

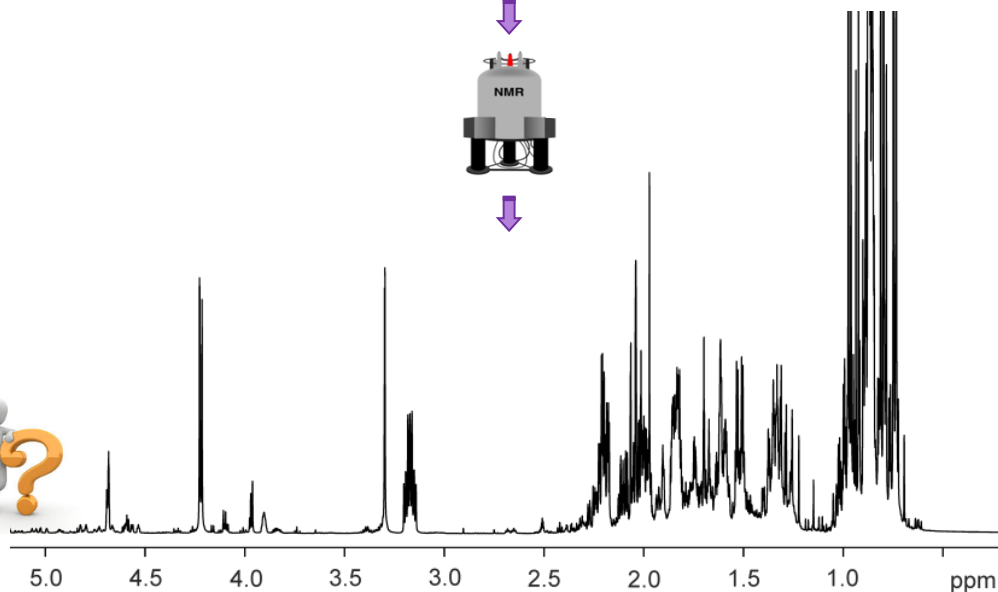
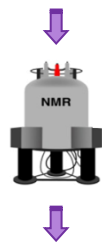
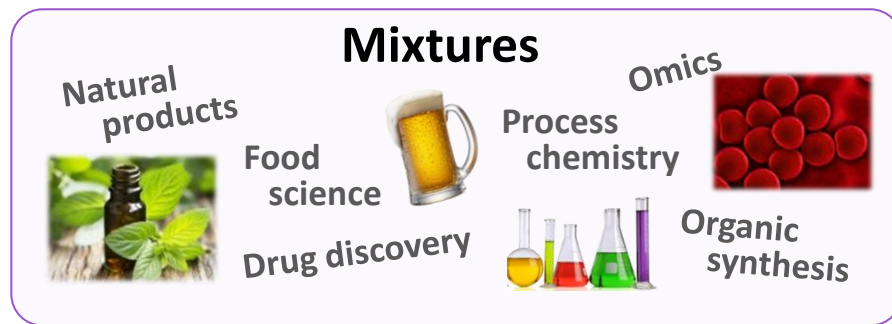
# Simplifying the analysis of mixtures

# New NMR methods and software

**Laura Castañar Acedo**  
NMR Methodology group  
The University of Manchester

Practical Applications of NMR in Industry Conference  
Hilton Head Island, South Carolina, USA

5<sup>th</sup> March 2019



## Information available

Chemical structure

Dynamics

Quantification

...

## Strengths

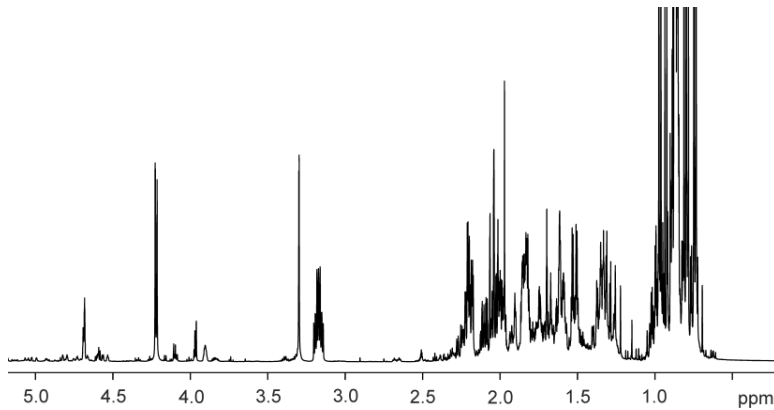
Non-destructive

Avoids physical separation

Individual species identification

...

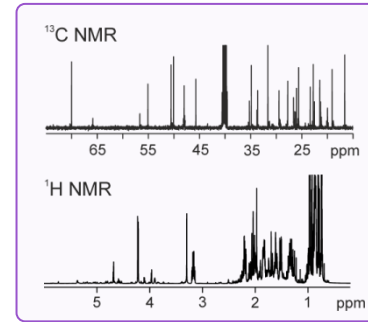
## 1D $^1\text{H}$ NMR & Mixture analysis



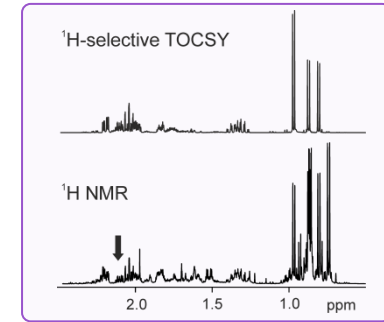
- ✓ The most abundant nuclei
- ✓ High sensitivity
- ✓ Structural information richness
- ✗ Signal overlap
- ✗ Low resolution spectra

## Alleviate overlap: classical strategies

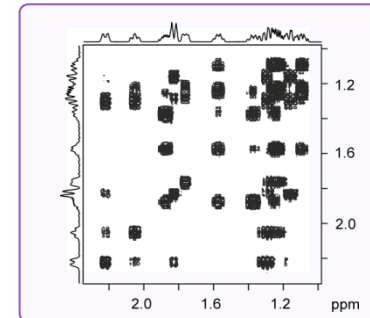
### Other nuclei



### Spectral editing

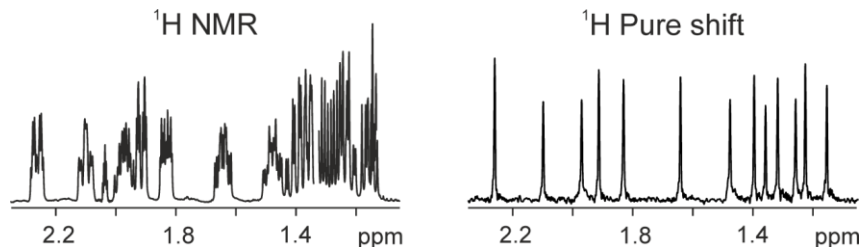


### nD NMR



# Alleviate overlap: recent strategies

## Pure shift NMR

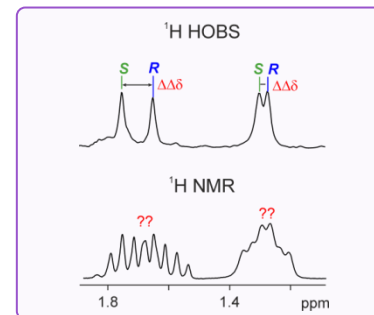


Peak positions determined solely  
by chemical shifts

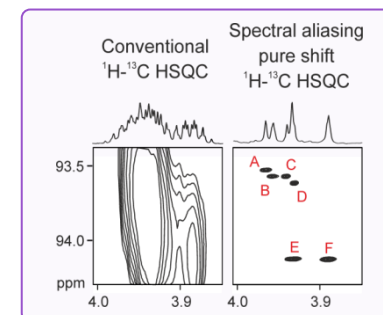
- ✓ Reduced spectral complexity
- ✓ Enhanced signal resolution
- ✓ Easier spectral analysis

*eMagRes* **2014**, *3*, 295; *Magn. Reson. Chem.* **2015**, *53*, 399  
*Prog. Nucl. Mag. Res. Sp.* **2015**, *86*, 1; *Magn. Reson. Chem.* **2017**, *55*, 47

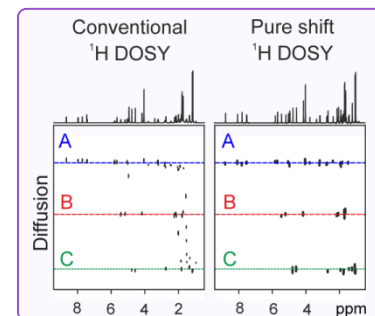
## Pure shift NMR of mixtures



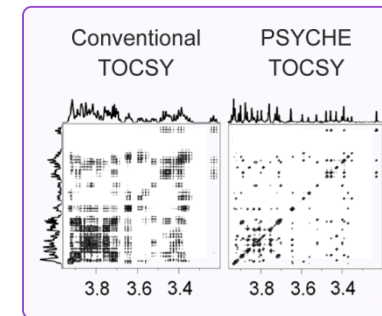
*ChemPhysChem* **2014**, *15*, 854  
*Chem. Commun.* **2014**, *50*, 2512  
*Magn. Reson. Chem.* **2018**, *56*, 876



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

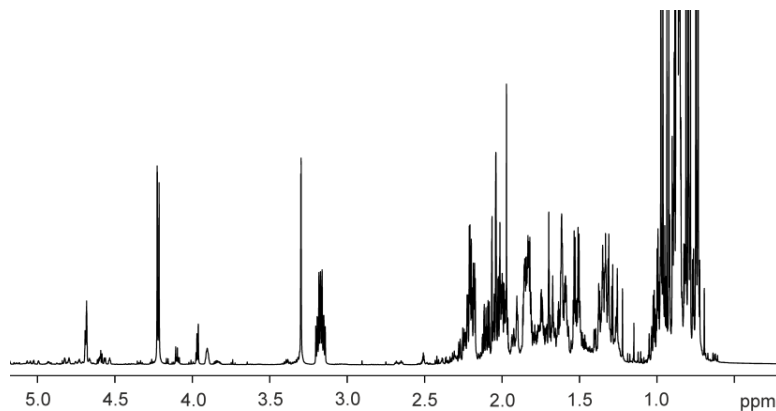


*Chem. Commun.* **2007**, 933  
*Chem. Eur. J.* **2014**, *20*, 11171  
*Chem. Commun.* **2014**, *50*, 4073



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

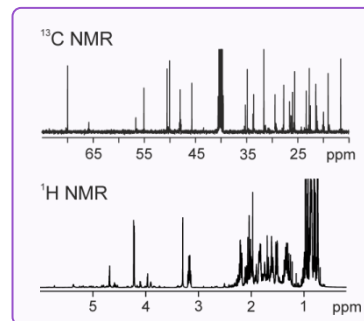
## 1D $^1\text{H}$ NMR & Mixture analysis



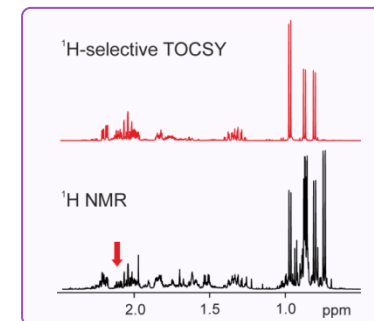
- ✓ The most abundant nuclei
- ✓ High sensitivity
- ✓ Structural information richness
- ✗ Signal overlap
- ✗ Low resolution spectra

## Strategies for alleviating overlap

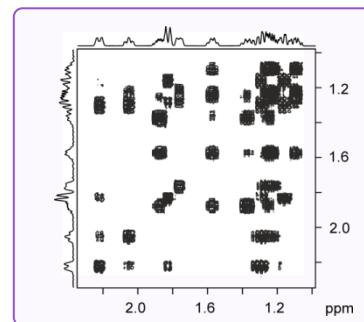
### Other nuclei



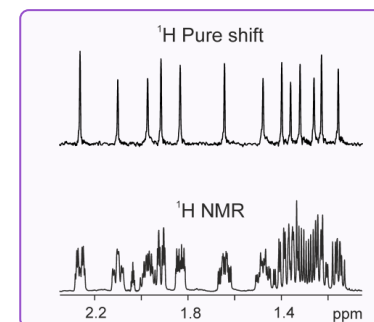
### Spectral editing



### nD NMR

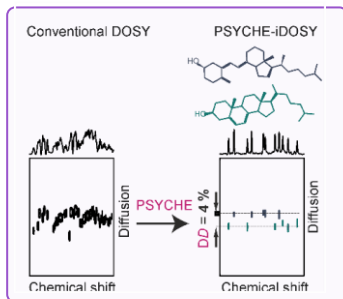


### Pure shift NMR

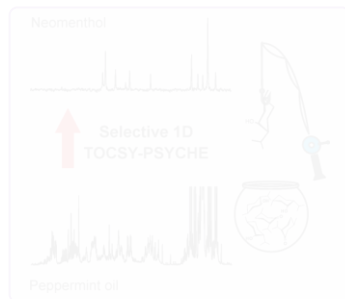


## New NMR methods

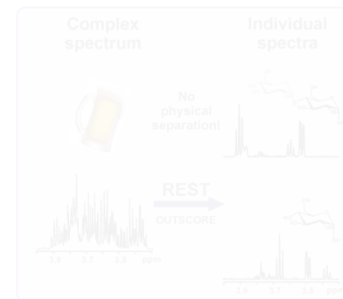
### PSYCHE-iDOSY



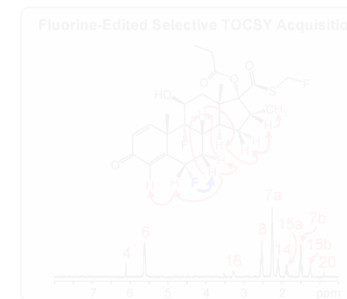
### TOCSY-PSYCHE



### REST



### FESTA



## New NMR software

### GNAT

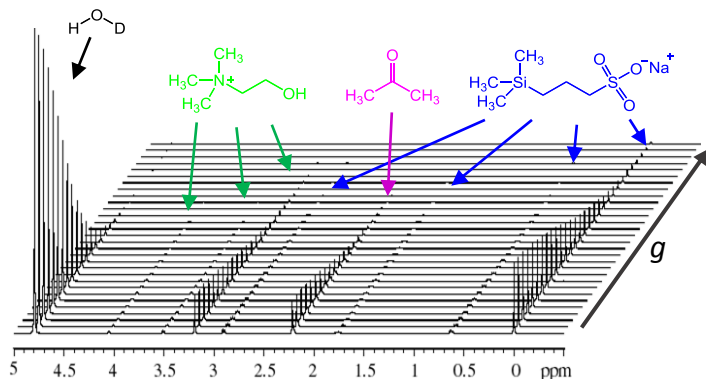


### MAGNATE



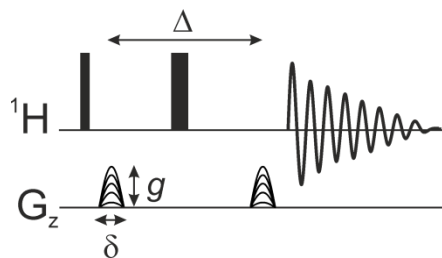
## Diffusion NMR

## Diffusion array



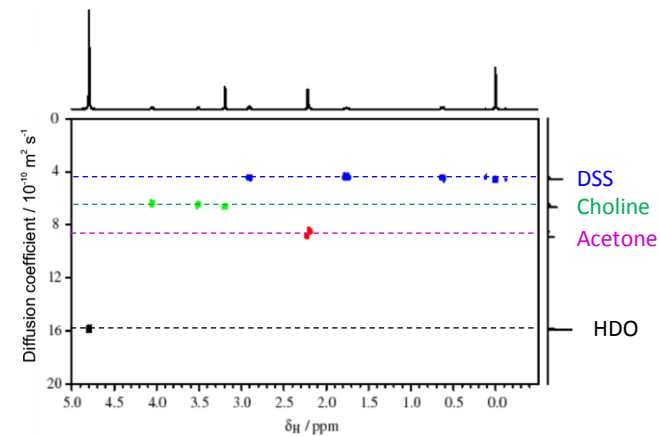
$$S(g) = S_0 e^{-D\gamma^2 \delta^2 g^2 \Delta'}$$

Stejskal-Tanner



Prototype sequence

## Diffusion-Ordered Spectroscopy (DOSY)



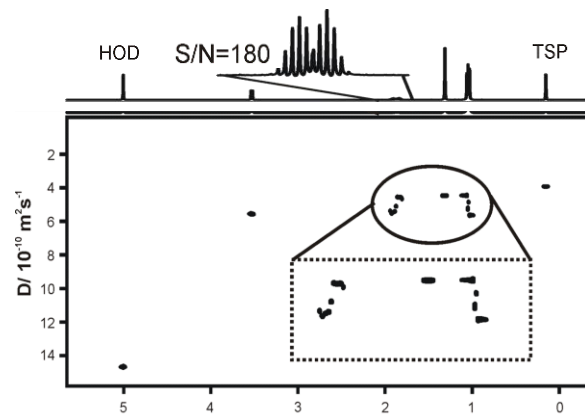
All signals from a given molecule:

- have same diffusion behaviour

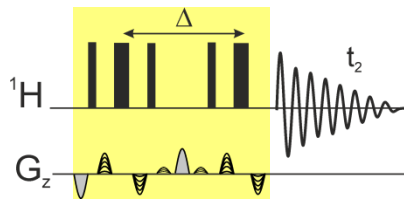
Signals overlap **x** - should appear at the same  $D$

# Diffusion NMR

## Conventional DOSY



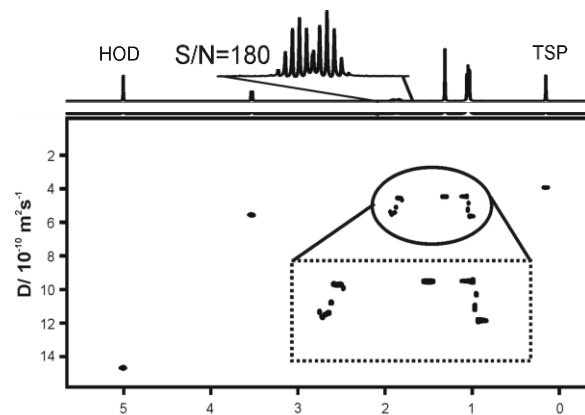
- ✗ Signals overlap
- ✗ Misleading peaks



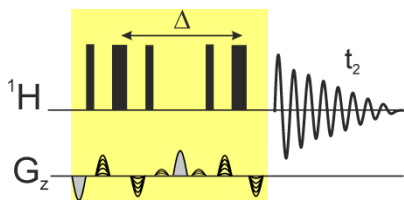


## Diffusion &amp; Pure shift NMR

## Conventional DOSY



- ✗ Signals overlap
- ✗ Misleading peaks

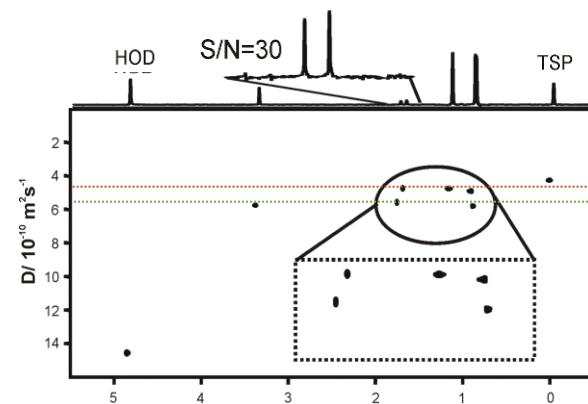


*Chem. Commun.* **2007**, 933  
*Chem. Eur. J.* **2014**, *20*, 11171

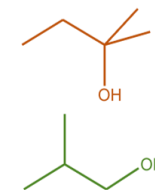
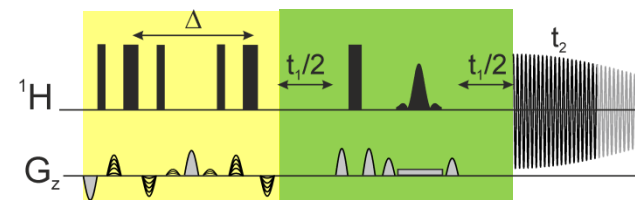
Zangger-Sterk (ZS)

**Low sensitivity**  
**PFG spatial variation**

## Pure shift DOSY

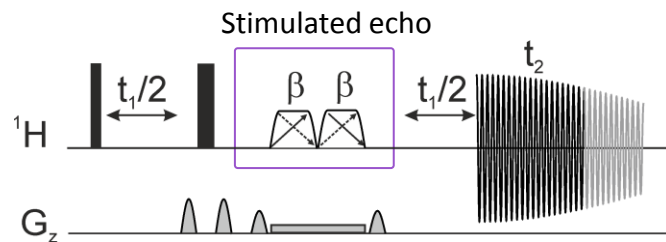


- ✓ Higher signal resolution
- ✓ Accurate  $D$  measurements



## PSYCHE-DOSY experiment

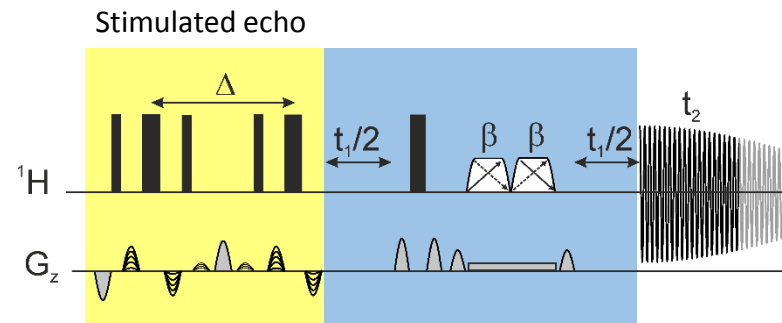
### PSYCHE



### PSYCHE benefits

- ✓ Higher sensitivity
- ✓ Less sensitive to spatial non-uniformity of PFG

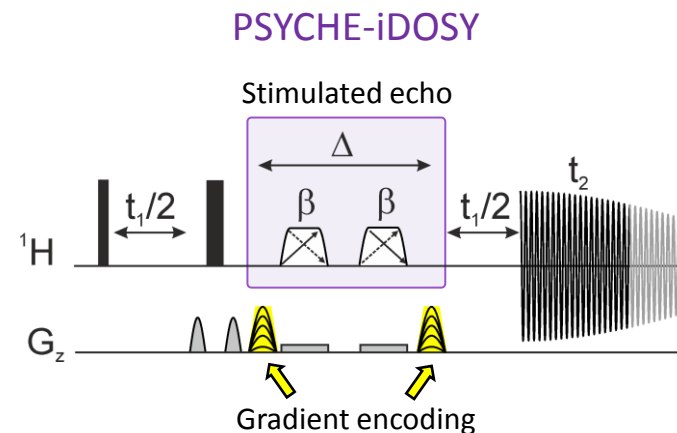
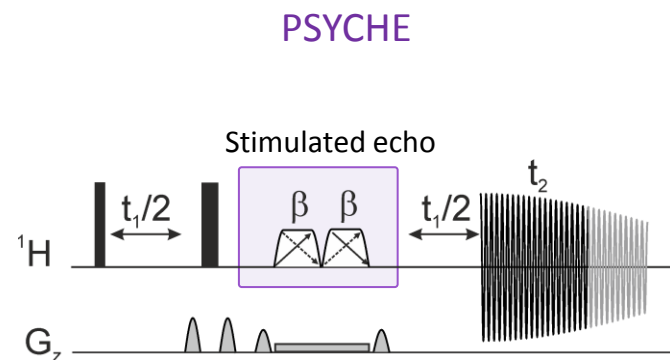
### Oneshot PSYCHE



### Oneshot PSYCHE

- Oneshot: diffusion encoding
- PSYCHE: Inverts active spins

## PSYCHE-iDOSY experiment



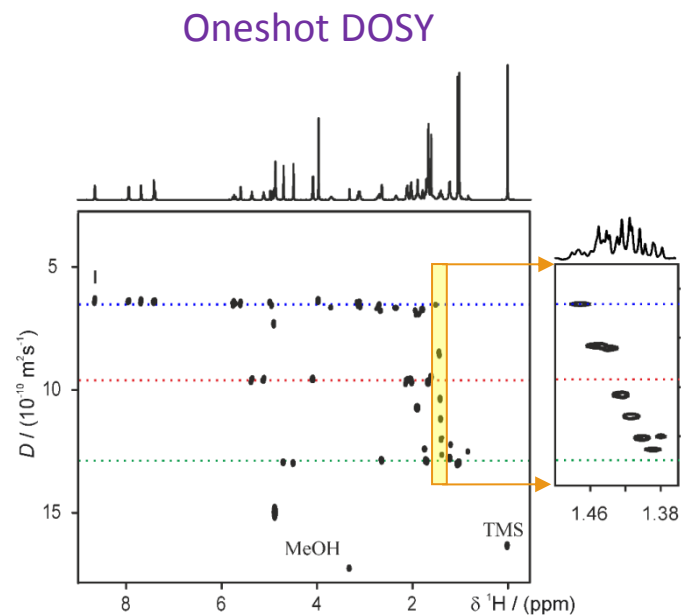
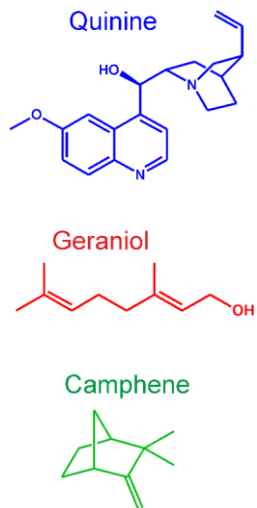
### PSYCHE benefits

- ✓ Higher sensitivity
- ✓ Less sensitive to spatial non-uniformity of PFG

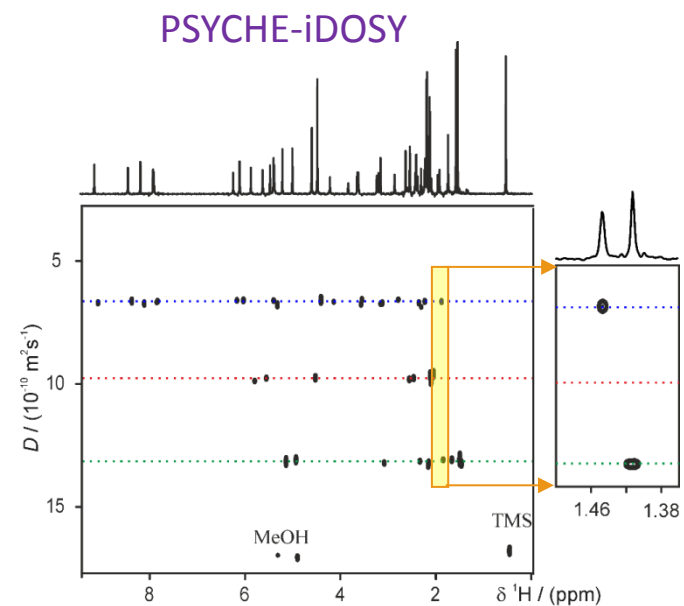
### PSYCHE element

- Inverts active spins
- Provides internal diffusion encoding (iDOSY)

## Pure shift DOSY & Mixtures



- ✗ Signal overlap
- ✗ Misleading peaks

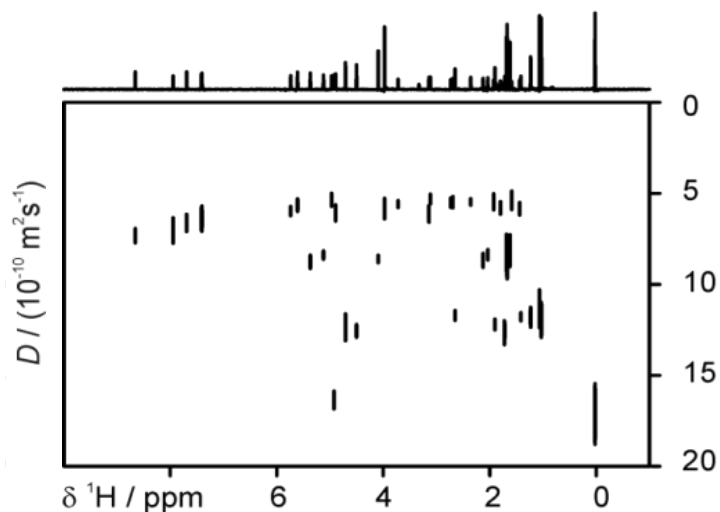


- ✓ Spectral simplification
- ✓ Higher signal resolution

## Pure shift DOSY & Resolution

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

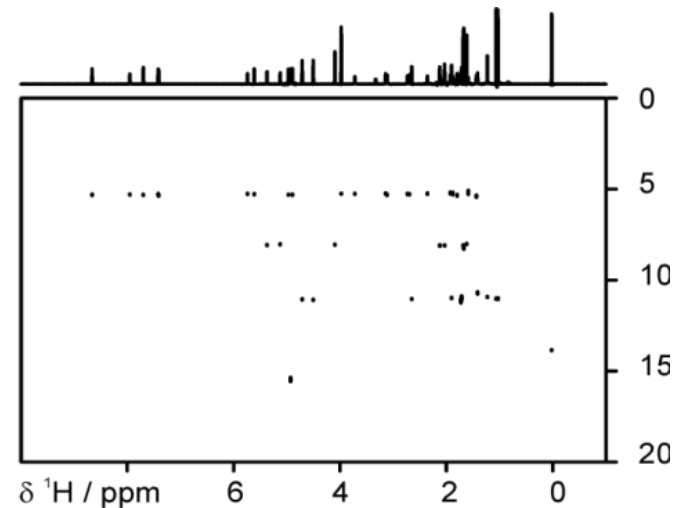
Oneshot ZS



✗ Low SNR      ✗ Many pulses & gradients

**Poor resolution**

PSYCHE-iDOSY

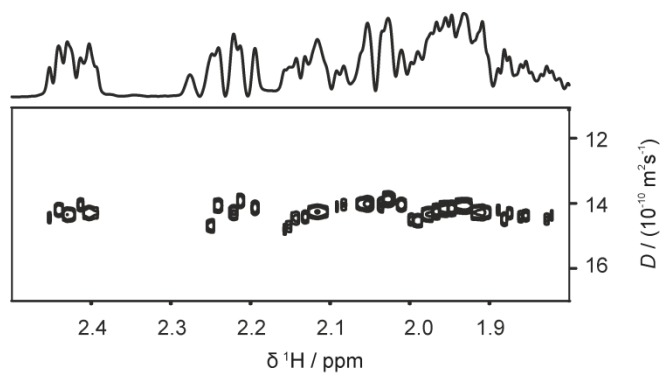


✓ Higher SNR      ✓ Fewer pulses & gradients

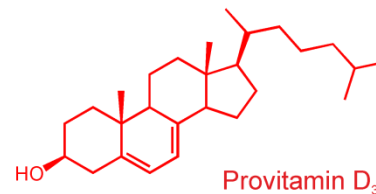
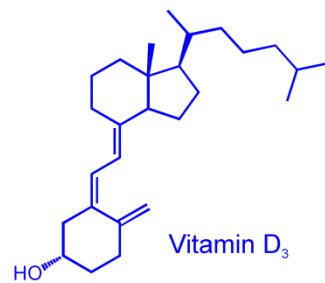
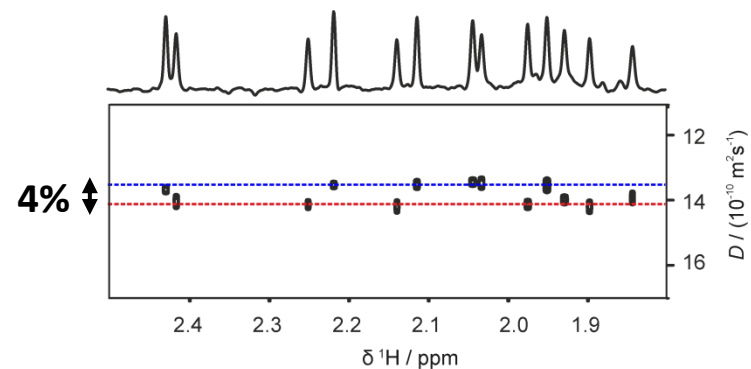
**Ultrahigh resolution**

## Ultrahigh resolution DOSY

Oneshot DOSY

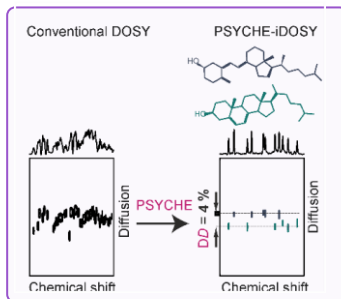


PSYCHE-iDOSY

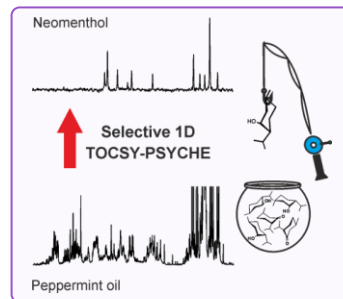


## New NMR methods

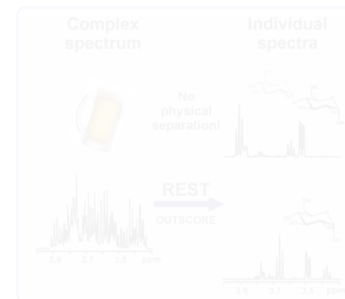
### PSYCHE-iDOSY



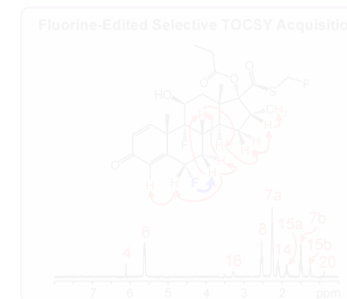
### TOCSY-PSYCHE



### REST



### FESTA



## New NMR software

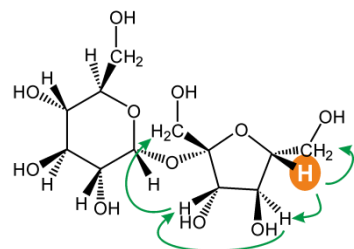
### GNAT



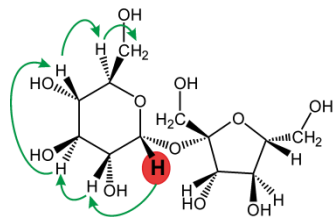
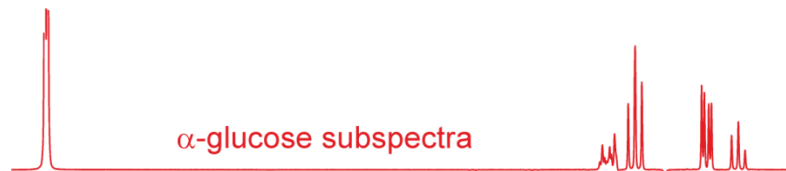
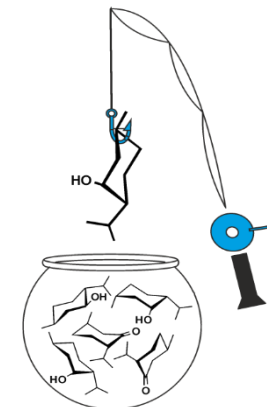
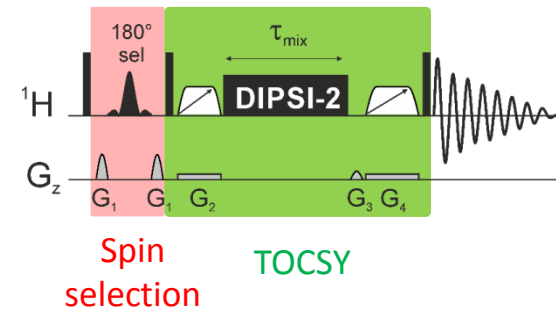
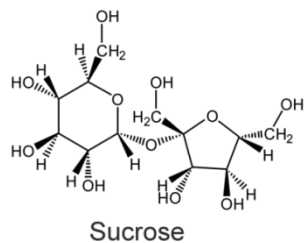
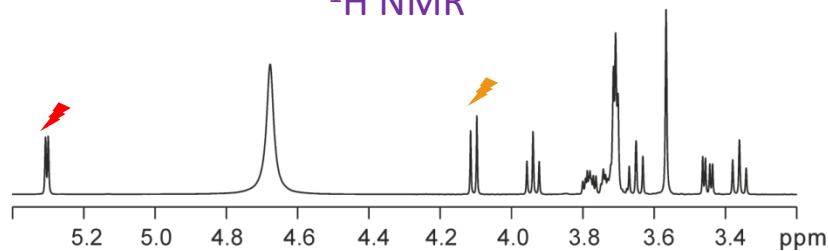
### MAGNATE



## Spectral factorization by homonuclear editing: selective 1D TOCSY



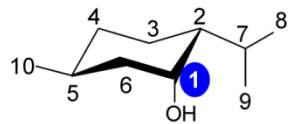
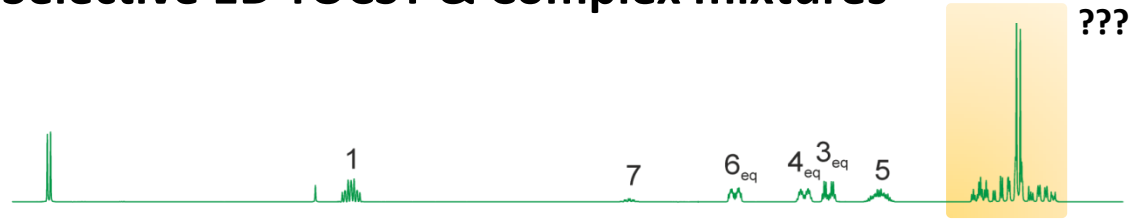
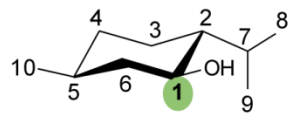
## Selective 1D TOCSY

 $\beta$ -fructose subspectra $\alpha$ -glucose subspectra $^1\text{H}$  NMR

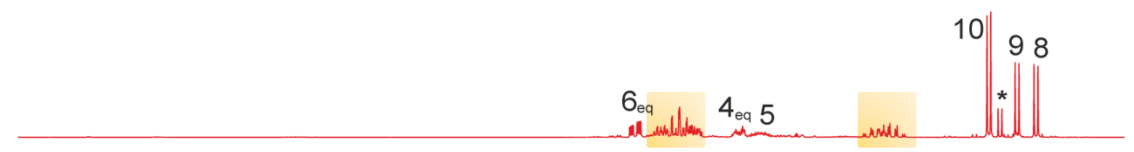
*J. Am. Chem. Soc.* **1985**, *107*, 7197  
*Magn. Reson. Chem.* **1995**, *33*, 156  
*Chem. Commun.* **1999**, 1319  
*Angew. Chem. Int. Ed.* **2003**, *42*, 3938



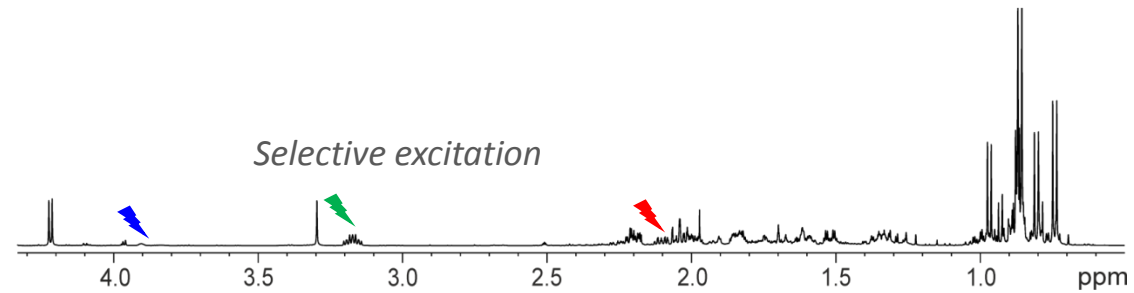
# Selective 1D TOCSY & Complex mixtures



Selective TOCSY

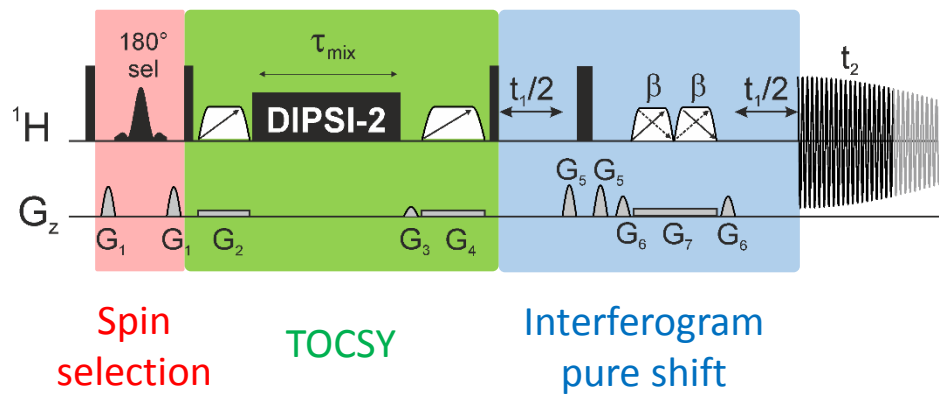


Peppermint oil



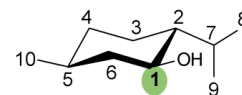
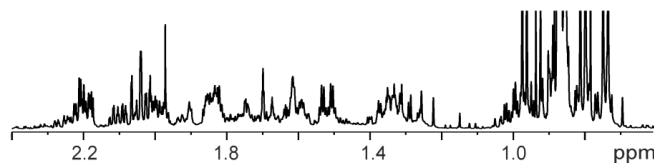
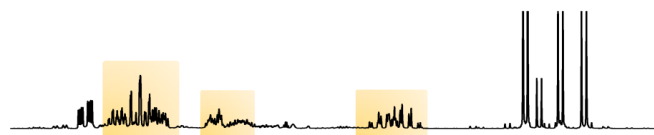
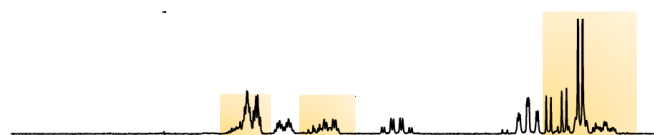
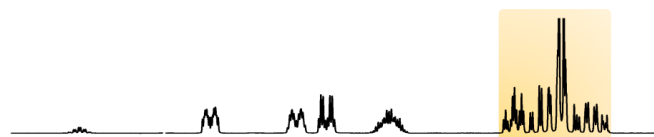
<sup>1</sup>H NMR

## Selective pure shift 1D TOCSY-PSYCHE experiment

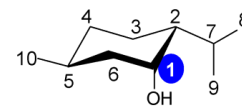


# Ultrahigh resolution pure shift selective TOCSY

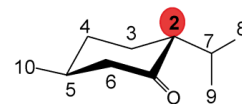
Conventional



Menthol



Neomenthol

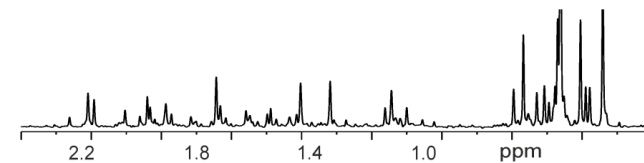
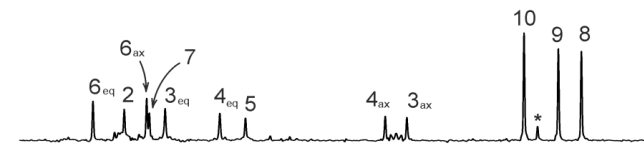
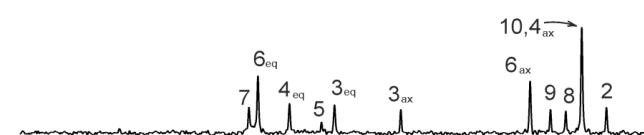
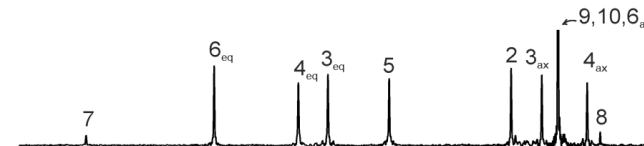


Menthone



Peppermint oil

Pure shift

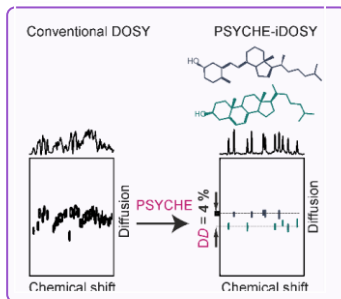


Selective TOCSY

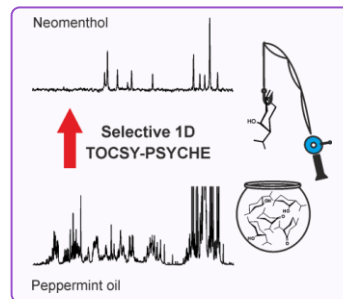
<sup>1</sup>H NMR

## New NMR methods

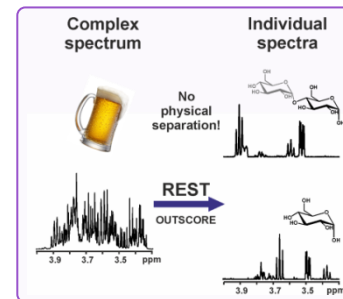
## PSYCHE-iDOSY



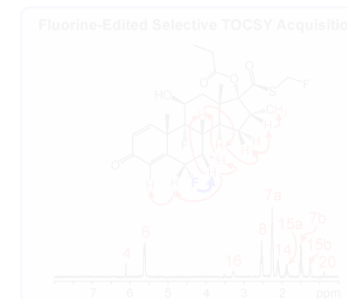
## TOCSY-PSYCHE



## REST

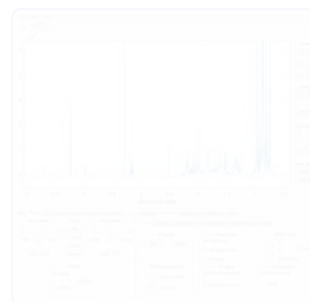


## FESTA



## New NMR software

## GNAT

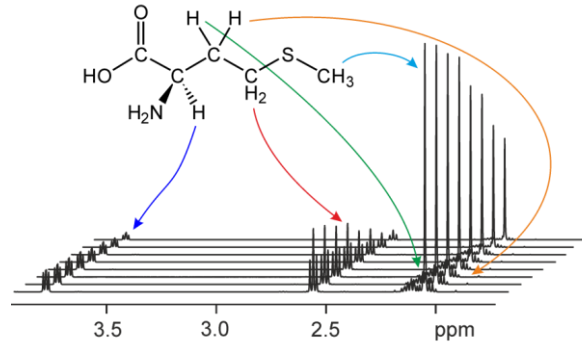


## MAGNATE



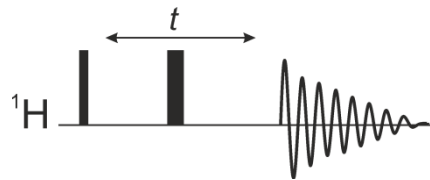
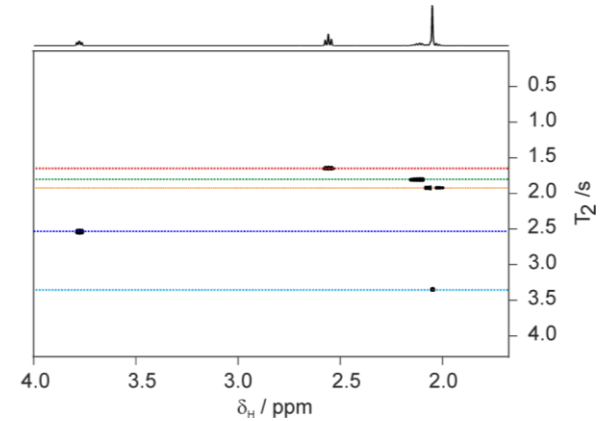
## Relaxation NMR

## Relaxation array



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

## Relaxation-Ordered Spectroscopy (ROSY)



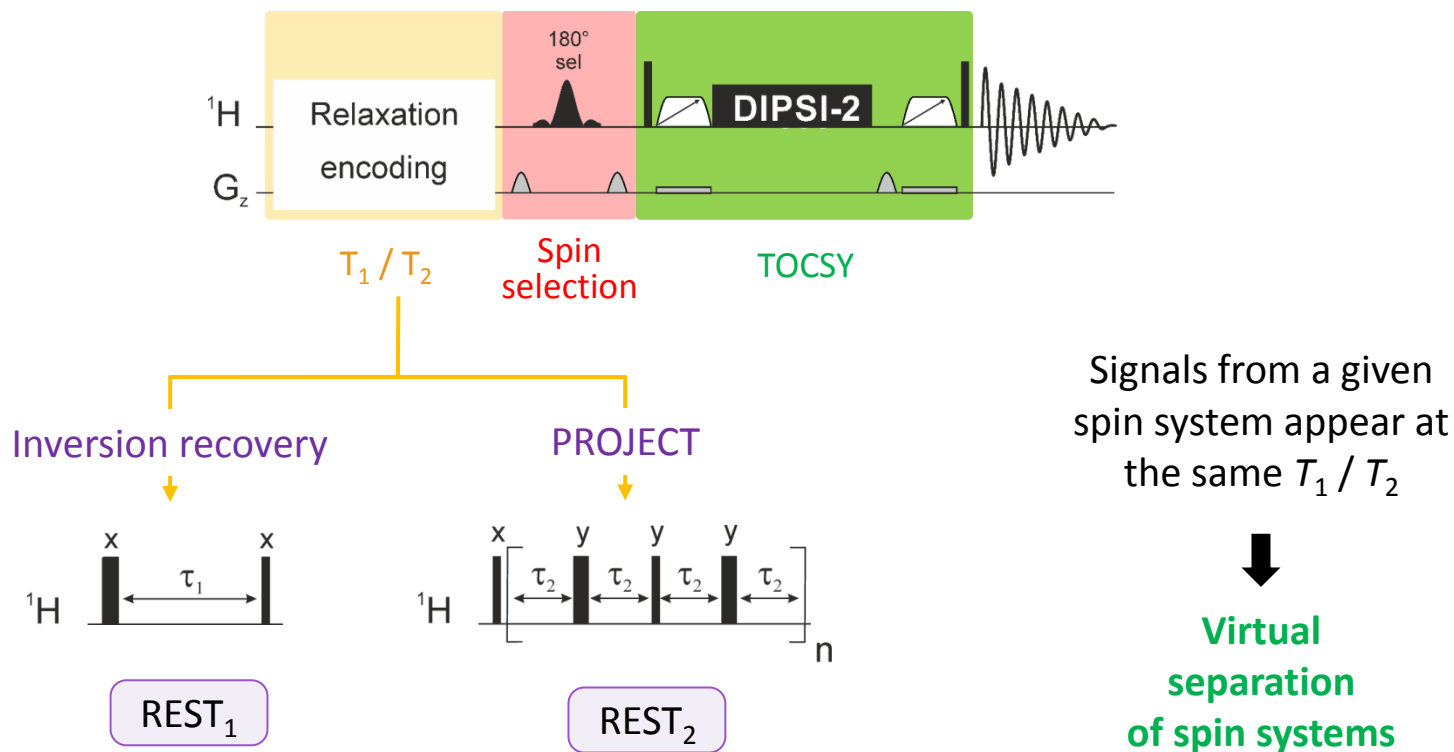
Prototype sequence

**NO virtual  
separation  
of components**

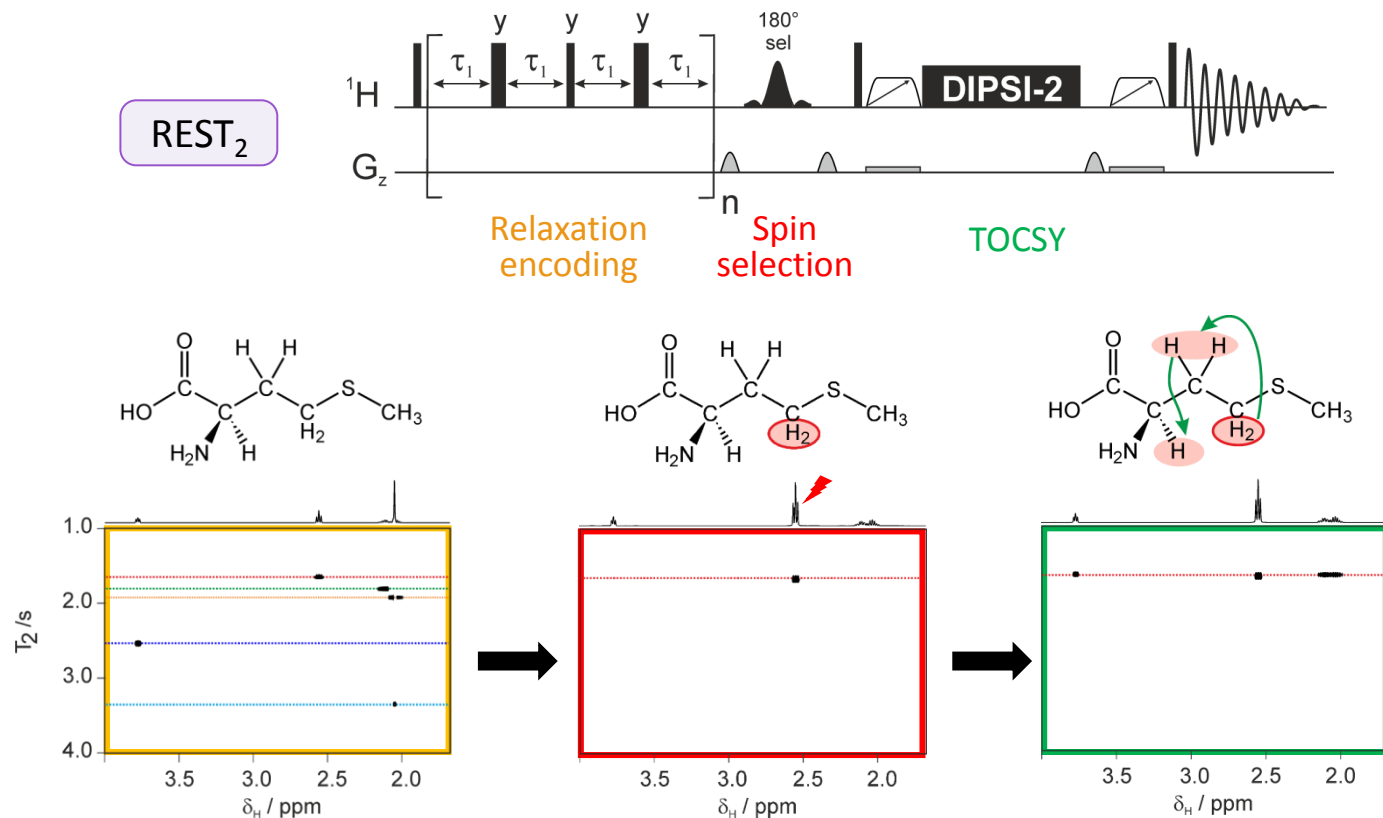
Signals from a given molecule:

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

# Relaxation-encoded selective TOCSY (REST) experiment

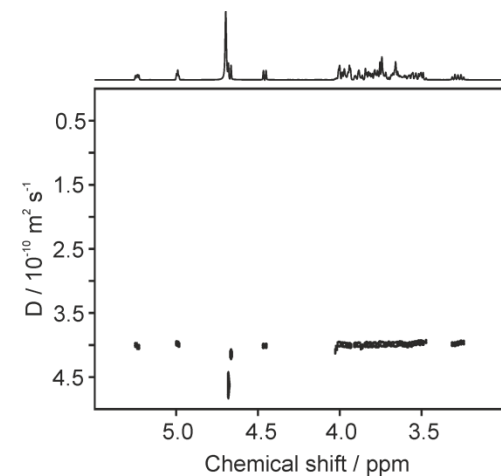


# Relaxation-encoded selective TOCSY (REST) experiment



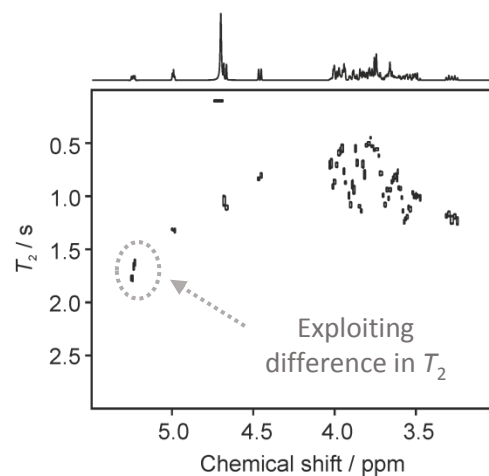
## REST &amp; Sugars

Oneshot DOSY

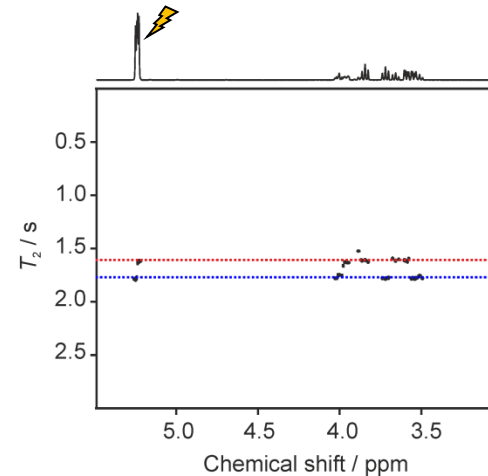


All sugar signals  
show the same  $D$

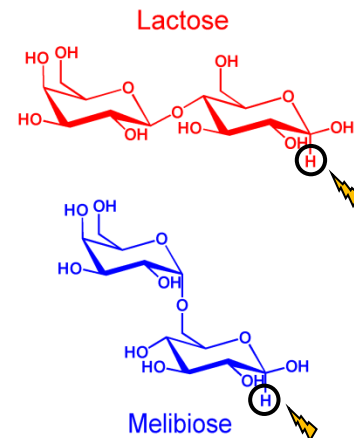
PROJECT ROSY



All sugar signals  
show different  $T_2$

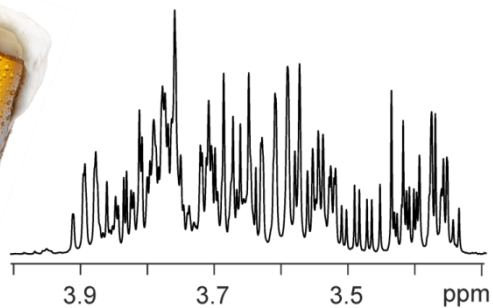
REST<sub>2</sub> ROSY

Virtual separation  
of components





## REST &amp; Beer



Complex Mixture

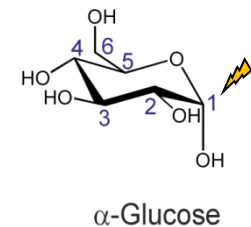
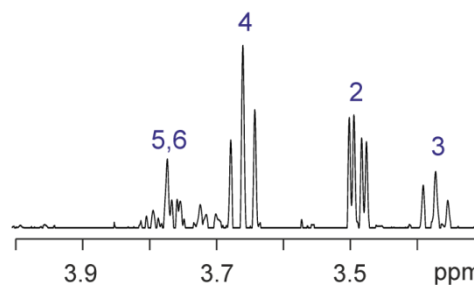
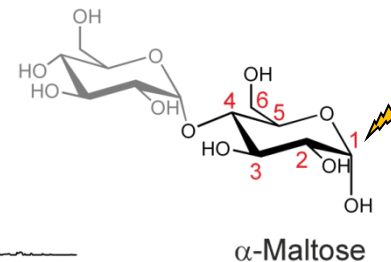
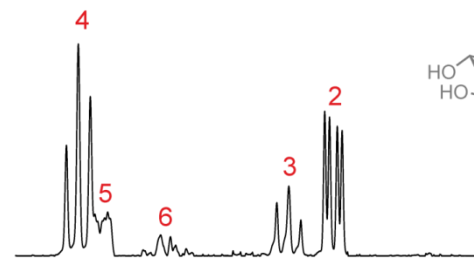
REST<sub>2</sub>



OUTSCORE

(Optimized Unmixing of  
True Spectra for  
COmponent REsolution)

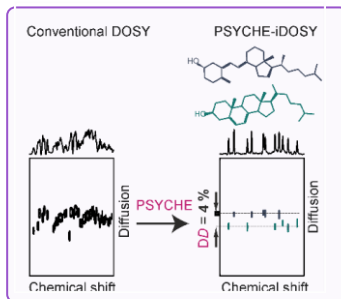
*Chem. Commun.* **2013**, 49, 10510



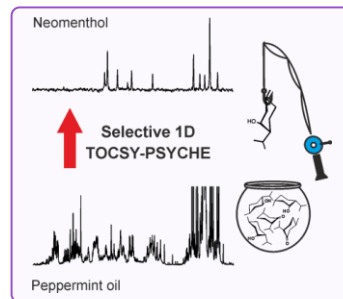
Virtual separation  
of components

## New NMR methods

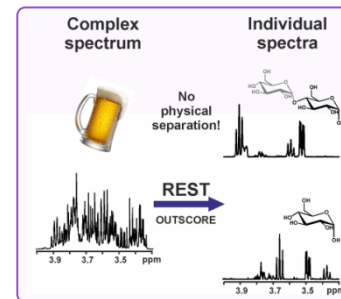
## PSYCHE-iDOSY



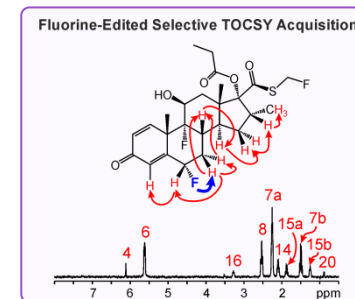
## TOCSY-PSYCHE



## REST



## FESTA



## New NMR software

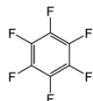
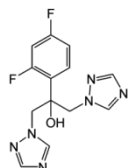
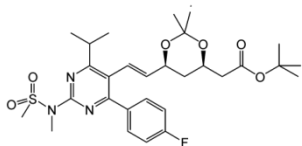
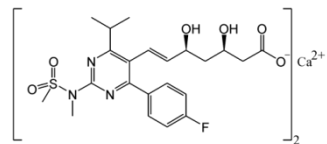
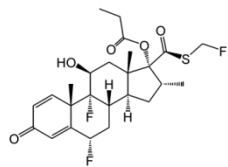
## GNAT



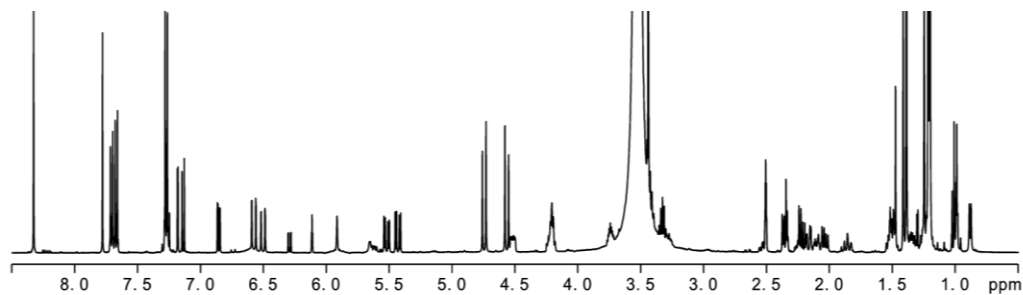
## MAGNATE



## Mixtures containing fluorinated species

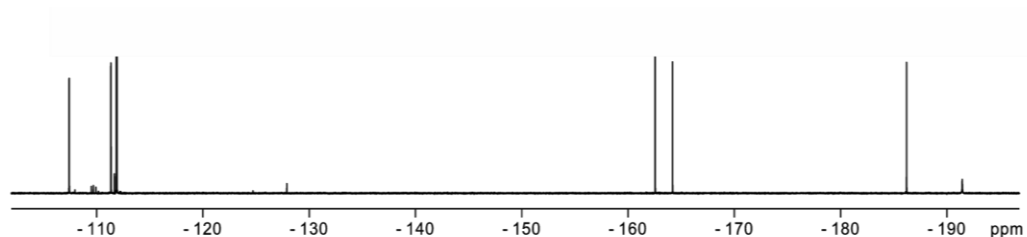


$^1\text{H}$  NMR



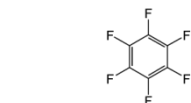
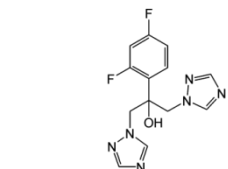
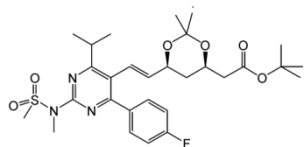
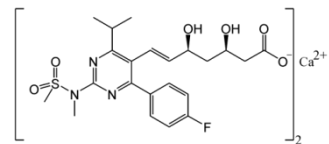
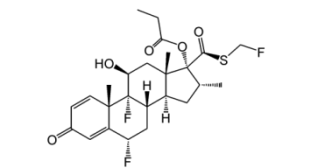
- ✗ Signal overlap
- ✗ Component information
- ✗ Structural information

$^{19}\text{F}$  NMR

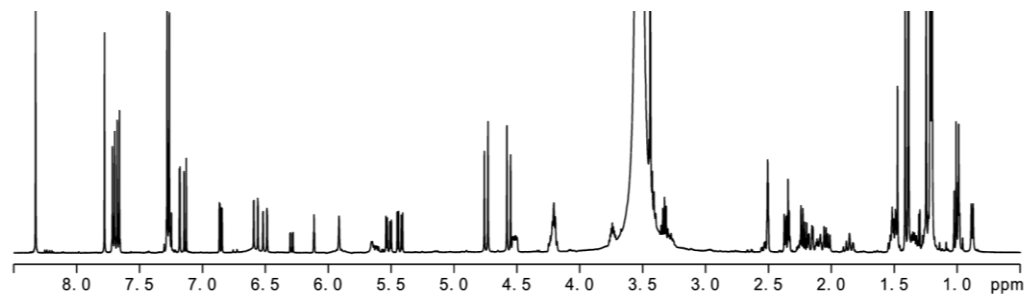


- ✓ Spectral resolution
- ✗ Component information
- ✗ Structural information

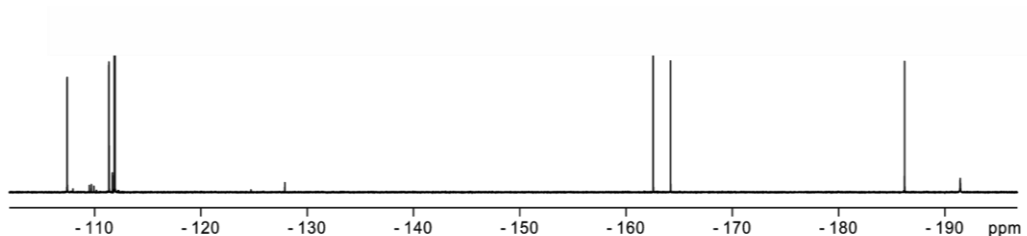
# Mixtures containing fluorinated species



$^1\text{H}$  NMR



$^{19}\text{F}$  NMR

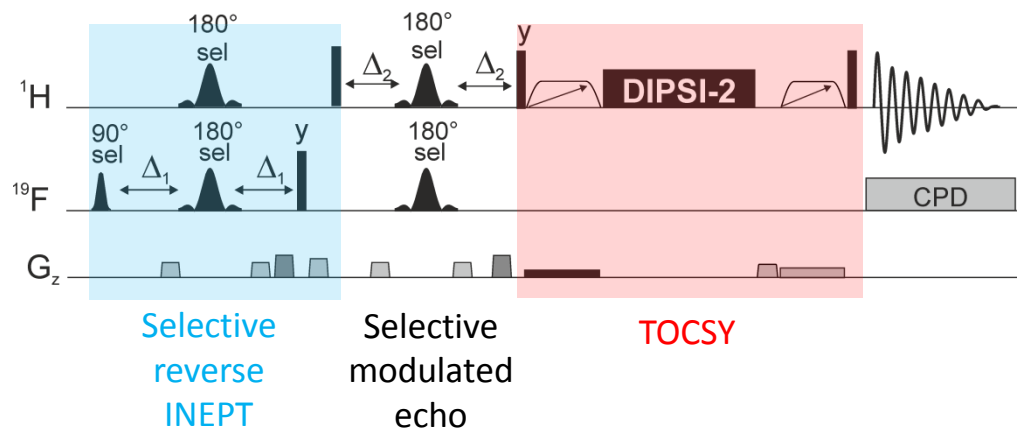


Structural richness

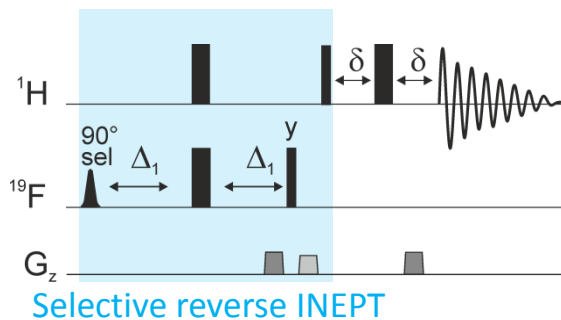
Synergy

High spectral resolution

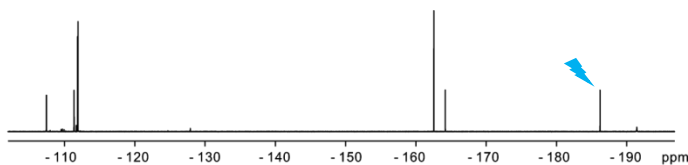
# Fluorine-edited selective TOCSY Acquisition (FESTA) experiment



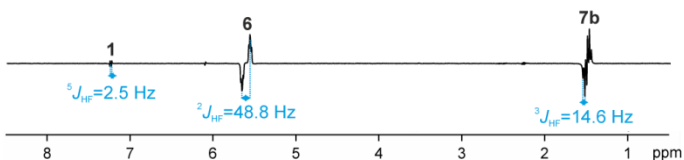
# Fluorine-edited selective TOCSY Acquisition (FESTA) experiment



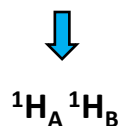
$^{19}\text{F}$  NMR



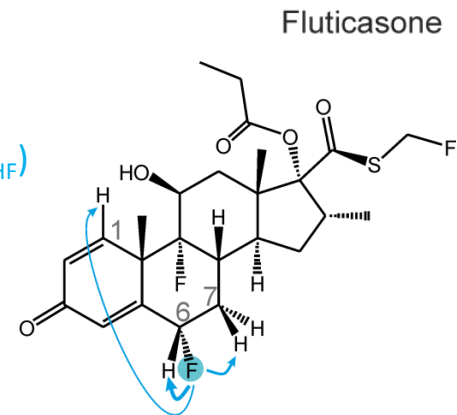
$^1\text{H}$  SRI



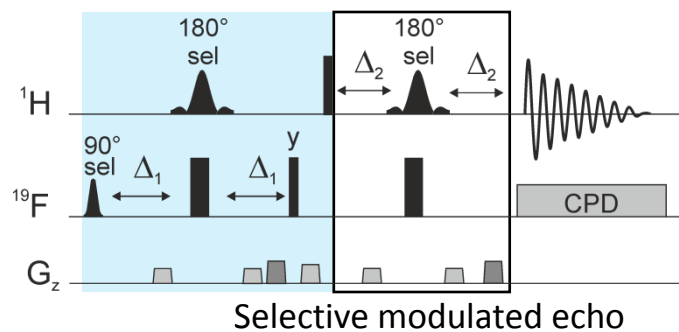
$^{19}\text{F}$  Spin selection



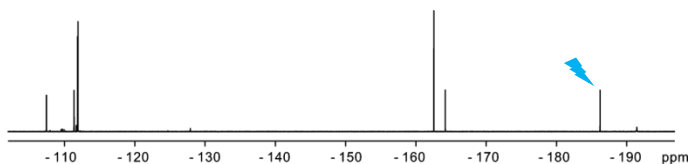
Heteronuclear transfer ( $J_{\text{HF}}$ )



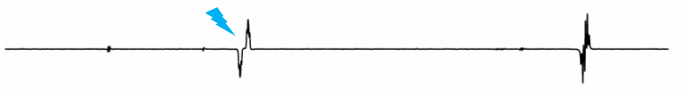
# Fluorine-edited selective TOCSY Acquisition (FESTA) experiment



$^{19}\text{F}$  NMR



$^1\text{H}$  SRI



$^1\text{H}$  SRI-SME



$^{19}\text{F}$  Spin selection



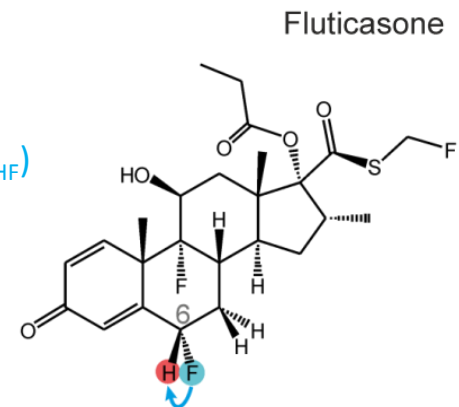
Heteronuclear transfer ( $J_{\text{HF}}$ )

$^1\text{H}_A$   $^1\text{H}_B$

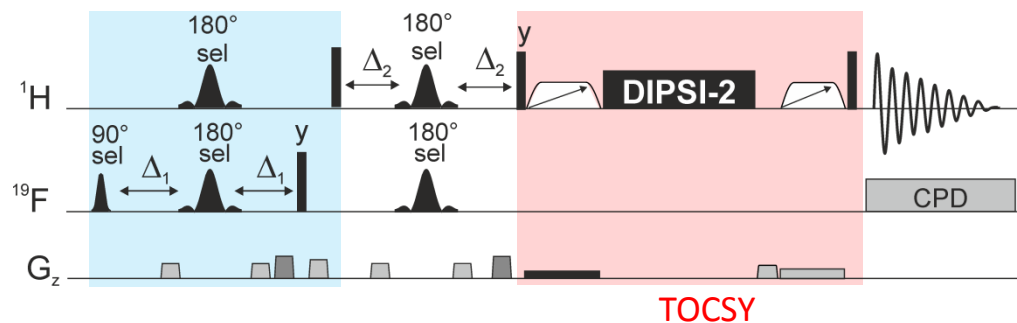


Spin selection

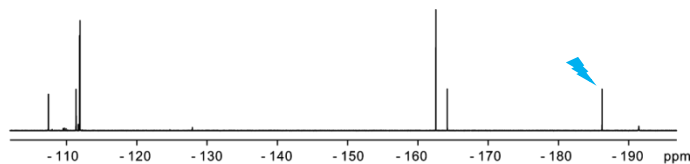
$^1\text{H}_A$



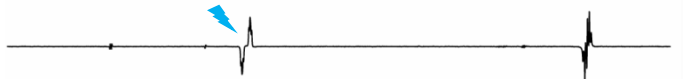
# Fluorine-edited selective TOCSY Acquisition (FESTA) experiment



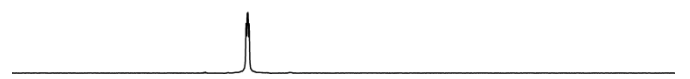
$^{19}\text{F}$  NMR



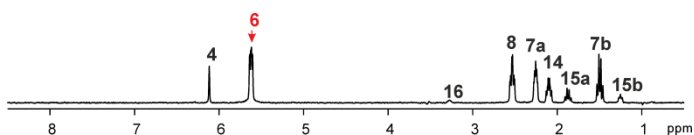
$^1\text{H}$  SRI



$^1\text{H}$  SRI-SME



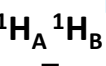
$^1\text{H}$  FESTA



$^{19}\text{F}$  Spin selection



Heteronuclear transfer ( $J_{\text{HF}}$ )



Spin selection

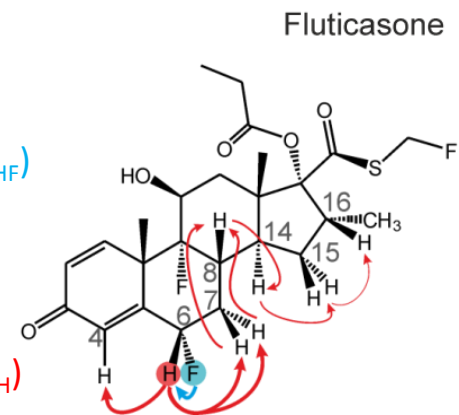


$^{1}\text{H}_\text{A}$

Homonuclear transfer ( $J_{\text{HH}}$ )

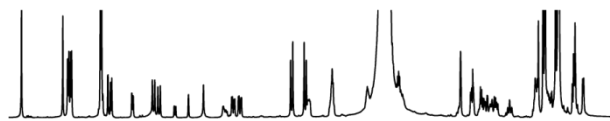
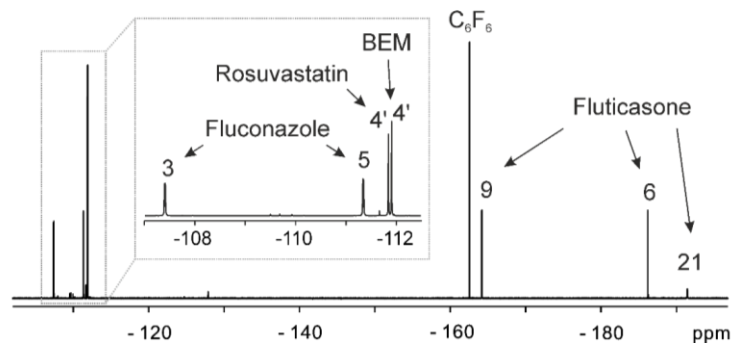
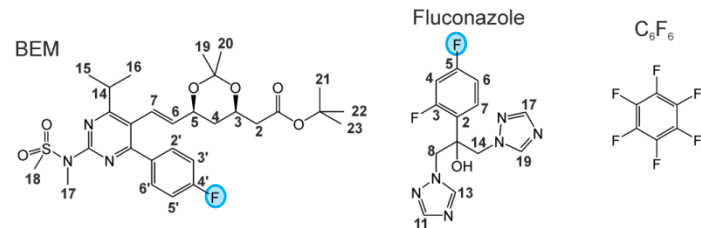
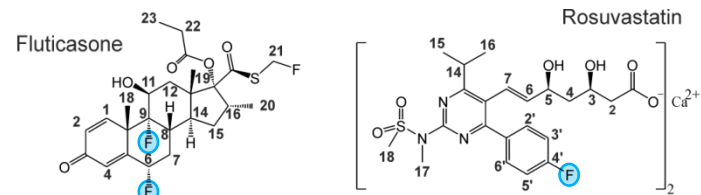
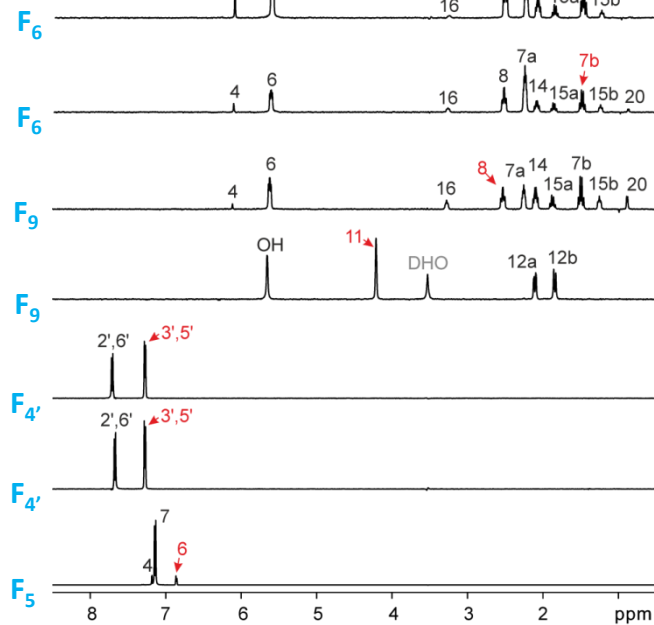


$^{1}\text{H} \rightarrow ^{1}\text{H} \rightarrow ^{1}\text{H} \rightarrow \dots$



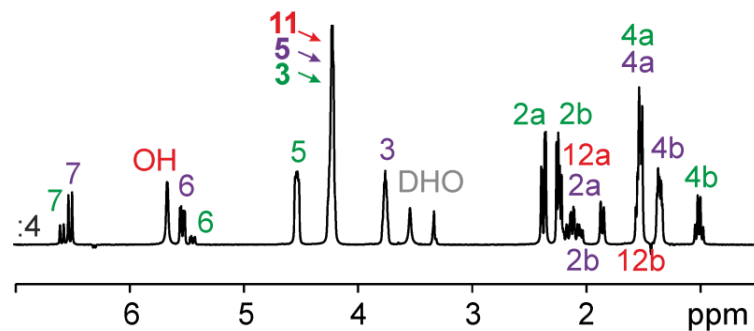


## FESTA &amp; Drug mixture

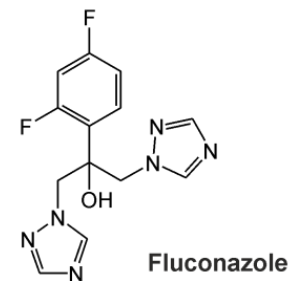
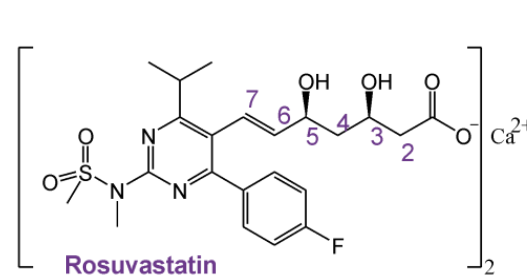
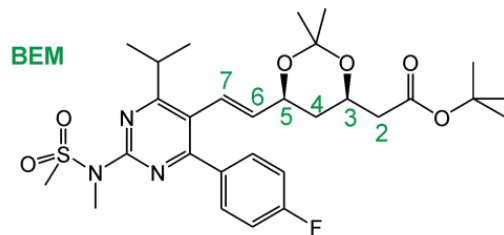
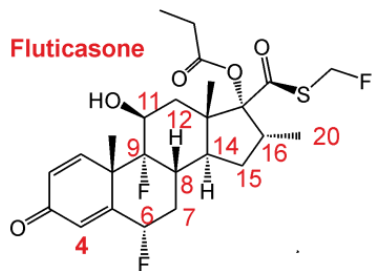
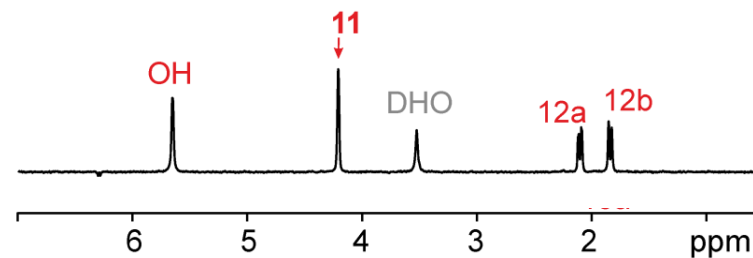
 $^1\text{H}$  NMR $^1\text{H}$  FESTA

## FESTA &amp; Drug mixture

Selective 1D TOCSY

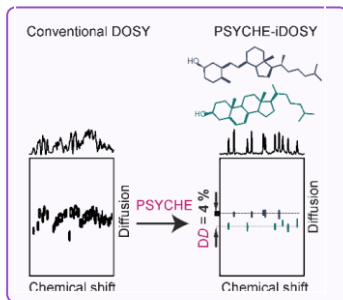


FESTA

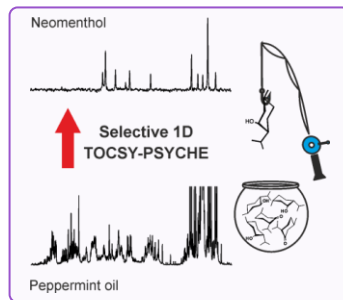


# New NMR methods

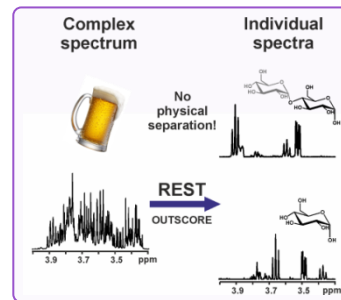
## PSYCHE-iDOSY



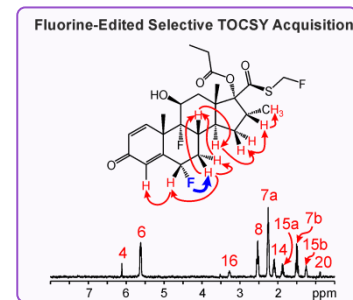
## TOCSY-PSYCHE



## REST

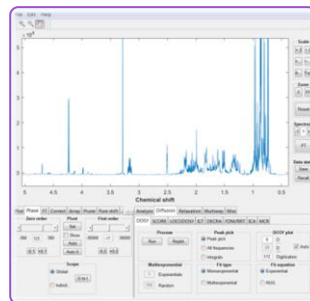


## FESTA



# New NMR software

## GNAT

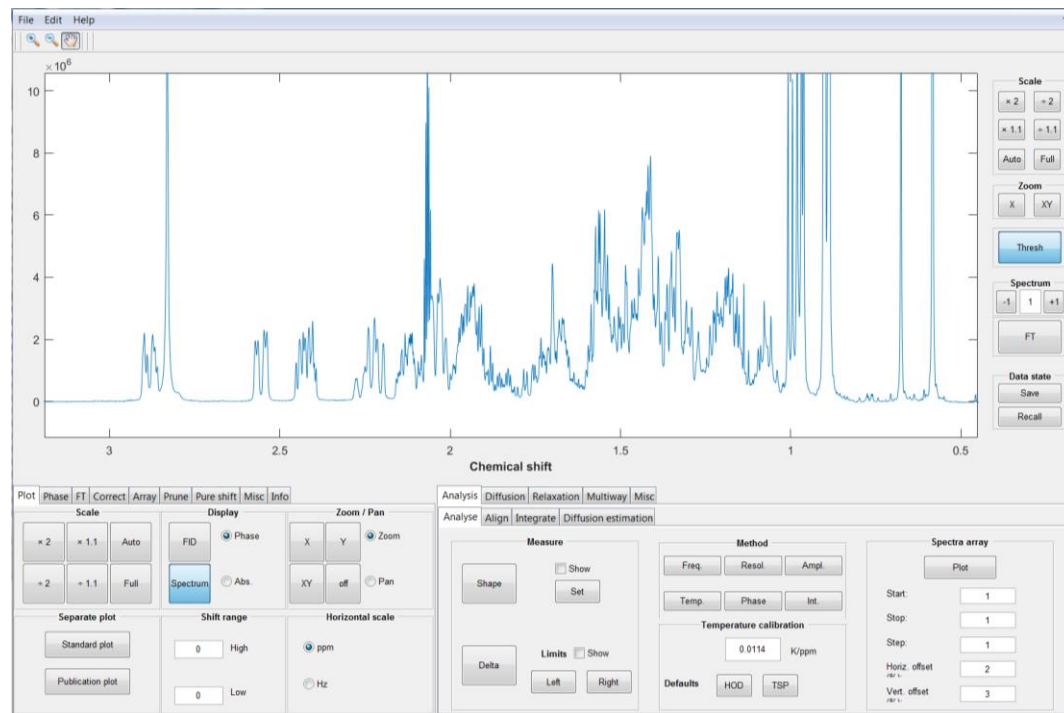
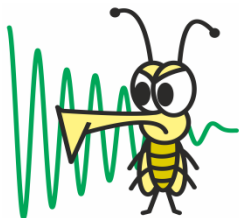


## MAGNATE



## 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)



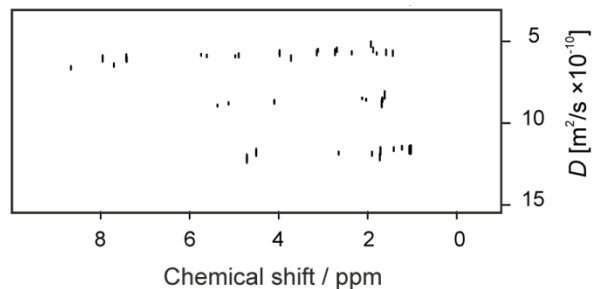
Main window of the graphical interface of the GNAT



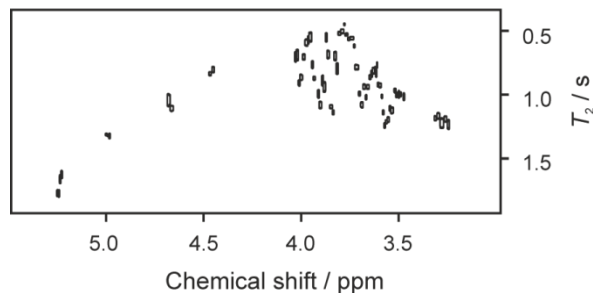
# GNAT & Mixture analysis

## Univariate methods

Diffusion (DOSY)



Relaxation (ROSY)



**Diffusion related parameters**

Calculate diffusion parameters by pulse sequence type

Monopolar  Bipolar

$\Delta$  0.1665  $\Delta'$  0.16575  $\delta$  0.0021

$\gamma$  267524618.573  $\tau$  0.001570

dosyconstant 52313110435.0967

	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) 0.0022212

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

Revert to original values

Delays (vdlst)		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

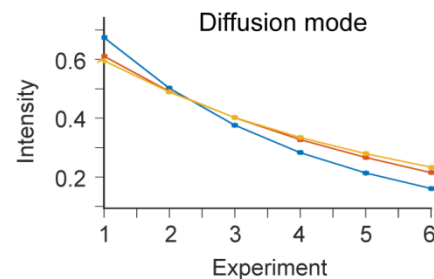
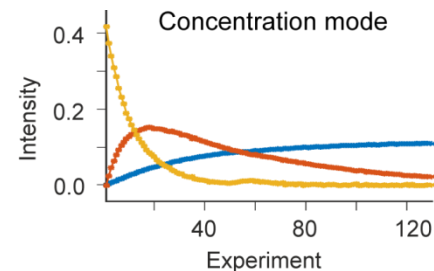
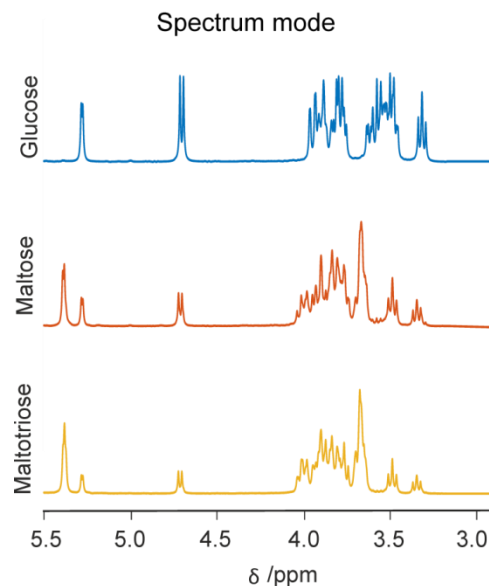
LOCODOSY

DECRA

FDM/RRT

PARAFAC

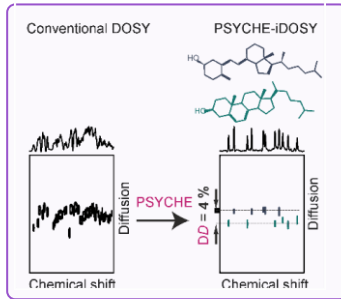
...



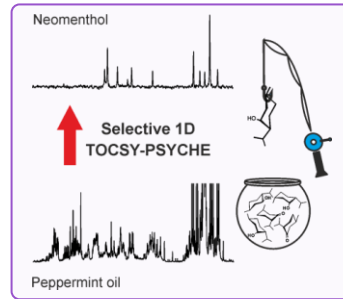
PARAFAC analysis of the hydrolysis of maltotriose. Diffusion NMR experiments were performed continuously over the course of the reaction (*Anal. Chem.* **2009**, *81*, 8119)

## New NMR methods

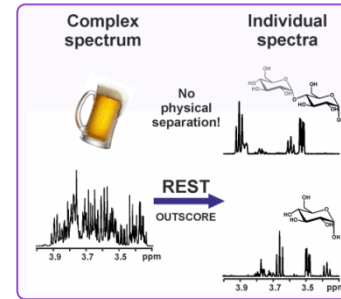
### PSYCHE-iDOSY



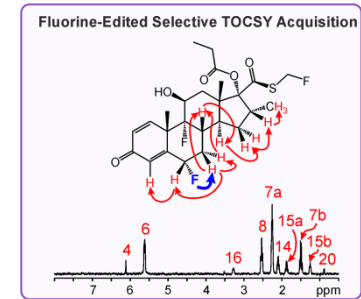
### TOCSY-PSYCHE



### REST

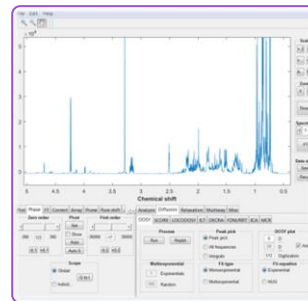


### FESTA

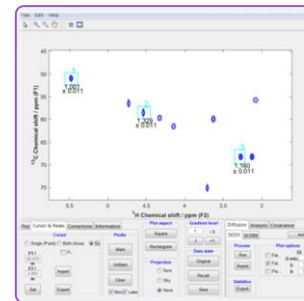


## New NMR software

### GNAT

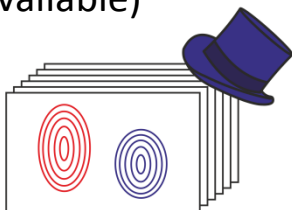


### MAGNATE

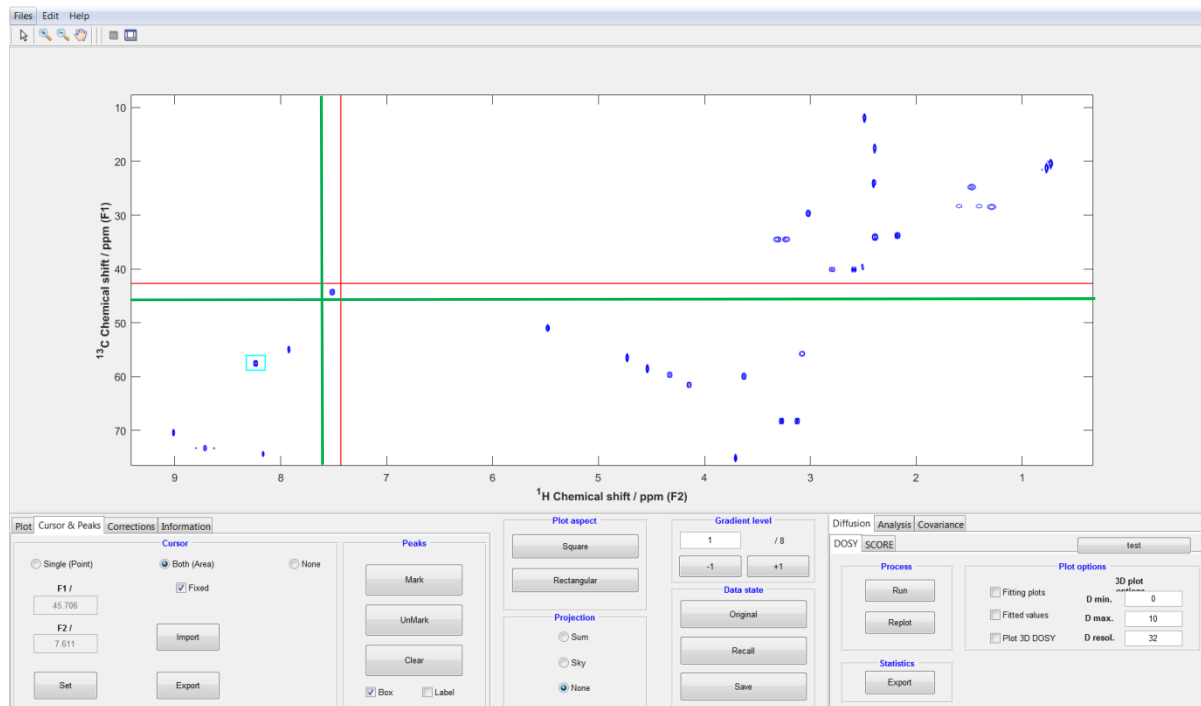


# Multidimensional Analysis for the GNAT Environment (MAGNATE)

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



maGNATE



Main window of the graphical interface of the MAGNATE

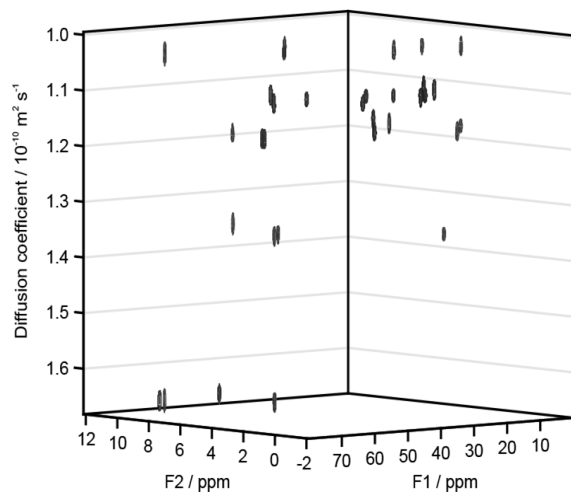




# MAGNATE & Mixture analysis

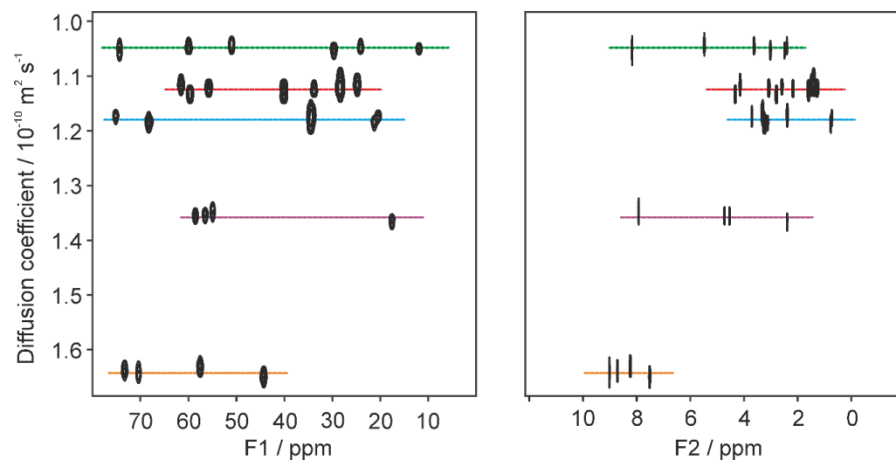
## Univariate methods

### 3D DOSY



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

### 2D Projections

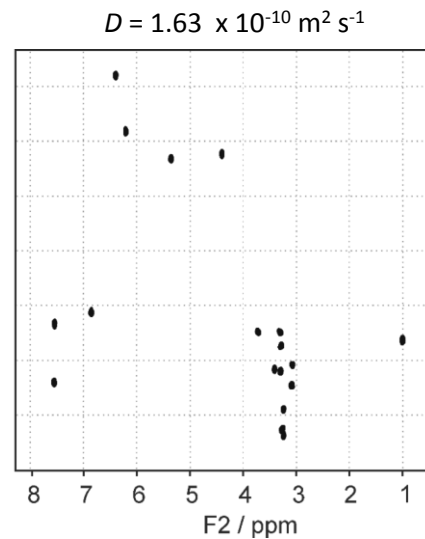
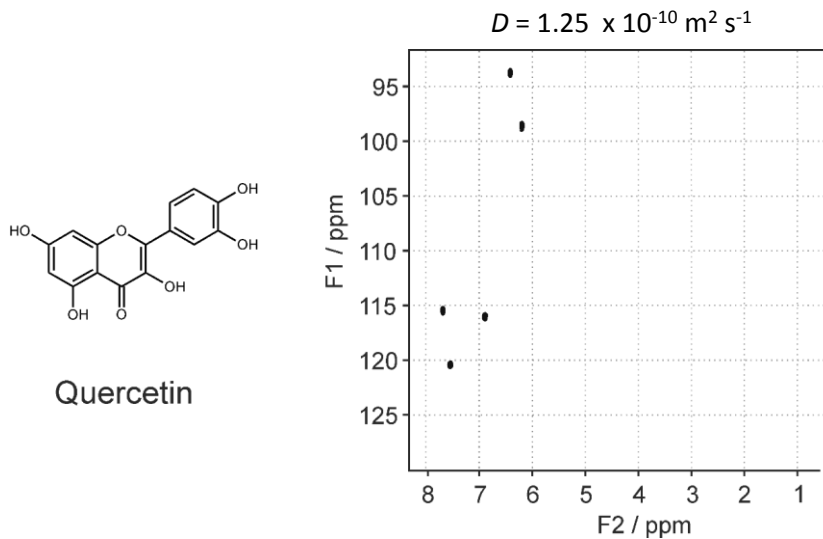


2D projection along  $^1\text{H}$  dimension

2D projection along  $^{13}\text{C}$  dimension

## MAGNATE &amp; Mixture analysis

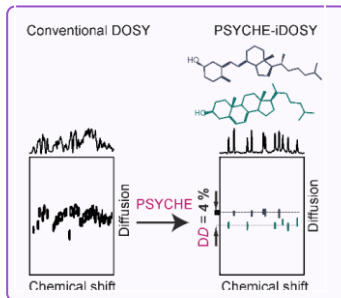
Multivariate methods



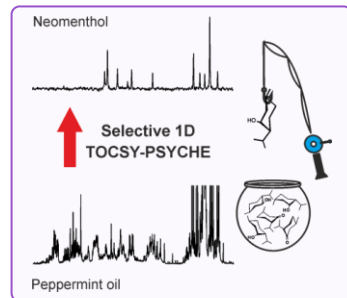
2D HSQC spectra obtained from OUTSCORE analysis of the Oneshot-HSQC data for the components of a mixture of flavonoids

## New NMR methods

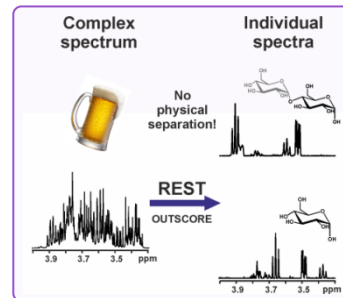
## PSYCHE-iDOSY



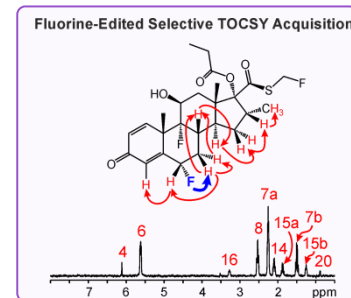
## TOCSY-PSYCHE



## REST

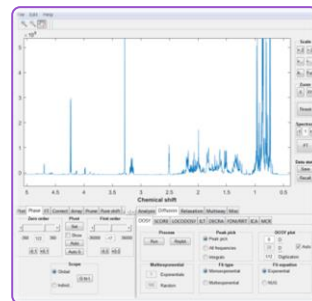


## FESTA

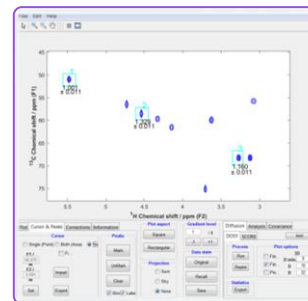


## New NMR software

## GNAT



## MAGNATE



# Manchester NMR Methodology Group

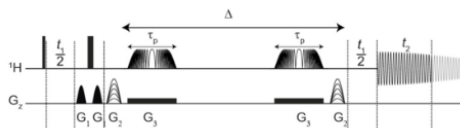
<https://nmr.chemistry.manchester.ac.uk/>



### Pulse Sequences

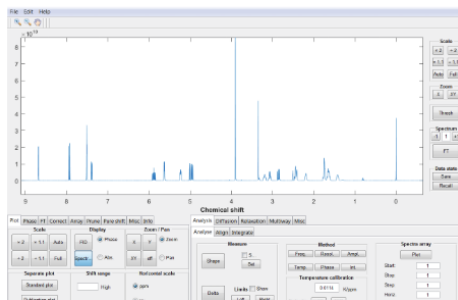
We are currently preparing many of our pulse sequences, parameter sets, example datasets and processing macros for the website. Some are available [here](#) but if you would like to use any of the other the sequences, as described in the [publications](#) section, please email us. The majority of sequences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



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

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