DOSY NMR

Theory and Practice

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What is DOSY NMR?

Diffusion **O**rdered **S**pectroscop**Y**

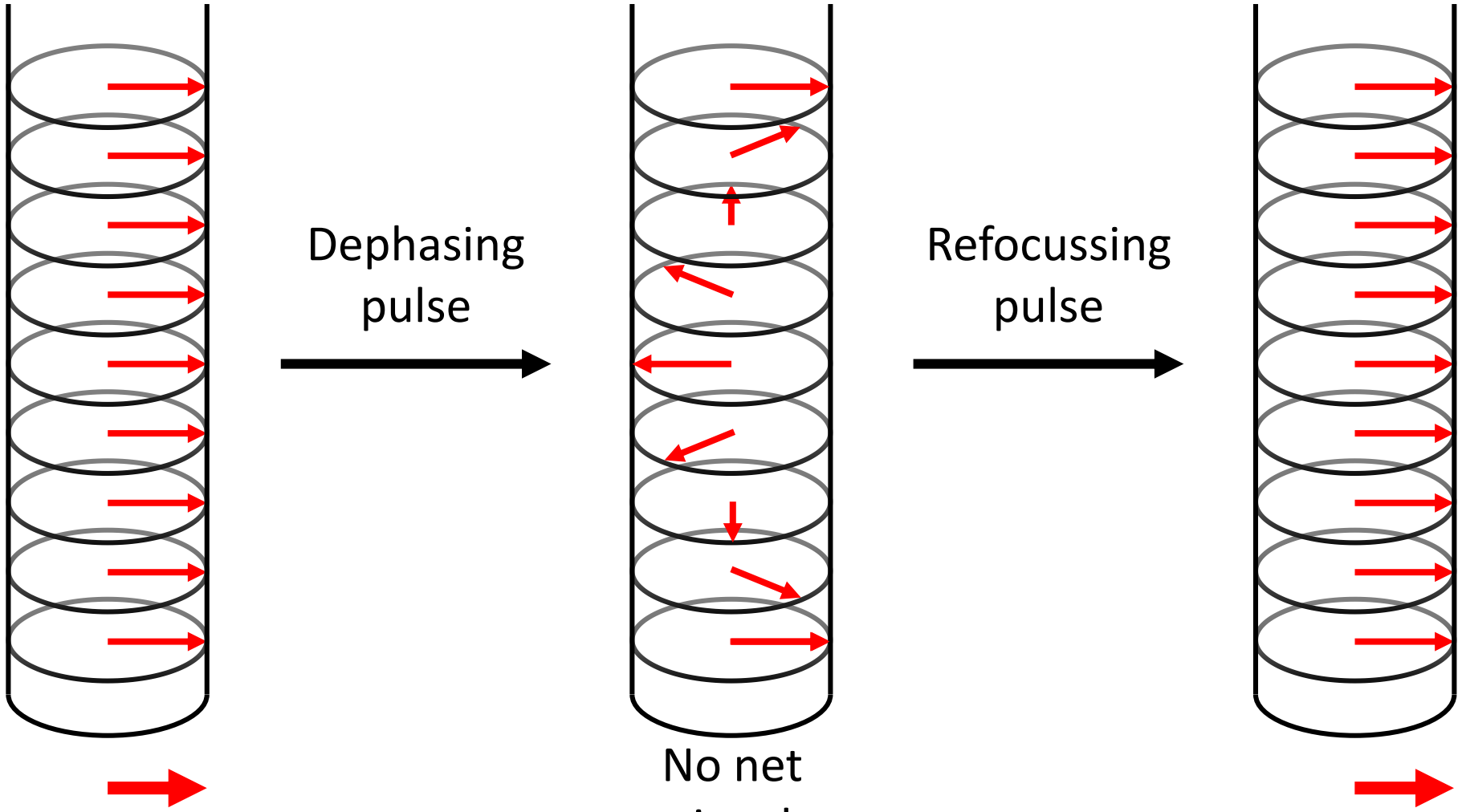
Can be used to differentiate between species of varying sizes in the same spectrum.

1D, **2D** or 3D NMR Technique

^{1}H, ^{19}F, ^{31}P …

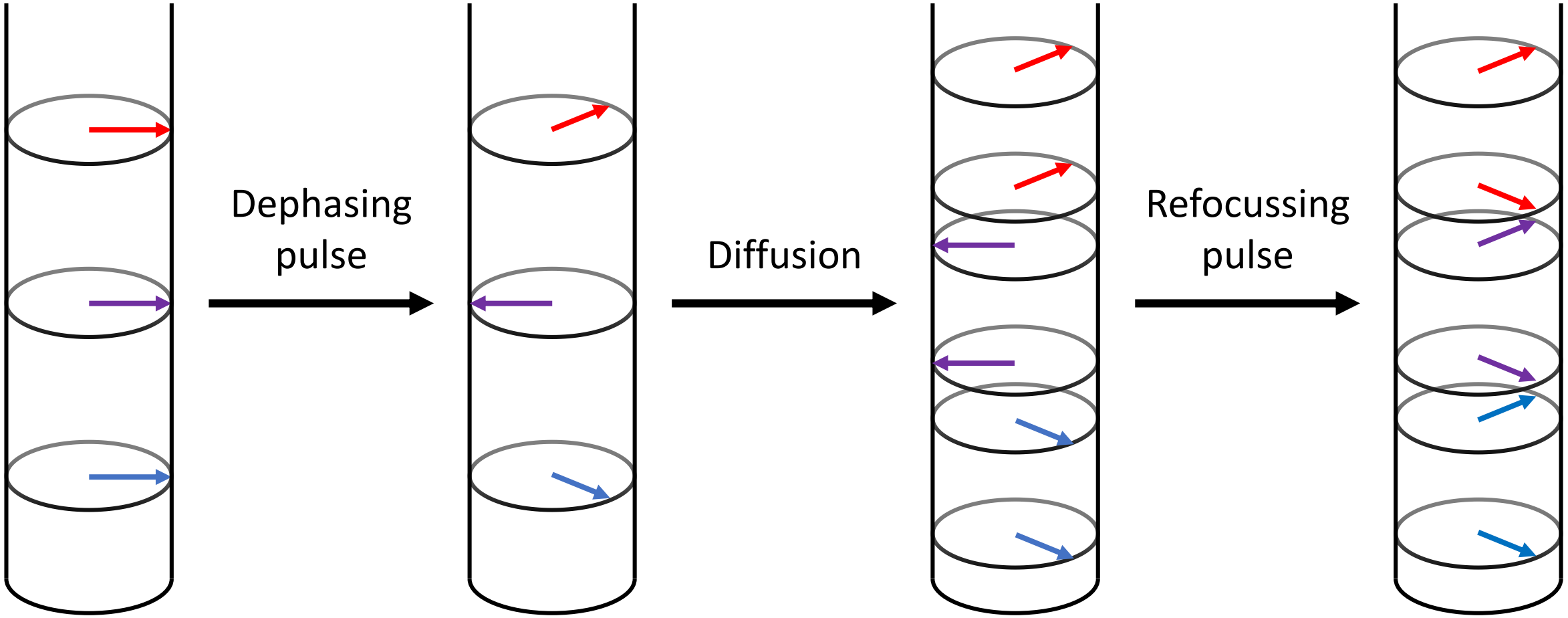
How does it work?

- Pulsed Field Gradients



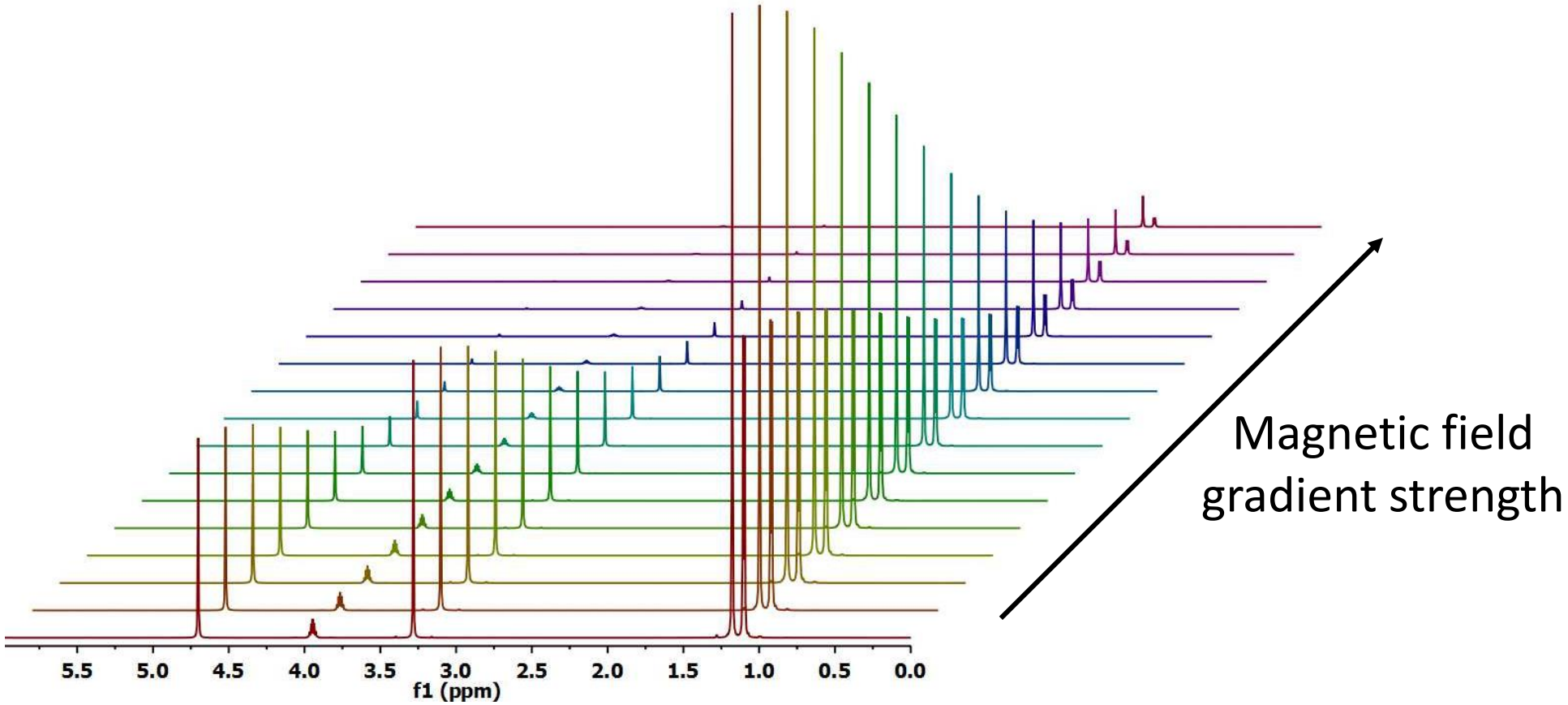
How does it work?

- The effect of diffusion:



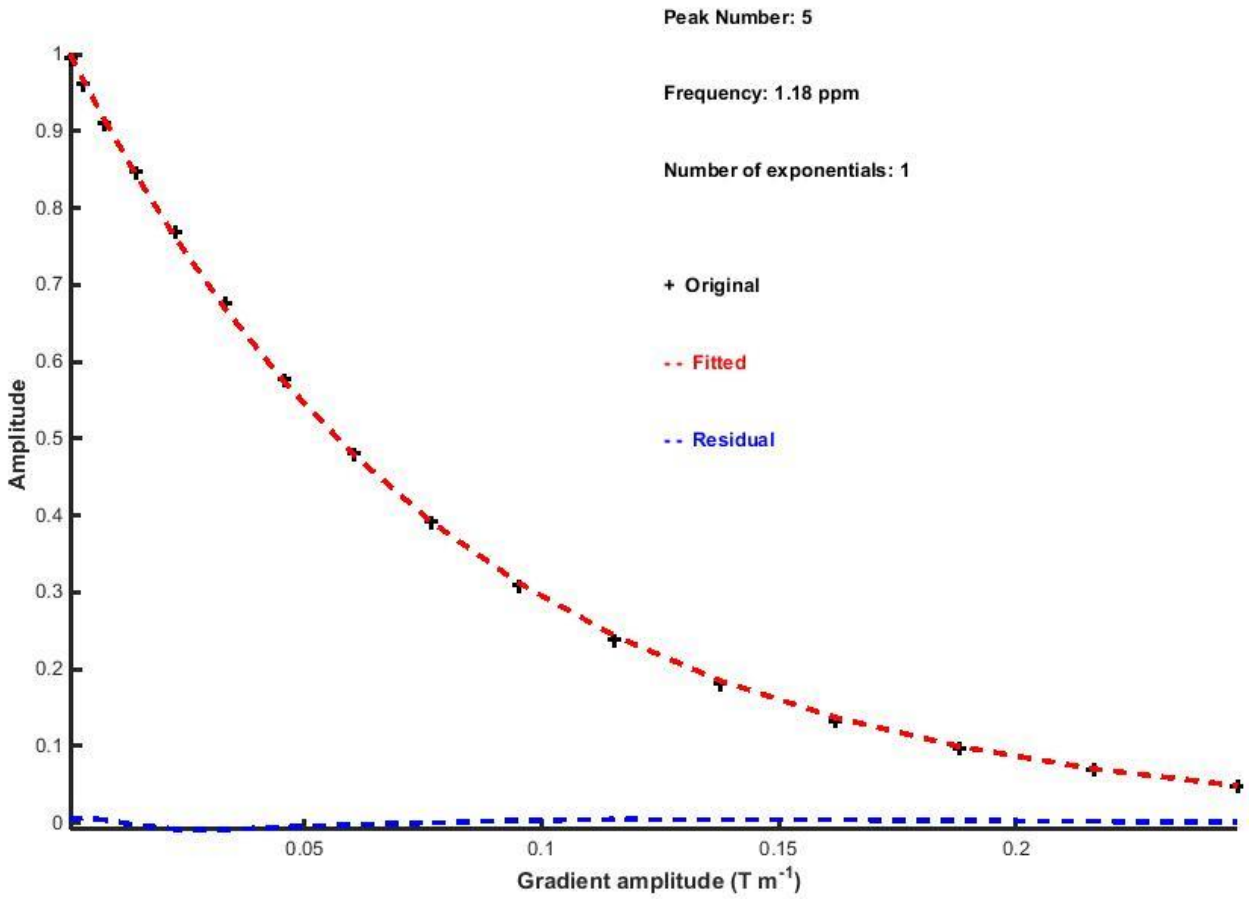
What is measured?

- Normally the gradient field strength is increased over 16 increments



Gaussian Distribution

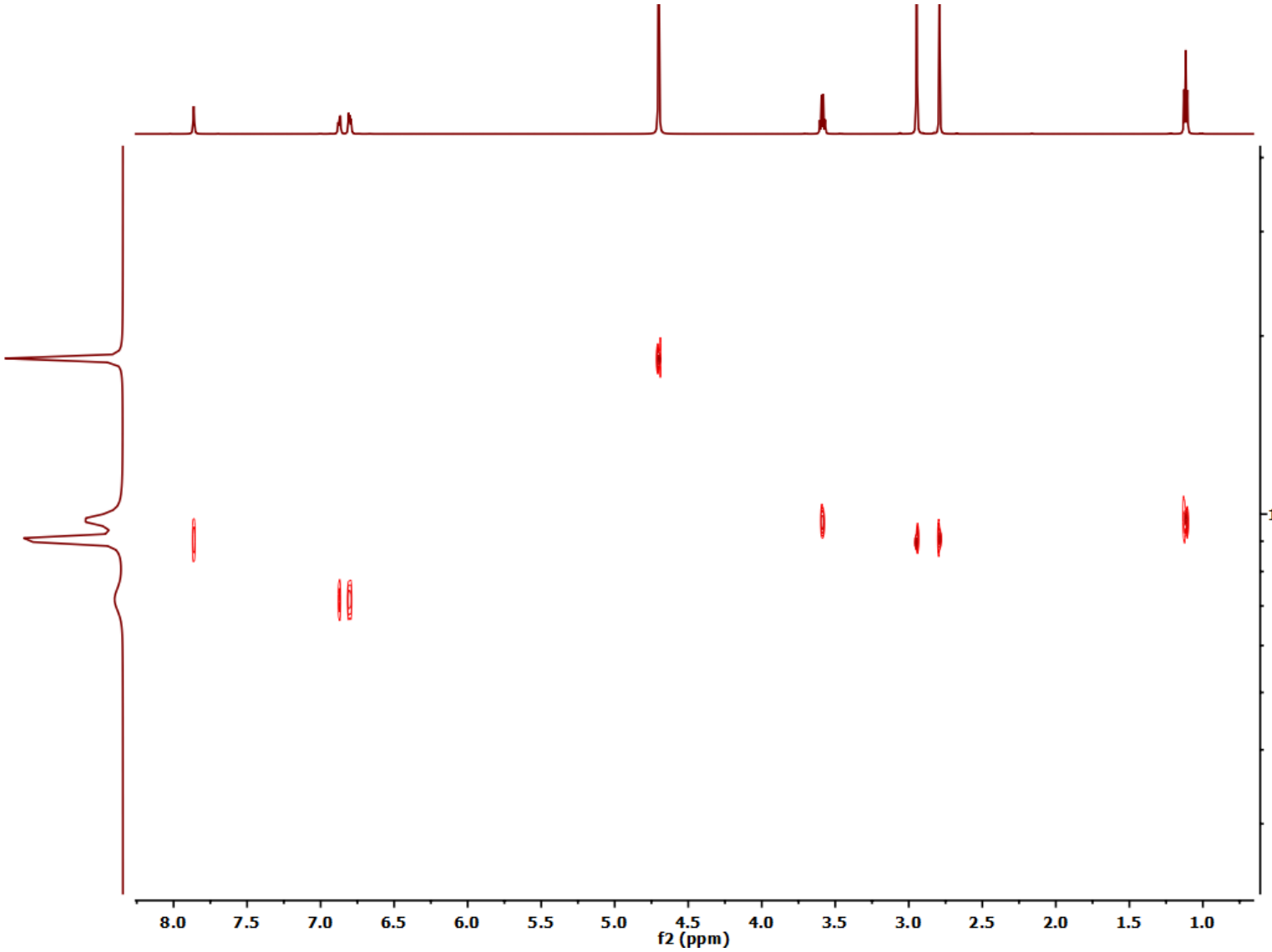
- The peak intensities can be fitted to a Gaussian decay to give the diffusion coefficient (D)



$$I = I_0 e^{-DQ}$$

$$Q = \gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)$$

Diffusion Coefficient



Larger diffusion coefficient – smaller molecule

Y-axis = Diffusion coefficient ($cm^2\ s^{-1}$)

Smaller diffusion coefficient – larger molecule

Stokes-Einstein Equation

Assuming the molecule is **spherical** in shape:

$$\mathrm{D} = \frac{k_\mathrm{B}\mathrm{T}}{6\pi\eta\mathrm{r_s}}$$

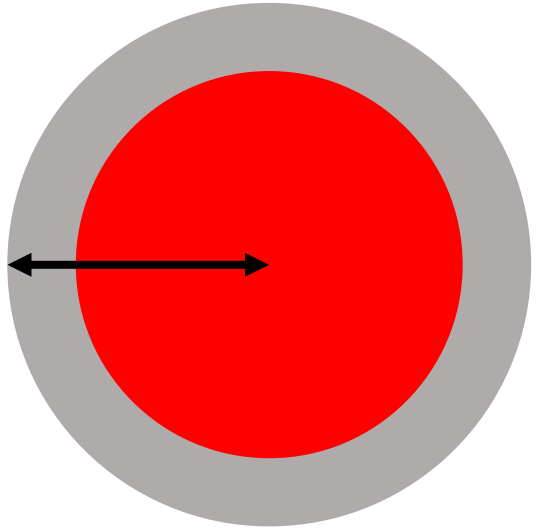
Where:

- D = Diffusion Coefficient ($m^2\ s^{-1}$ but often given in $cm^2\ s^{-1}$)
- k_B = Boltzmann Constant ($1.4 \times 10^{-23}\ m^2\ kg\ s^{-2}\ K^{-1}$)
- T = Temperature (K, usually 300 K in Edinburgh's NMR spectrometers)
- η = Viscosity (Pa s = $kg\ s^{-1}\ m^{-1}$ but often given in mPa s)
- r_s = Hydrodynamic radius of the molecule (m)

Hydrodynamic Radius

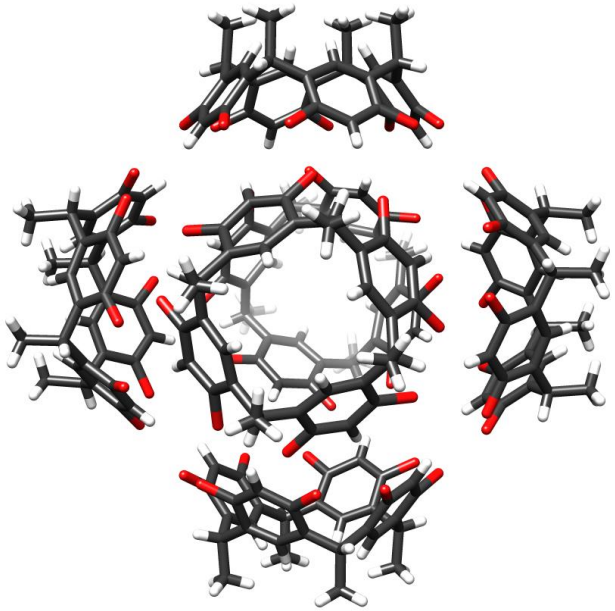
- The Stokes-Einstein equation assumes **perfect spheres** that are larger than solvent molecules
  - Large non-spherical compounds diffuse slower
  - Very small compounds diffuse faster
- Radius of the compounds includes **solvation sphere**
  - Different solvents will give different values
  - Different solvents will have different interactions

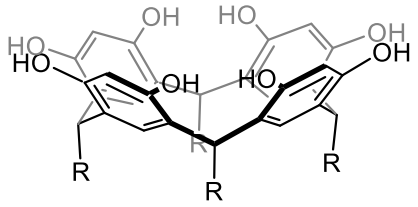
Hydrodynamic Radius (r_s)



Calculating hydrodynamic radius

Can calculate the hydrodynamic radius using Einstein-Stokes equation:





Resorcin[4]arene

20 mM in *d*-Chloroform

$$D = \frac{k_B T}{6\pi\eta r_s}$$

Which rearranges to:

$$r_s = \frac{k_B T}{6\pi\eta D}$$

Where:

$$k_B = 1.4 \times 10^{-23}\ \mathrm{J\ K^{-1}}$$
$$T = 300\ \mathrm{K}$$
$$\eta = 0.58\ \mathrm{mPa\ s}$$
$$D = 2.47 \times 10^{-6}\ \mathrm{cm^2\ s^{-1}}$$

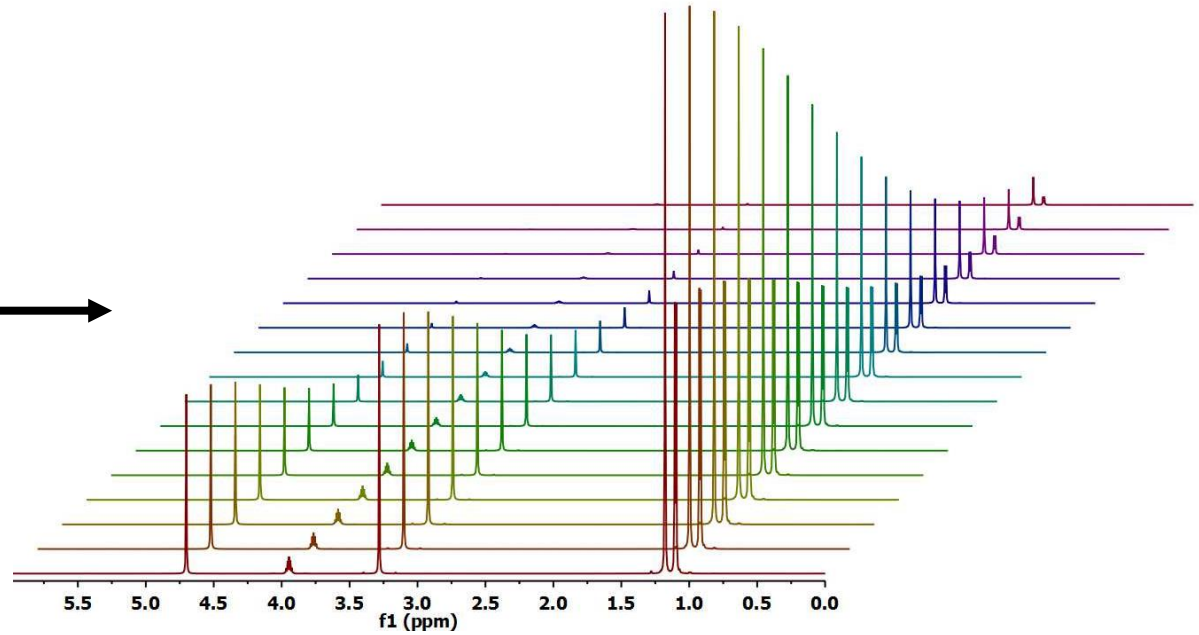
Therefore:

$$r_s = \frac{1.4 \times 10^{-23} \times 300}{6\pi \times 0.58 \times 10^{-3} \times 2.47 \times 10^{-10}} = 15.4\ \text{Å}$$

Processing the data (in MestReNova)

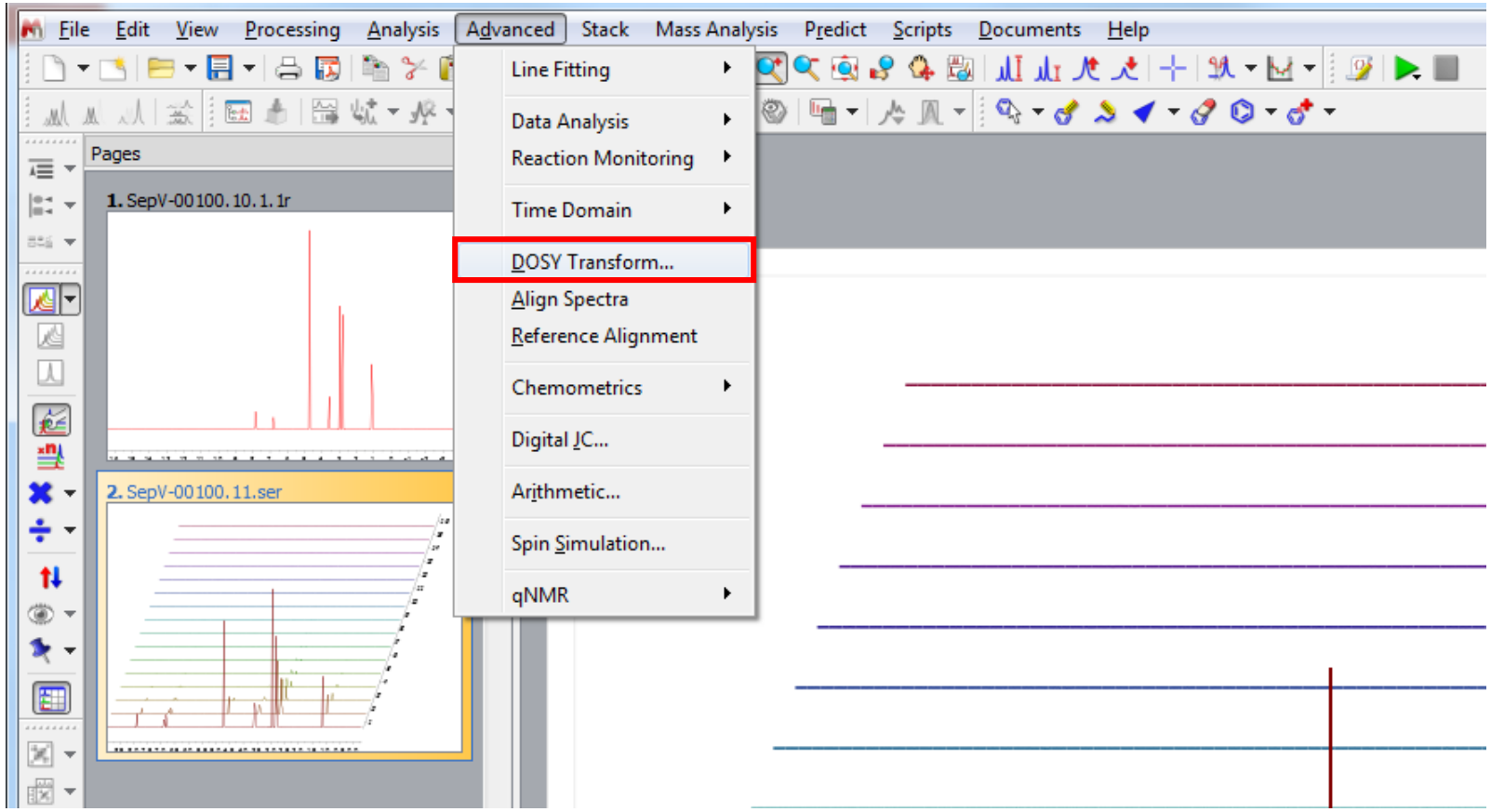
- Open the “ser” file

Name	Date modified	Type	Size
pdata	28/11/2017 08:56	File folder	
acqu	03/09/2015 11:02	File	9 KB
acqu2	03/09/2015 11:02	File	1 KB
acqu2s	03/09/2015 11:02	File	1 KB
acqum	03/09/2015 11:02	File	1 KB
acqus	03/09/2015 11:02	File	9 KB
audita	03/09/2015 11:02	Text Document	1 KB
difflist	03/09/2015 11:02	File	1 KB
format.temp	03/09/2015 11:02	TEMP File	4 KB
orig	03/09/2015 11:02	File	1 KB
prosol_History	03/09/2015 11:02	File	0 KB
pulseprogram	03/09/2015 11:02	File	7 KB
scon2	03/09/2015 11:02	File	1 KB
ser	03/09/2015 11:02	File	2,048 KB
shimvalues	03/09/2015 11:02	File	3 KB
specpar	03/09/2015 11:02	File	3 KB
uxnmr.info	03/09/2015 11:02	INFO File	5 KB
uxnmr.par	03/09/2015 11:02	PAR File	23 KB



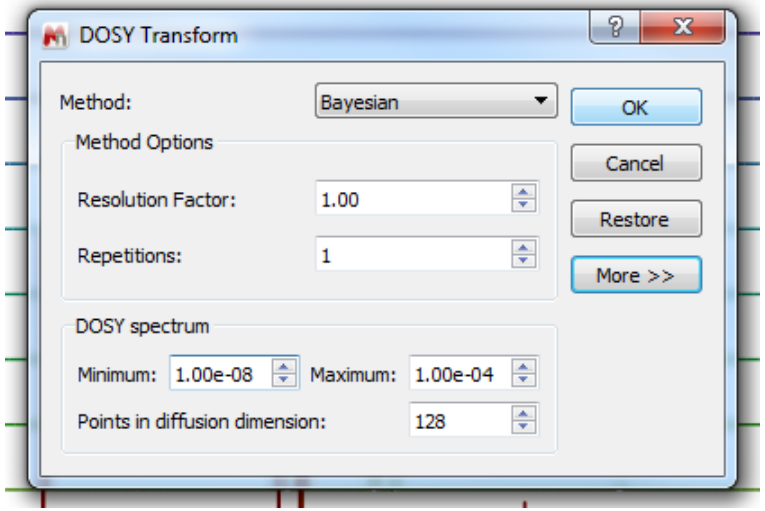
Processing the data (in MestReNova)

- Advanced > DOSY Transform…



Processing the data (in MestReNova)

Generally, the default settings should work well



Resolution factor:

- Start at 1

Repetitions:

- Start at 1

Try to avoid increasing these too much – may lead to artefact peaks

Minimum/maximum:

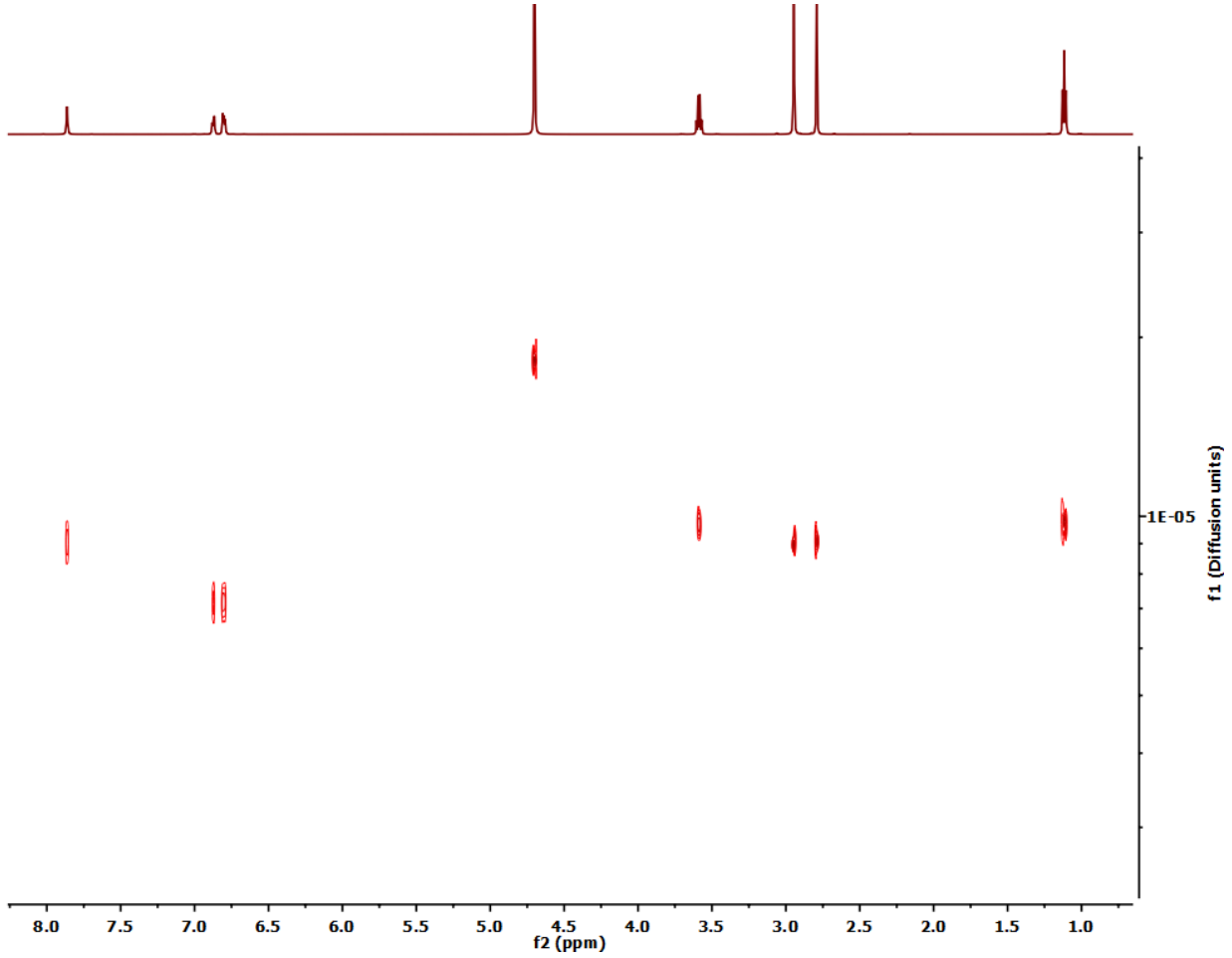
- Range of diffusion coefficient to be processed for

Points in diffusion dimension:

- Increasing may lead to a better resolved spectrum but will be computationally more expensive – Edinburgh's DOSYs have 512 points
  - Use 128, 256 or 512

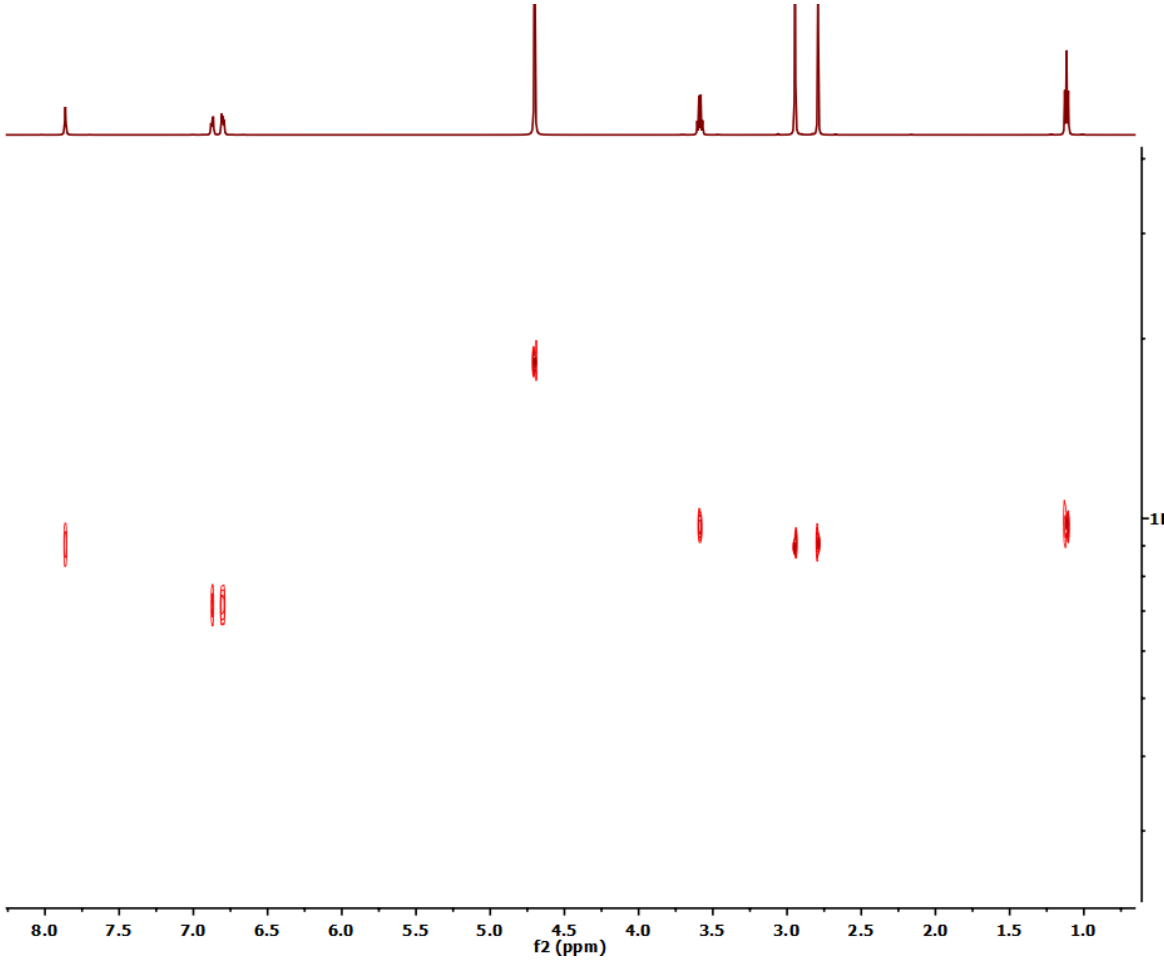
Processing the data (in MestReNova)

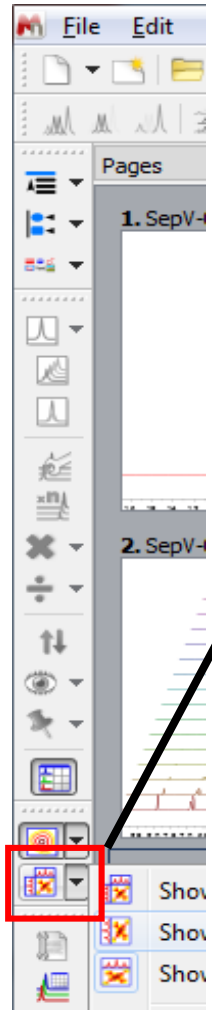
- Processed data

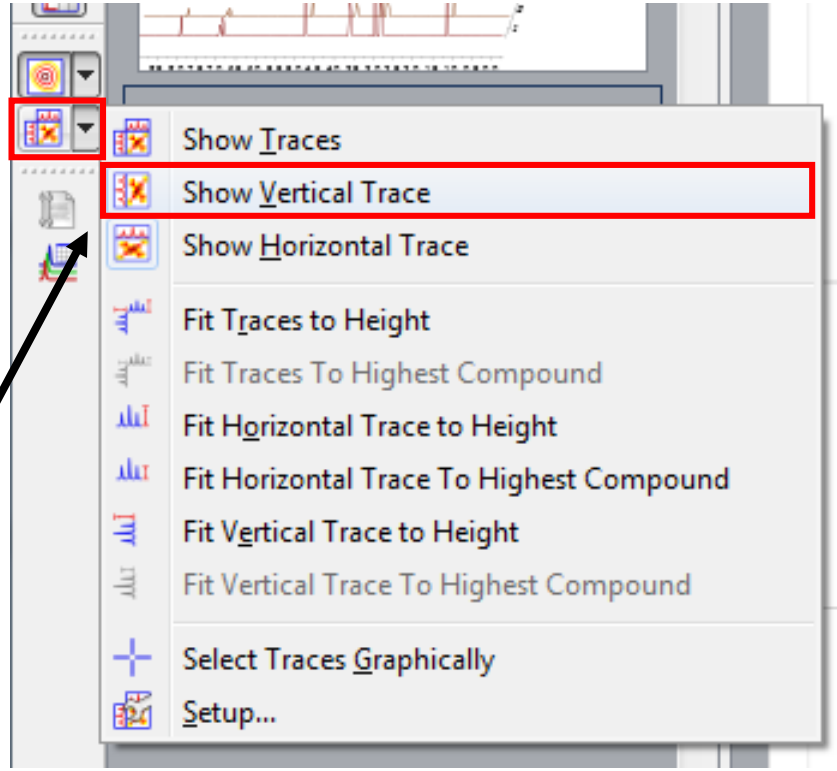


Processing the data (in MestReNova)

- Showing the vertical trace

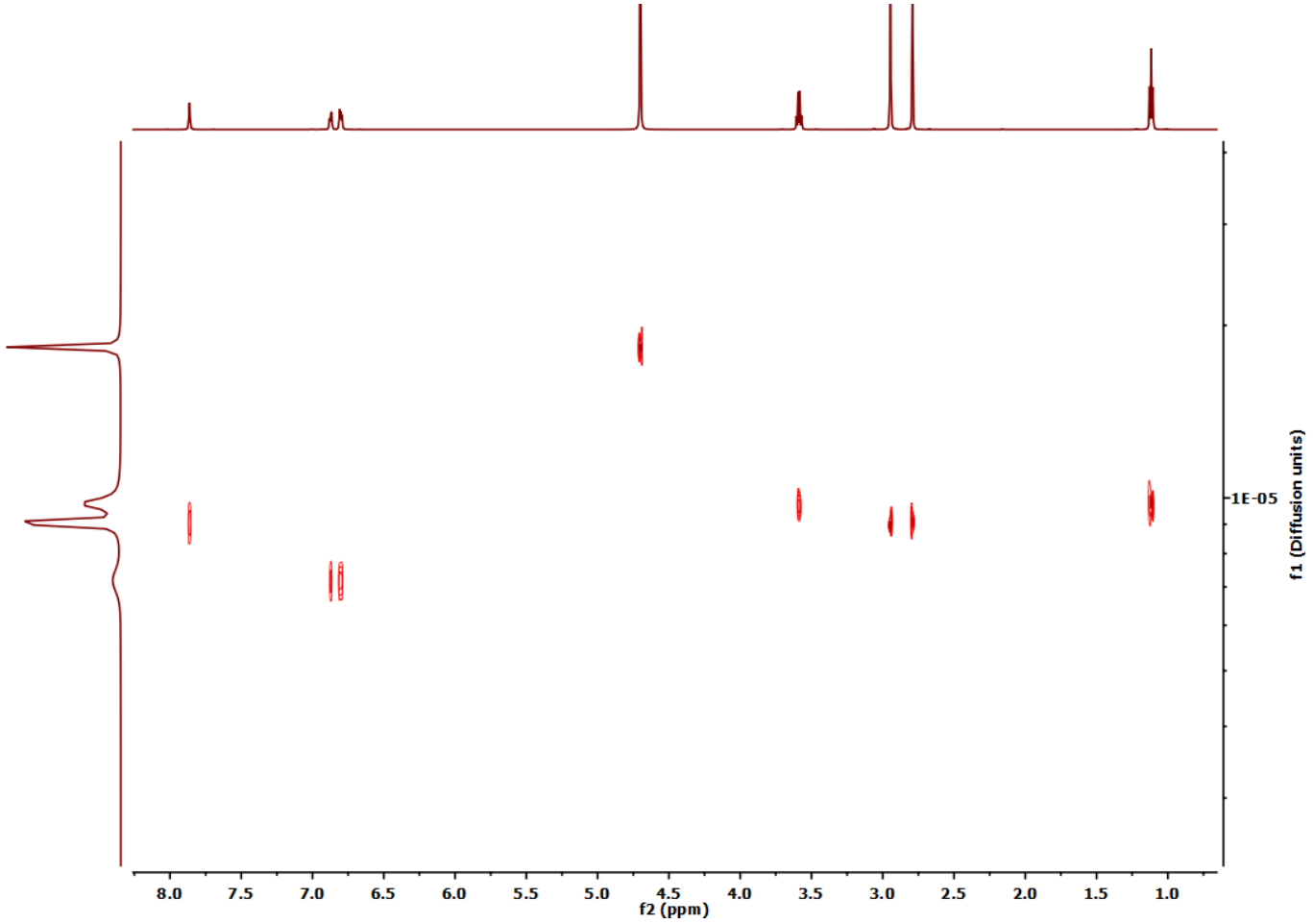






Identification of mixtures

- Four different sized species present in the spectrum



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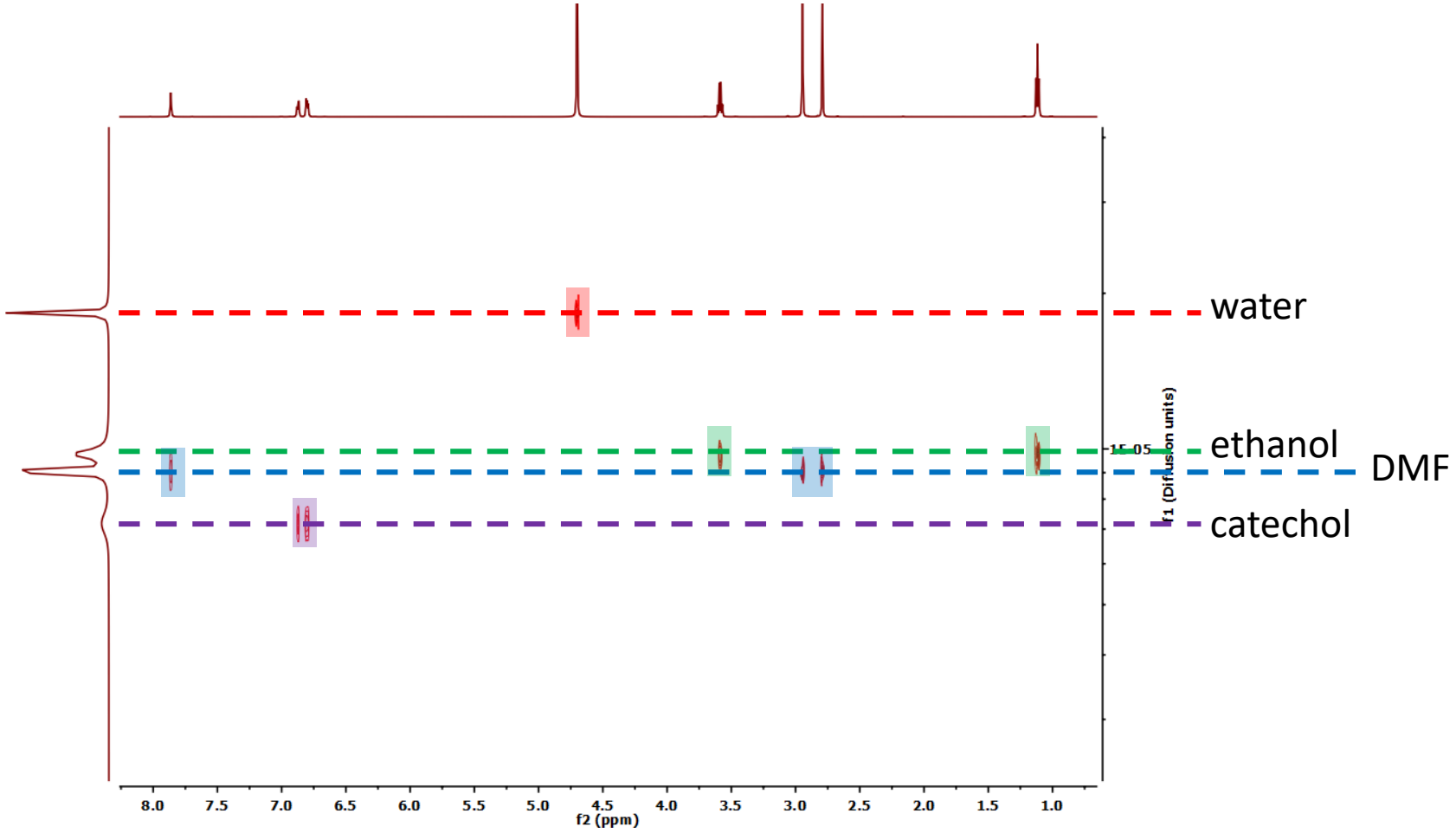
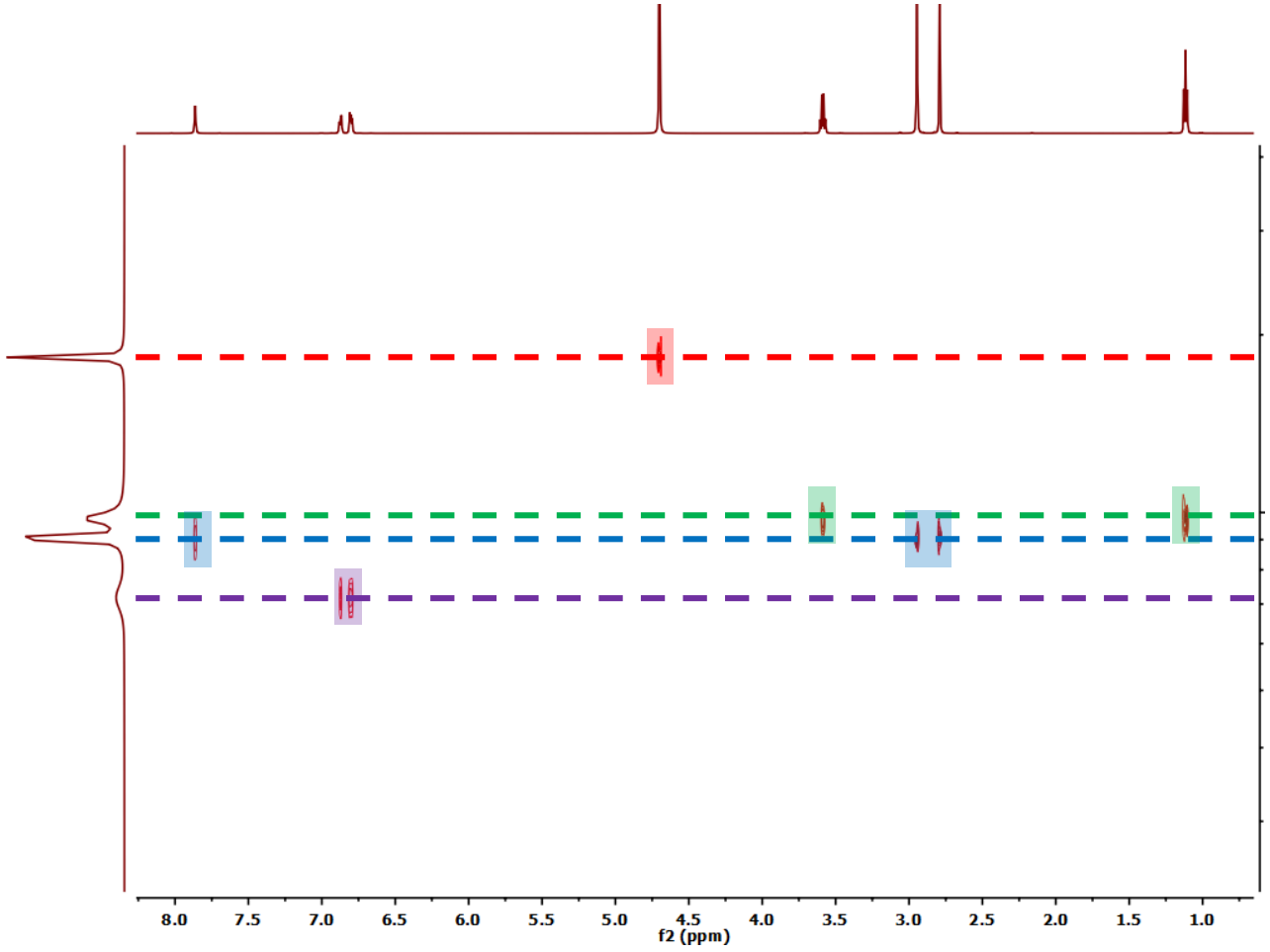
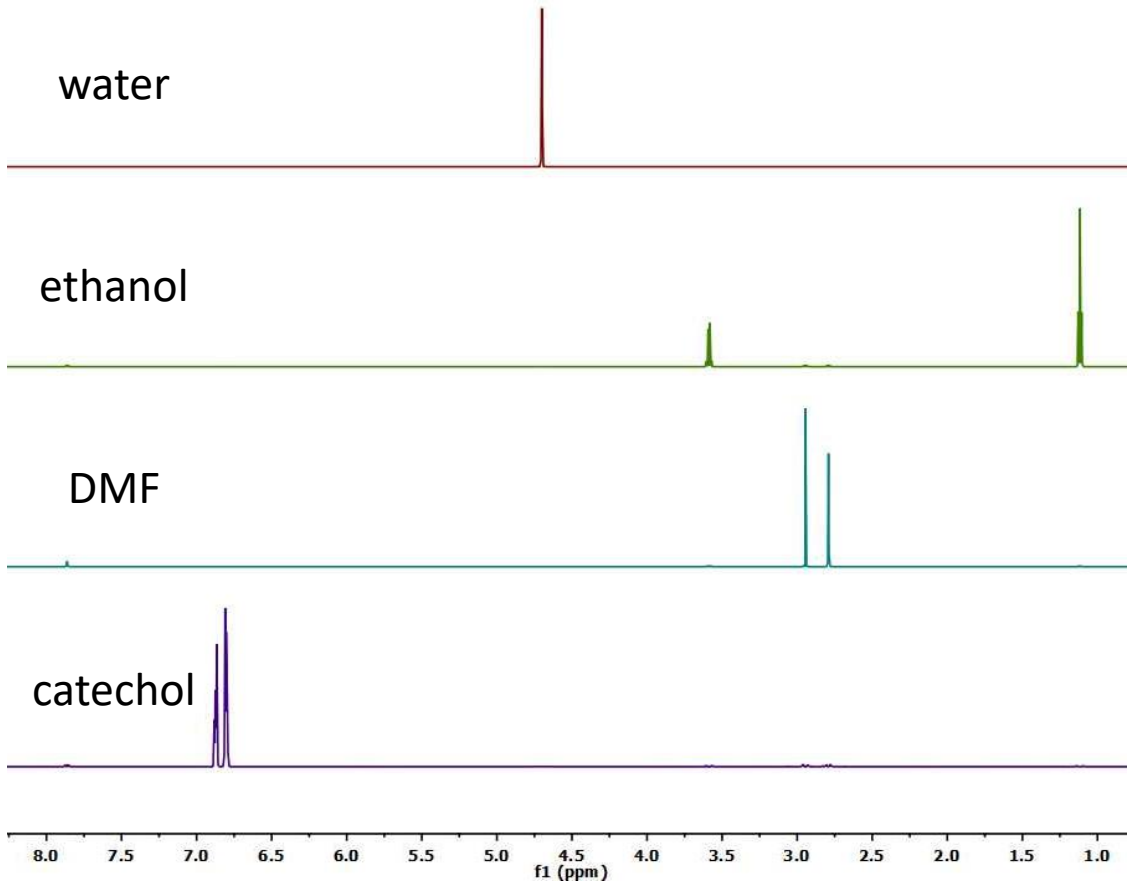


Identification of mixtures

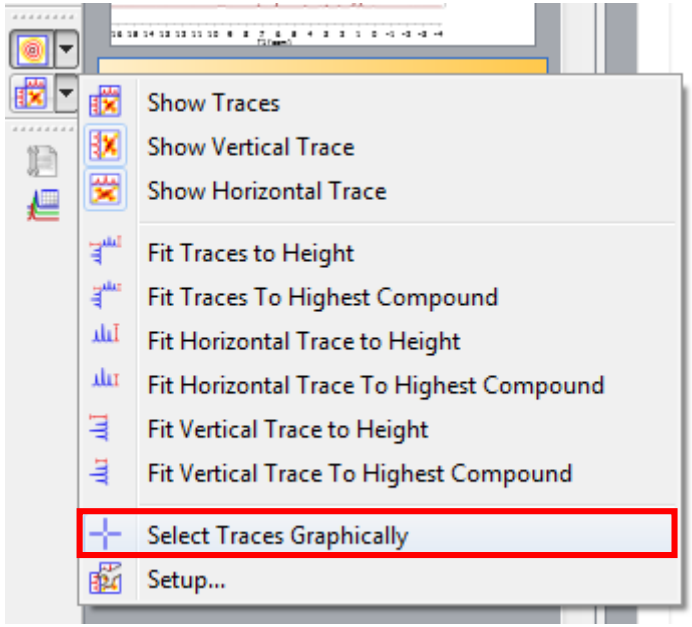
- Can abstract ^{1}H NMR spectra from DOSY plot

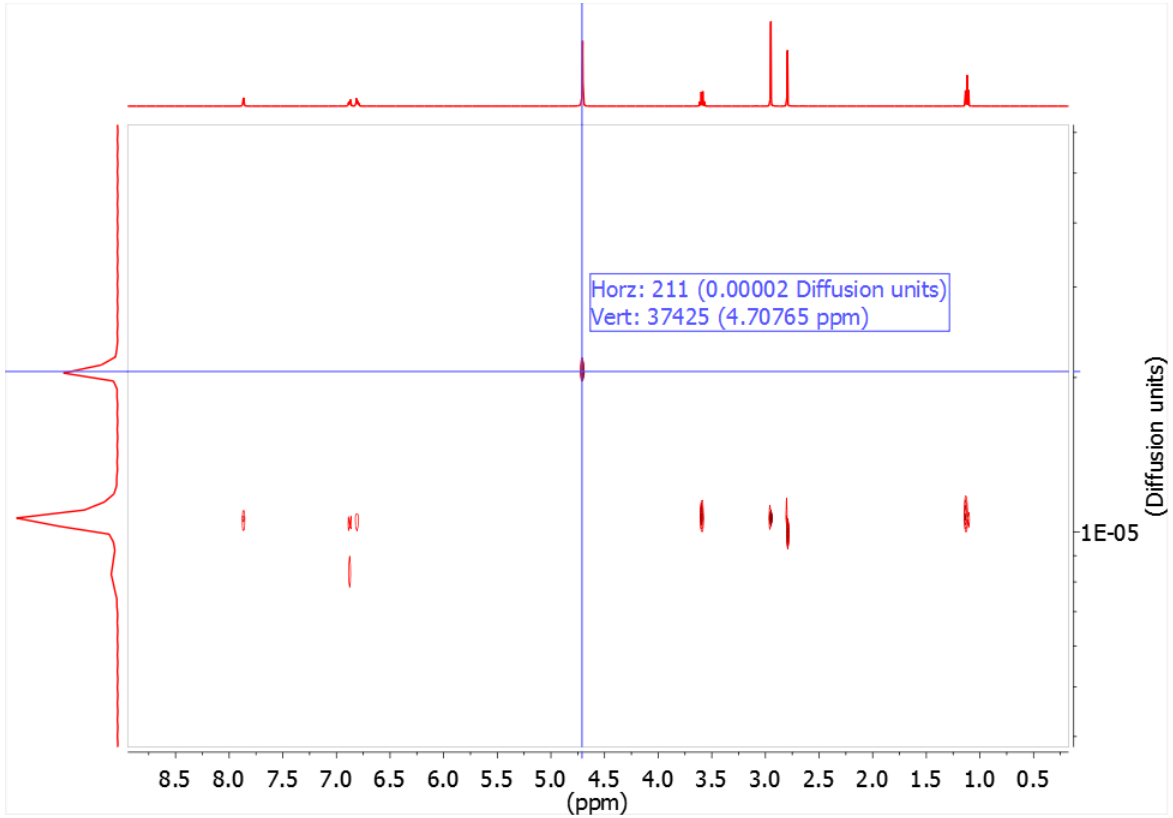




Abstracting spectra (using MestReNova)

- Can select peaks correlating to individual diffusion coefficients





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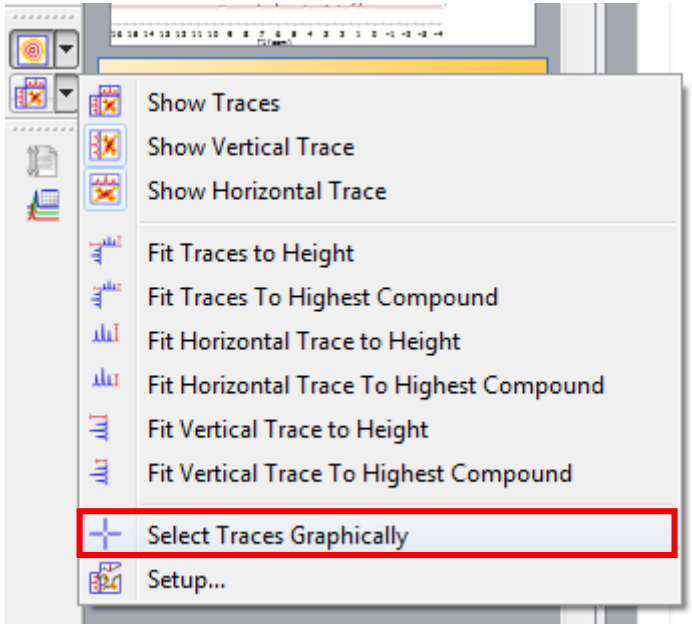

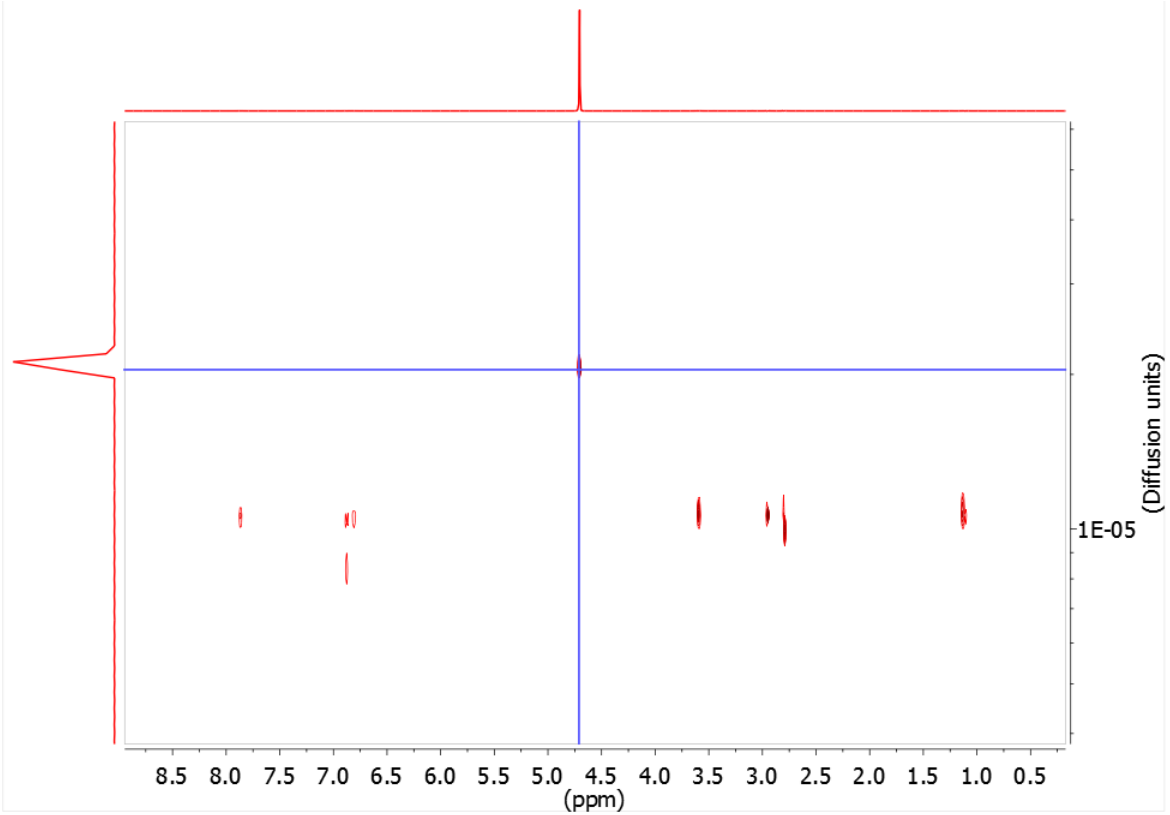

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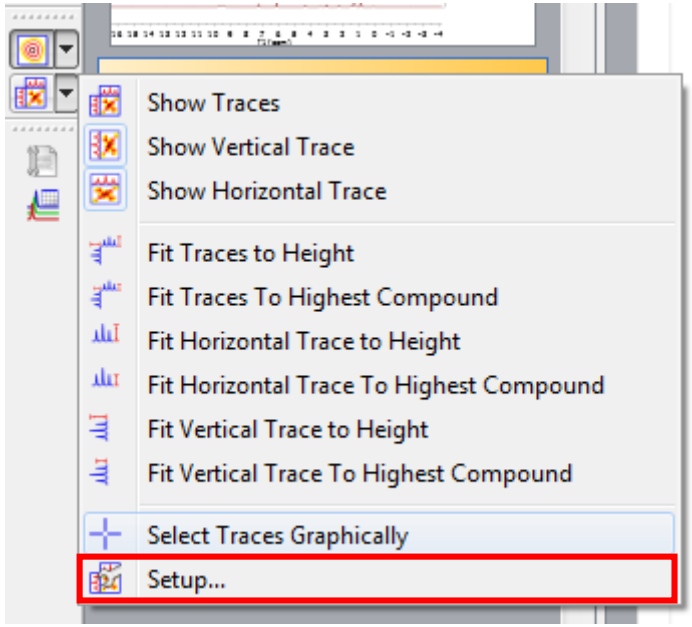
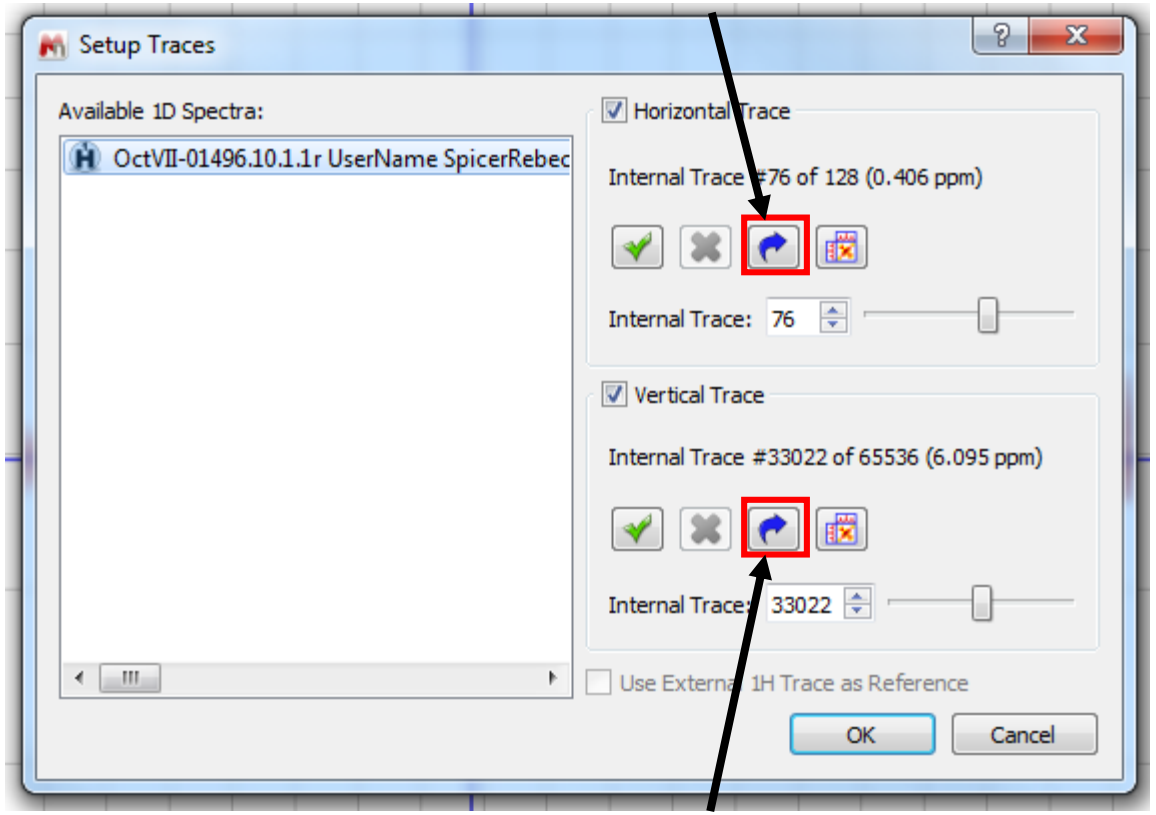


Abstract horizontal trace



Abstract vertical trace

Practical Considerations

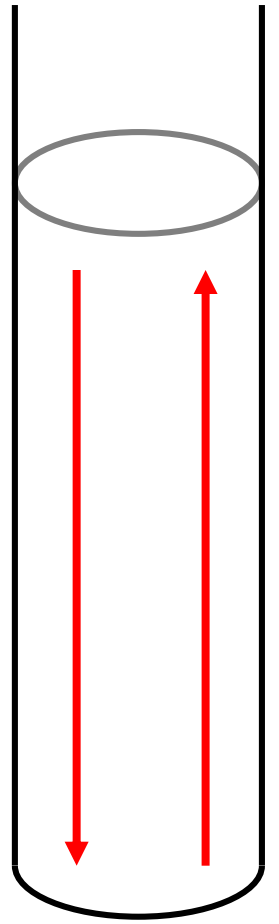
- Concentration
- Convection
- Overlapping peaks
  - Programs

Concentration

- Use roughly 10 mM minimum
  - Weaker samples are undetectable or cause artefact peaks during processing
- Longer experiments are available with more field strength increments
  - Ava500 preferential for ^{1}H DOSY over Ava600 and Ava400
  - 128 increments (2 h 20 min night DOSY) instead of 16 (20 min day DOSY)

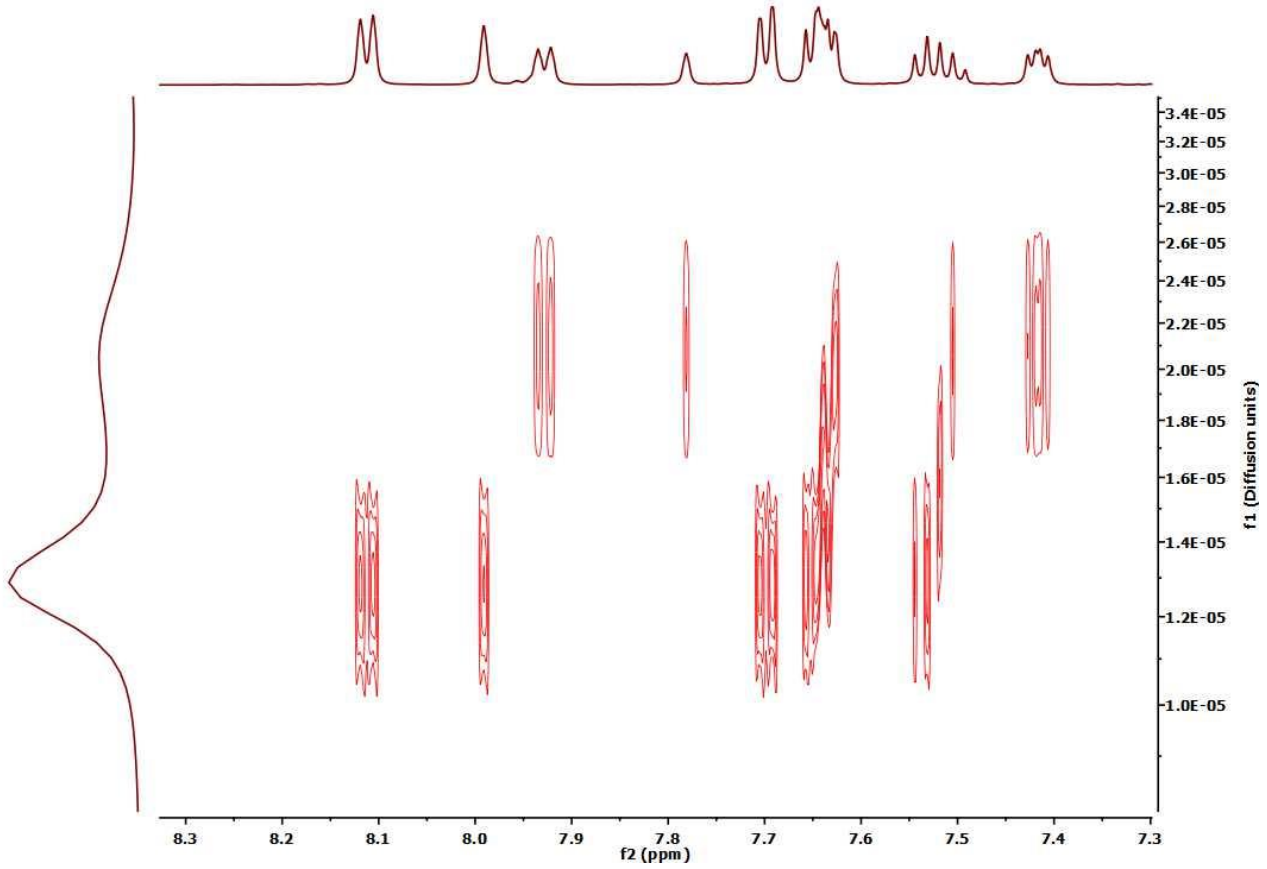
Convection

- Convection currents can disrupt diffusion
  - Use the lowest volume required
  - Use thick walled NMR tubes
- Very minimal at room temperature
  - Unless using low boiling solvent
  - Need to consider for higher/variable temperature (VT) experiments



Overlapping Peaks

- Difficult to solve multi-exponential decay



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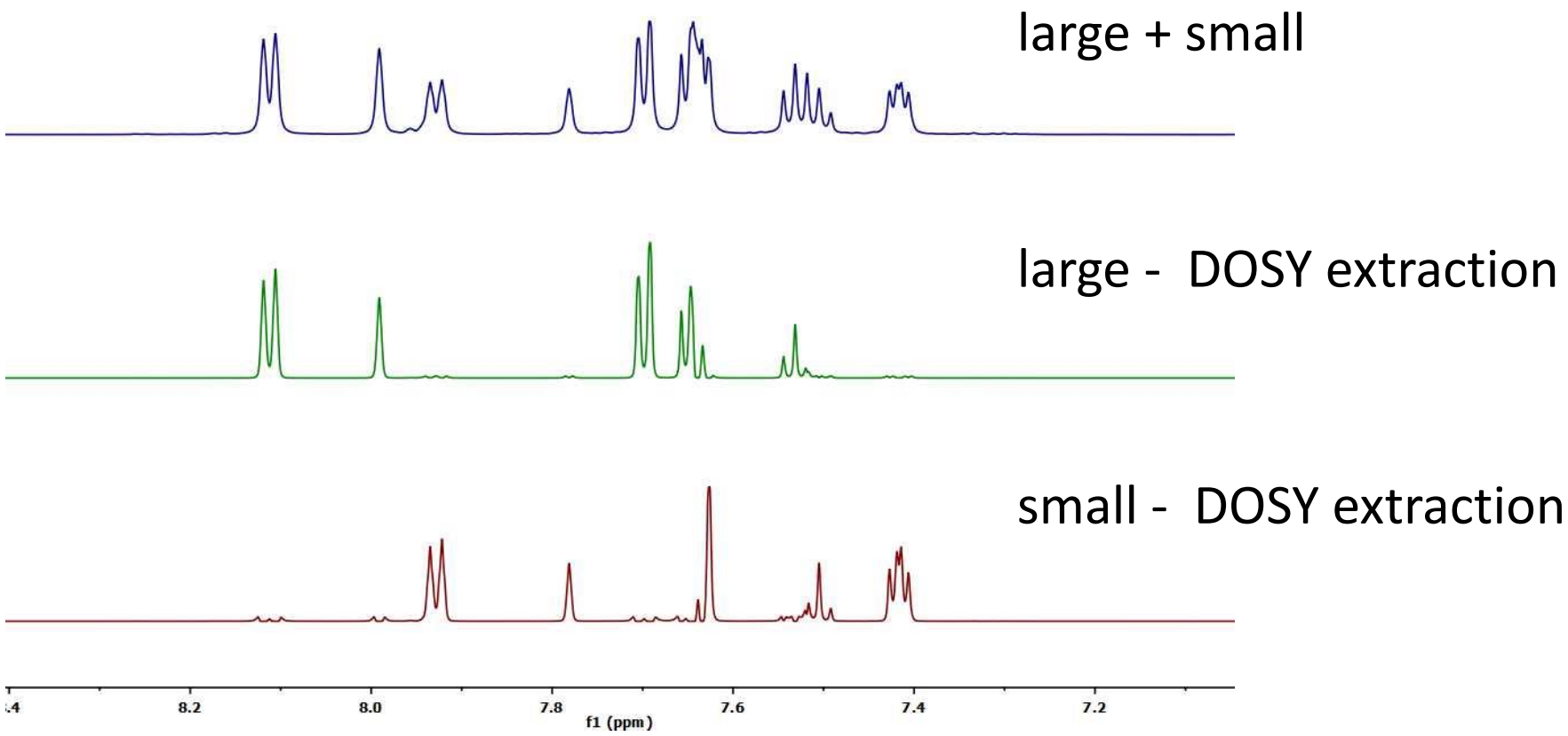


Overlapping Peaks

- Multi-exponential fitting functions are available
- Can vary the different parameters to see if it improves resolution
- Pureshift-DOSY
  - Time consuming and has sensitivity issues

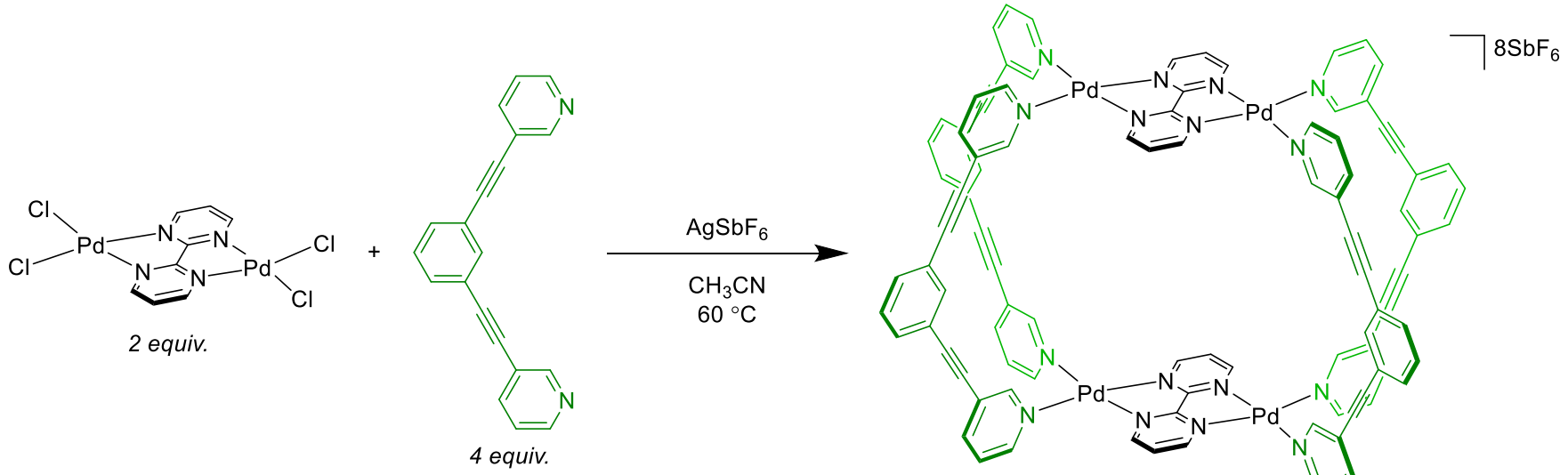
Processing

- Multiple programs are available
  - MestReNova
  - Topspin
  - Dynamic Centre
  - DOSY Toolbox

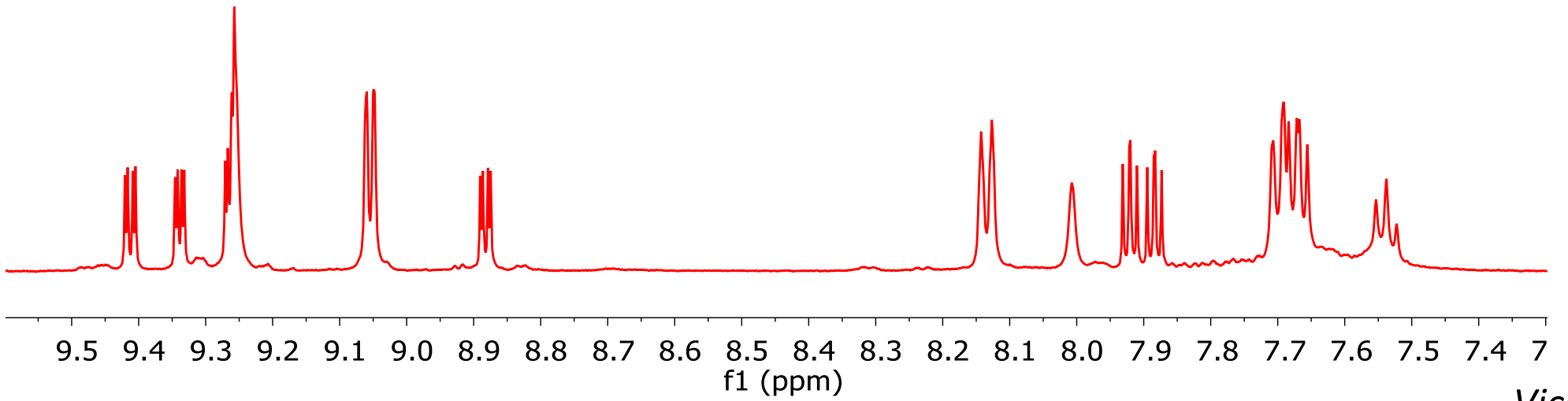
Applications

- Identification of mixtures
  - Crude reaction mixtures
- Supramolecular interactions
  - Dimerisation processes
  - Self-assembly processes

Application: Successful supramolecular assembly?



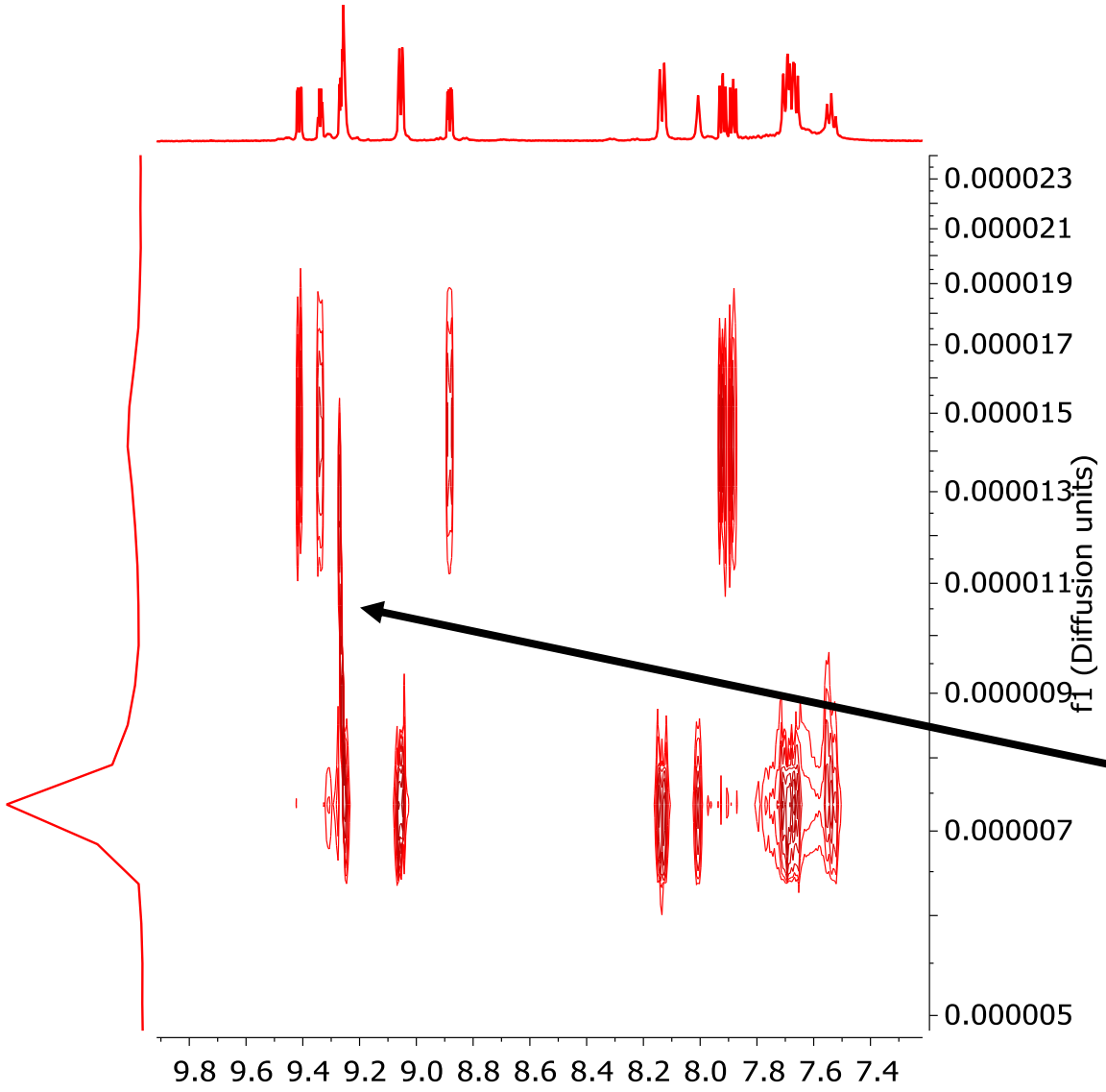
Expect 9 ^{1}H NMR signals



More than 9 signals observed…

Vicente Marti Centelles

Application: Successful supramolecular assembly?

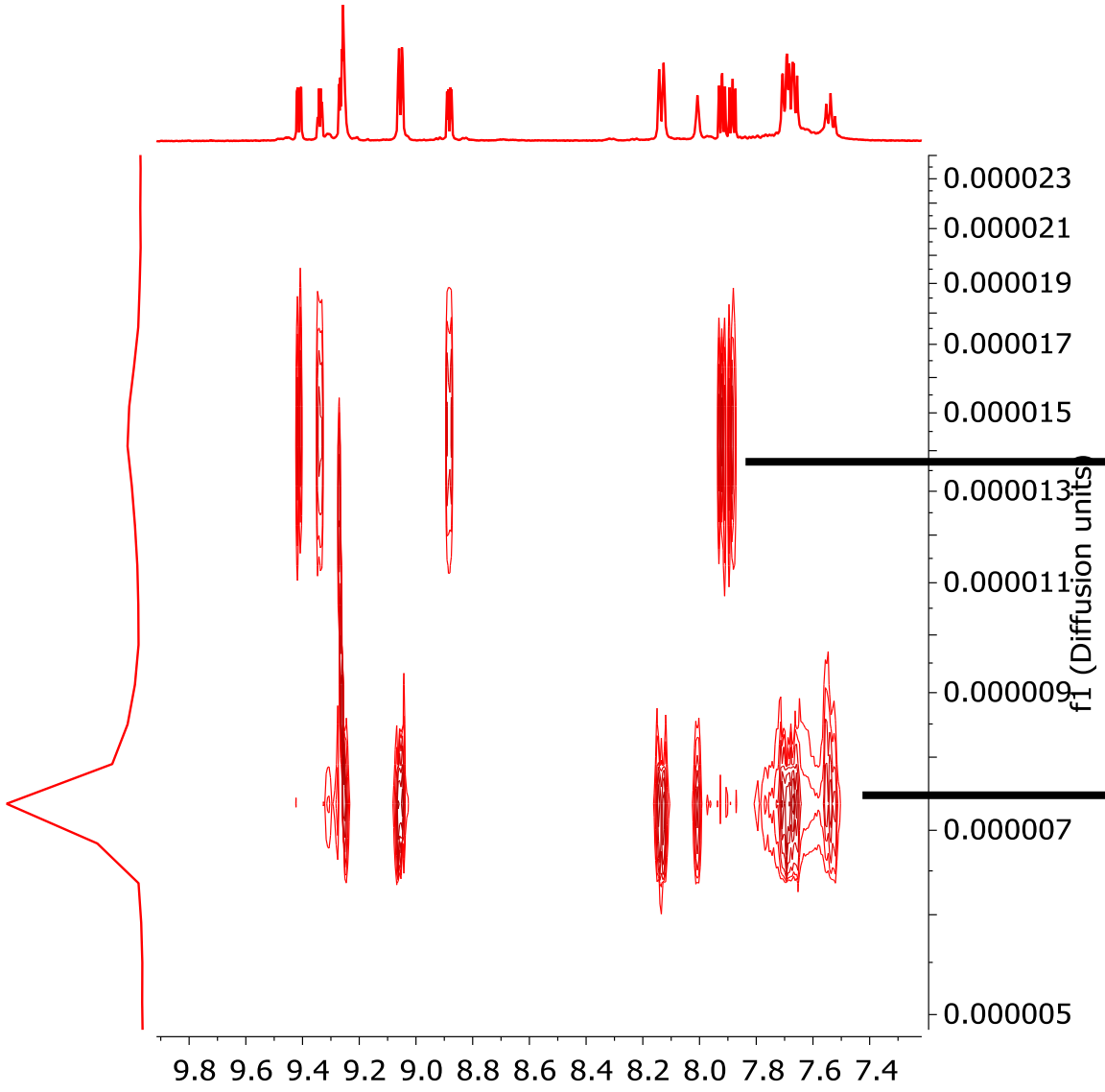


DOSY NMR indicated two different species present

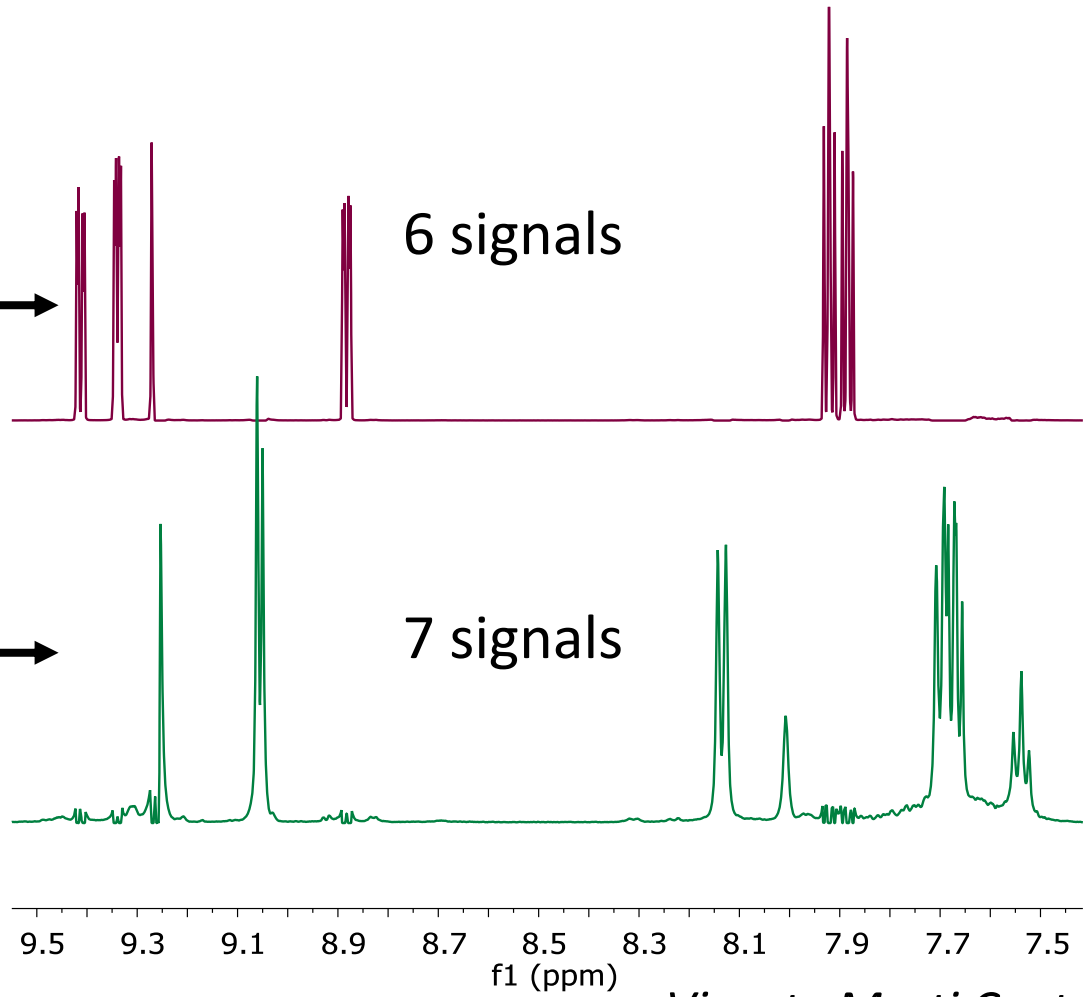
Two overlapping peaks give a broad signal across both species

Vicente Marti Centelles

Application: Successful supramolecular assembly?

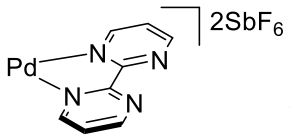


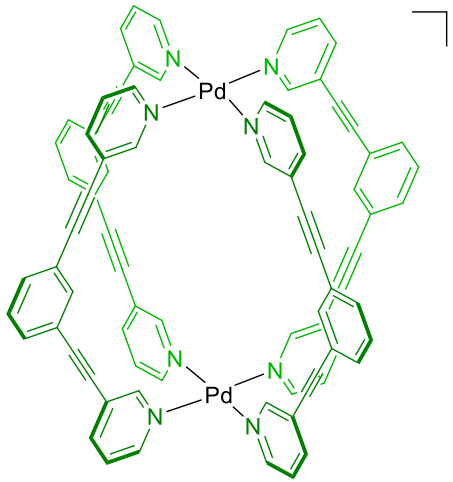
Abstracted NMR spectra:



Vicente Marti Centelles

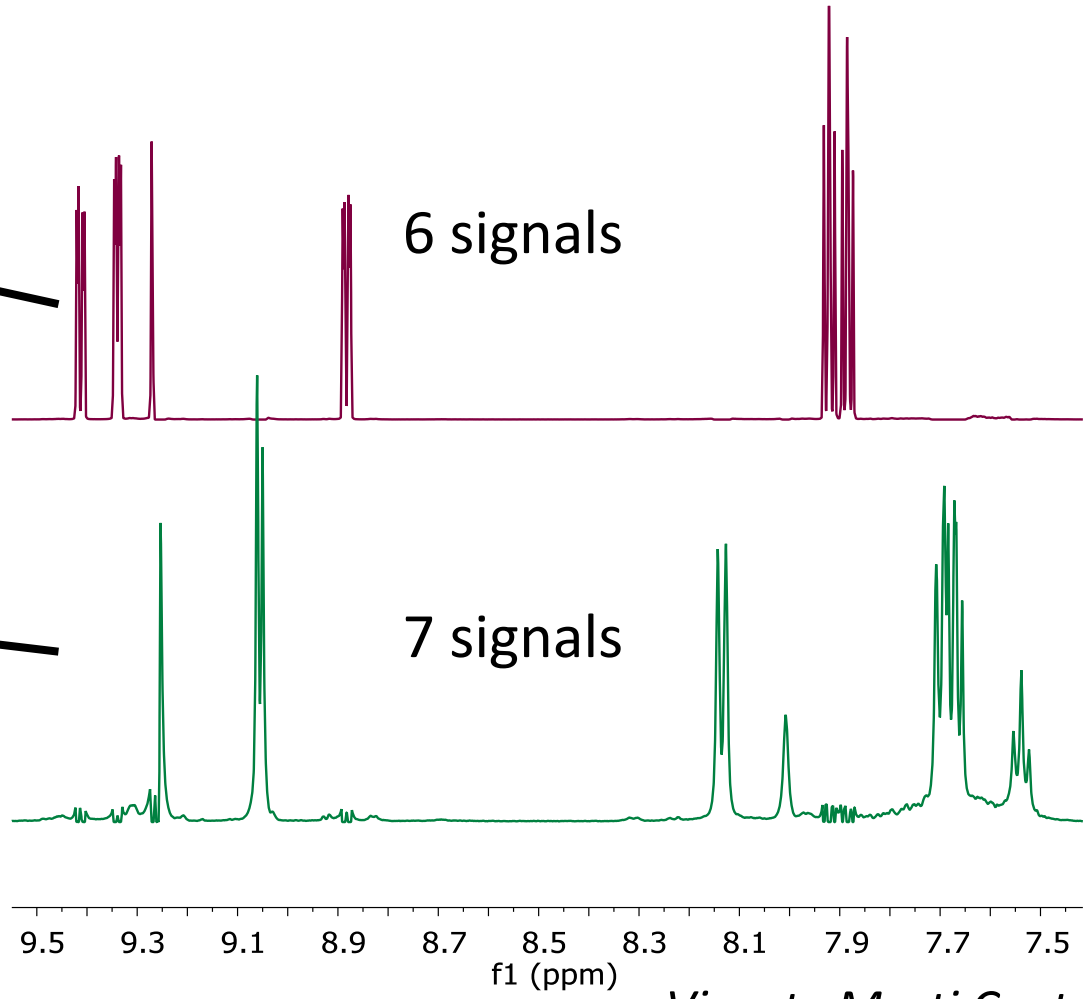
Application: Successful supramolecular assembly?





Abstracted NMR spectrum matches previously acquired spectrum of Pd_2L_4 assembly

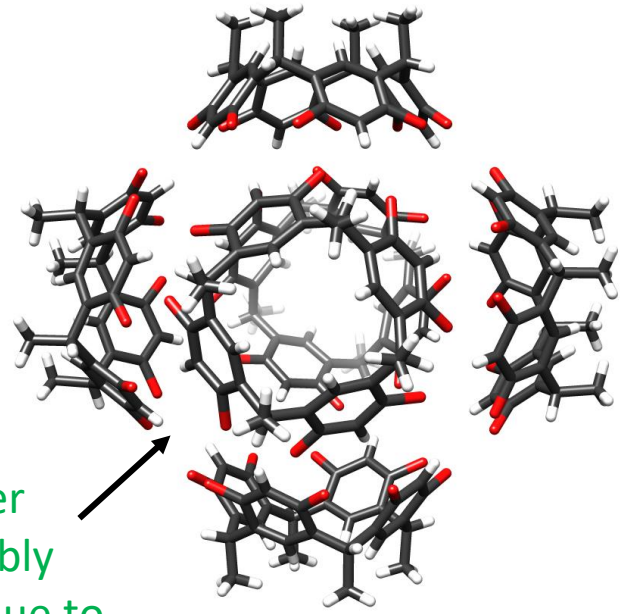
Abstracted NMR spectra:



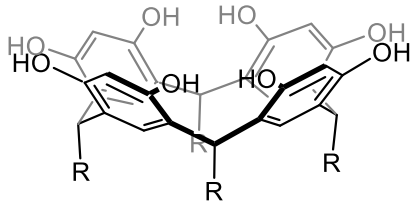
Vicente Marti Centelles

Application: Effect of solvent on hydrodynamic radius

Can calculate the hydrodynamic radius using Einstein-Stokes equation:



Larger assembly formed due to hydrogen bonding



Resorcin[4]arene

20 mM

Solvent	***d*-Chloroform**	***d_2*-Dichloromethane**	***d_6*-Acetone**
Viscosity (mPa s)	0.58	0.45	0.32
Diffusion coefficient ($cm^2\ s^{-1}$)	2.47×10^{-6}	2.47×10^{-6}	7.89×10^{-6}
Hydrodynamic radius (Å)	15.4	19.8	8.7

Hydrogen bonds formed between resorin[4]arene and residual water – formation of resorcin[4]arene hexamer in solution

Disrupts hydrogen bonding – monomeric resorcin[4]arene in solution

Automation

- These experiments are all set up in automation and generally give good results.

^{1}H DOSY NMR	^{19}F DOSY NMR
Use Ava500	Use Pro500
C DOSY 1H DOSY (16) 20 min day	C DOSY 19F DOSY (120) 2h night
C DOSY.long 1 H DOSY (128) 2 h 20 min night	

But if the results are bad **just ask** and

the parameters can be adjusted!

Useful Review Article

Pulsed-field gradient nuclear magnetic resonance measurements (PFG NMR) for diffusion ordered spectroscopy (DOSY) mapping

Analyst, 2017, **142**, 3771–3796

Thank you for listening!