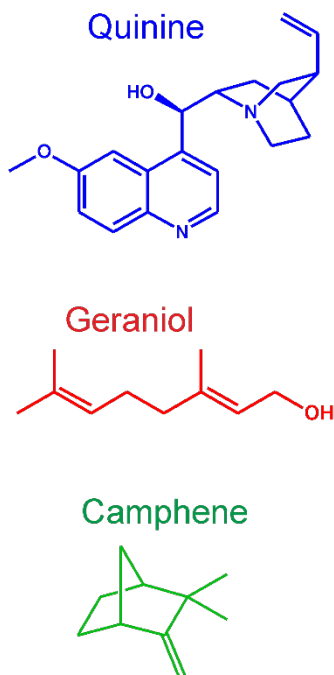


- ✗ Signal overlap
- ✗ Misleading peaks

PSYCHE-iDOSY

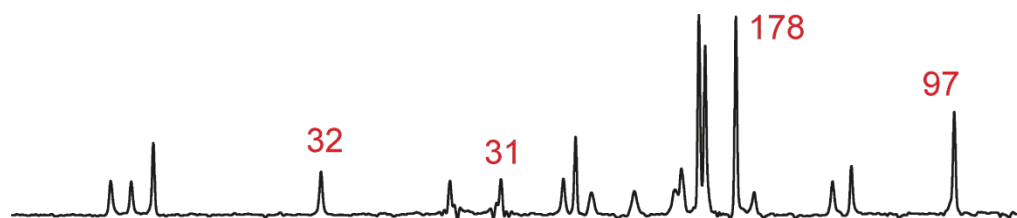


- ✓ Spectral simplification
- ✓ Higher signal resolution

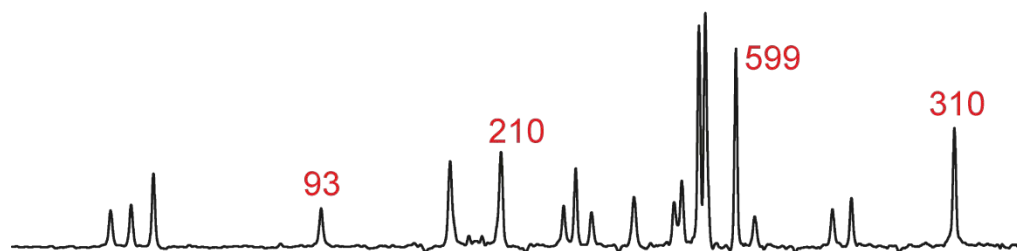


Pure shift DOSY & Sensitivity

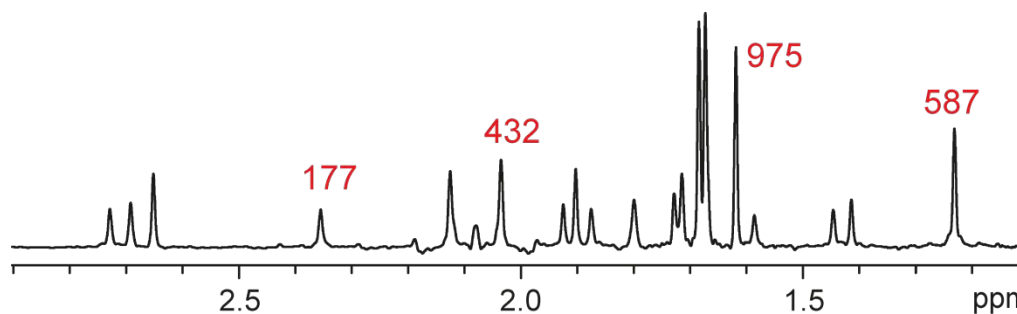
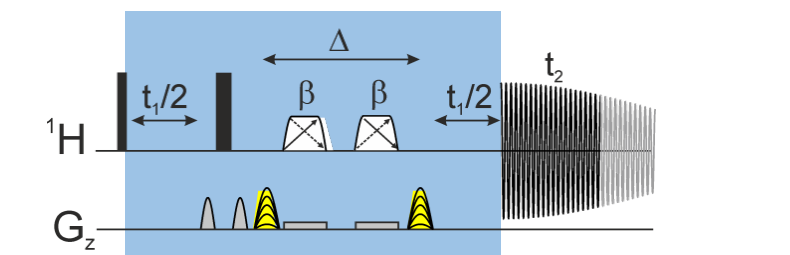
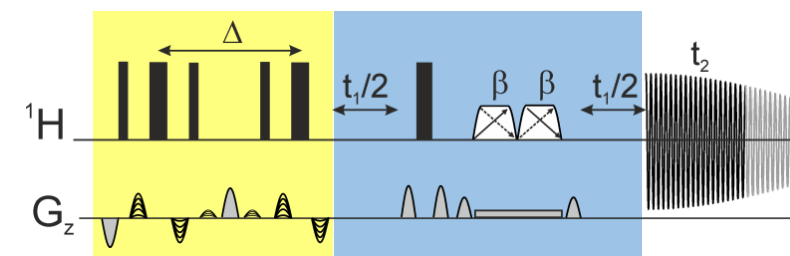
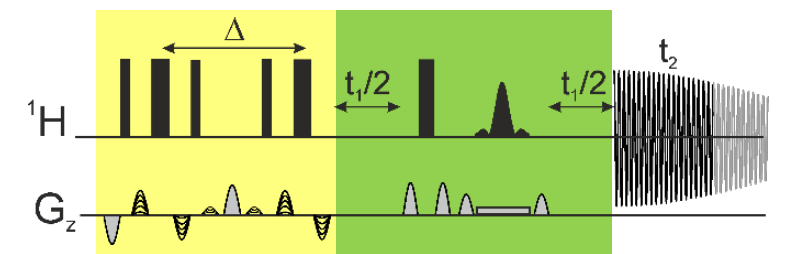
Oneshot ZS

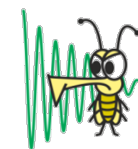


Oneshot PSYCHE



PSYCHE-iDOSY

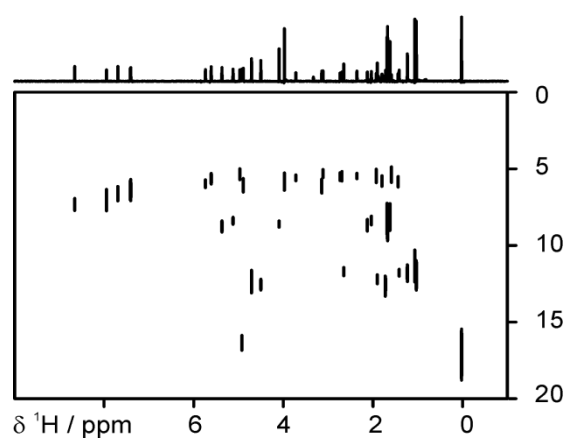
¹H spectra taken from the first increment of each experiment



Pure shift DOSY & Resolution

Resolution in the diffusion domain \rightarrow Determined by the uncertainties in D \rightarrow Estimated in the fitting

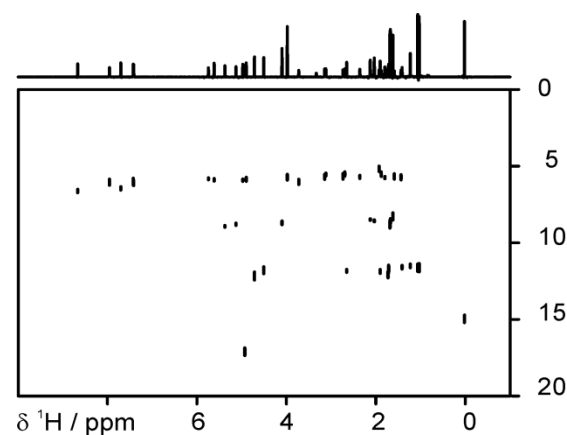
Oneshot ZS



Low SNR
Many pulses & gradients
PFG spatial non-uniformity

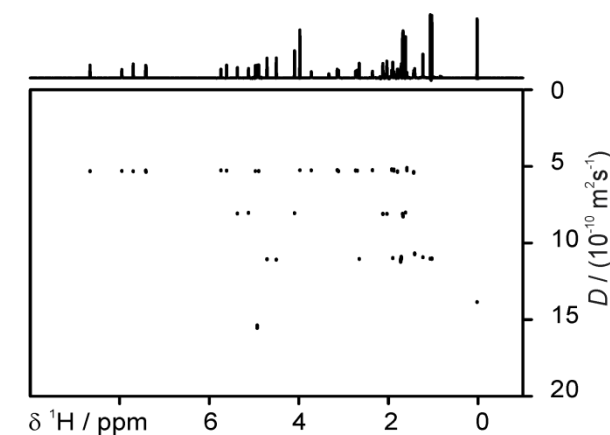
Poor resolution in the diffusion dimension

Oneshot PSYCHE



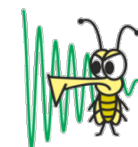
Moderate SNR
Many pulses & gradients

PSYCHE-iDOSY



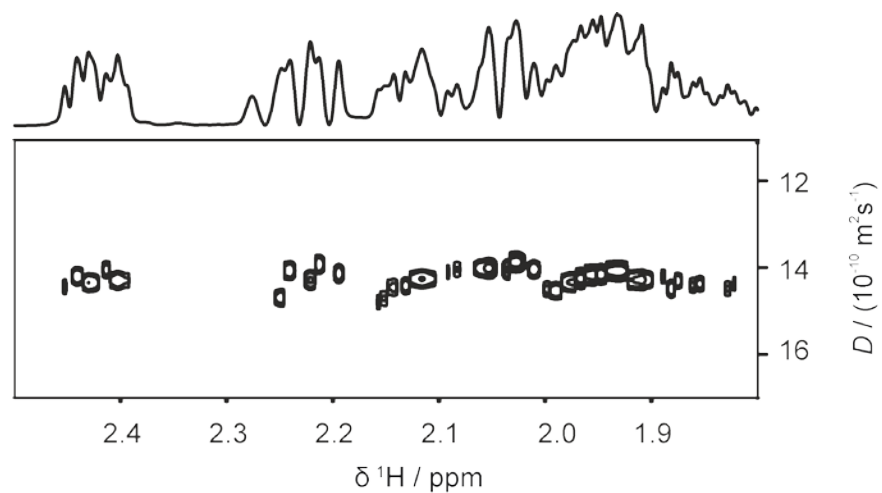
Higher SNR
Fewer pulses & gradients

Ultrahigh resolution in the diffusion dimension

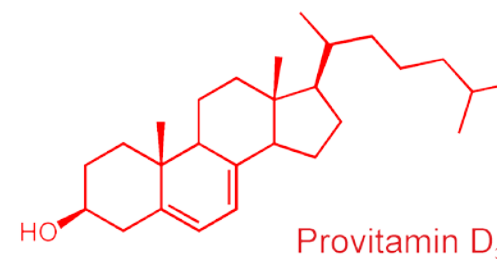
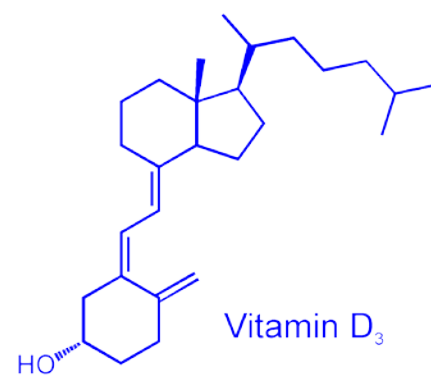
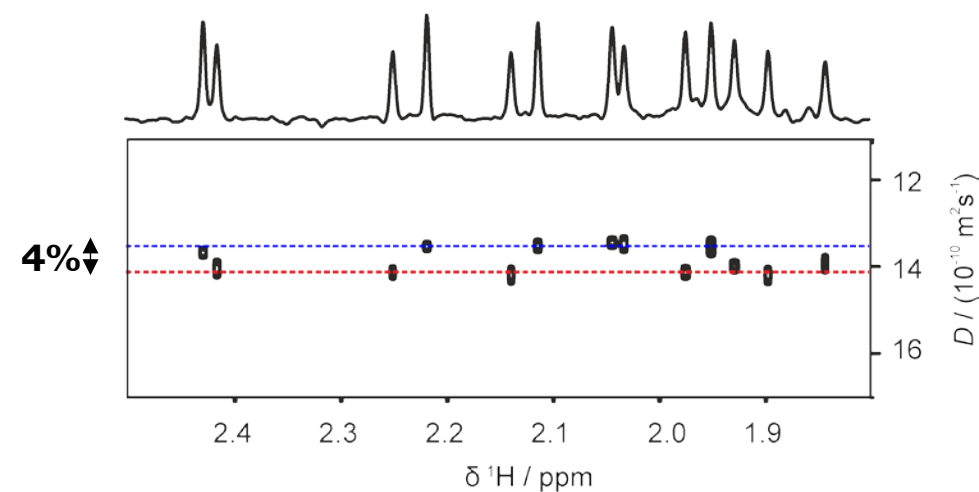


Ultrahigh resolution DOSY

Oneshot DOSY

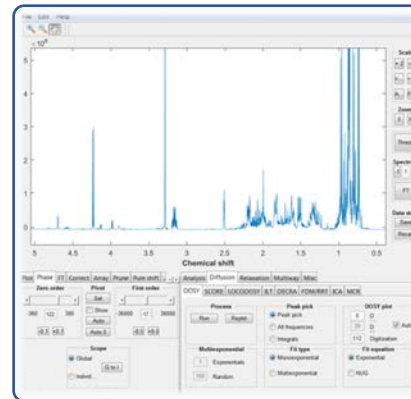


PSYCHE-iDOSY

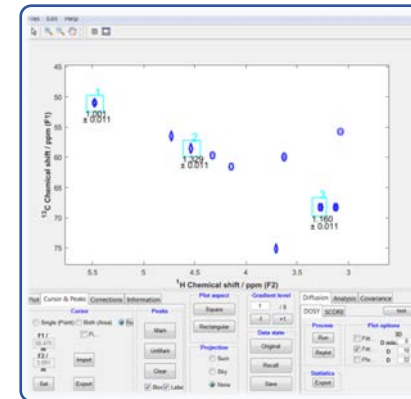


New NMR software

GNAT

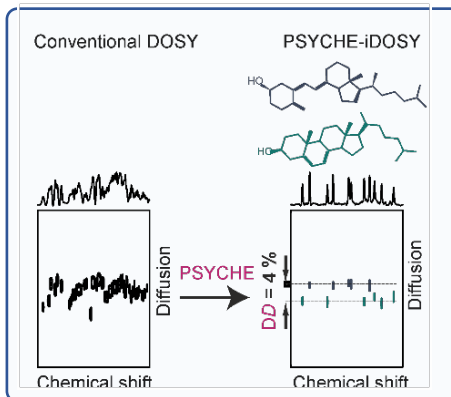


MAGNATE

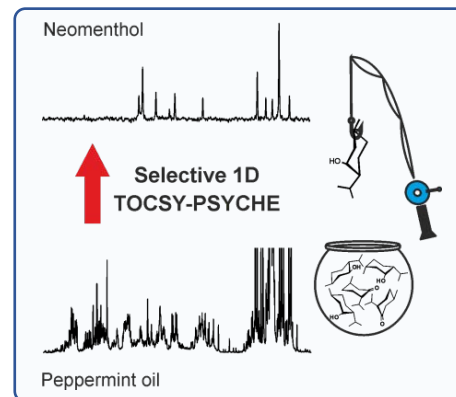


New NMR methods

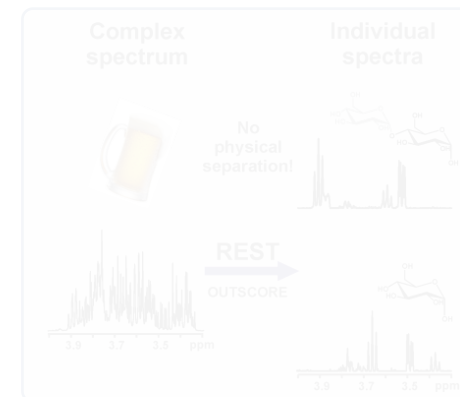
PSYCHE-iDOSY



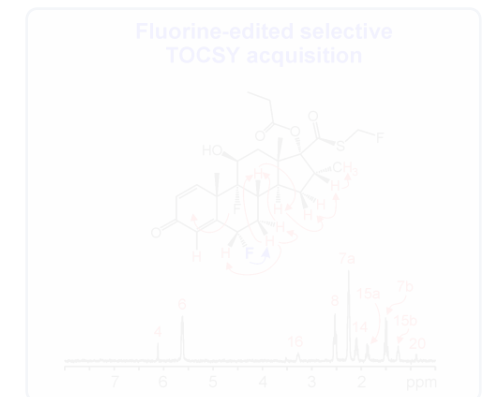
Selective 1D TOCSY-PSYCHE



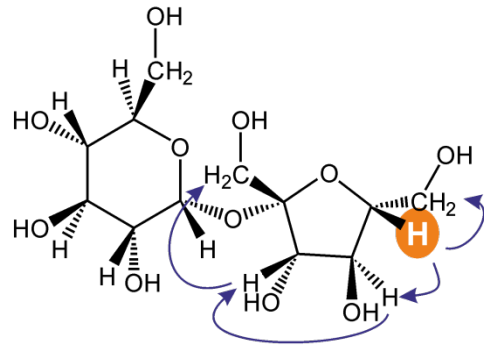
REST



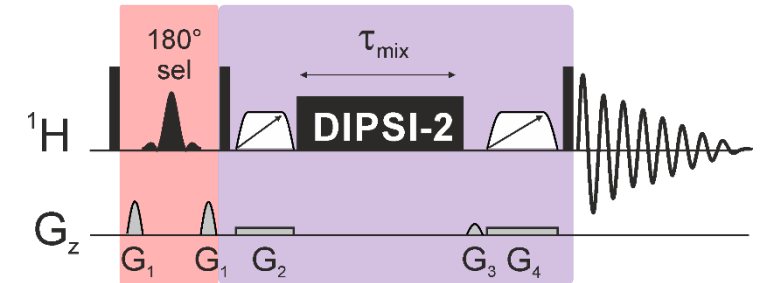
FESTA



Spectral factorization by homonuclear editing

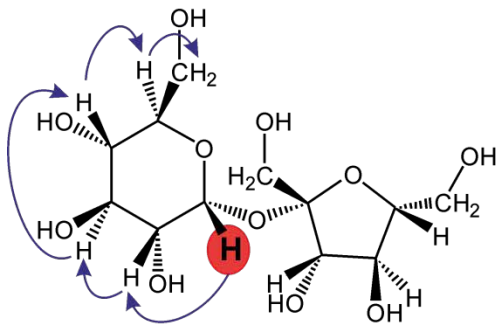


β -fructose subspectra

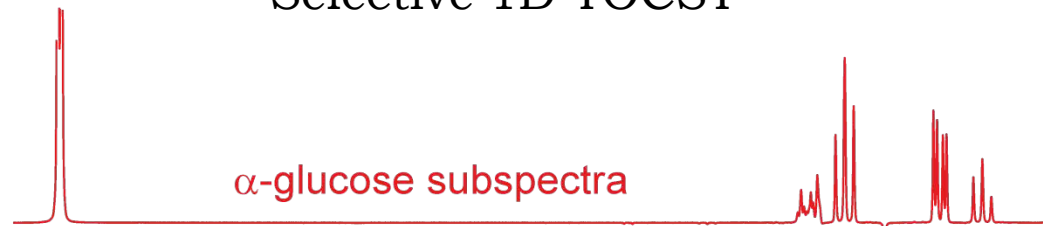


Spin selection

TOCSY

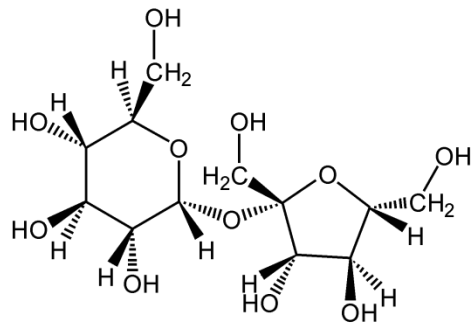
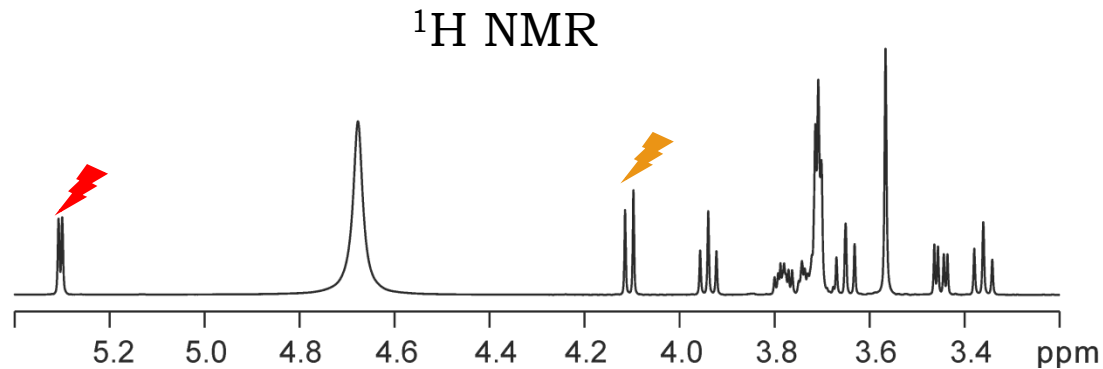


α -glucose subspectra

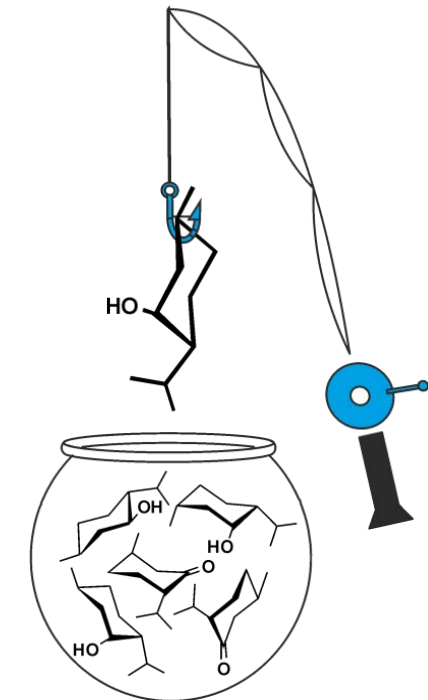


Selective 1D TOCSY

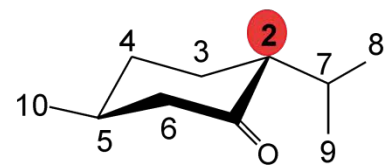
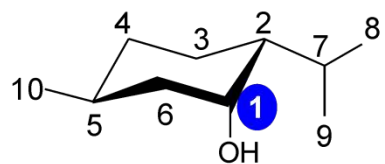
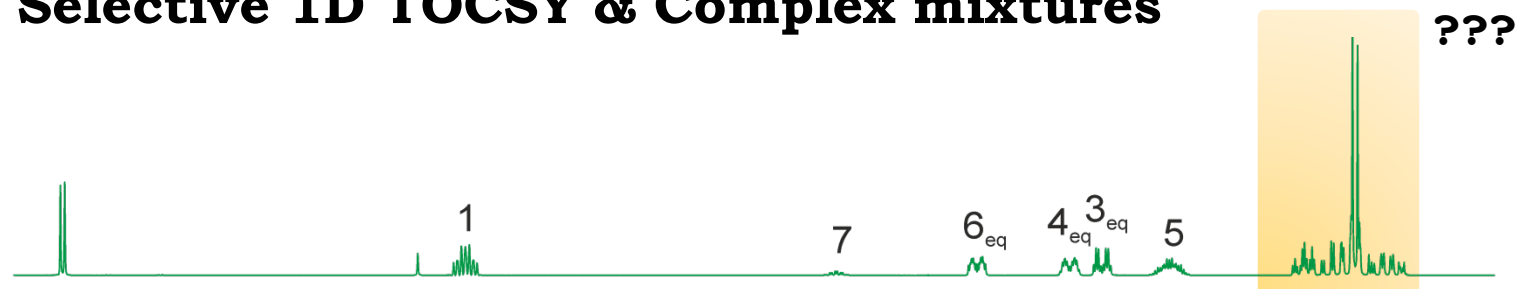
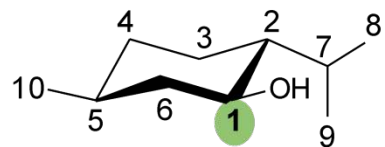
^1H NMR



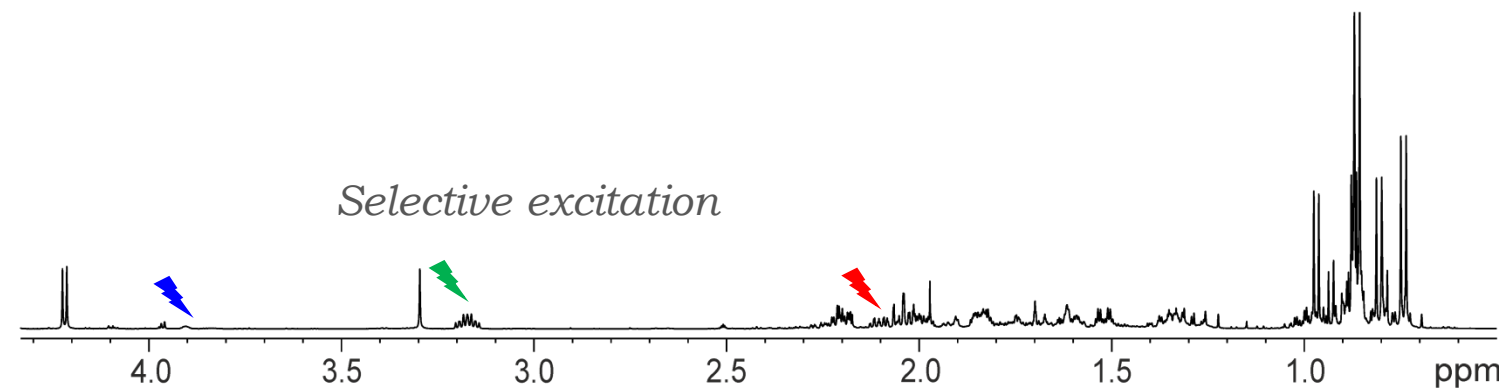
Sucrose



Selective 1D TOCSY & Complex mixtures



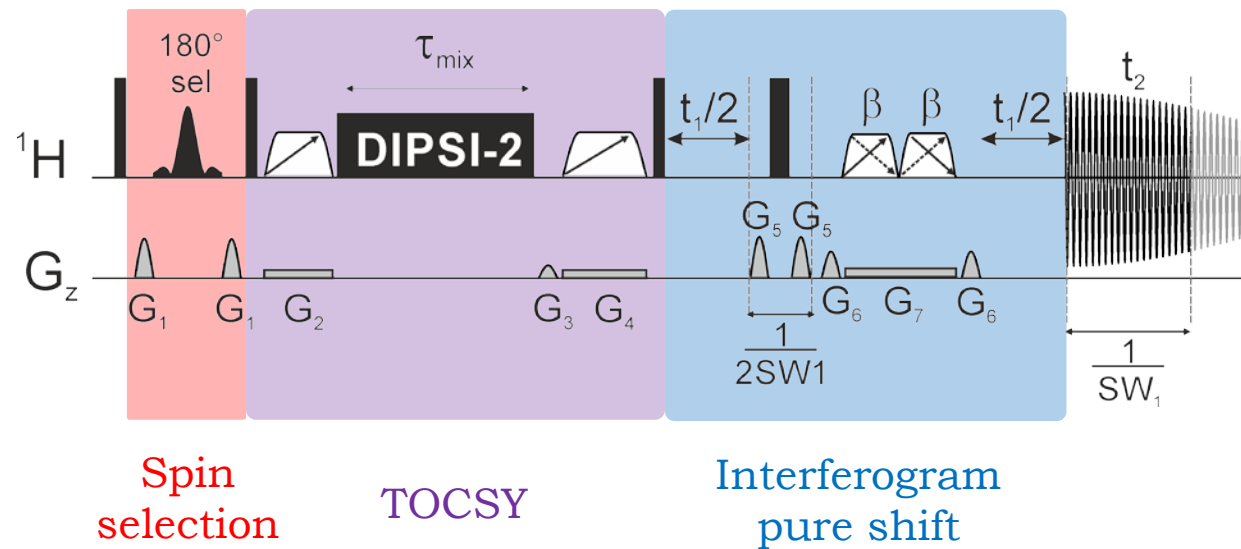
Peppermint oil



Selective TOCSY

¹H NMR

Selective pure shift 1D TOCSY-PSYCHE experiment

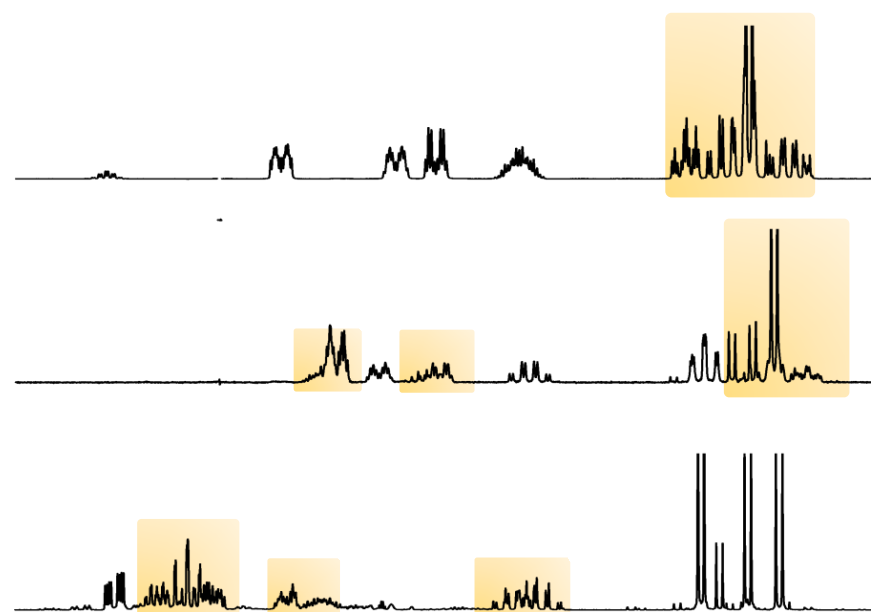


Ultrahigh resolution pure shift selective TOCSY

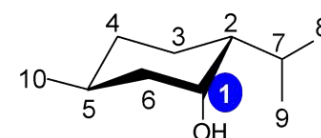
Conventional

Pure shift

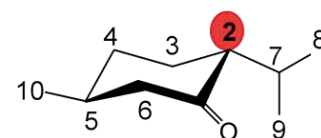
Selective TOCSY



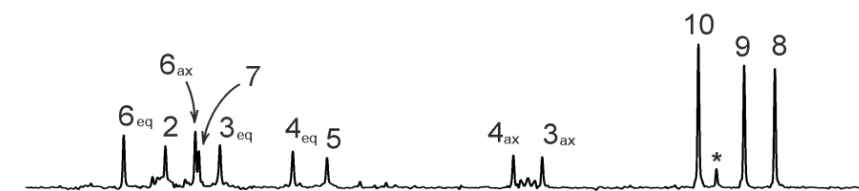
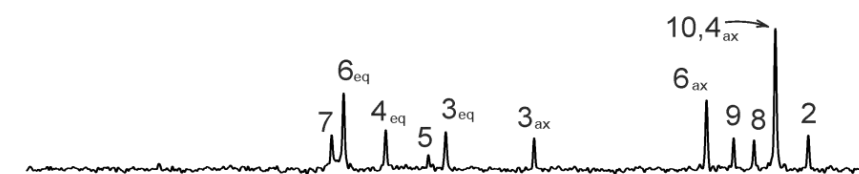
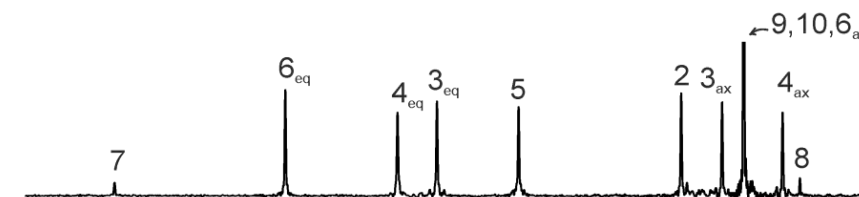
Menthol



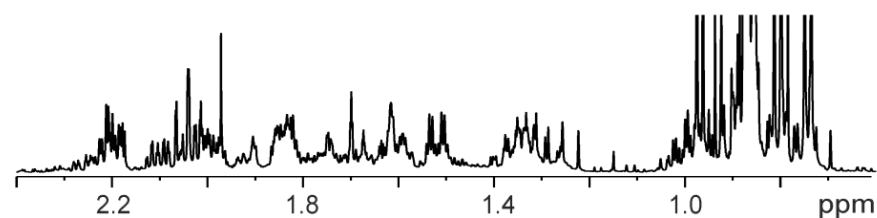
Neomenthol



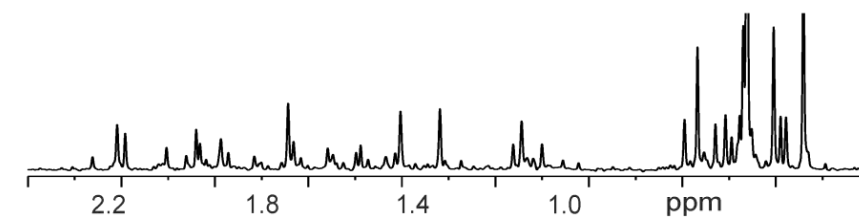
Menthone



1H NMR

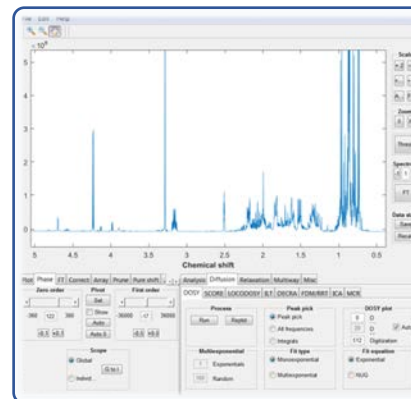


Peppermint oil

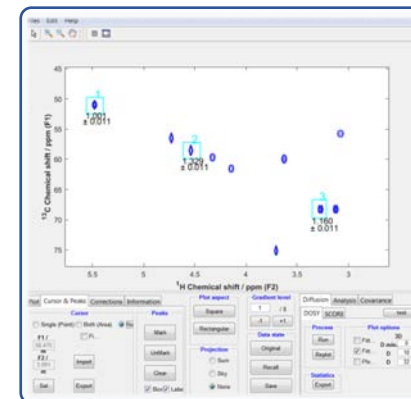


New NMR software

GNAT

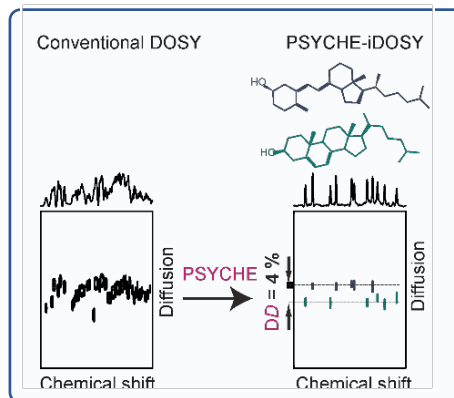


MAGNATE

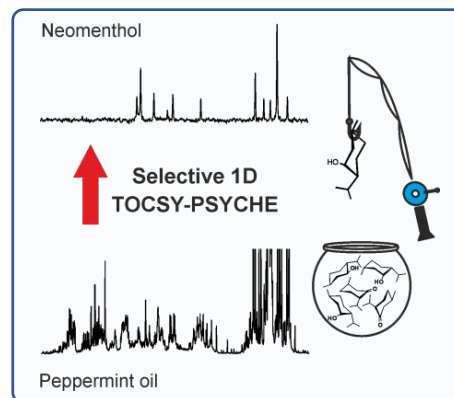


New NMR methods

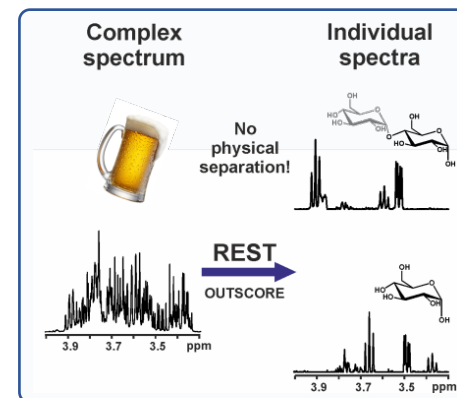
PSYCHE-iDOSY



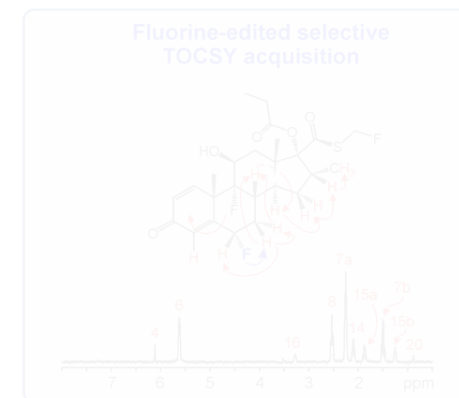
Selective 1D TOCSY-PSYCHE

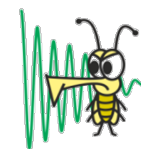


REST



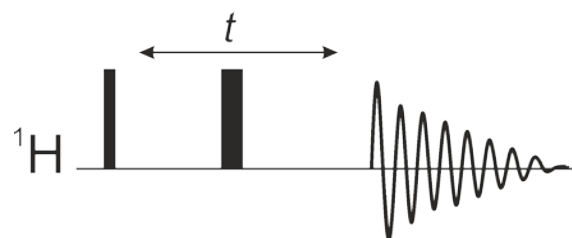
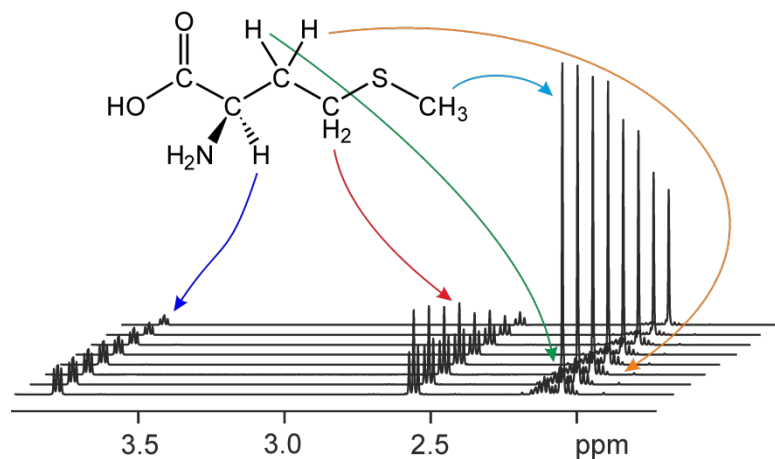
FESTA





Relaxation NMR

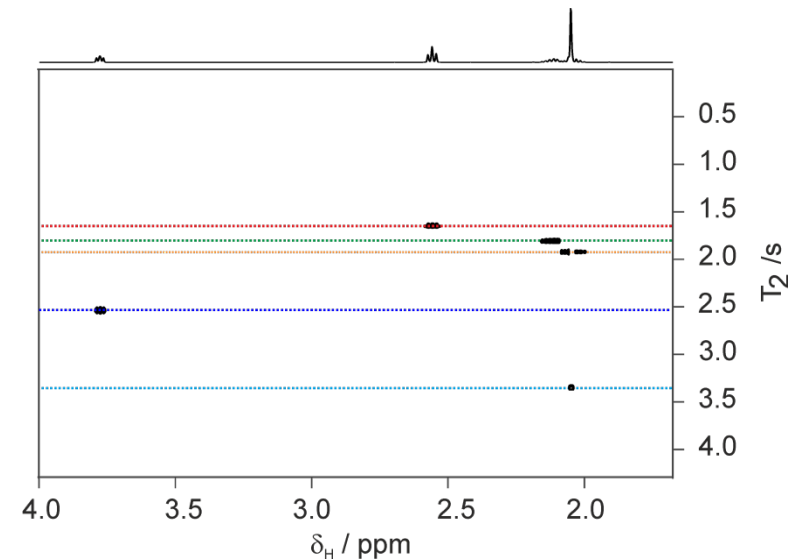
Relaxation array



Prototype sequence

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

Relaxation-Ordered Spectroscopy (ROSY)

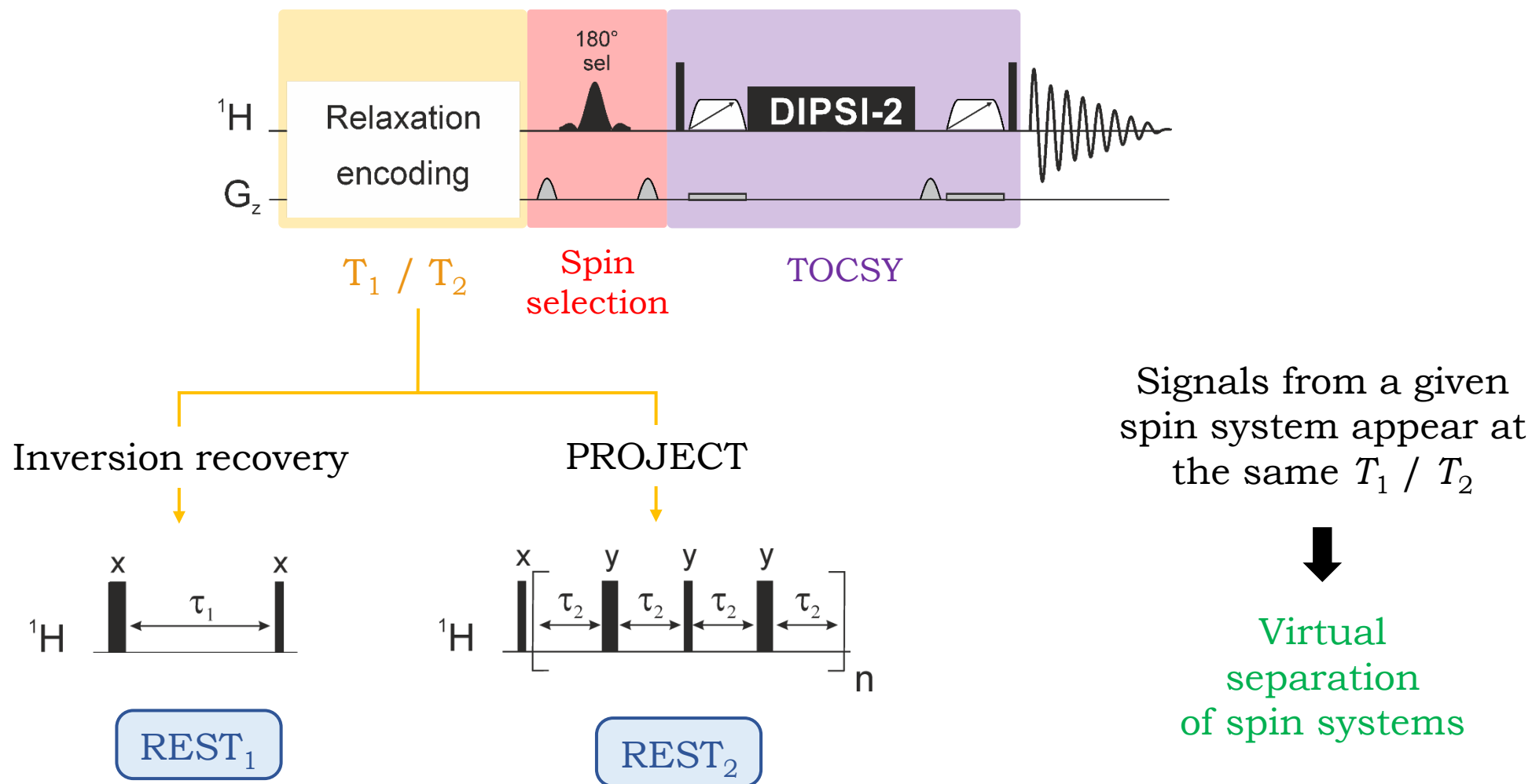


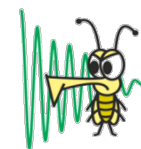
Signals from a given molecule:

- have different relaxation behaviour
- appear at different T_1 / T_2

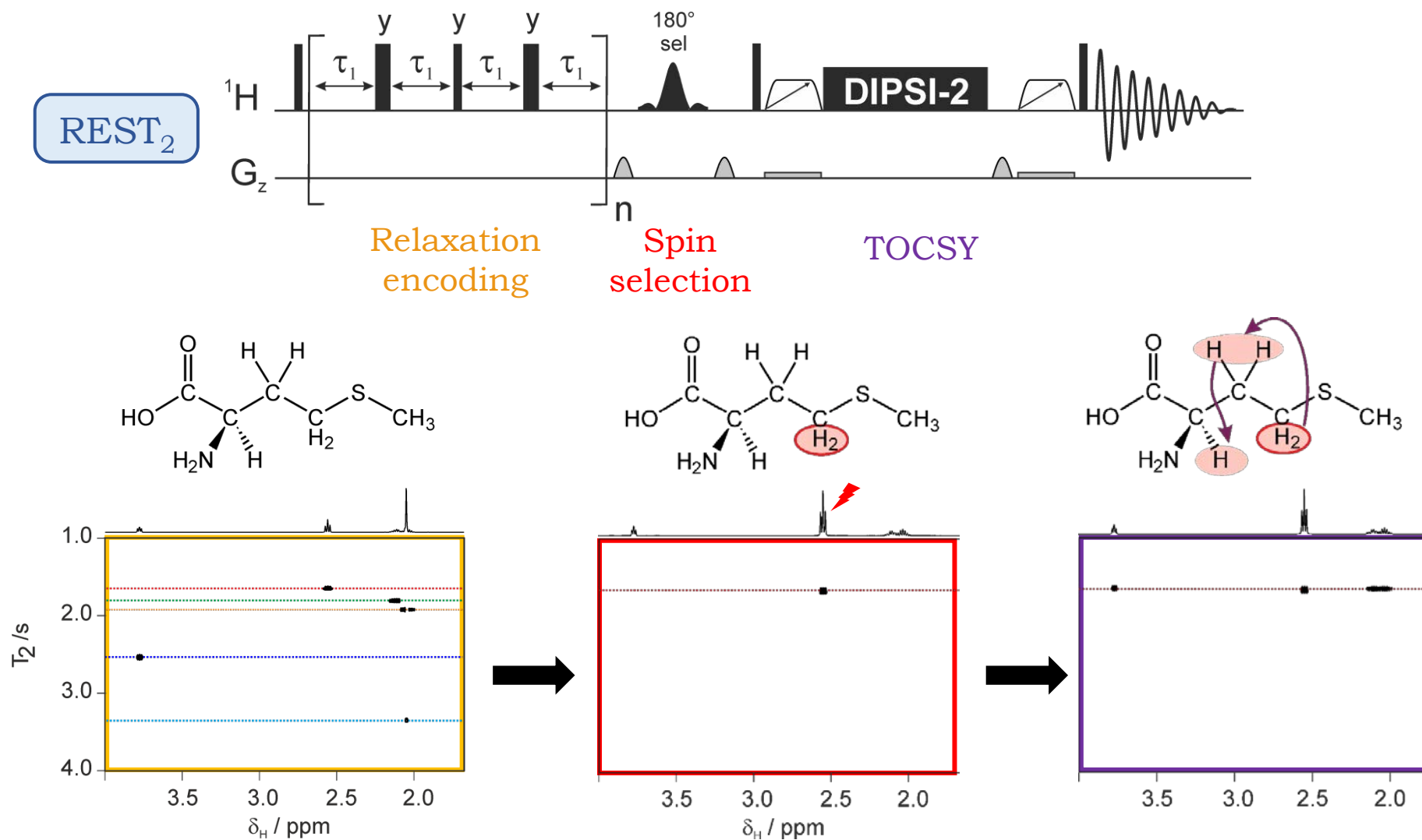
**NO virtual
separation
of components**

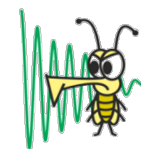
Relaxation-encoded selective TOCSY (REST) experiment



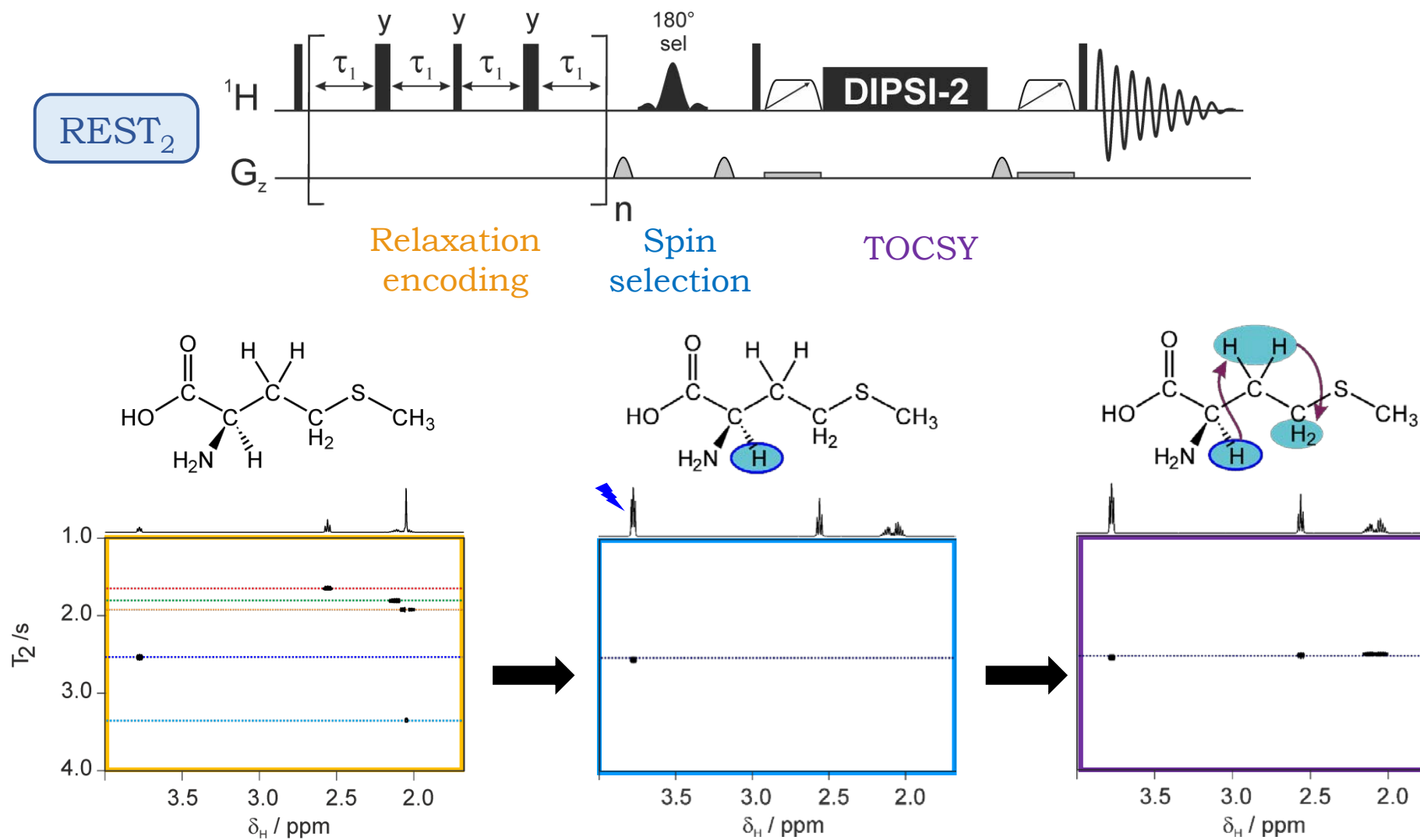


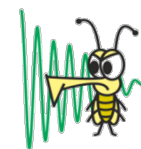
Relaxation-encoded selective TOCSY (REST) experiment





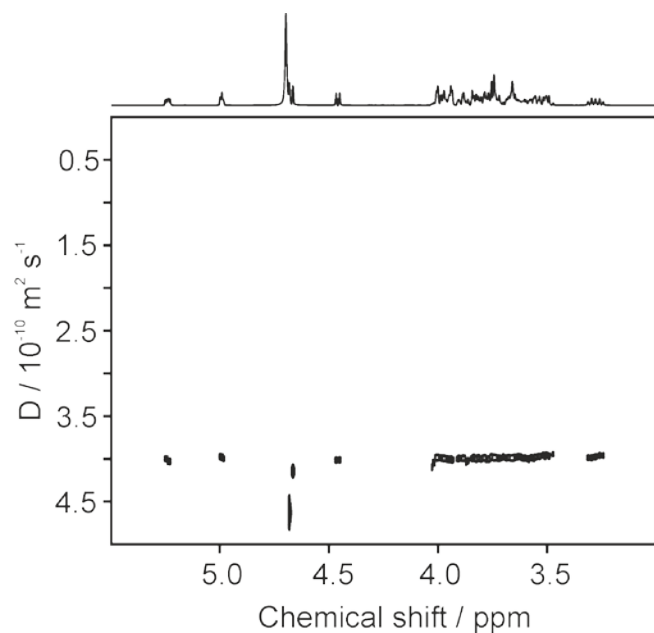
Relaxation-encoded selective TOCSY (REST) experiment





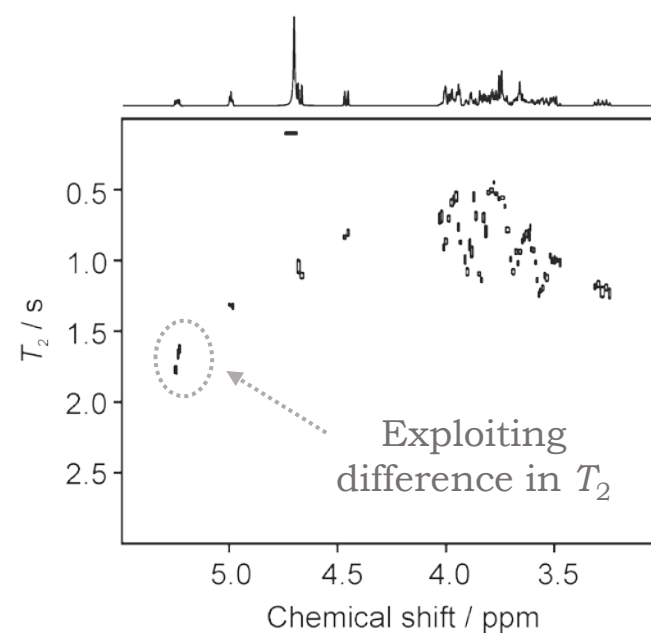
REST & Sugars

Oneshot DOSY



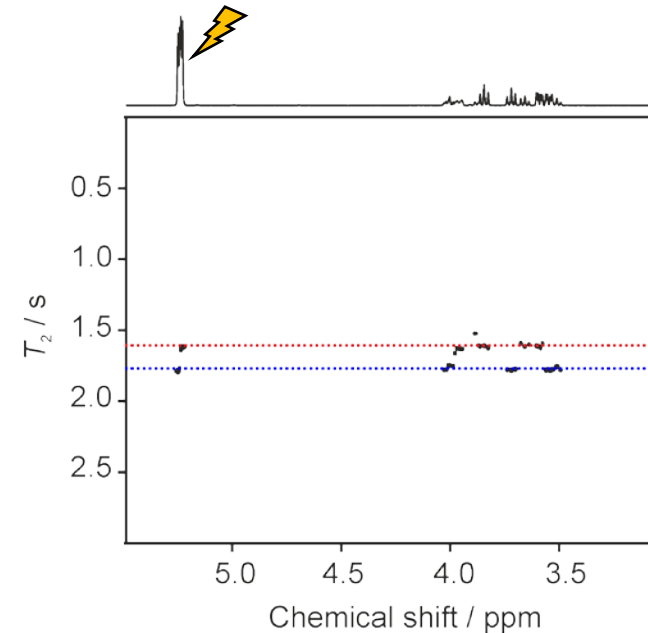
All sugar signals
show the same D

PROJECT ROSY

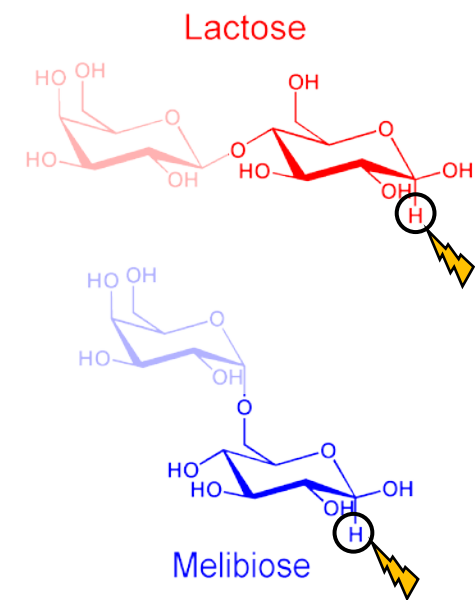


All sugar signals
show different T_2

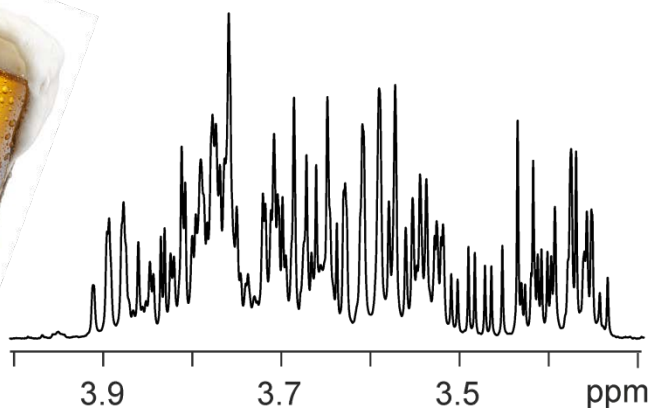
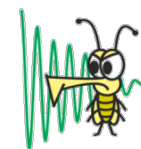
REST₂ ROSY



Virtual separation
of components



REST & Beer



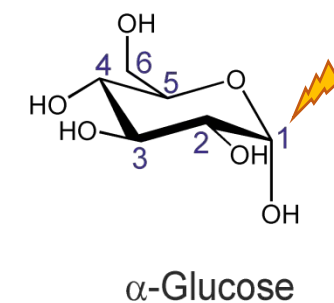
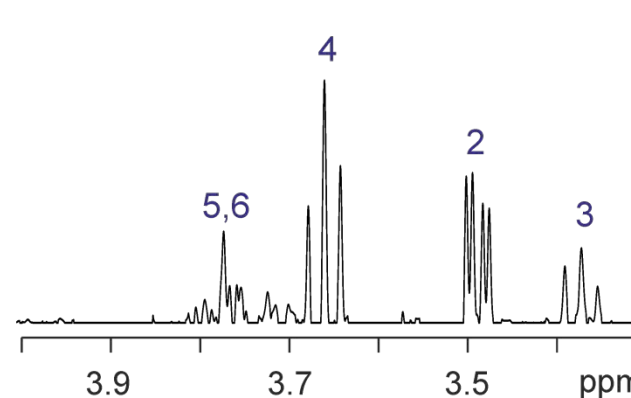
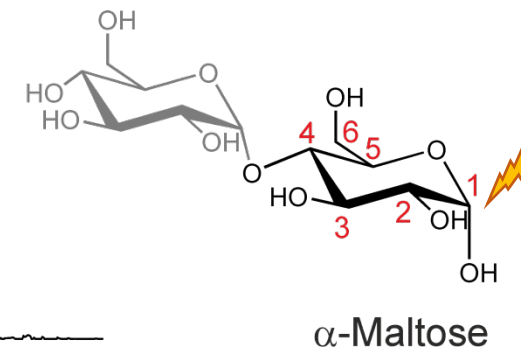
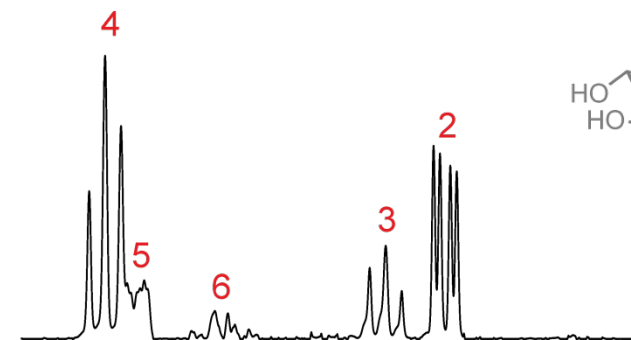
Complex Mixture

REST₂

OUTSCORE

(Optimized Unmixing of
 True Spectra for
 Component RESolution)

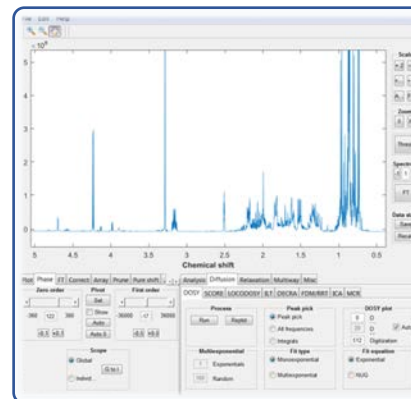
Chem. Commun. **2013**, 49, 10510



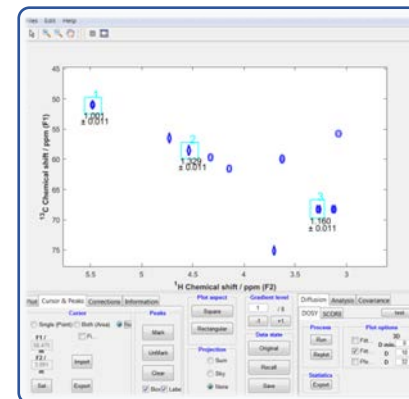
Virtual separation
 of components

New NMR software

GNAT

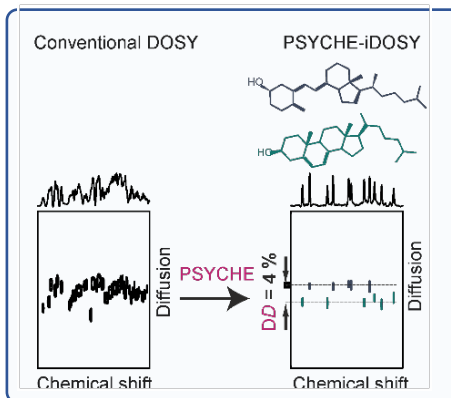


MAGNATE

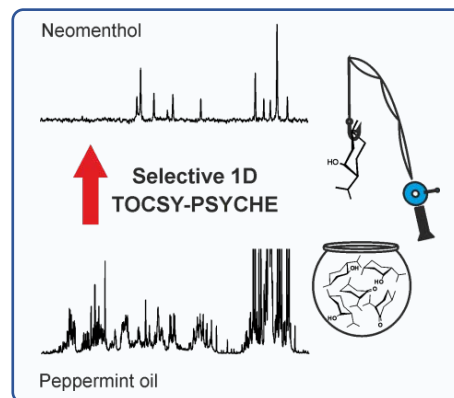


New NMR methods

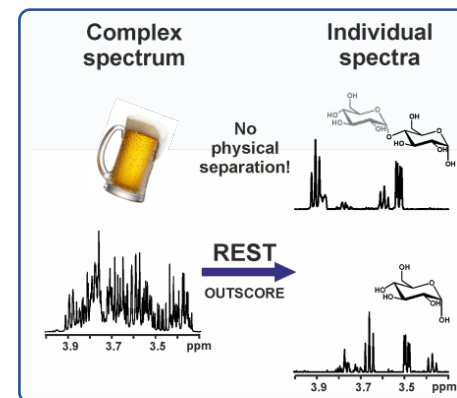
PSYCHE-iDOSY



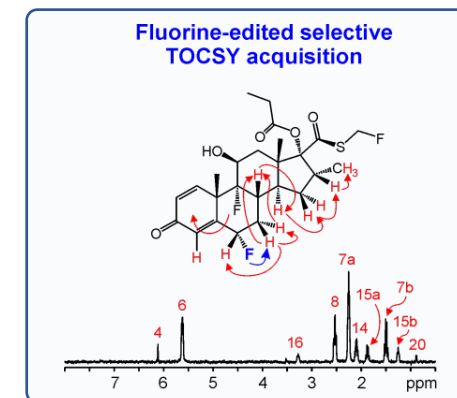
Selective 1D TOCSY-PSYCHE



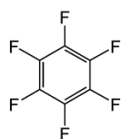
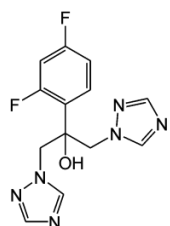
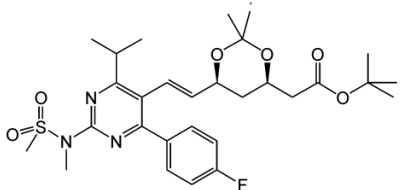
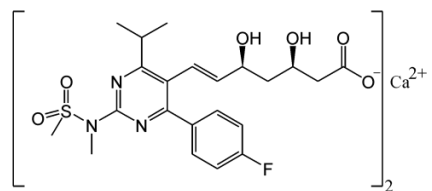
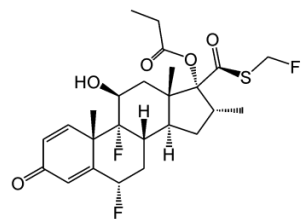
REST



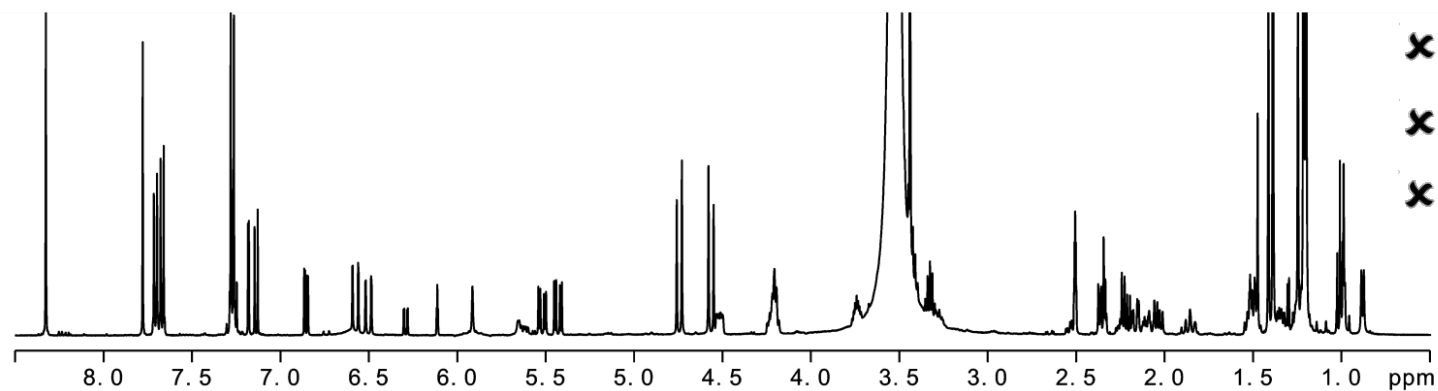
FESTA



Mixtures containing fluorinated species

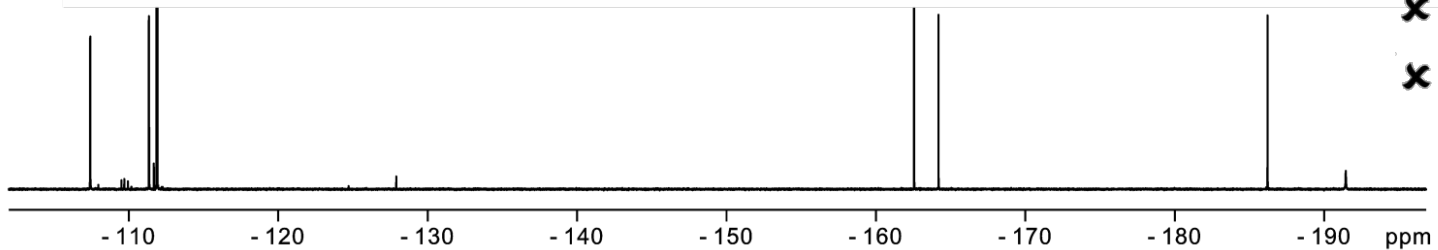


^1H NMR



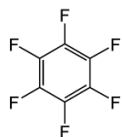
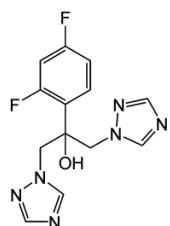
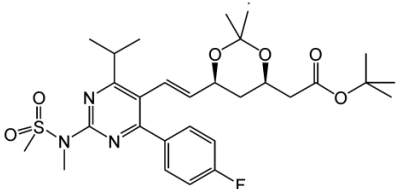
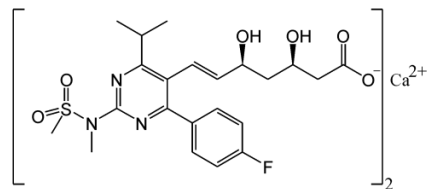
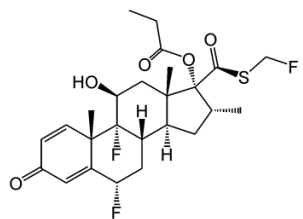
- ✗ Signal overlap
- ✗ Component information
- ✗ Structural information

^{19}F NMR

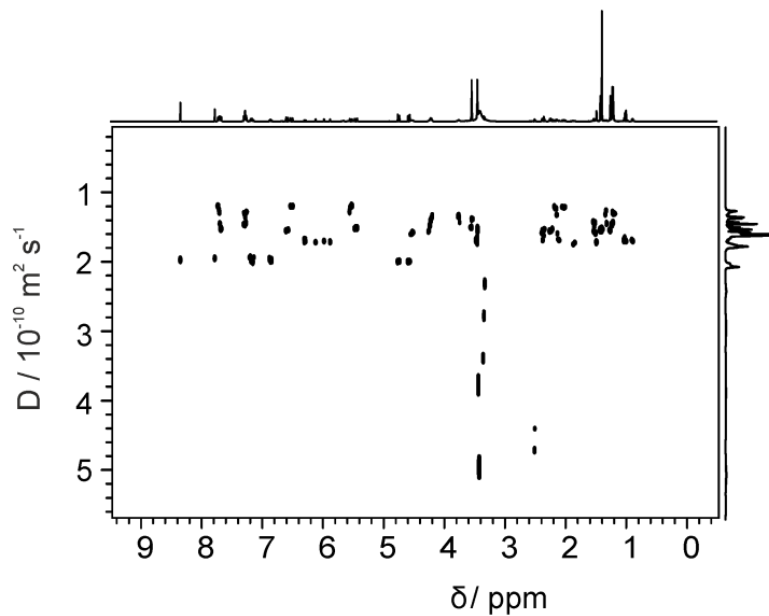


- ✓ Spectral resolution
- ✗ Component information
- ✗ Structural information

Mixtures containing fluorinated species

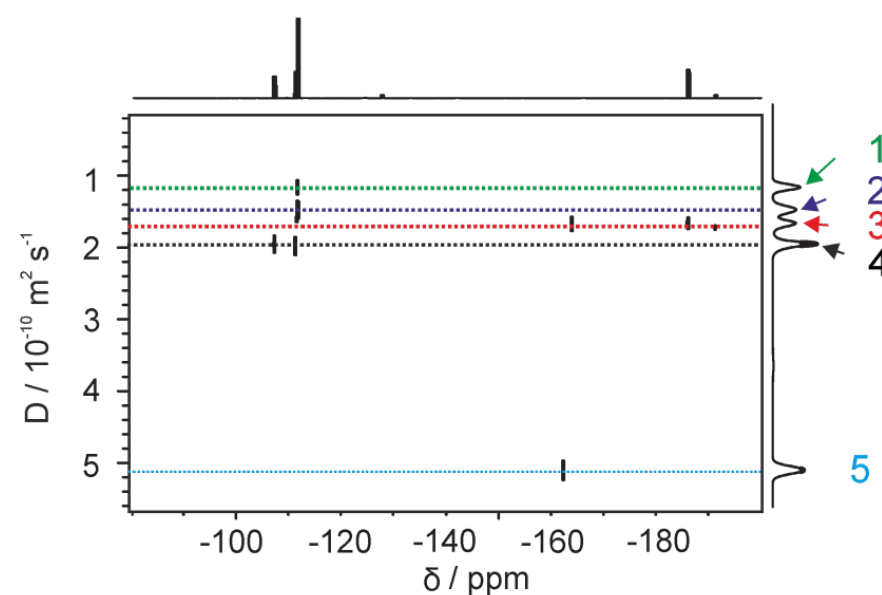


^1H DOSY



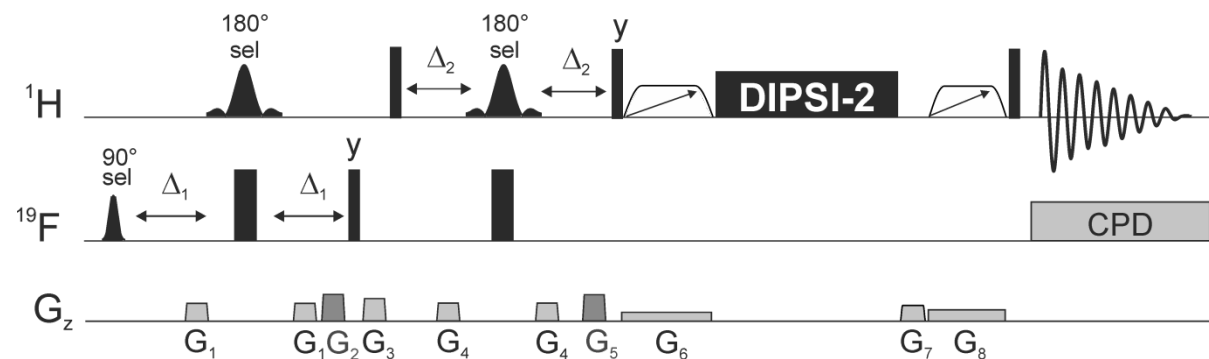
- ✗ Signal overlap
- ✗ Component information
- ✗ Structural information

^{19}F DOSY

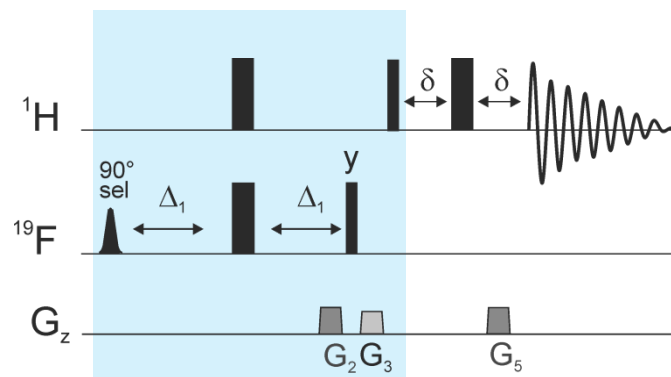


- ✓ Spectral resolution
- ✓ Component information
- ✗ Structural information

Fluorine-edited selective TOCSY Acquisition (FESTA) experiment

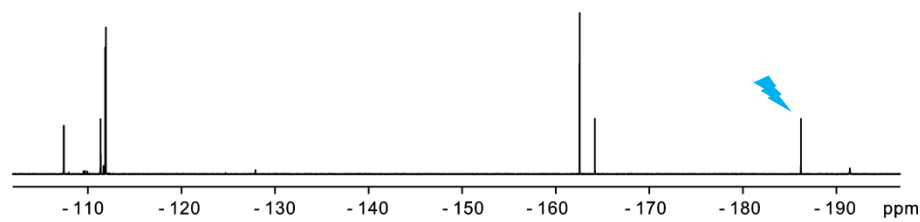


Fluorine-edited selective TOCSY Acquisition (FESTA) experiment



Selective reverse INEPT

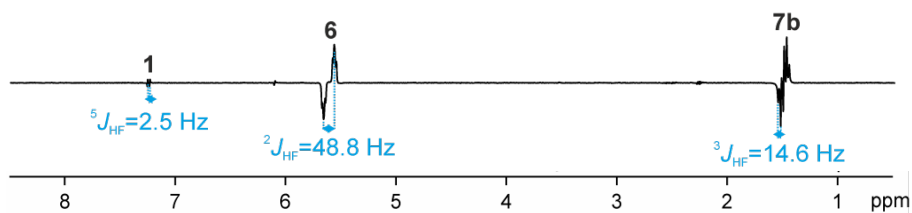
^{19}F NMR



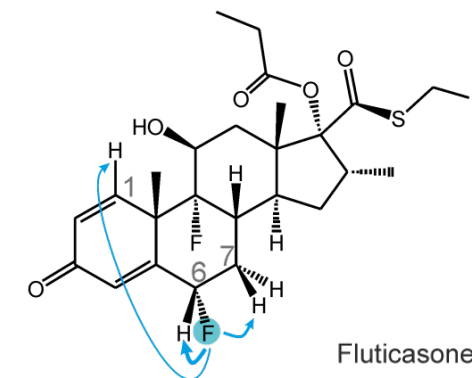
^{19}F Spin selection

Heteronuclear transfer (J_{HF})

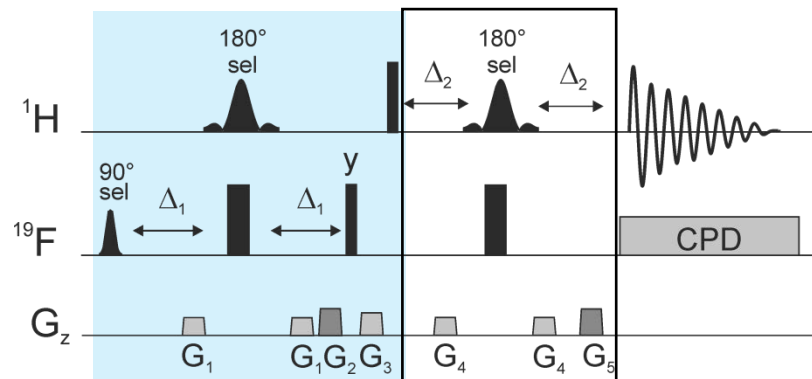
^1H SRI



$^1\text{H}_\text{A}$ $^1\text{H}_\text{B}$

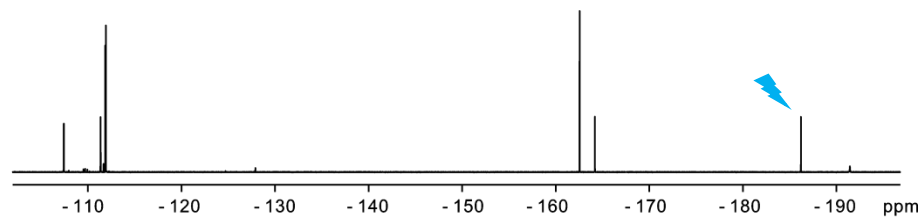


Fluorine-edited selective TOCSY Acquisition (FESTA) experiment



Selective modulated echo

^{19}F NMR

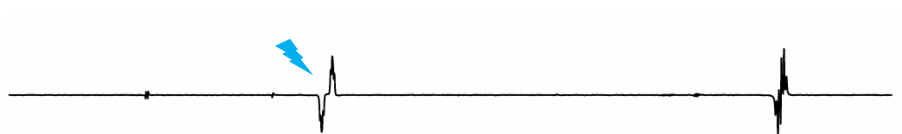


^{19}F Spin selection



Heteronuclear transfer (J_{HF})

^1H SRI

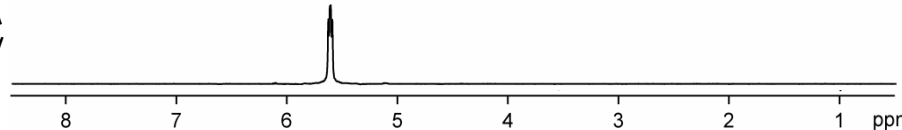


$^1\text{H}_\text{A}$ $^1\text{H}_\text{B}$

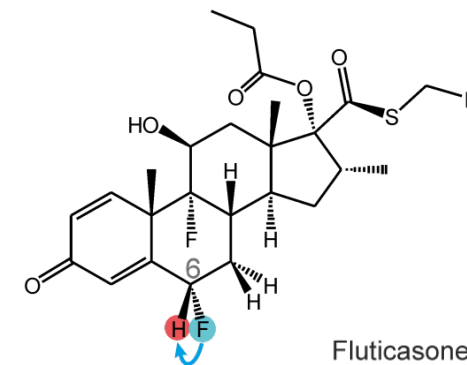


Spin selection

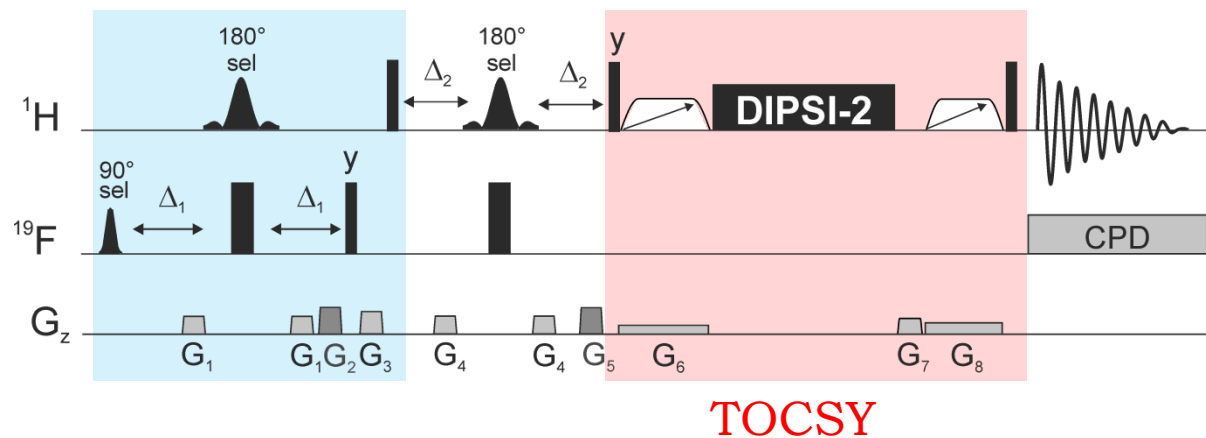
^1H SRI-SME



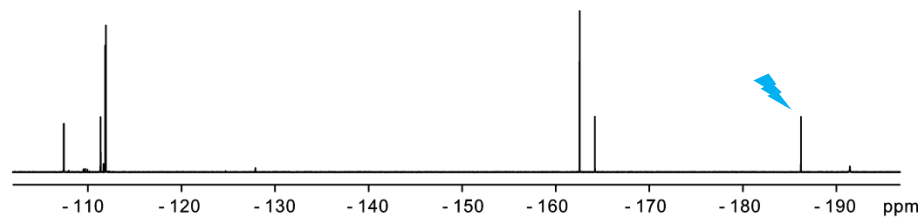
$^1\text{H}_\text{A}$



Fluorine-edited selective TOCSY Acquisition (FESTA) experiment



¹⁹F NMR

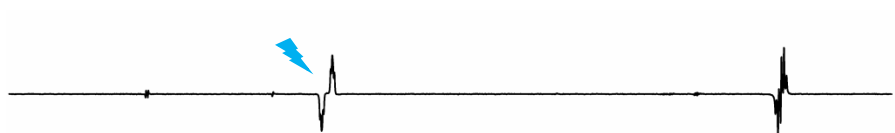


¹⁹F Spin selection



Heteronuclear transfer (J_{HF})

¹H SRI

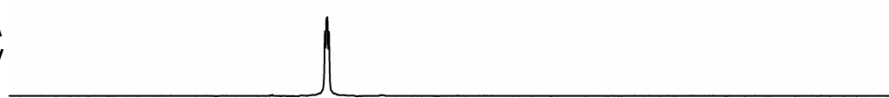


¹H_A ¹H_B



Spin selection

¹H SRI-SME

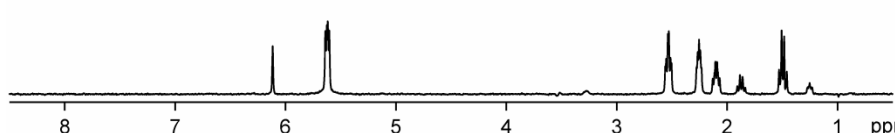


¹H_A

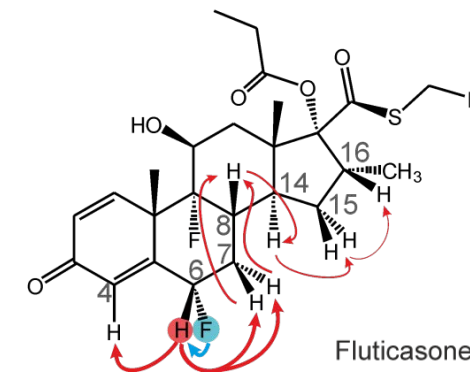


Homonuclear transfer (J_{HH})

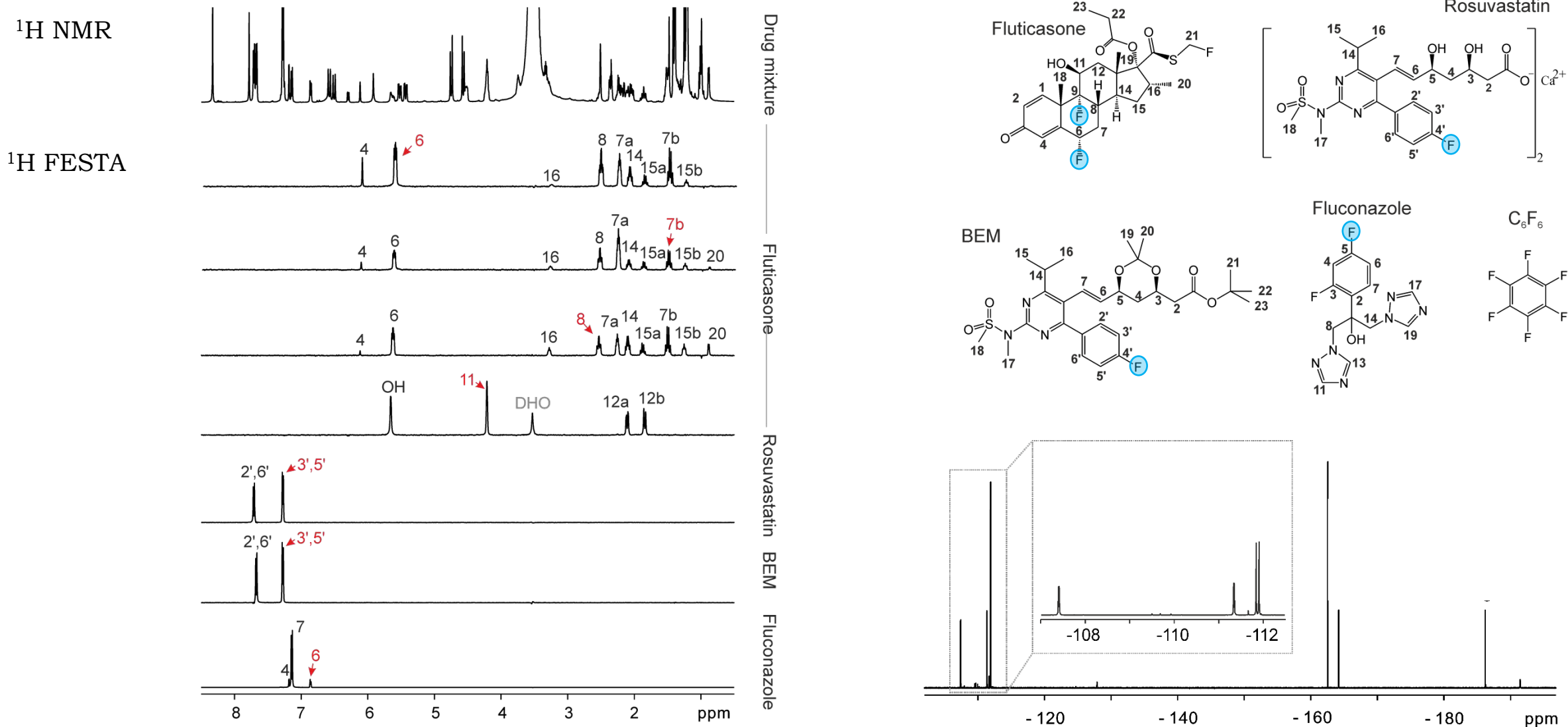
¹H FESTA



¹H → ¹H → ¹H → ...



FESTA & Drug mixture

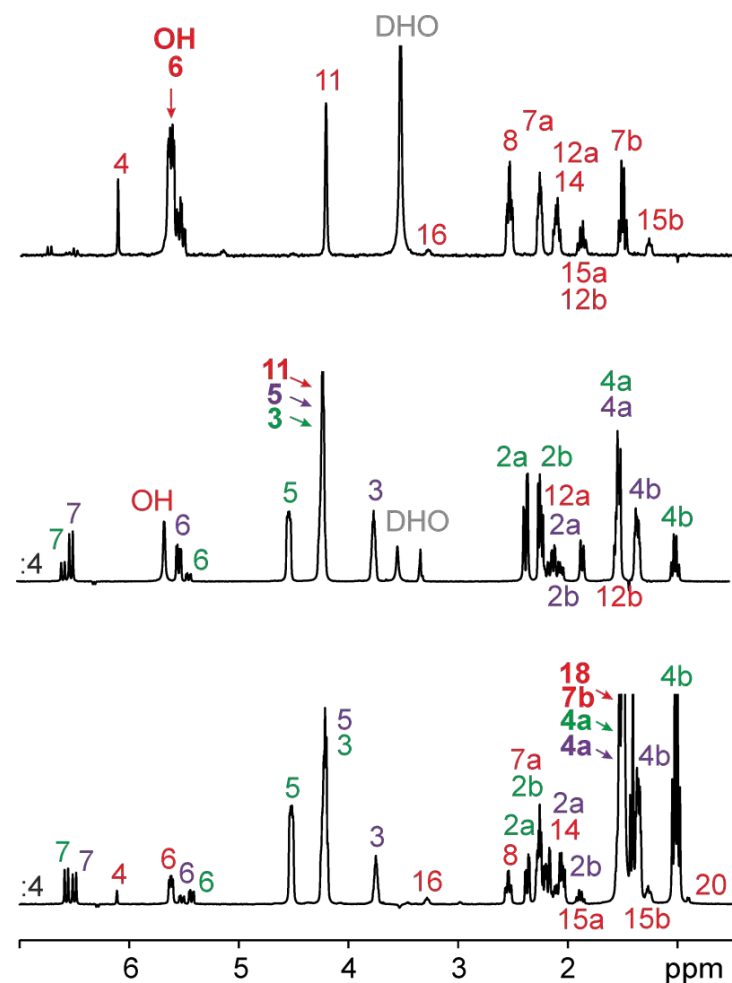


L. Castañar, P. Moutzouri, T. M. Barbosa, C. F. Tormena, R. Rittner, A. R. Phillips, S. R. Coombes, M. Nilsson, G. A. Morris

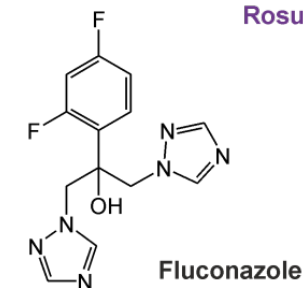
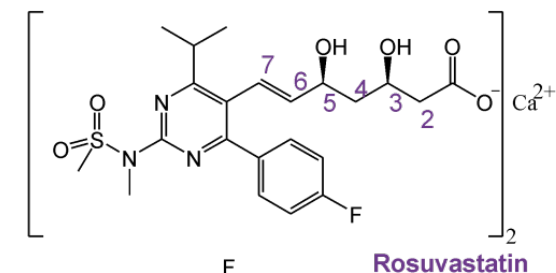
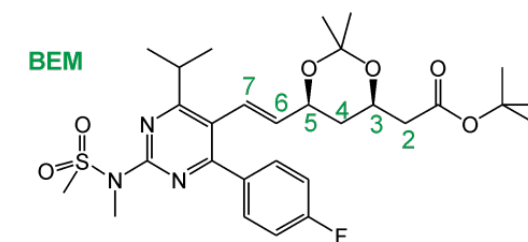
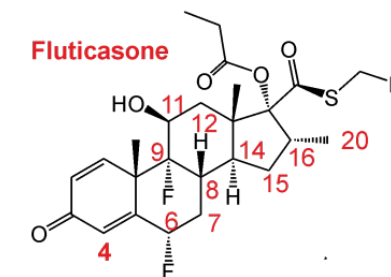
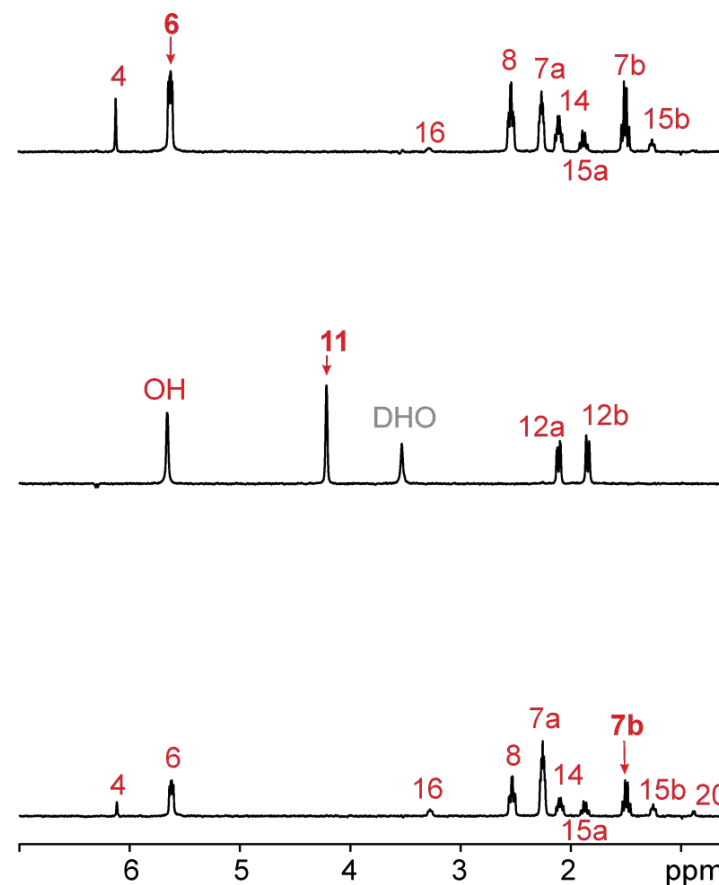
Anal. Chem. **2018**, *90*, 5445

FESTA & Drug mixture

Selective 1D TOCSY

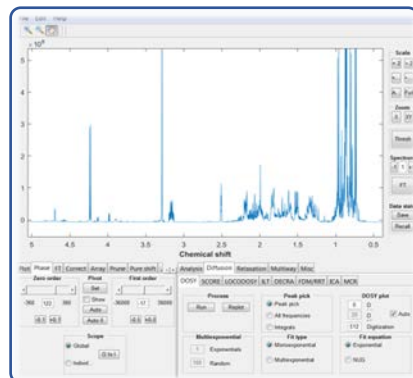


FESTA



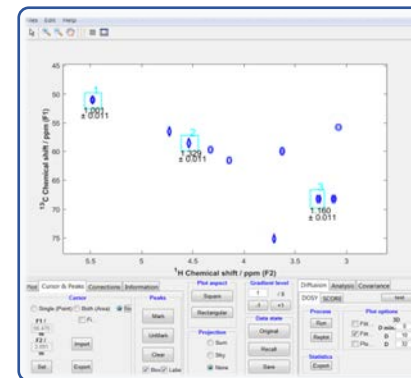
New NMR software

GNAT



Magn. Reson. Chem. **2018**

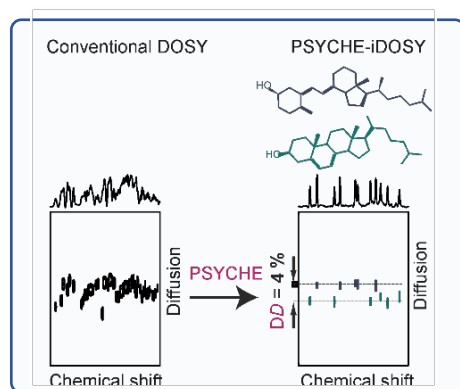
MAGNATE



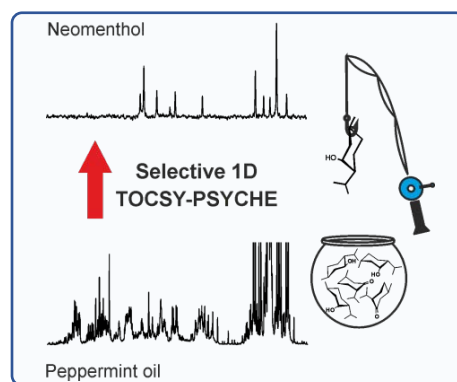
in preparation

New NMR methods

PSYCHE-iDOSY

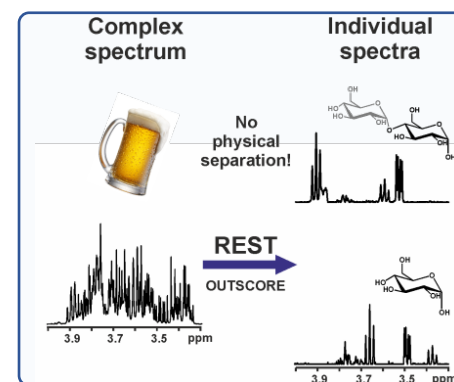


Angew. Chem. Int. Ed. **2016**,
55, 15579

Selective 1D
TOCSY-PSYCHE

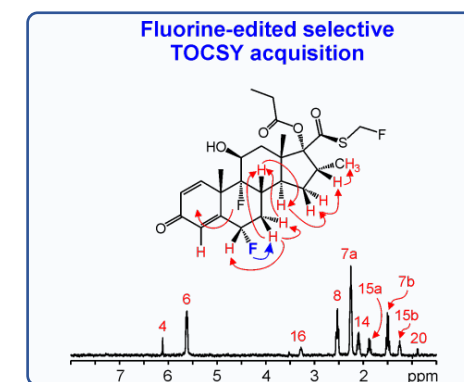
RSC Adv. **2016**, 6, 100063

REST



Chem. Commun. **2017**, 53, 7461

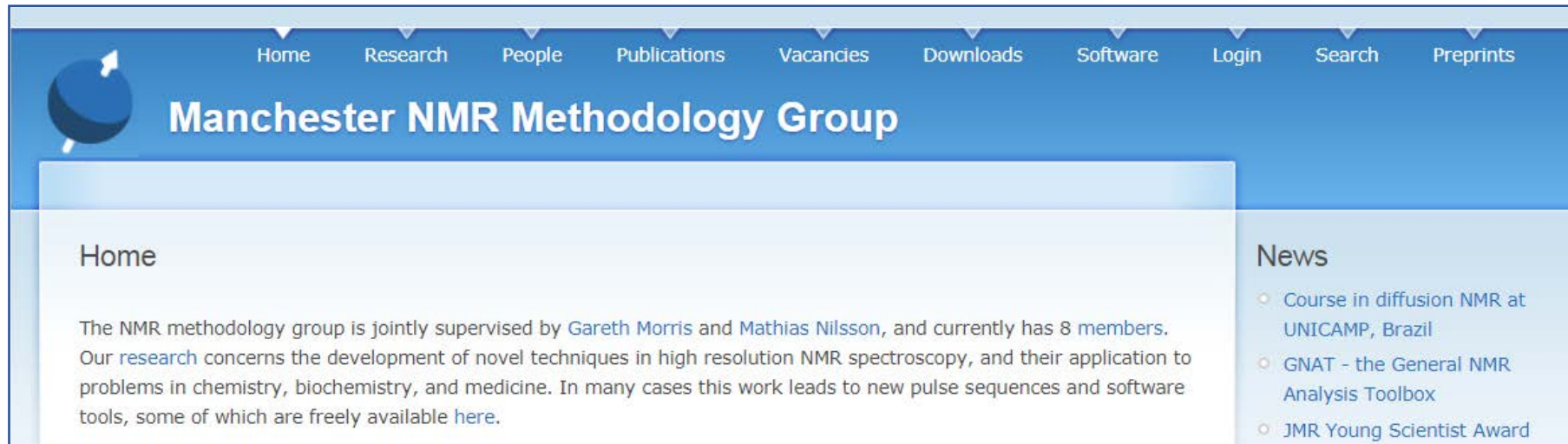
FESTA



Anal. Chem. **2018**, 90, 5445

Manchester NMR Methodology Group

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

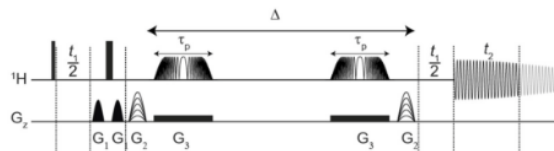


The screenshot shows the website's navigation menu with links for Home, Research, People, Publications, Vacancies, Downloads, Software, Login, Search, and Preprints. The main heading is "Manchester NMR Methodology Group". Below this, there is a "Home" section with a paragraph about the group's supervision and research focus. To the right, a "News" section lists three items: a course in diffusion NMR at UNICAMP, Brazil; GNAT - the General NMR Analysis Toolbox; and the JMR Young Scientist Award.

Pulse Sequences

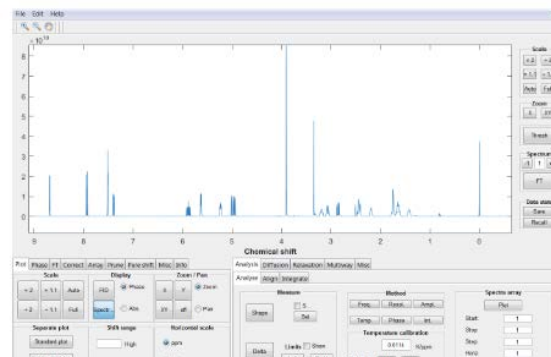
We are currently preparing many of our pulse sequences, parameter sets, example datasets and processing macros for the website. Some are available [here](#) but if you would like to use any of the other the sequences, as described in the [publications](#) section, please email us. The majority of sequences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

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AstraZeneca



**Thank you very much
for your attention!**

Laura Castañar Acedo

NMR Methodology group

The University of Manchester

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