MANCHESTER 1824
The University of Manchester

Introduction to diffusion NMR

Mathias Nilsson

NMR Methodology Group
University of Manchester

UNICAMP. Campinas, Brazil
April 17th , 2018

Diffusion NMR: today's programme

9.00-9.50	Introduction and Theory
9.50-10.10	Break
10:10-11.00	Acquisition, Analysis and Practicalities
11.00-11.30	Questions and Answers
11.30-14.00	Lunch
14.00-14.50	Advanced experiments
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15.00-15.50	Introduction to the GNAT Processing software
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17.00-17.30	Conclusion and open discussion

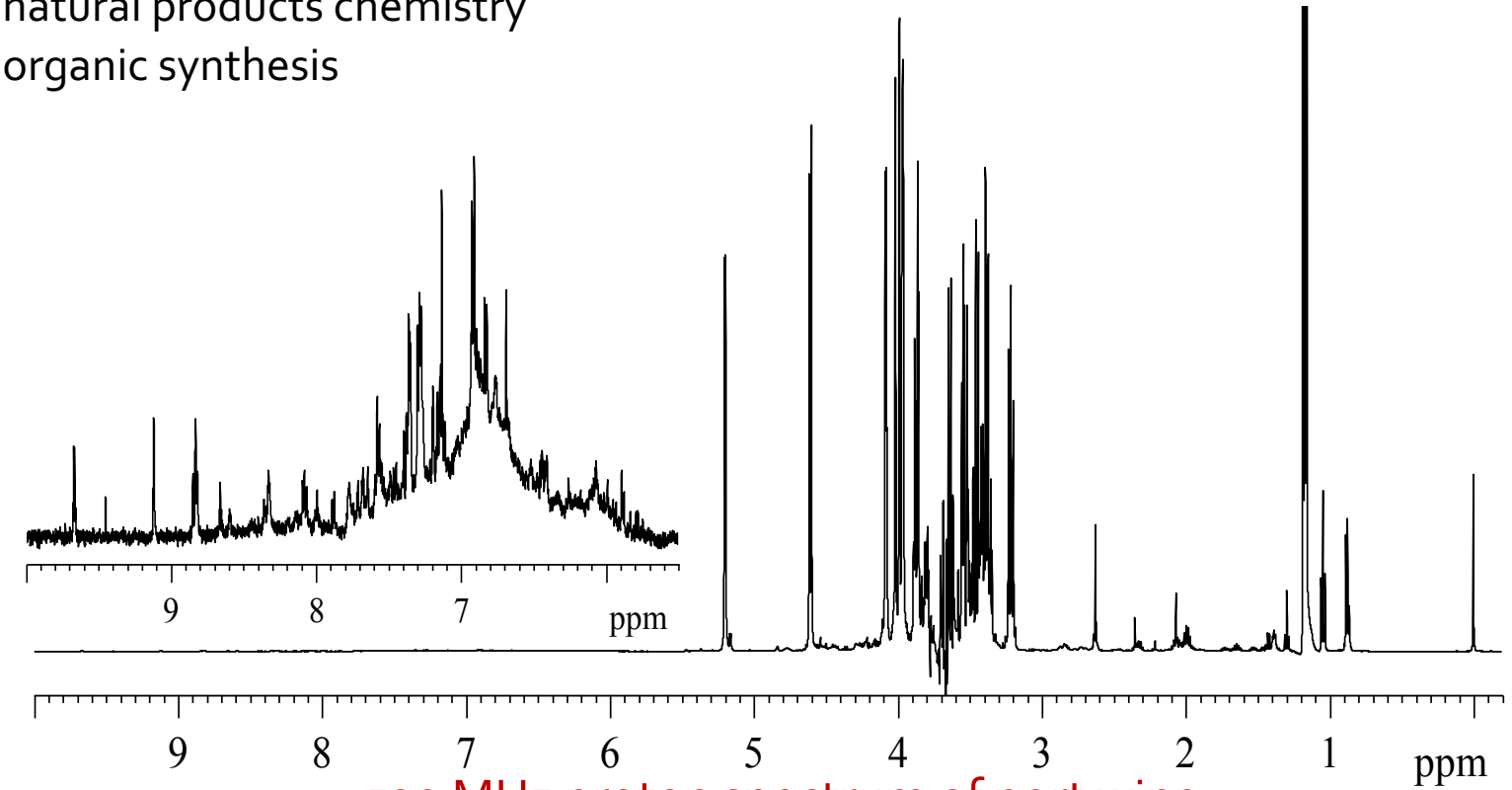
mixture analysis by NMR

applications

metabolomics
drug development
process chemistry
food science
natural products chemistry
organic synthesis

pros/cons

+ structural information
+ nondestructive
– low(ish) sensitivity
– usually needs separation (e.g. LC-NMR)



500 MHz proton spectrum of port wine

J. Agric. Food Chem. **2004**, 52, 3736

Diffusion NMR

Which species contribute to this spectrum

.

500 MHz ^{1}H NMR spectrum of Red Bull energy drink (sugar freee)

J. Am. Chem. Soc. **2011**. 133, 7640–7643

Diffusion NMR: information

Diffusion is central in much of chemistry and science in general. It is important in a large variety of topics including mass transport, reactivity, kinetics, separation science, nano-technology, hydrodynamics, inter-molecular dynamics, motional restriction etc.
In this course we will focus directly on how to measure diffusion by NMR, how to process the data and some of its applications:

Relative diffusion information -> separation/identification of NMR spectra from different components in a mixture. Diffusion-ordered spectroscopy (DOSY)

Absolute diffusion information -> size estimation of molecules and aggregates

Manipulating diffusion -> identification of components and binding/interaction

Diffusion NMR: suggested reading

Recommended Texts

Claridge, *High-Resolution NMR Techniques in Organic Chemistry*, 3rd ed., Elsevier, 2016 (Chapter 10 on Diffusion NMR)

Callaghan, Translational Dynamics and Magnetic Resonance. Principles of Pulsed Gradient Spin Echo NMR. Oxford, 2011

Advanced topics

Price, *NMR Studies of Translational Motion: Principles and Applications*, Cambridge University Press, 2009

And references given on slides

Brownian motion

Molecules in solution move at random because of collisions. How far they move on average in a given length of time t depends on the diffusion coefficient D: the root-mean-square displacement for diffusion in three dimensions is $\sqrt{6Dt}$

The diffusion coefficient

At a given temperature T, the diffusion coefficient D depends on the solvent viscosity η and the solute hydrodynamic radius a according to the Stokes-Einstein equation (k is the Boltzmann constant):

$$D = \frac{kT}{6\pi\eta a}$$

The equation is valid for solute molecules **at infinite dilution** diffusing through a **continuum solvent** (i.e. where the solvent molecules are much smaller than the solute).

the diffusion coefficient

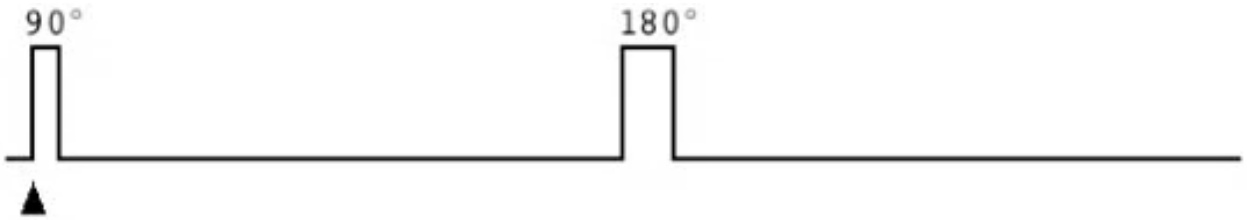
$$D = \frac{kT}{6\pi\eta a}$$

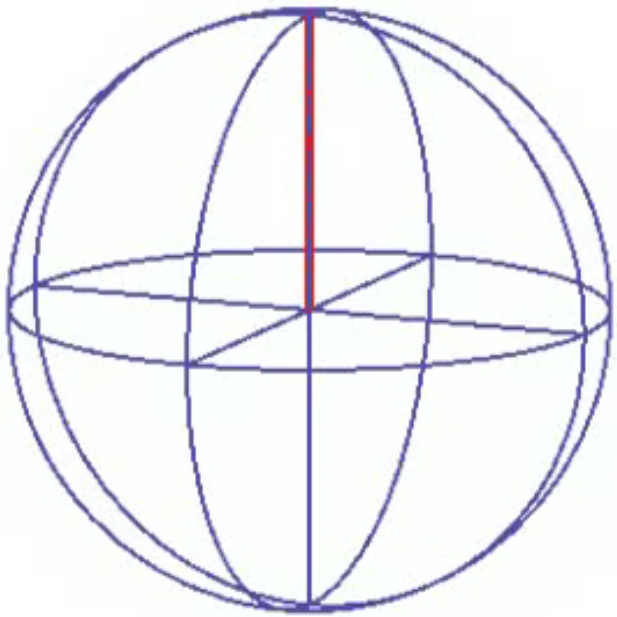
The hydrodynamic radius a is the effective average radius of the solvated solute molecules, and will depend on the molar mass MW. Assuming similar chemistries (i.e. constant density)

- for a spherical molecule such as a globular protein, $D \propto (MW)^{-1/3}$
- for a 'random coil' polymer or a flat disk, $D \propto (MW)^{-1/2}$
- for a rigid linear molecule $D \propto (MW)^{-1}$

In practice D will also depend on concentration, molecular shape, interactions etc.

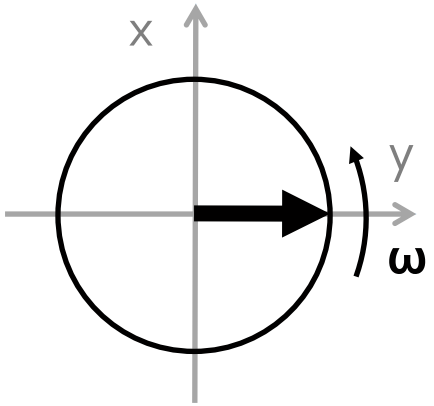
The spin echo





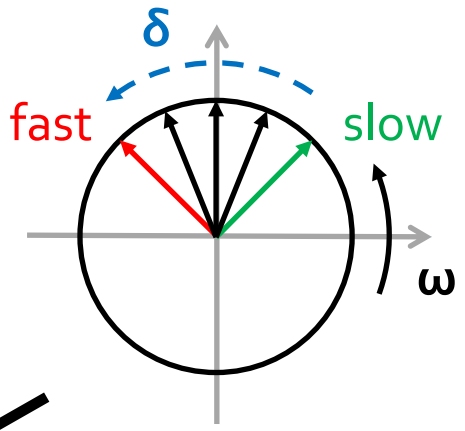
A 90° pulse about the X axis takes the magnetization M down to the Y axis; it then precesses about Z for a period τ, is rotated by 180° about the Y axis, and then precesses for a further period τ, ending up back at the Y axis.

spin echo: refocusing the chemical shift



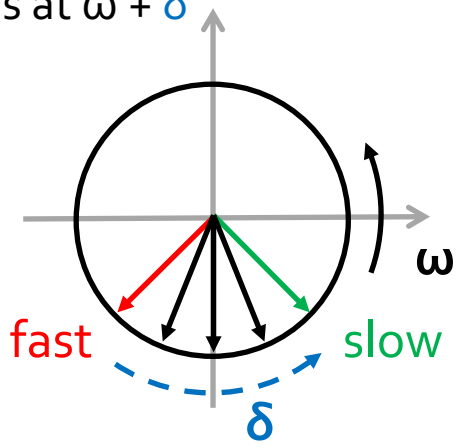
After the initial excitation (90°about -x) ; magnetization from all spins align along y-axis and rotates at ω + δ

τ



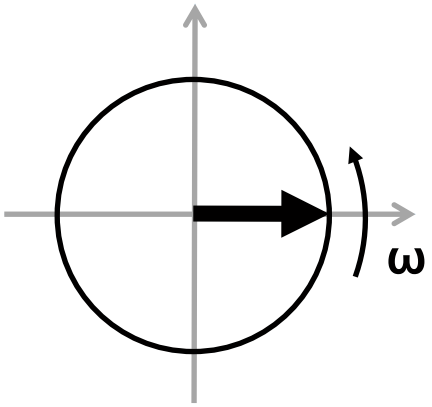
After a delay (τ) the spin vectors have acquired a phase difference proportional to δ

180° pulse about y



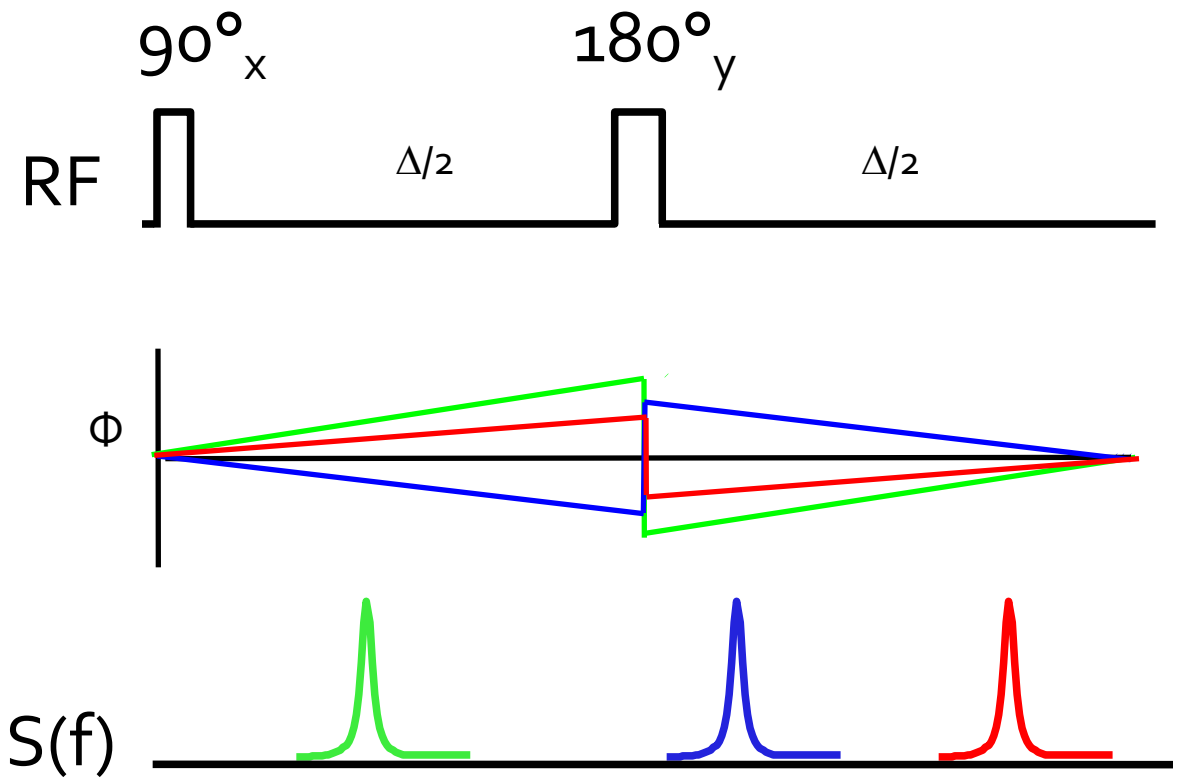
An 180° rotation inverts the positions of spin vectors

τ



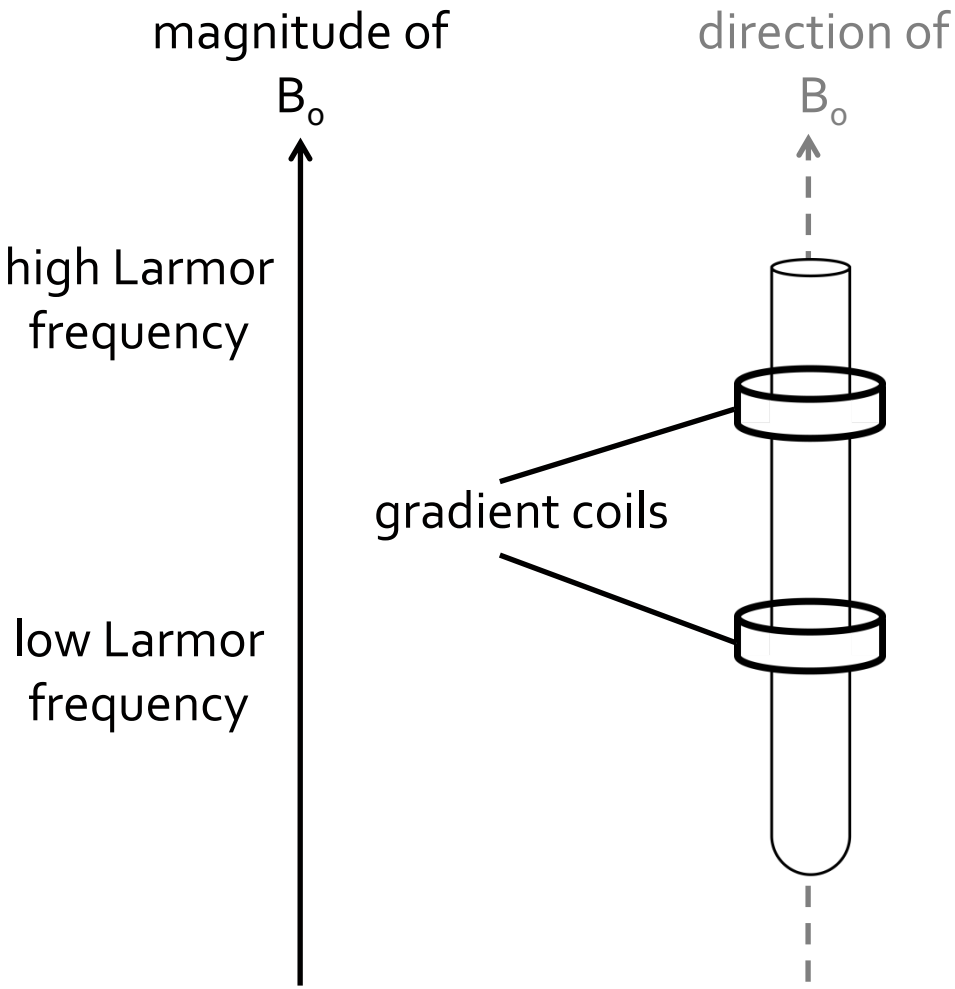
After a second delay (τ) the spin vectors refocus at the y-axis

the spin echo: phase history



Refocussing condition: $\int_0^t G * dt = 0$

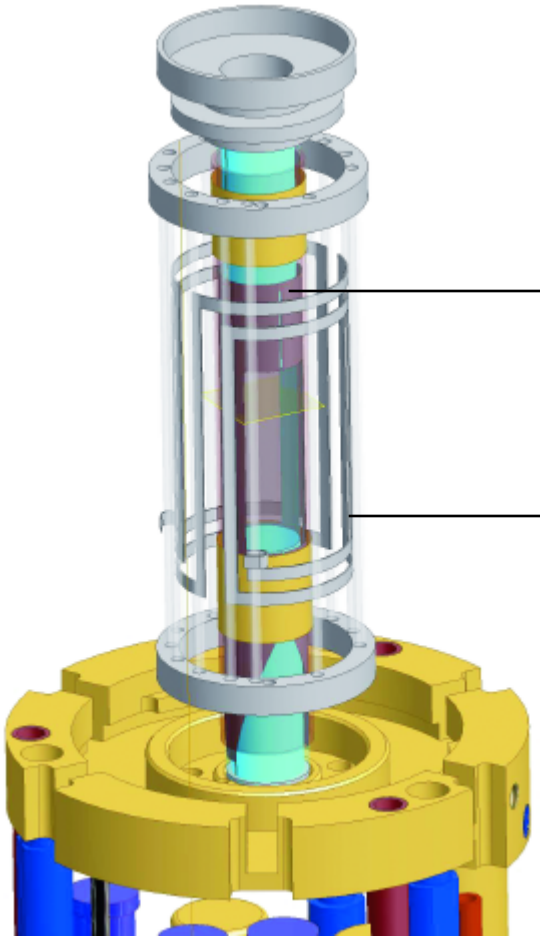
magnetic field gradients



The strength of the magnetic field is varied so that the Larmor frequency is [linearly] dependent on z position.

$$\nu_L = \frac{\gamma B_0}{2\pi} + G_z z$$

The NMR probe (inverse)

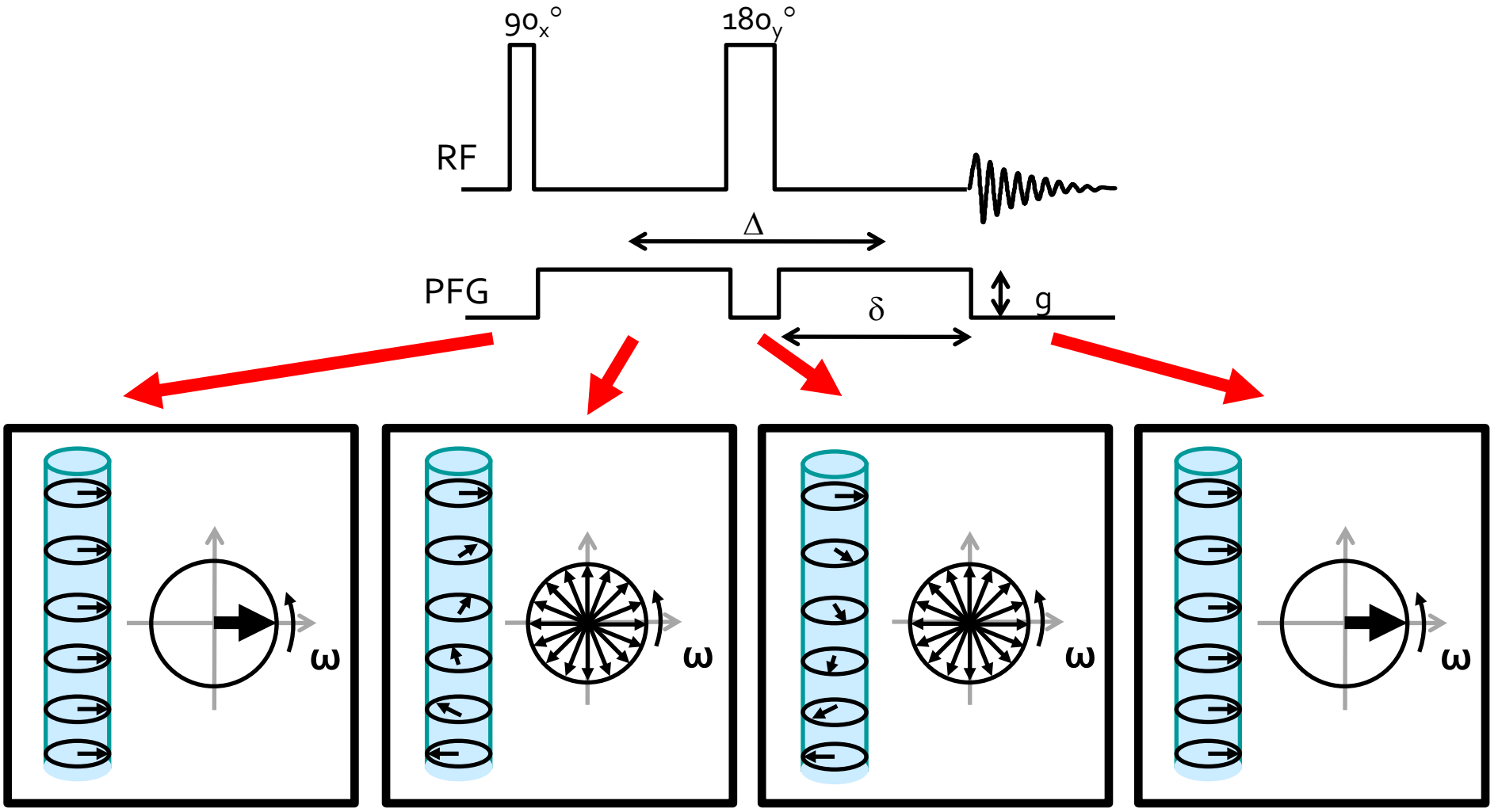


^{1}H coil

X-coil

Gradient coil

dephasing of magnetisation



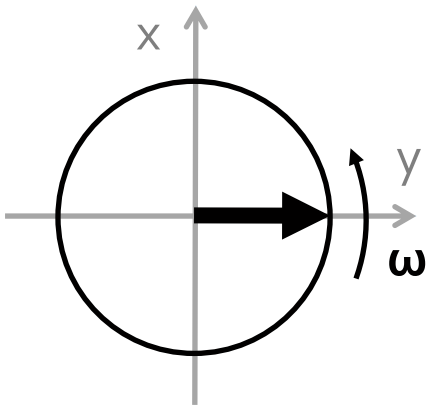
Larmour frequency is constant over z

z dependent frequency

Inversion of spins

All [non moving] spins refocused

gradient spin echo: refocusing the spatially dependent phase



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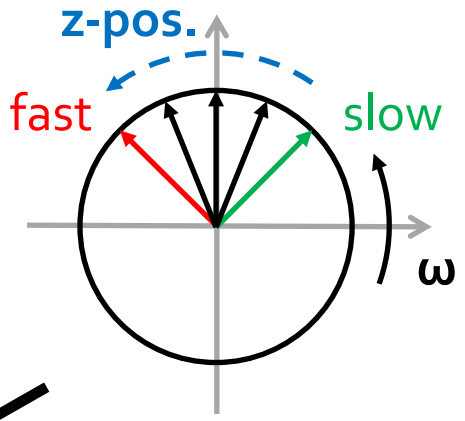

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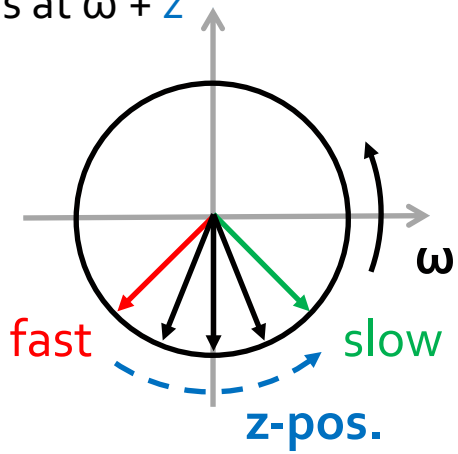

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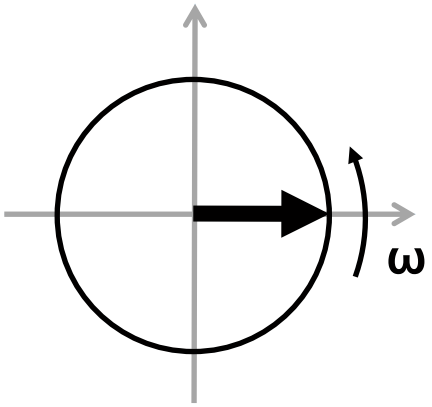
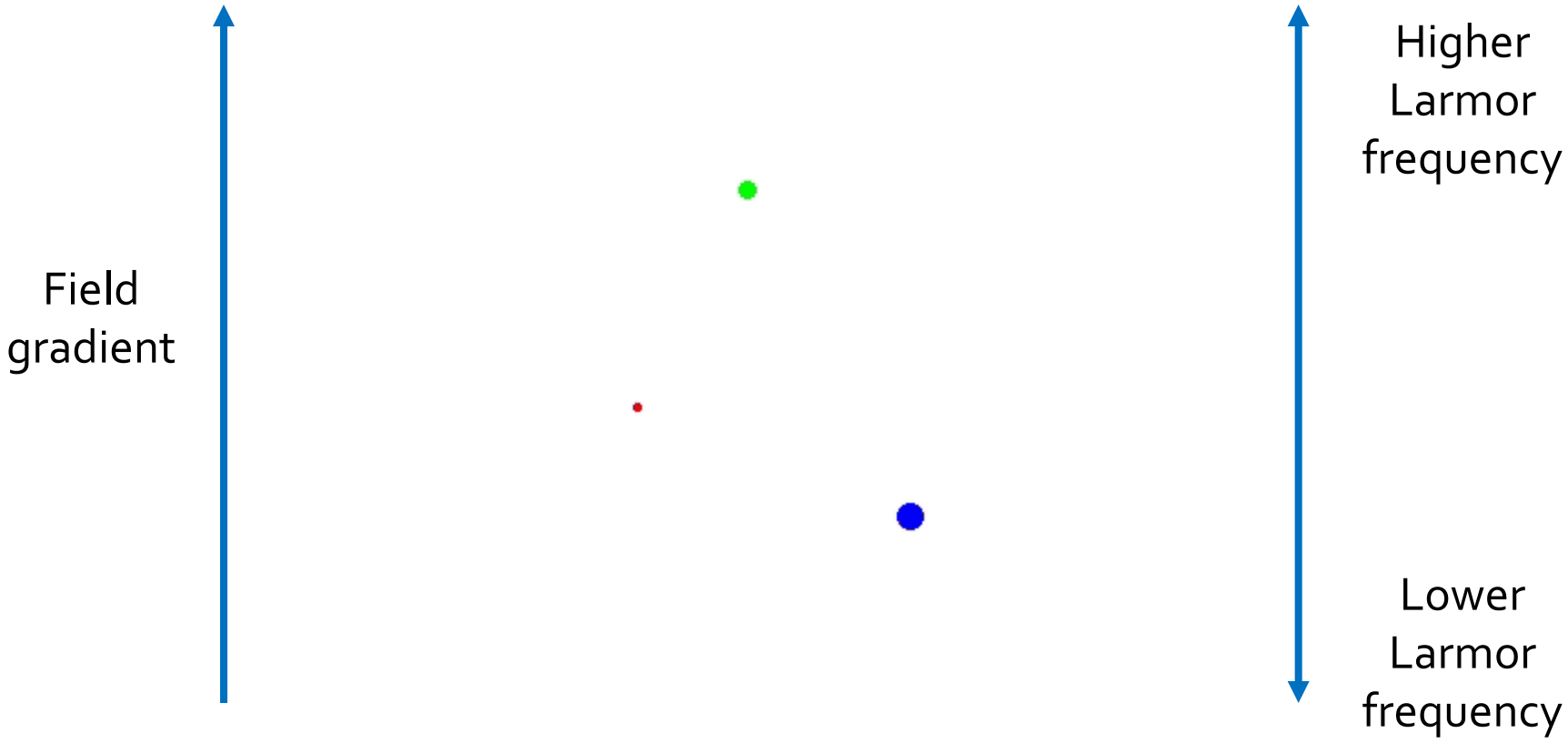


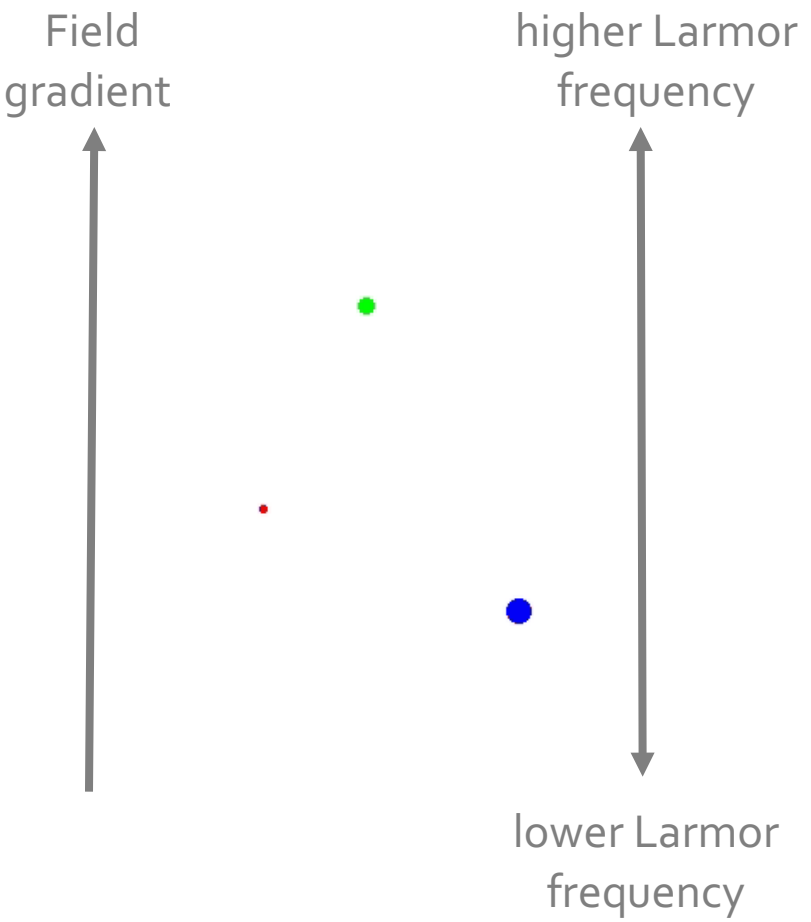
After a second delay (τ) the spin vectors refocus at the y-axis

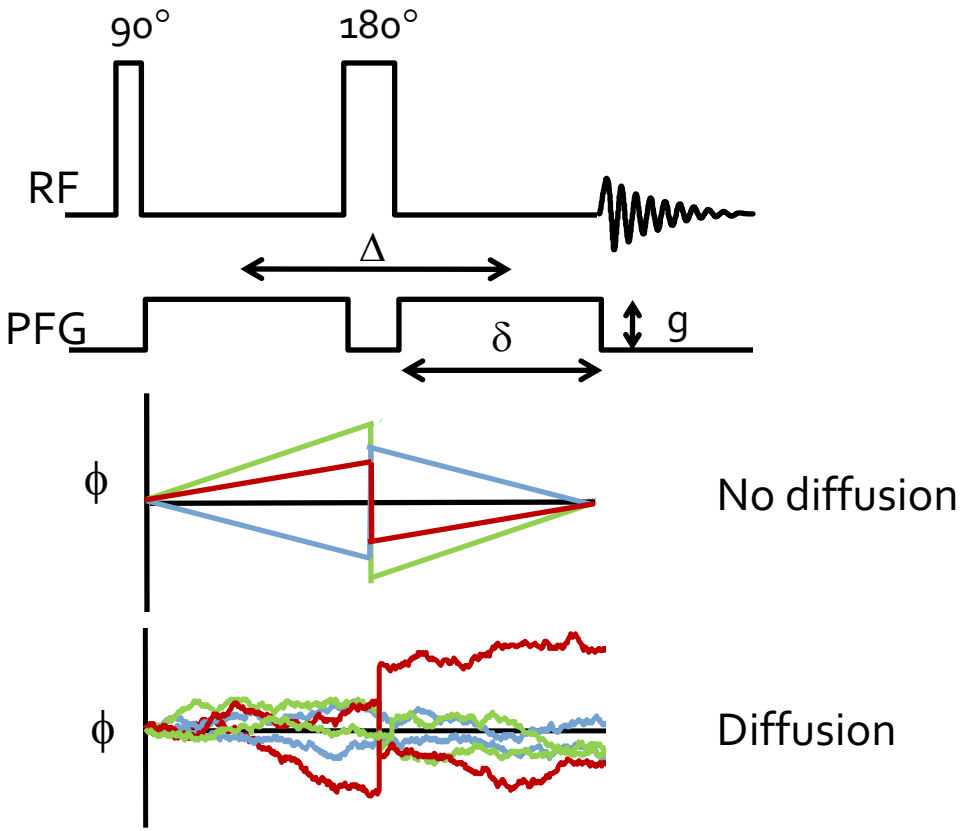
Brownian motion during a spin echo



In the presence of a field gradient, diffusion during Δ causes spins to lose phase coherence, attenuating the spin echo at a rate that depends on gradient strength (*G*) and diffusion coefficient (*D*)

diffusion encoding in a spin echo





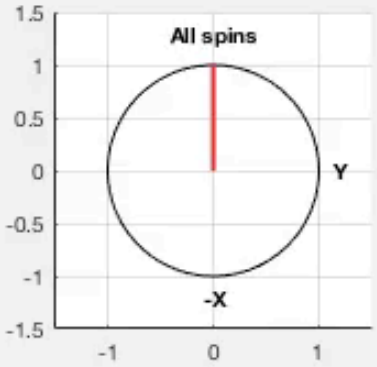
Brownian motion causes the signal to attenuate

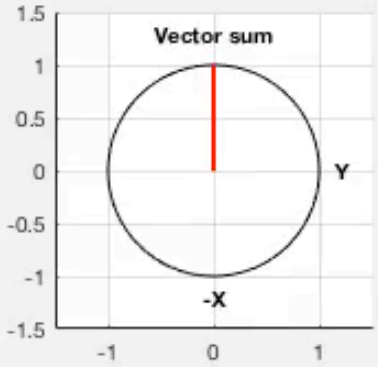
$$S(g) = S_0 e^{-D\gamma^2\delta^2 g^2 \Delta'}$$

$$\Delta' = \Delta - \delta/3$$

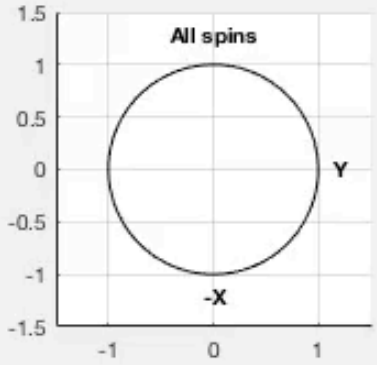
Reversible vs. irreversible loss of coherence

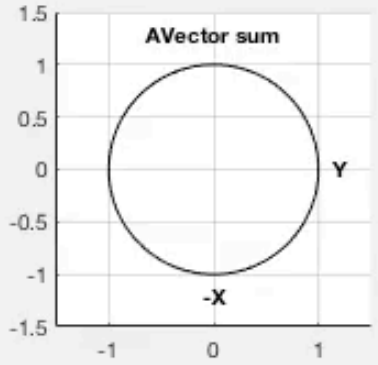
Reversible: different, but static, positions of each spin in the sample during a gradient





After 90°pulse



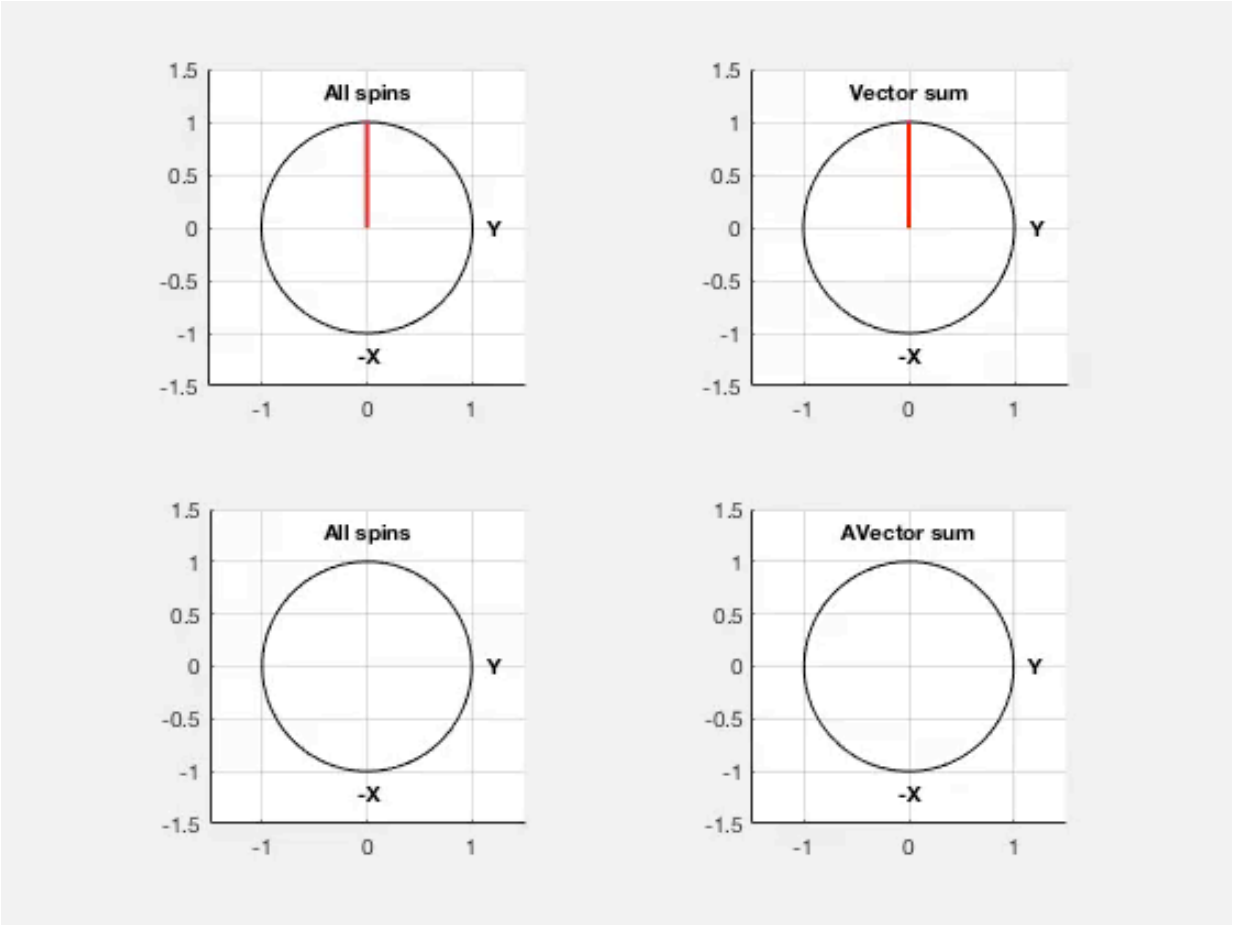


After 180°pulse

The loss of phase coherence in Mx/My
caused by B_0 inhomogeniety (e.g. a controlled gradient) is reversible

Reversible vs. irreversible loss of coherence

Reversible: random change of positions of each spin in the sample during a gradient



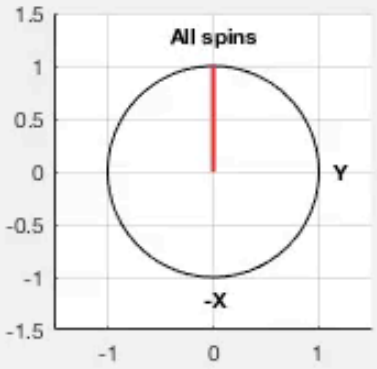
After 90°pulse

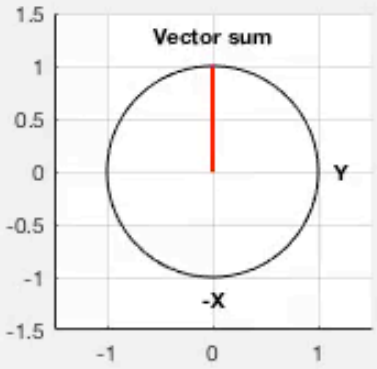
After 180°pulse

Brownian motion causes irreversible loss of phase coherence

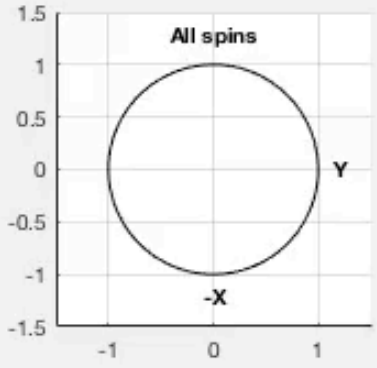
Reversible vs. irreversible loss of coherence

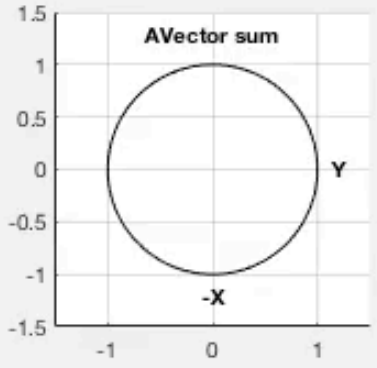
Both together





After 90°pulse

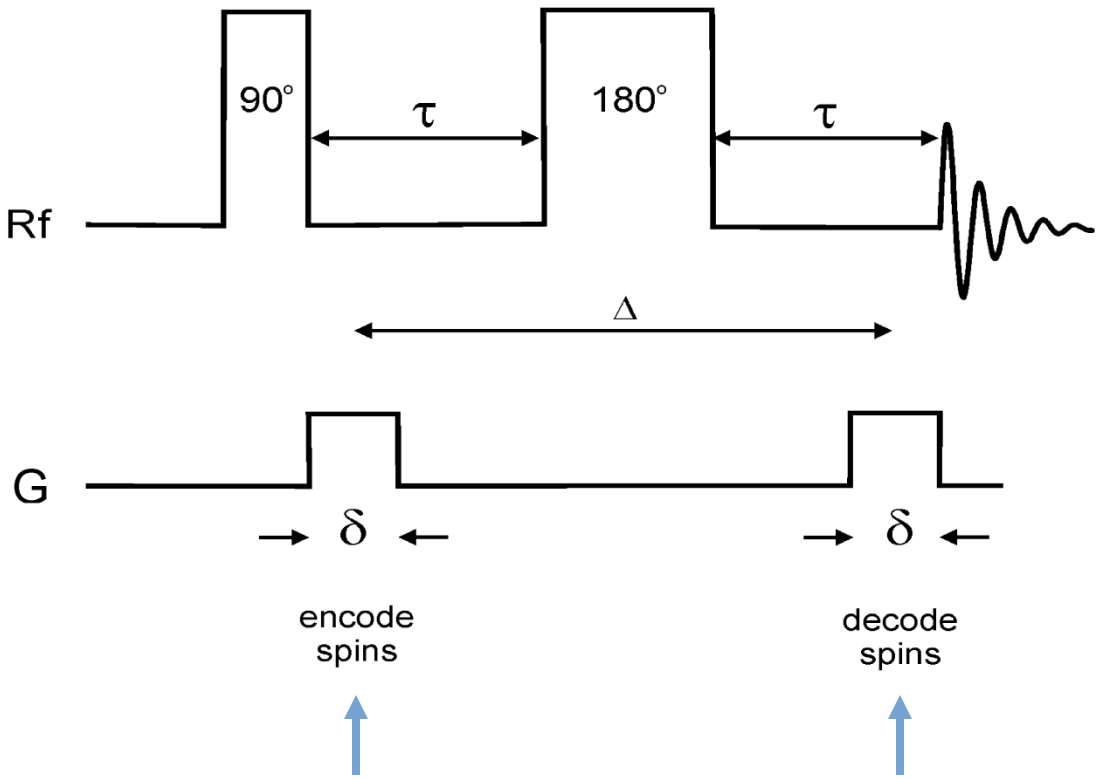




After 180°pulse

measuring diffusion with NMR

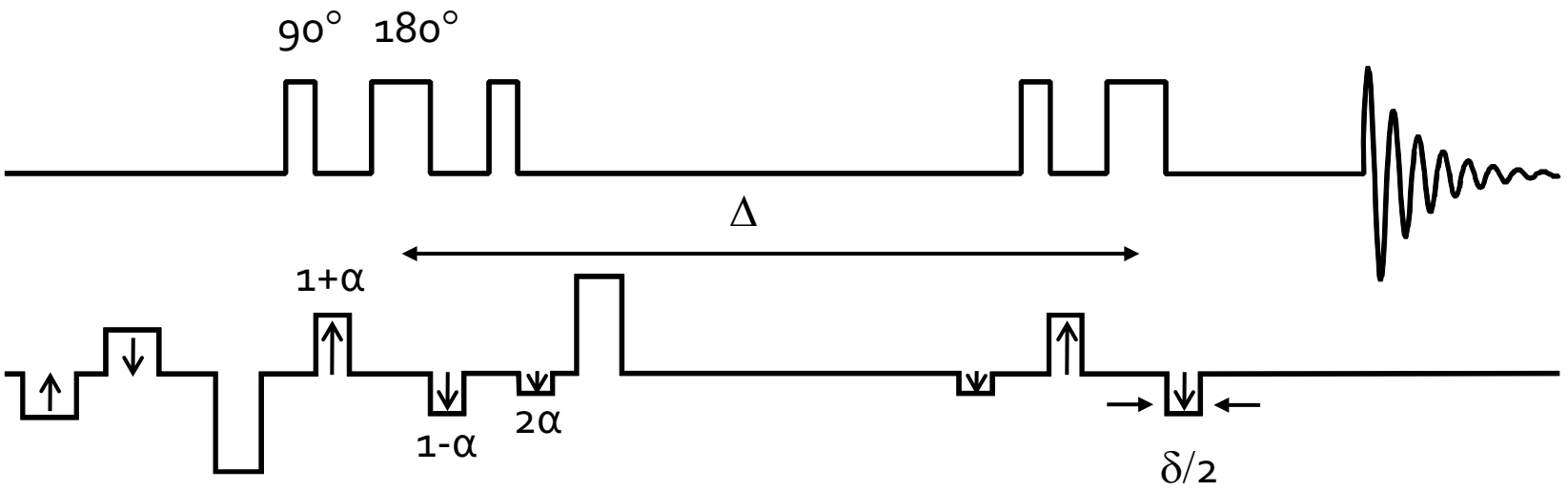
the pulsed field gradient spin echo (PFGSE)



spin phases scrambled

phases unscrambled
if spins haven't moved

A practical pulse sequence: "Oneshot"



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

$$\Delta' = \Delta - \frac{\delta(\alpha^2 - 2)}{6} - \frac{\tau(\alpha^2 - 1)}{2}$$

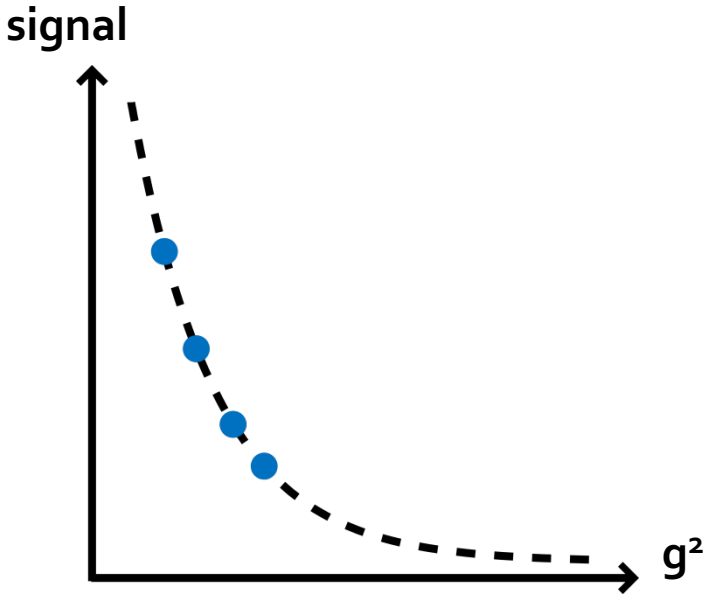
needs only 1 transient per increment; minimum time < 1 min

Magn. Reson. Chem. **2002**, 40, S147

An example

A ^{1}H PFGSTE experiment was carried out on a polymer sample, using gradient pulses δ of 5 ms duration and a diffusion delay Δ of 0.5 s. The signal varied with gradient strength as follows:

g/(T m^{-1})	signal/arbitrary units
0.2	0.93
0.28	0.64
0.35	0.41
0.40	0.29



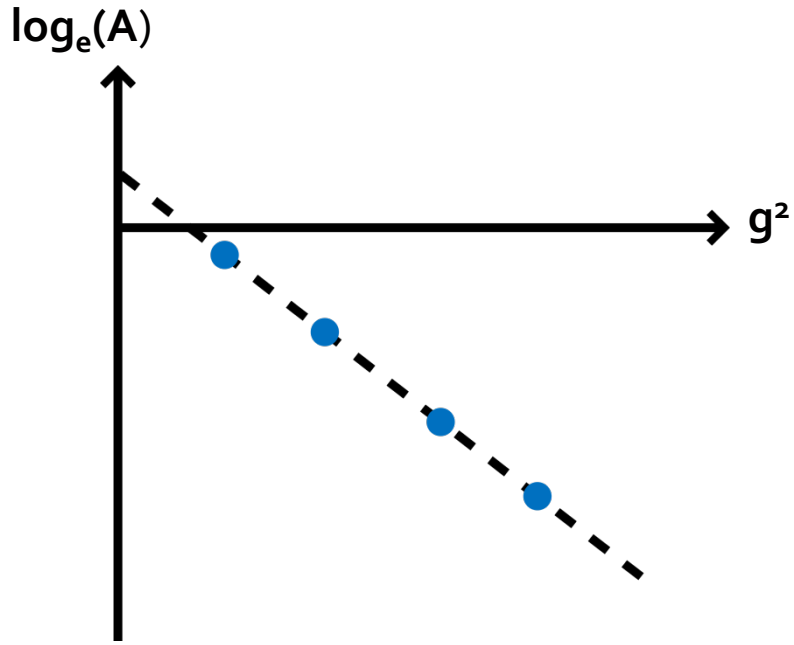
Calculate the diffusion coefficient of the polymer. Fit to the Stejskal-Tanner equation

$$A(g) = \left(\frac{S(g)}{S(0)} \right) = \exp\left(-D\gamma^2 g^2 \delta^2 \Delta' \right)$$

example continued

According to the Stejskal-Tanner equation, a plot of the natural logarithm of the attenuation $A = S(g)/S(0)$ against g^2 should be a straight line of slope $-\gamma^2\delta^2\Delta' D$.

$g^2/(\mathrm{T}^2\,\mathrm{m}^{-2})$	$\log_e(A)$
0.04	–0.07
0.0784	–0.45
0.123	–0.89
0.16	–1.24



The slope of the graph is –9.8, so $D = 9.8/((2.675 \times 10^8 \times 0.005)^2 \times 0.498)$

$= 1.1 \times 10^{-11}\ \mathrm{m}^2\ \mathrm{s}^{-1}$

In practice we typically use more gradient levels. Why?

How fast is diffusion really ?

Lord Kelvins experiment (world longest running)

Started at University of Glasgow in 1872 using 5.3 m cylinders with one differently colored solution in the top and bottom half respectively

Kelvin estimated about 10000 years for “perfect” mixing.

Was he right?

Was Lord Kelvin right?

Two differently coloured solutions are placed in the bottom and top half of a 5.3 m long tube and left to diffusion. Lord Kelvin estimated that it would take about 10000 years for 'perfect mixing'.

The diffusion coefficient of the coloured molecules are 7.5×10^{-10} m^2 s^{-1} at 25°C and the viscosity η of the solution is 1.1 mPa.

Hint: assume that 'perfect mixing' can be approximated by a mean distance travelled of 2.65 m in the z-direction.

Remember

The root-mean-square displacement, x, due to diffusion is given by:

$$x^2 = \alpha D t$$

Where D is the diffusion coefficient, t is time and α is a constant depending on dimensionality; α is 2, 4 or 6 for 1, 2 or 3 dimensional diffusion.

Answer

Was Lord Kelvin right?

The root-mean-square displacement (RSD), x, due to diffusion is given by:

$$x^2 = \alpha D t$$

Where *D* is the diffusion coefficient, *t* is time and α is a constant depending on dimensionality; α is 2, 4 or 6 for 1, 2 or 3 dimensional diffusion.

The time it takes for and RSD is hence:

$t=x^2/\alpha D$

$t=2.65^2/2*7.5 \times 10^{-10} = 4.68 \times 10^9$ seconds= 148 years

Lord Kelvin also wrongly estimated the age of the Earth to 20 million* years because he did not take convection into account. We will have a look at convection in the next lecture.

*todays accepted age is 4.5 billion years

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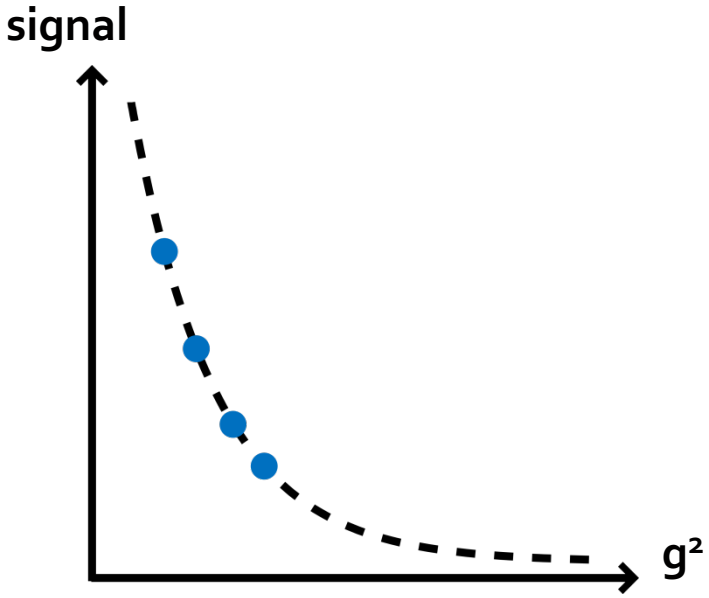

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constructing an HR-DOSY spectrum

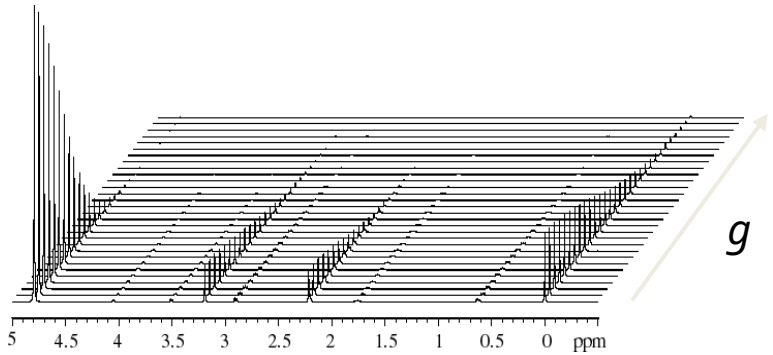
measure PFGS[T]E spectra as a function of *G*

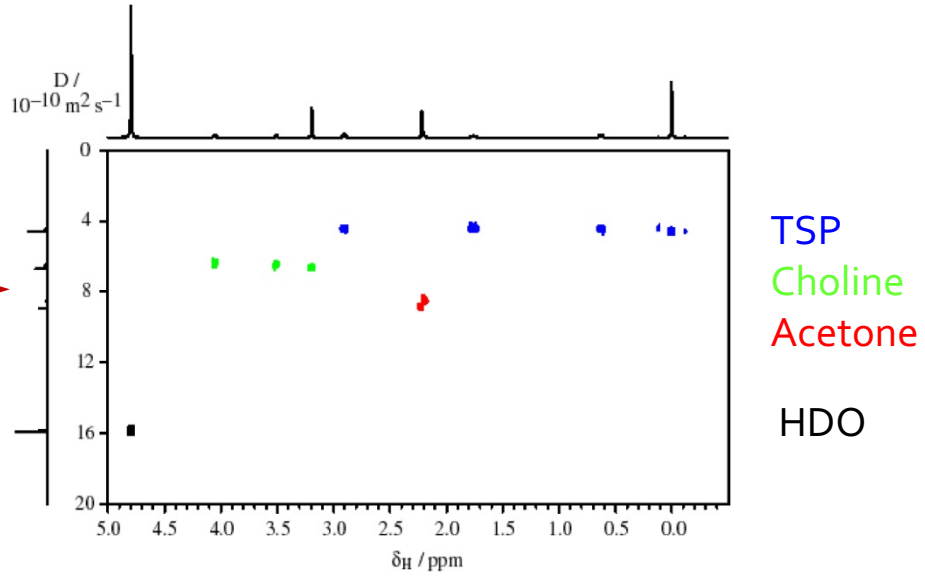
fit peak heights/areas to a single exponential to get diffusion coefficients *D*

extend 1D peaks into a second dimension, with Gaussian shapes centred on the *D*'s

peak widths in diffusion dimension determined by the standard errors σ_D

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

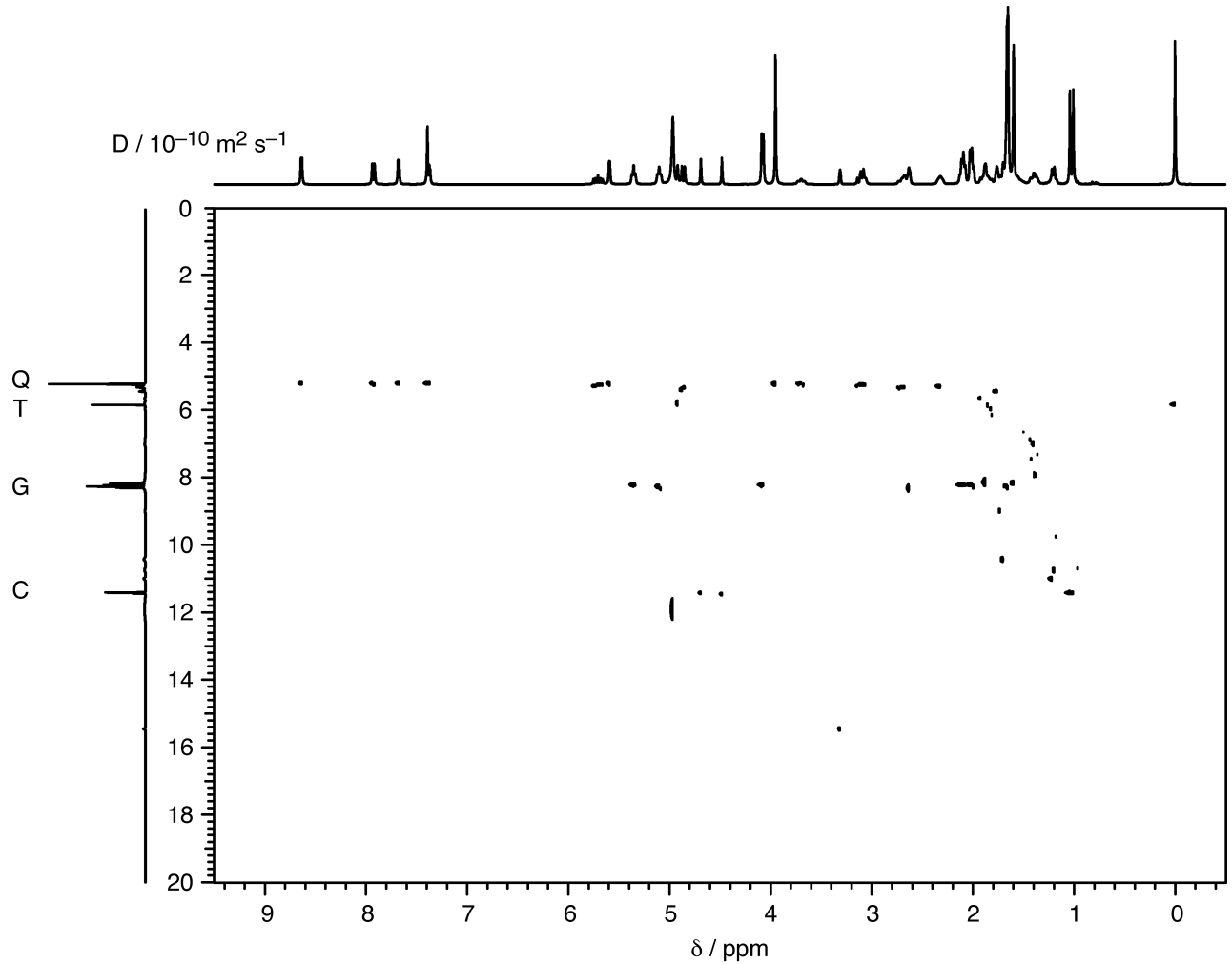




400 MHz ^{1}H DOSY spectrum of choline, acetone and DSS in D_2O

J. Magn. Reson. Ser. B **1995**, 108, 170

a ‘typical’ mixture



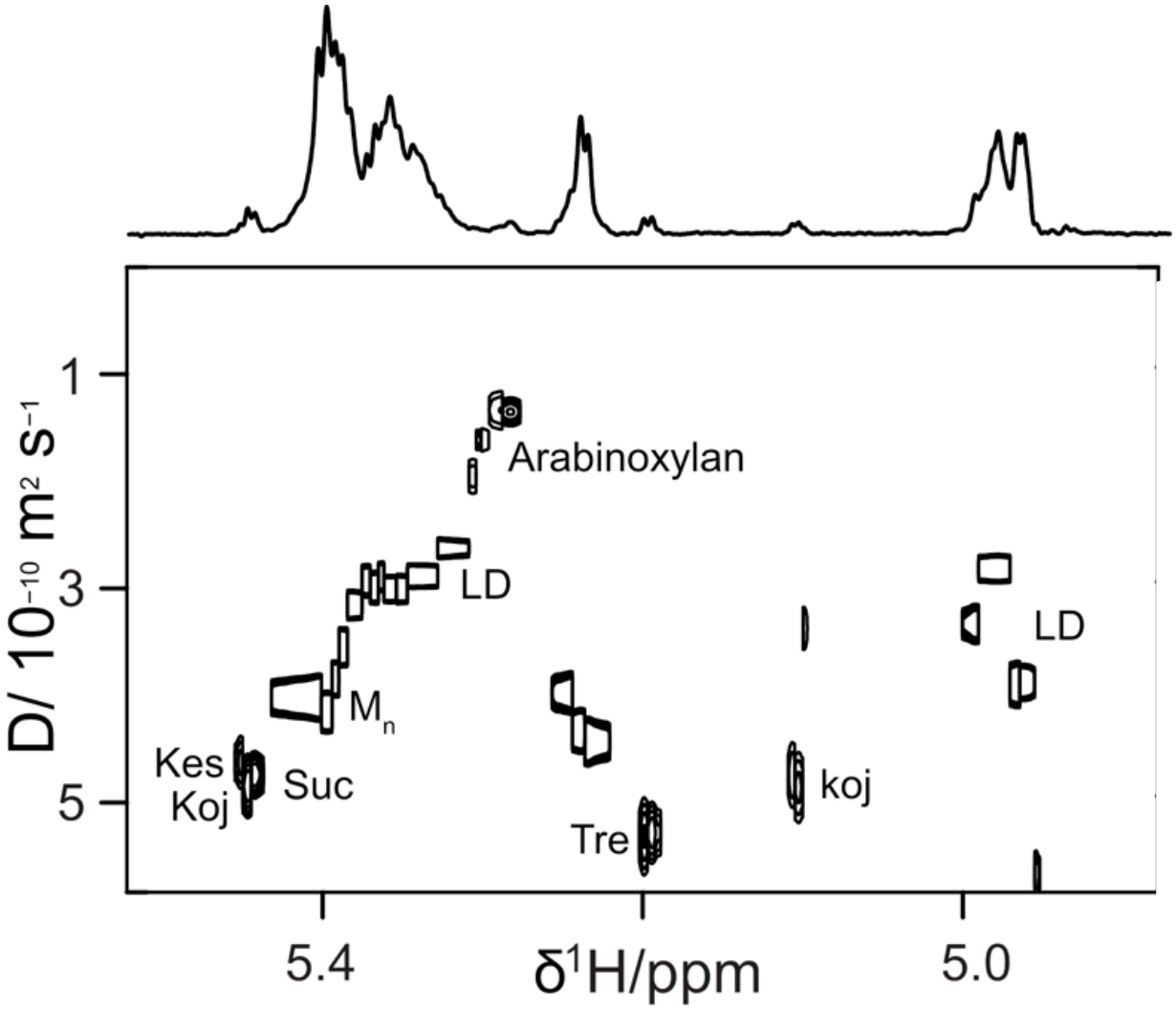
quinine

geraniol

camphene

400 MHz ^{1}H Oneshot DOSY spectrum of quinine, geraniol , camphene and TSP in CD_3OD. The least attenuated spectrum from the parent dataset is plotted at the top, and the integral (sum) projection onto the diffusion axis at the left.

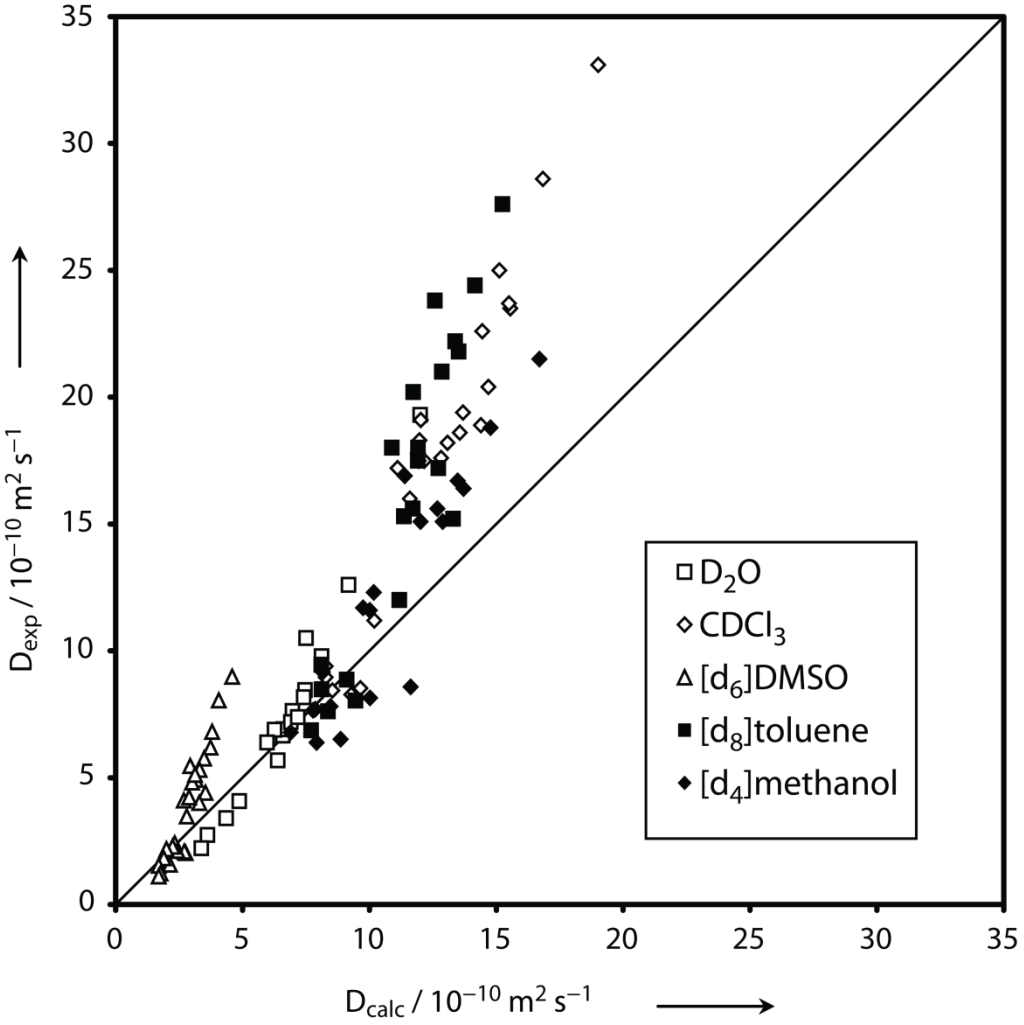
Lager beer: 800 MHz proton 2D DOSY spectrum



Anomeric region, containing of several oligo- and polysaccharides

Food Chemistry. **2013**, 150, 65

predicting D(MW): Stokes-Einstein

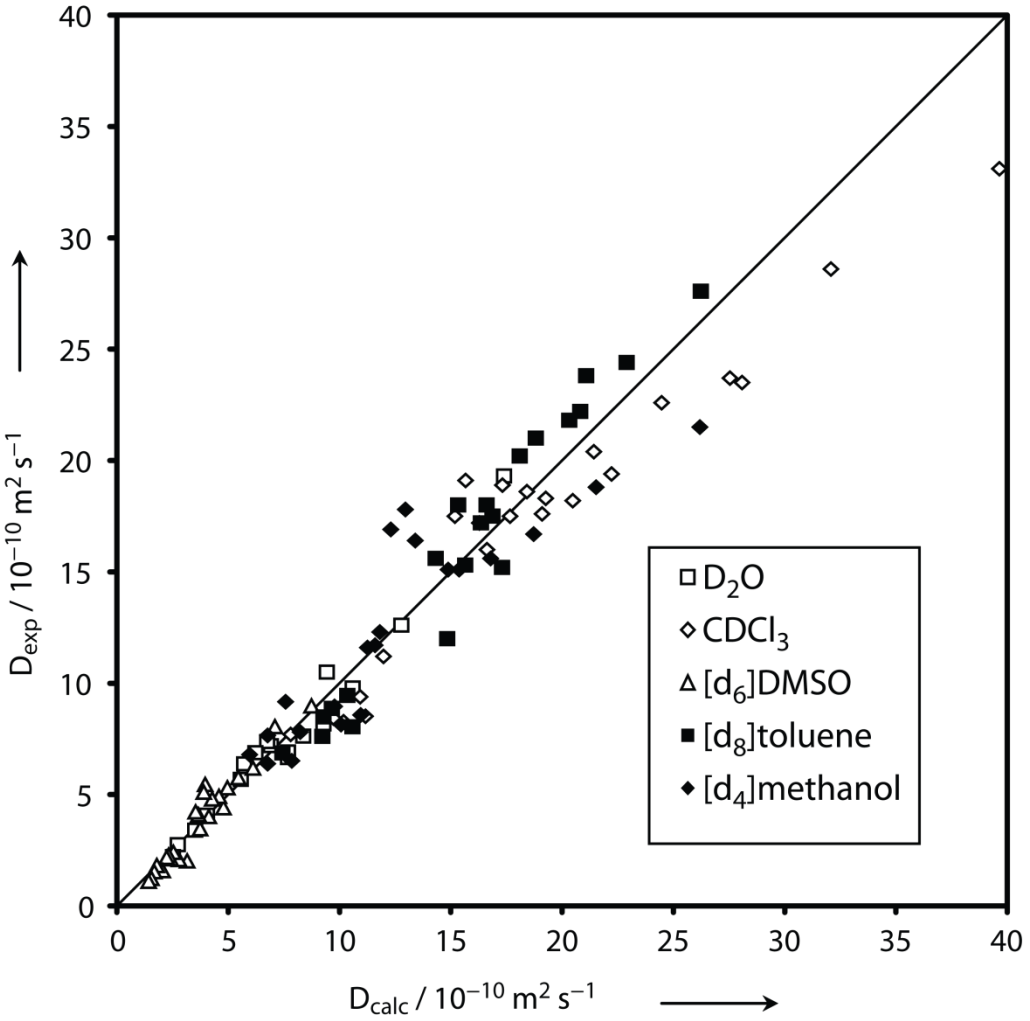


Balances thermal energy against friction

Assumes hard spheres (f=1) in a continuum fluid

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

predicting D(MW): proposed alternative



A constant effective density for all solutes

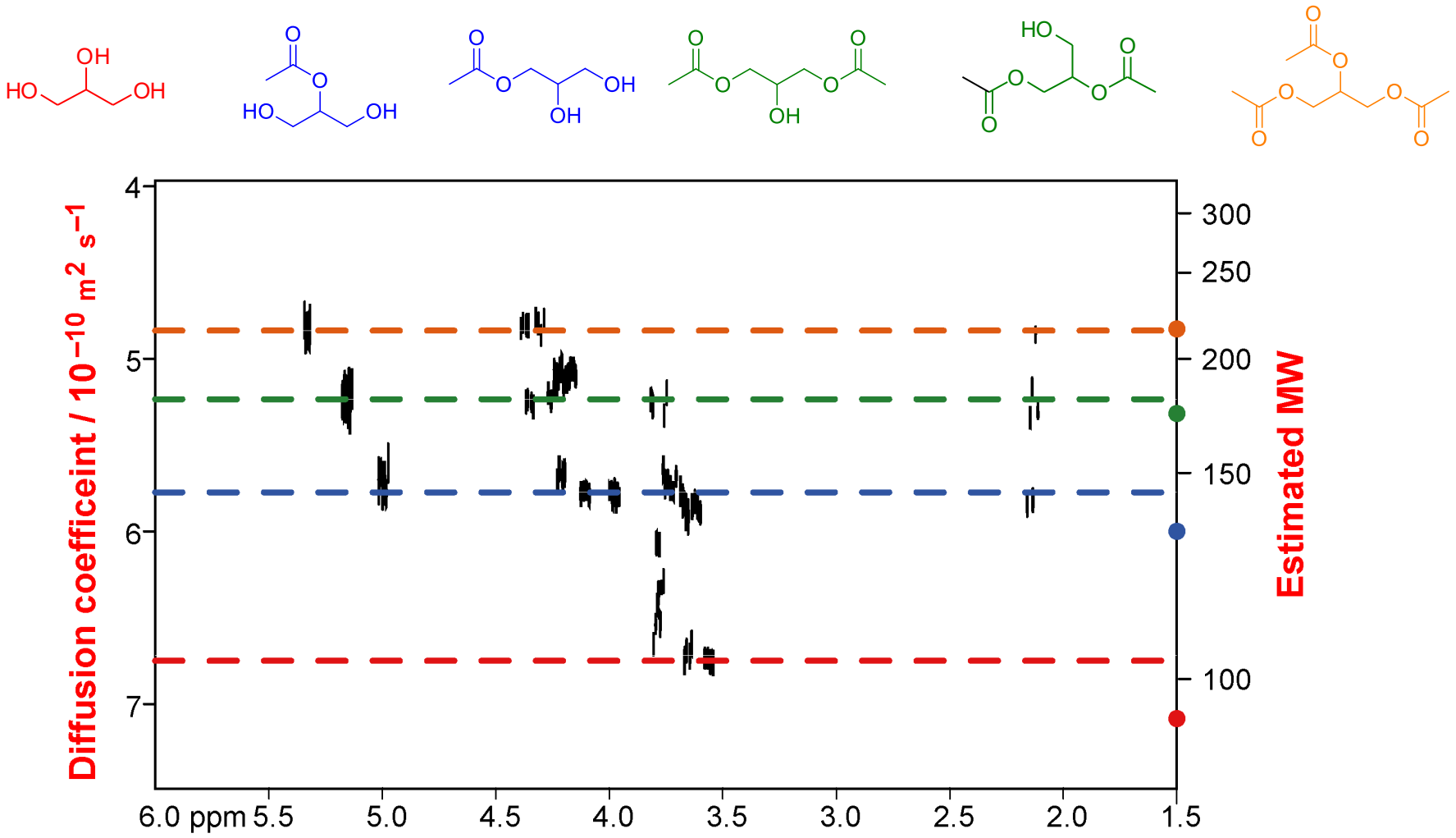
Combines theoretical and empirical approaches

Relatives sizes of solvent and solute molecules are used

$$D = \frac{k_B T\left(\frac{3\alpha}{2} + \frac{1}{1+\alpha}\right)}{6\pi\eta \sqrt[3]{\frac{3MW}{4\pi\rho_{eff} N_A}}}, \quad \text{where} \quad \alpha = \sqrt[3]{\frac{MW_S}{MW}}$$

Angew. Chem. Int. Ed. **2013**, 52, 3199

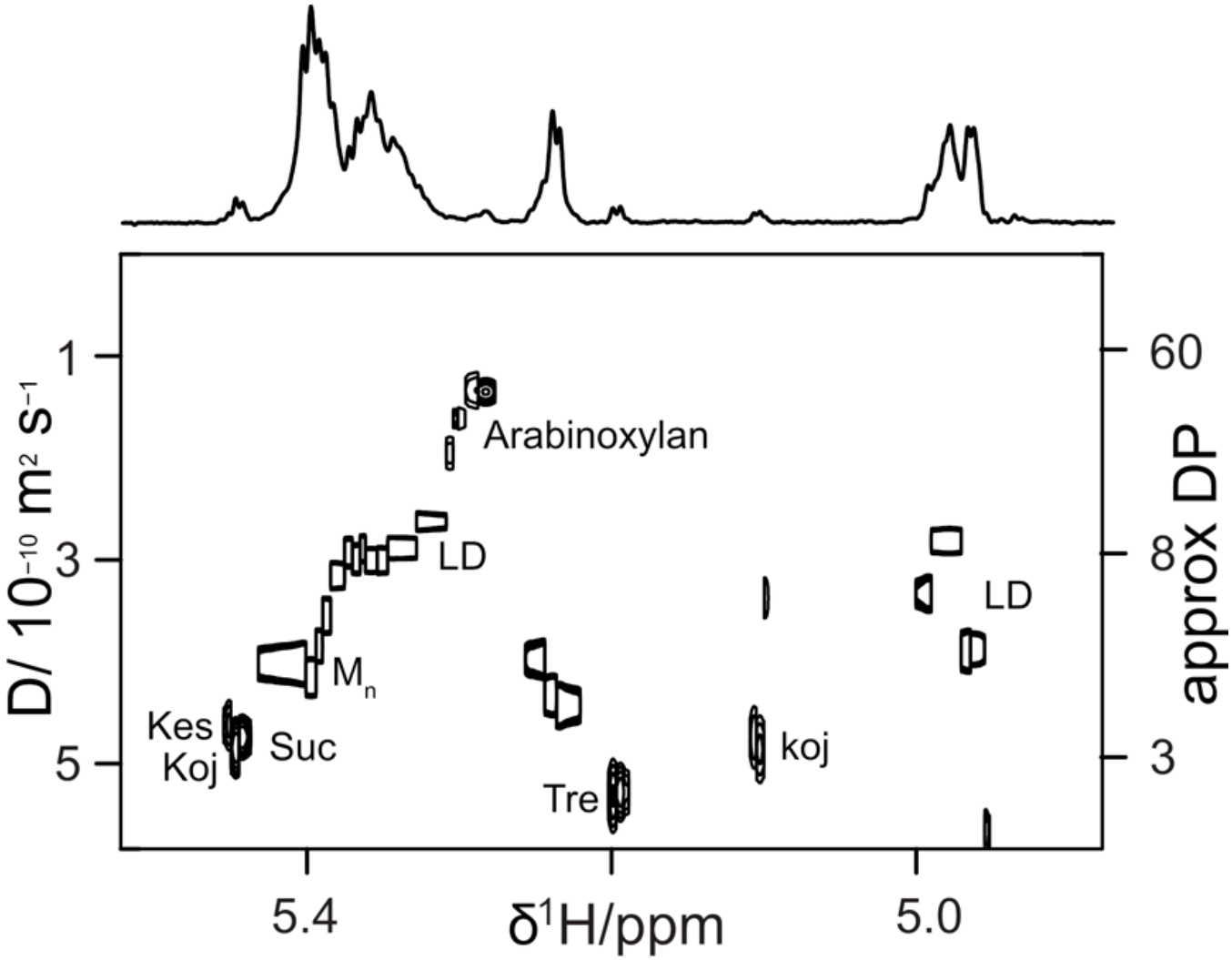
predicting D/MW: a MW scale



DOSY spectrum of crude "monoacetin", containing glycerol and mono-, di- and triacetin in D_2O.

Angew. Chem. Int. Ed. **2013**, 52, 3199

Lager beer: 800 MHz proton 2D DOSY spectrum



Anomeric region, estimation of degree of polymerisation of several oligo- and polysaccharides

Food Chemistry. **2013**, 150, 65

How to acquire a “DOSY” data set

Suggested setup

Use a spectral width wide enough

A significant proportion of clean baseline (maybe 1/3 of the spectrum on each side) helps in getting good baseline correction

Use 10-30 gradient levels (no need for a power of 2)

For a large range of diffusion coefficients and/or the use of advanced processing, choose a higher number of gradient increments.

Use equal steps in gradient squared

Bruker default is linear.

NB. More pulse sequence specific (e.g. Oneshot) is available in the Bruker download package on our homepage:

How to acquire a “DOSY” data set

Suggested setup

Attenuation between first and last increment should be about 70%

This is for monoexponential fitting i.e standard DOSY. For more advanced processing such as multiexponetial fit and multivariate methods (more on that after lunch) it can be useful to have a 95% attenuation.

Start with a minumum gradient value of 10% (Bruker default is 2%)

Too low gradient strengths often give poor results due to less efficient corehence selection and nonlinearity if gradient. (For Oneshot sequence don’t go beyond 80% or coherence selection may suffer, see Bruker package on our homepage)

The number of scans makes a difference (not just S/N)

For the Oneshot use 1 scan for a “quick and dirty” experiment, 4 scans for good quality and 16 Scans for a clean results (some improvement still with 32, 64, 128 and 256). For a bpp bruker sequence multiply this by 4.

Motion in NMR Experiments: the Bloch-Torrey equation

The Bloch-Torrey equation describes the evolution of transverse magnetization $M_+ = M_y - iM_x$ as a function of position **r** and time t in the presence of a field gradient **g** and of diffusion with coefficient *D* and/or flow with velocity **v**:

$$\frac{dM_+(\mathbf{r},t)}{dt} = -i\gamma \mathbf{r}.\mathbf{g}M_+ - M_+ / T_2 + D\nabla^2 M_+ - \nabla.\mathbf{v}M_+$$

free precession

relaxation

diffusion

flow

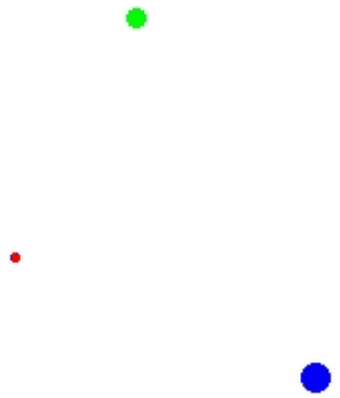
Diffusion measurements: interfering effects

Sample movements

- Vibrations
- Displacement

Liquid movements

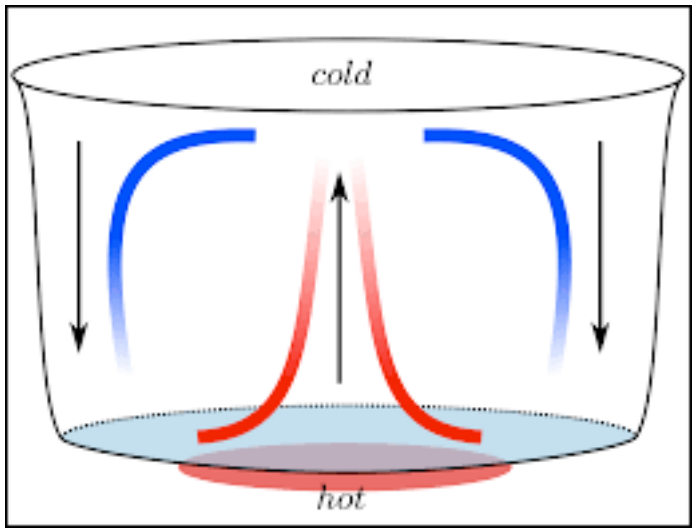
- Flow
- Convection



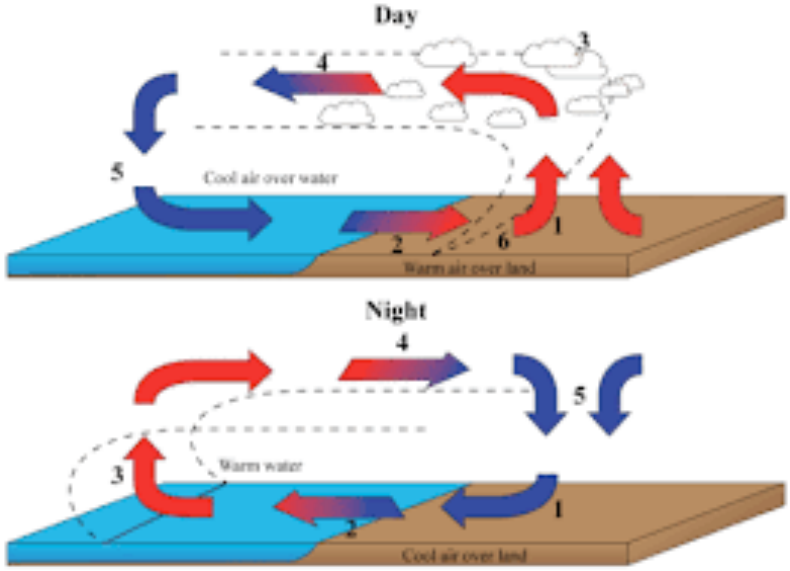
A PFG NMR diffusion experiment measure the movement of molecules, assuming it originates from diffusion, in a magnetic field gradient as a decrease in signal. Any other movement causes an interfering effect

Diffusion measurements: convection

Convection in
a heated pot



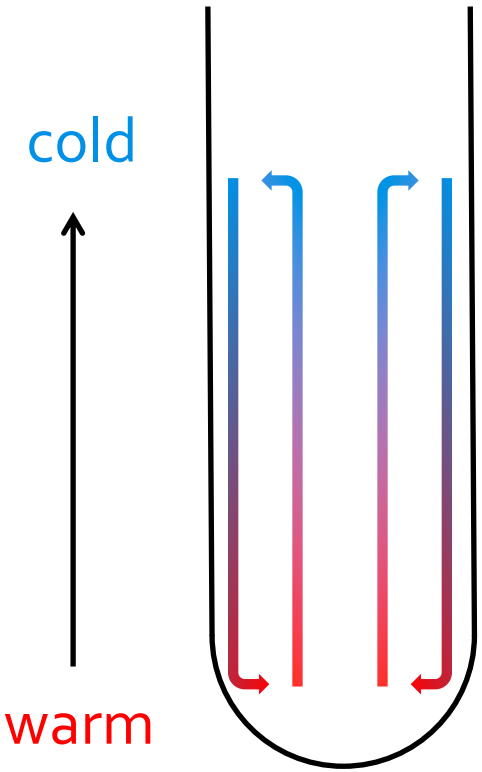
Convection at the
sea/land interface



Convection can occur when a system has different temperatures in different parts and the system strives towards equilibrium.

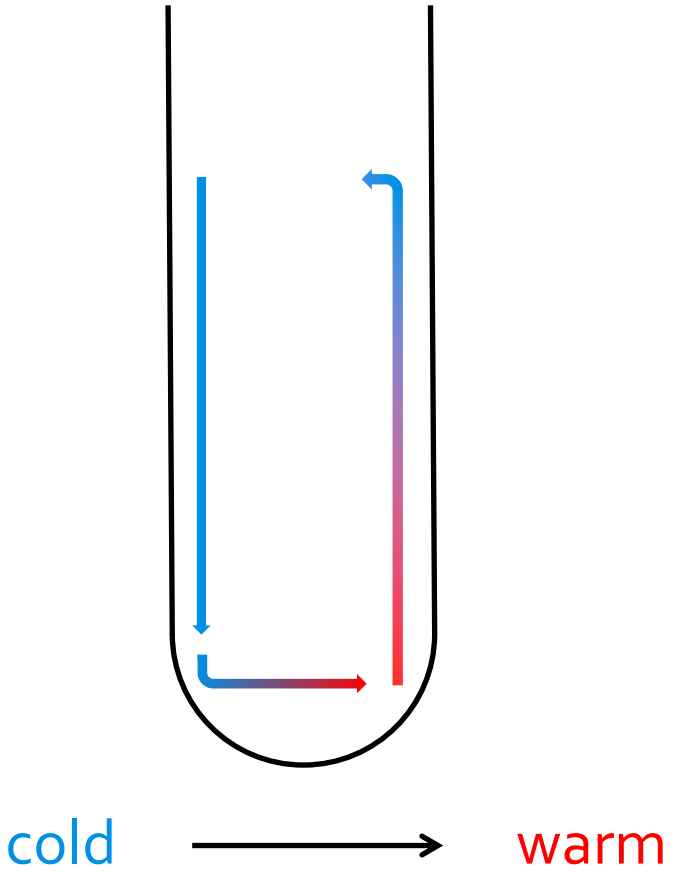
Convection in an NMR tube

Raleigh–Bérnard convection



Critical phenomenon

Hadley convection



Always present

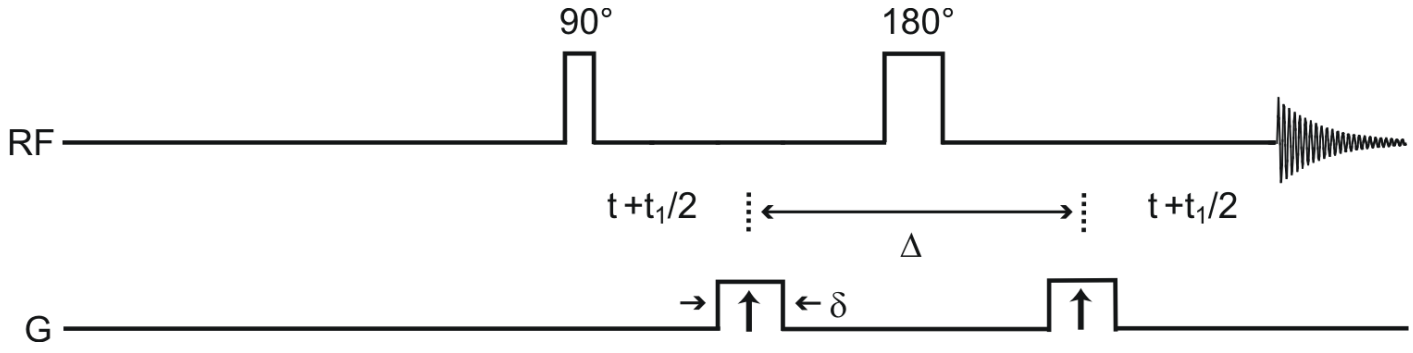
Convection in an NMR tube

Stejskal-Tanner equation modified for convection flow in an NMR tube

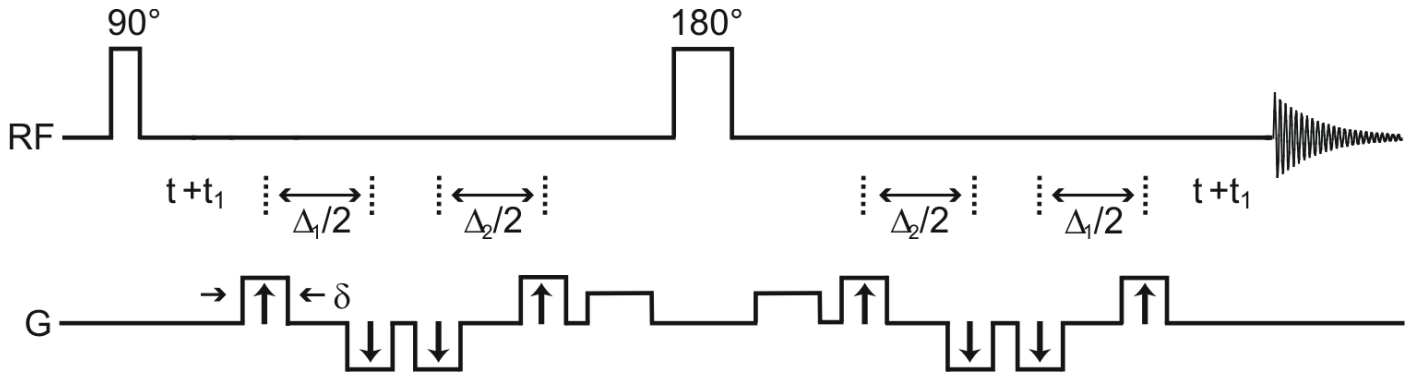
$$S(g) = S_0 e^{-D\gamma^2\delta^2 g^2 \Delta'} \cos D\gamma\delta g\Delta' v$$

Where γ is the magnetogyric ratio, *g* is the gradient pulse amplitude, and *d* is the gradient pulse width. Δ' is the effective diffusion and flow time and *v* is the flow velocity

Convection compensation



PFG Spin echo



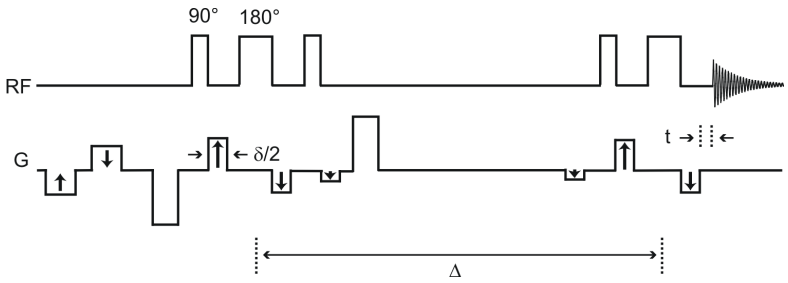
PFG Spin echo

Compensated

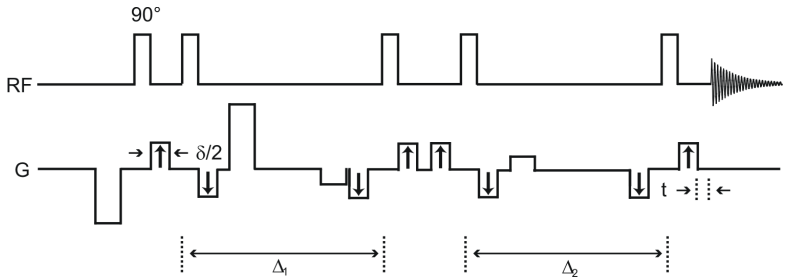
The effects of convection can be corrected to first order by dividing up the diffusion time () in different elements and using opposite polarities of the gradient pulses. The effect of flow in one diffusion element then cancels the effect in the other.

Convection compensation: pulse sequences

Double stimulated echo: lose 50% of signal and more phase cycling

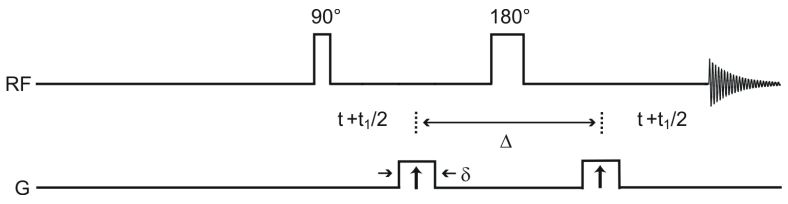


Oneshot

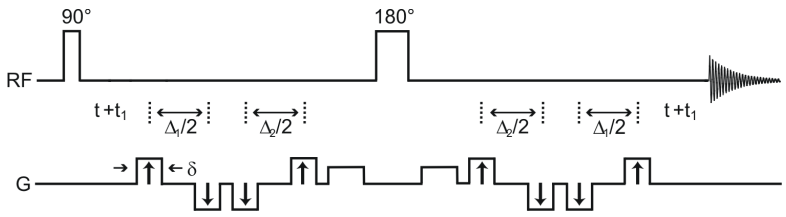


PFGDSTE - Compensated

Spin echo: no loss of signal or increased need for phase cycling



2DJ-IDOSY

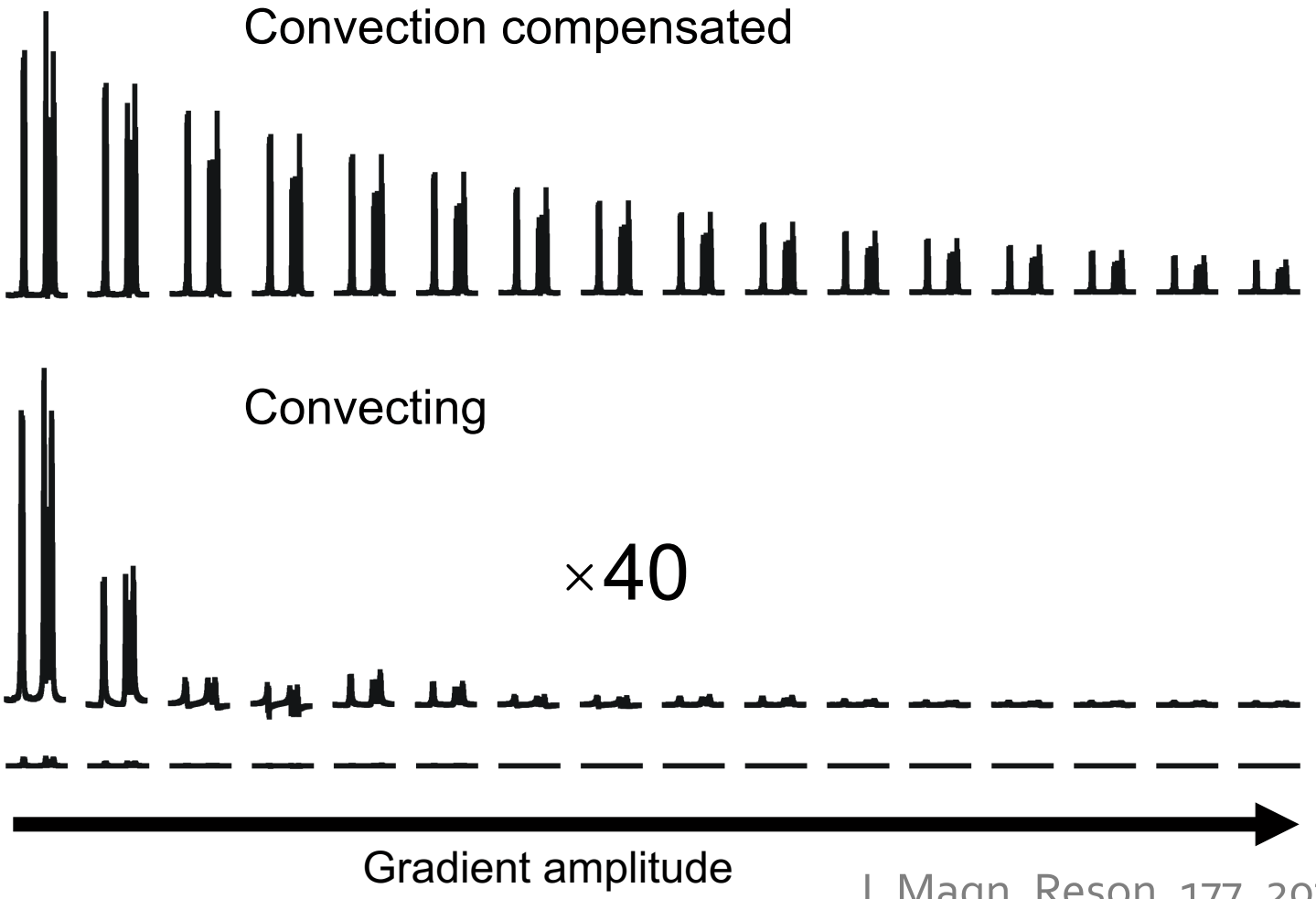


2DJ-IDOSY - Compensated

J. Magn. Reson. 177, 203 (2005)

Convection in an NMR tube

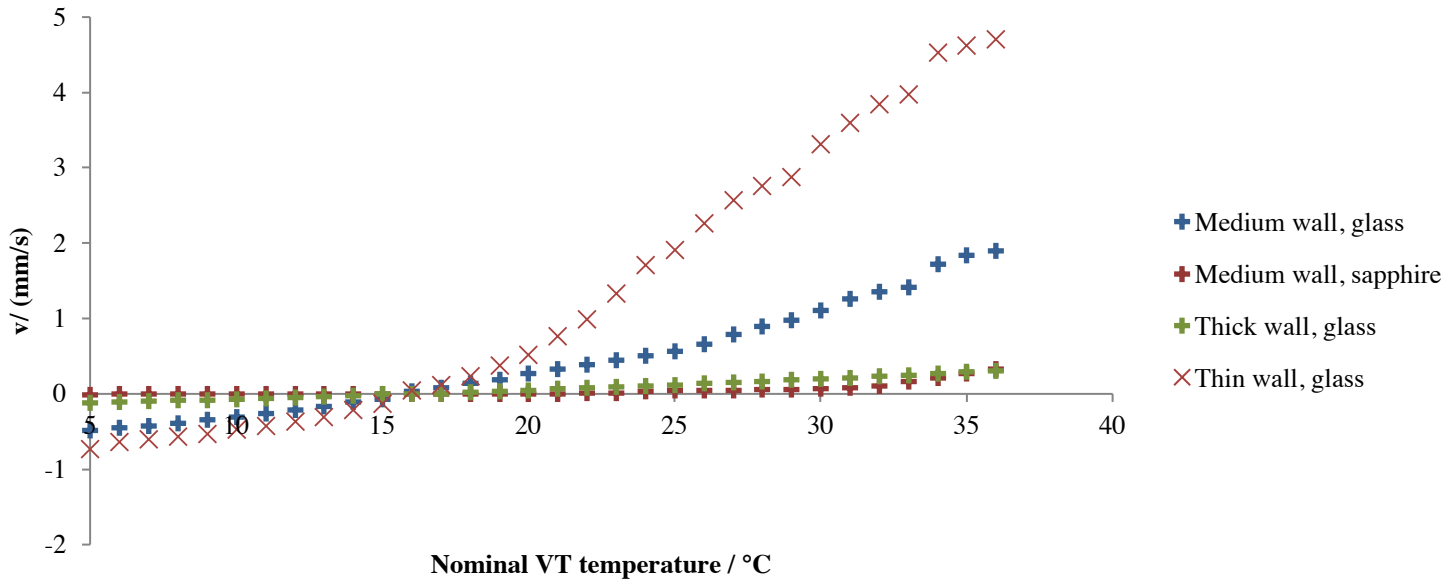
Aromatic signals from quinine (7.1 to 7.6 ppm) as a function of increasing gradient strength at 25 °C.



J. Magn. Reson. 177, 203 (2005)

Experimental measurements of convection velocity (1)

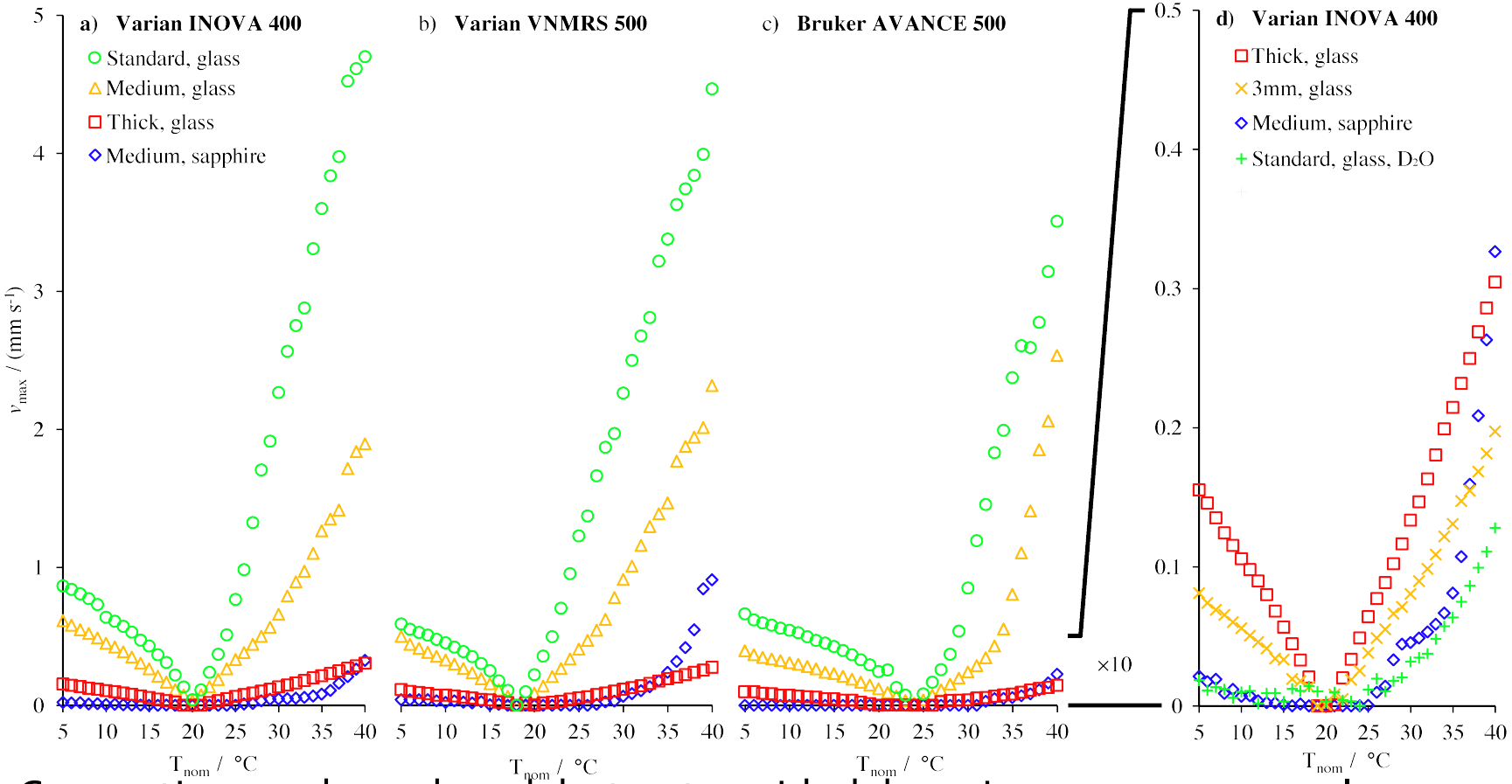
Common sense suggests that convection should only happen where the bottom of the sample as warmer than the top – a negative temperature gradient. Experiment shows that this is not the case: convection in a chloroform sample occurs both above and below the quiescent sample temperature.



Rayleigh-Bénard convection requires –d*T*/dz above a critical threshold, so cannot be responsible here – instead, we are seeing **Hadley convection** at lower temperatures, driven by *horizontal* temperature gradients.

Experimental measurements of convection velocity (2)

Similar results initially seen on 4 different spectrometers (**but that is not the whole story**)

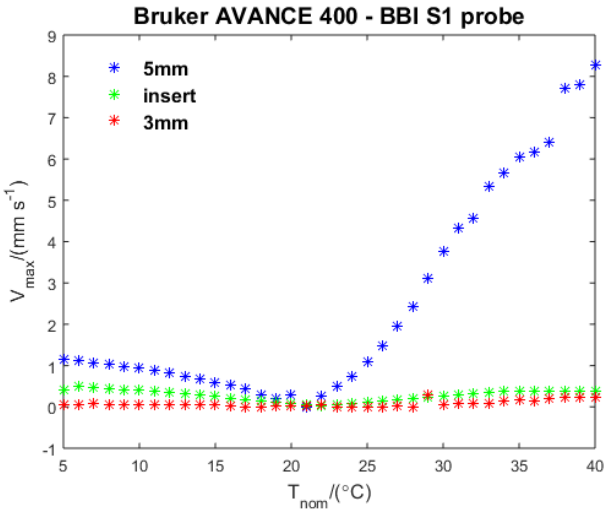


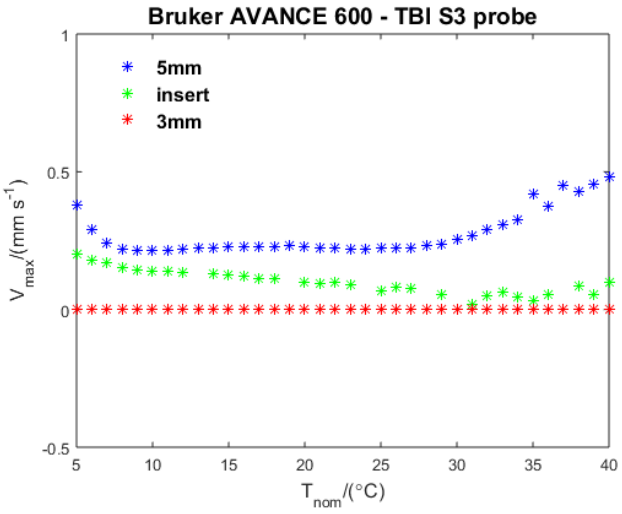
Convection can be reduced, but not avoided, by using narrower samples and/or using sapphire rather than glass; by using solvents with lower coefficient of thermal expansion/higher viscosity; and by reducing d*T*/d*x*,d*y*.

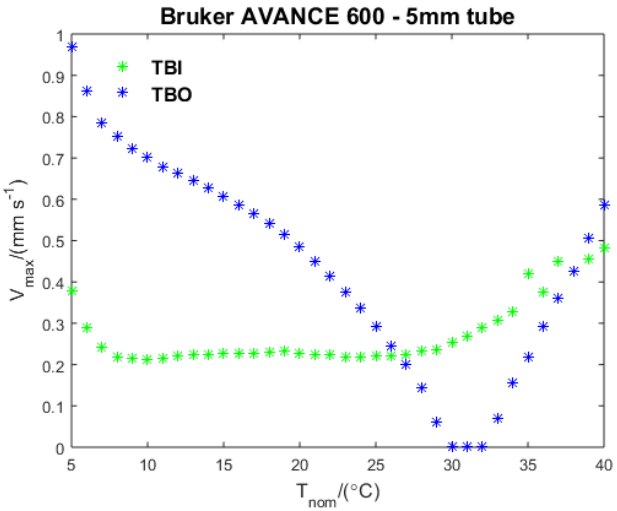
J. Magn. Reson. 252, 120 (2015)

Experimental measurements of convection velocity (3)

Other probes/spectrometers show more florid behavior
(all chloroform samples)







In some probes convection is always present at a problematic level (for a standard 5 mm tube using chloroform as solvent). It is still obvious that using a a restricted sample diameter suppresses convection very efficiently.

RSC Advances. 6, 95173 (2016)

How to minimise convection

Use a small (inner) diameter tube

This is probably the most effective method, but costs you (typically 50%) in sensitivity. A 3mm tube or a thick-walled 5mm tube are good choices.

Use a more viscous solvent

D_2O and DMSO are good choice. Solvent like chloroform convects very easily

Turn of the VT control (Not for cryoprobes!)

Leaving the probe to equilibrate a quiescent temperature minimizes temperature gradients.

Restrict the sample height

E.g. using a Shigemi tube. Significantly less effective than a small diameter but preserves more signal.

How to minimise convection

Use a sapphire tube

Expensive but the high heat conductivity helps reduce temperature gradients.

Increase the VT air flow

Helps reduce temperature gradients, but vibrations can disturb the measurements.

Spin the sample

Very efficient (reduces temperature gradients), but often gives messy results if the sequence timing is not matched with the rotation frequency.

Use convection compensated sequences

“Last resort” e.g. for high and low temperature experiments. Good but not perfect compensation. Costs 50% in sensitivity and requires a lot (64 scans) phase cycling.

Diffusion NMR: today's programme

9.00-9.50	Introduction and Theory
9.50-10.10	Break
10:10-11.00	Acquisition, Analysis and Practicalities
11.00-11.30	Questions and Answers
11.30-14.00	Lunch
14.00-14.50	Advanced experiments
14.50-15.00	Break
15.00-15.50	Introduction to the GNAT Processing software
15.50-16.00	Break
16.00-17.00	Hands on analysis using GNAT, with your own (or provided) data.
17.00-17.30	Conclusion and open discussion

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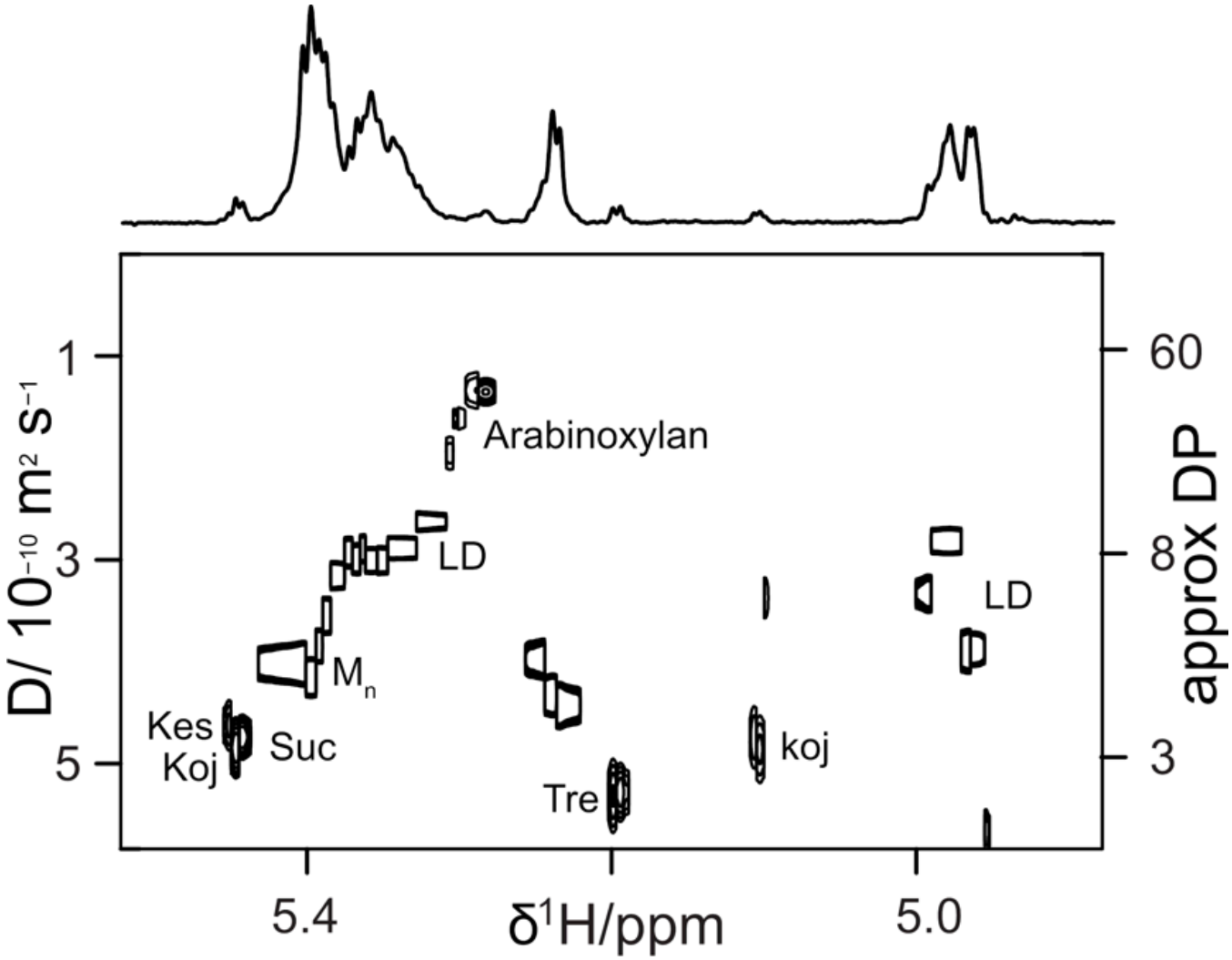
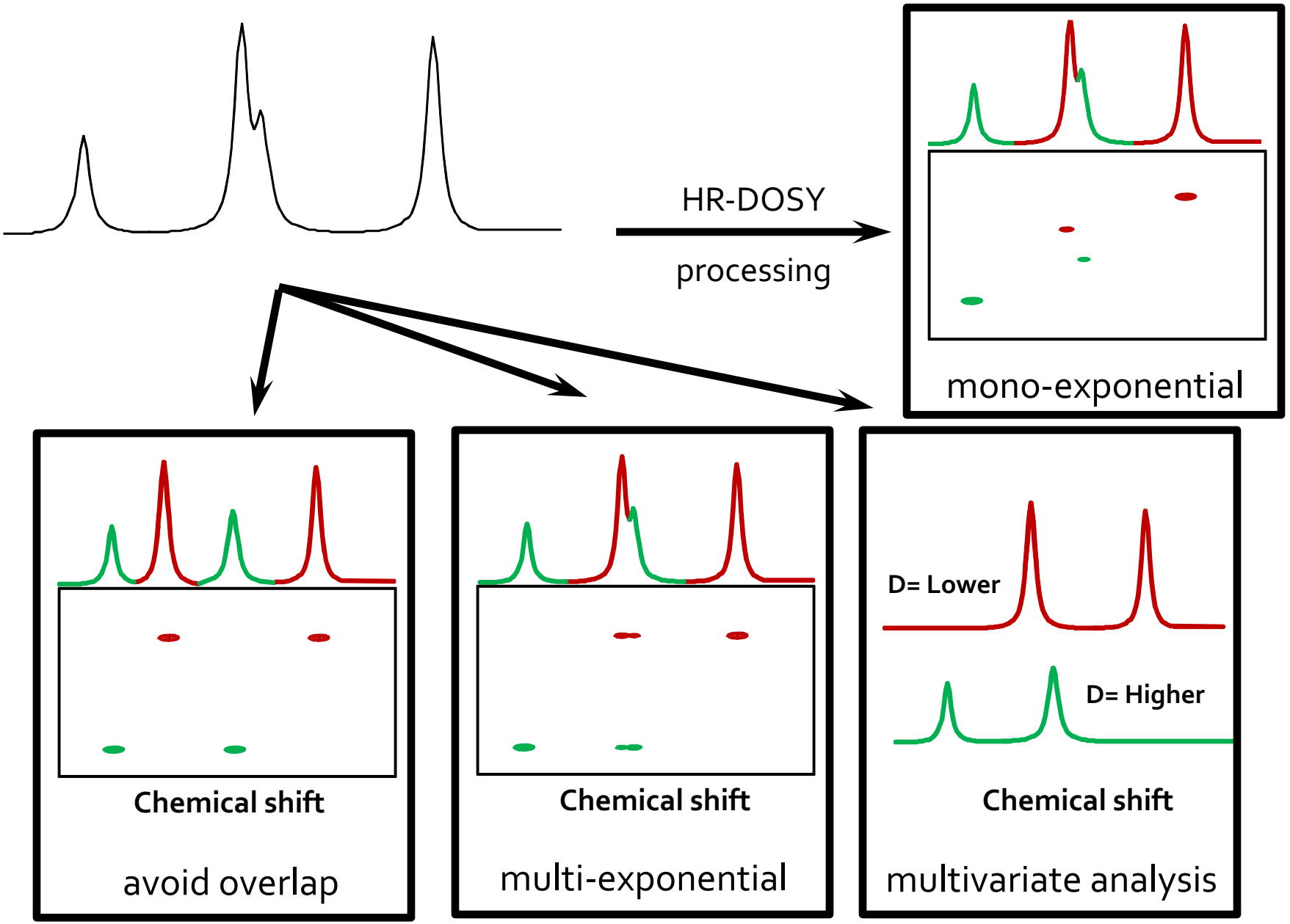
Lager beer: 800 MHz proton 2D DOSY spectrum



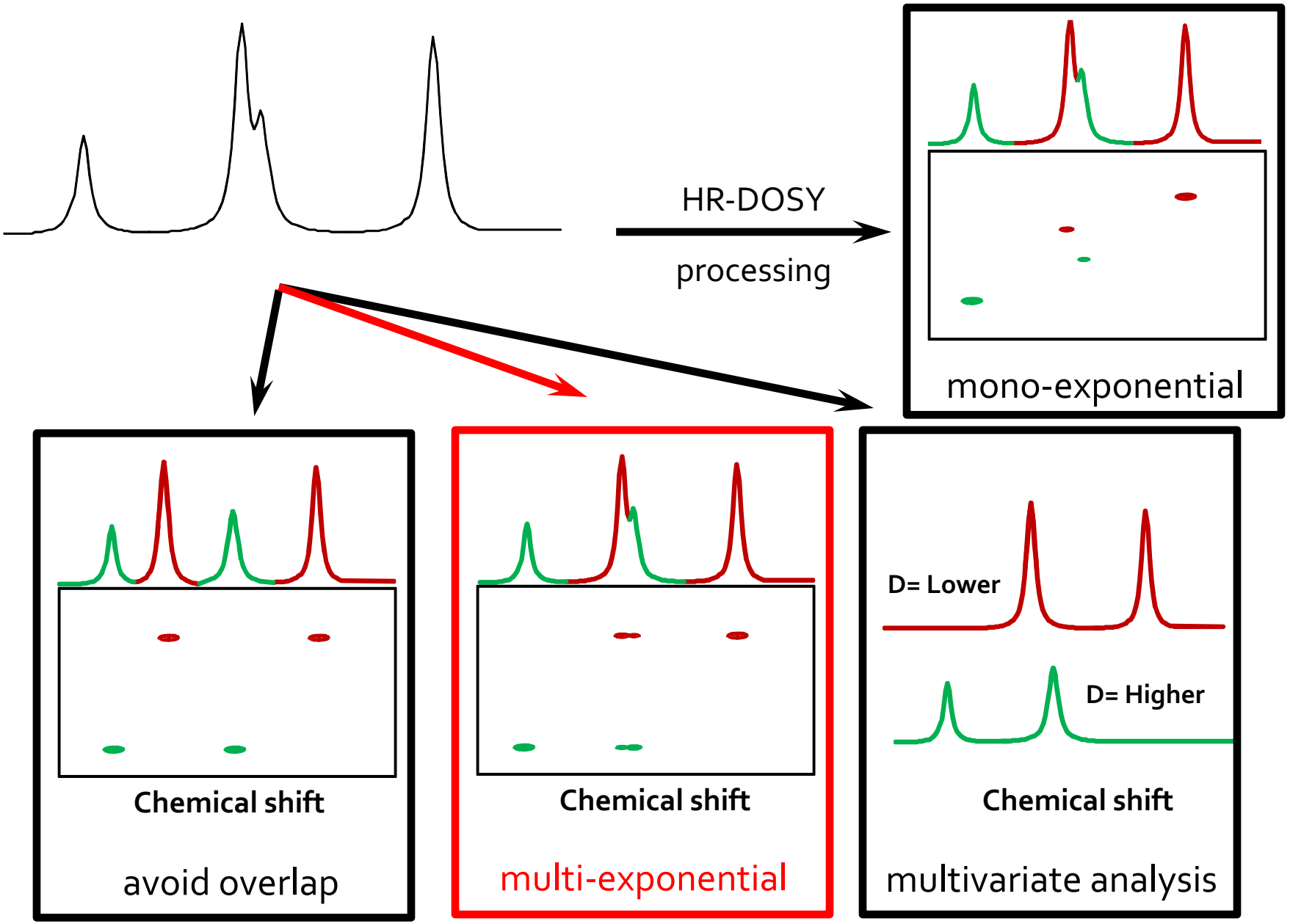
Anomeric region, estimation of degree of polymerisation of several oligo- and polysaccharides

Food Chemistry. **2013**, 150, 65

signal overlap in DOSY processing

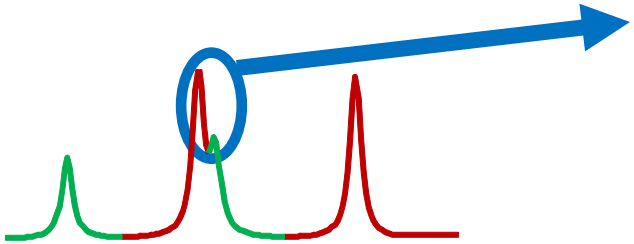


signal overlap in DOSY processing

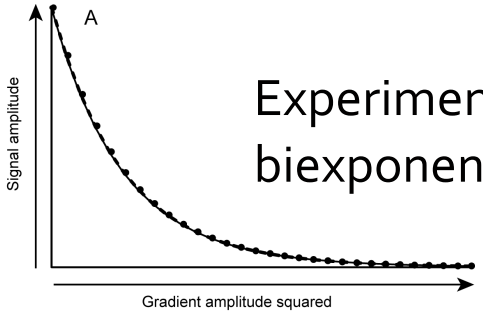


Resolving superimposed exponentials

Superimposed exponentials is a very difficult mathematical problem (ill-posed and numerically unstable).
It is only practically feasible with high signal to noise ratio and for a limited (2-3) number of exponentials.



Chemical shift

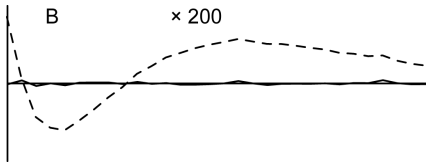


Experimental (noisy) biexponential decay

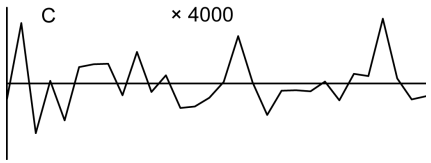
Residuals (**E**) are the fit (**F**) subtracted from the experimental data (**X**)

R = X-F

Residuals:



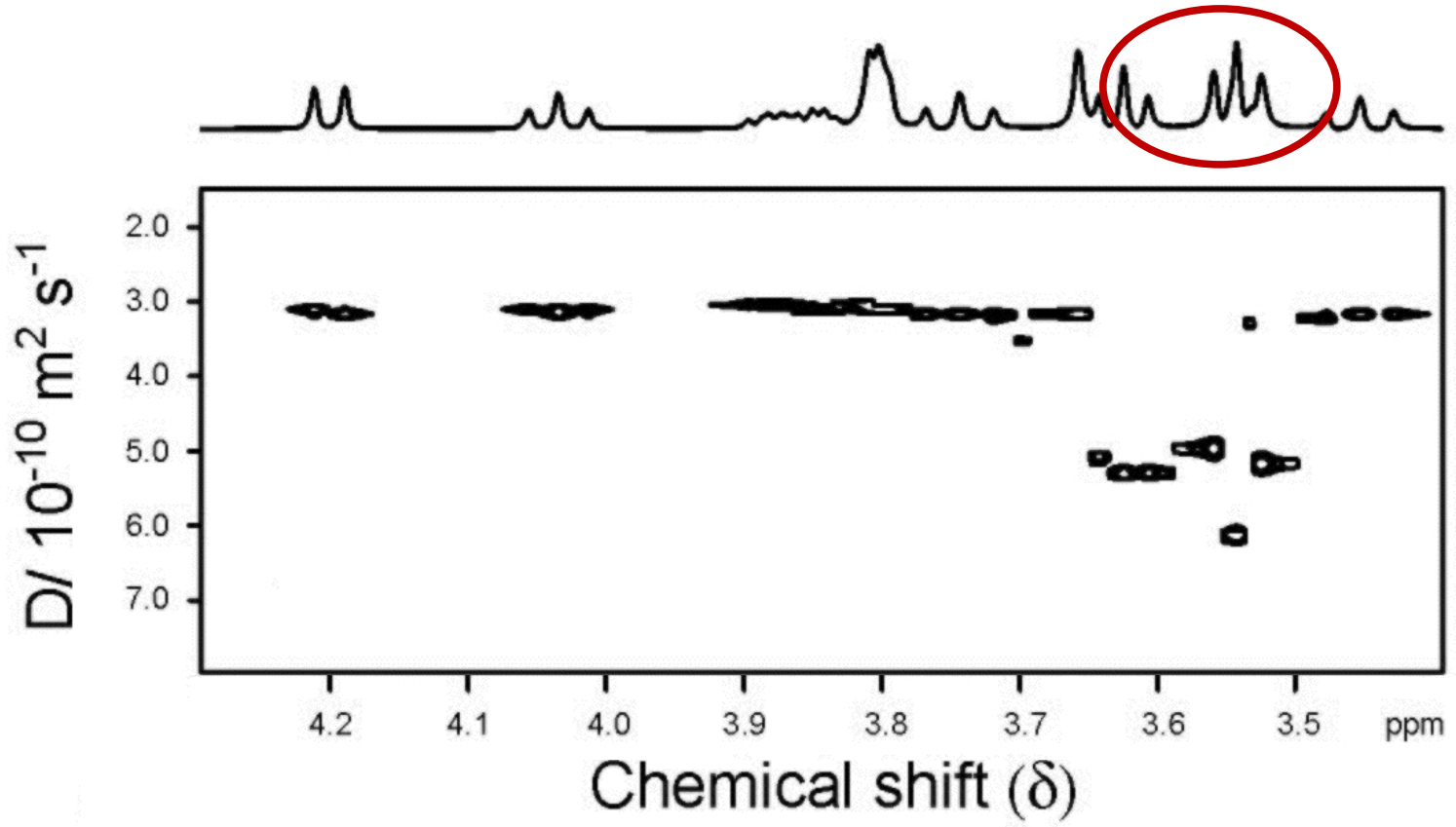
monoexponential fit



biexponential fit
only noise remaining

overlap in 2D DOSY: monoexponential fitting

400 MHz oneshot spectrum of sucrose, isopentanol and propan-1-ol in D_2O

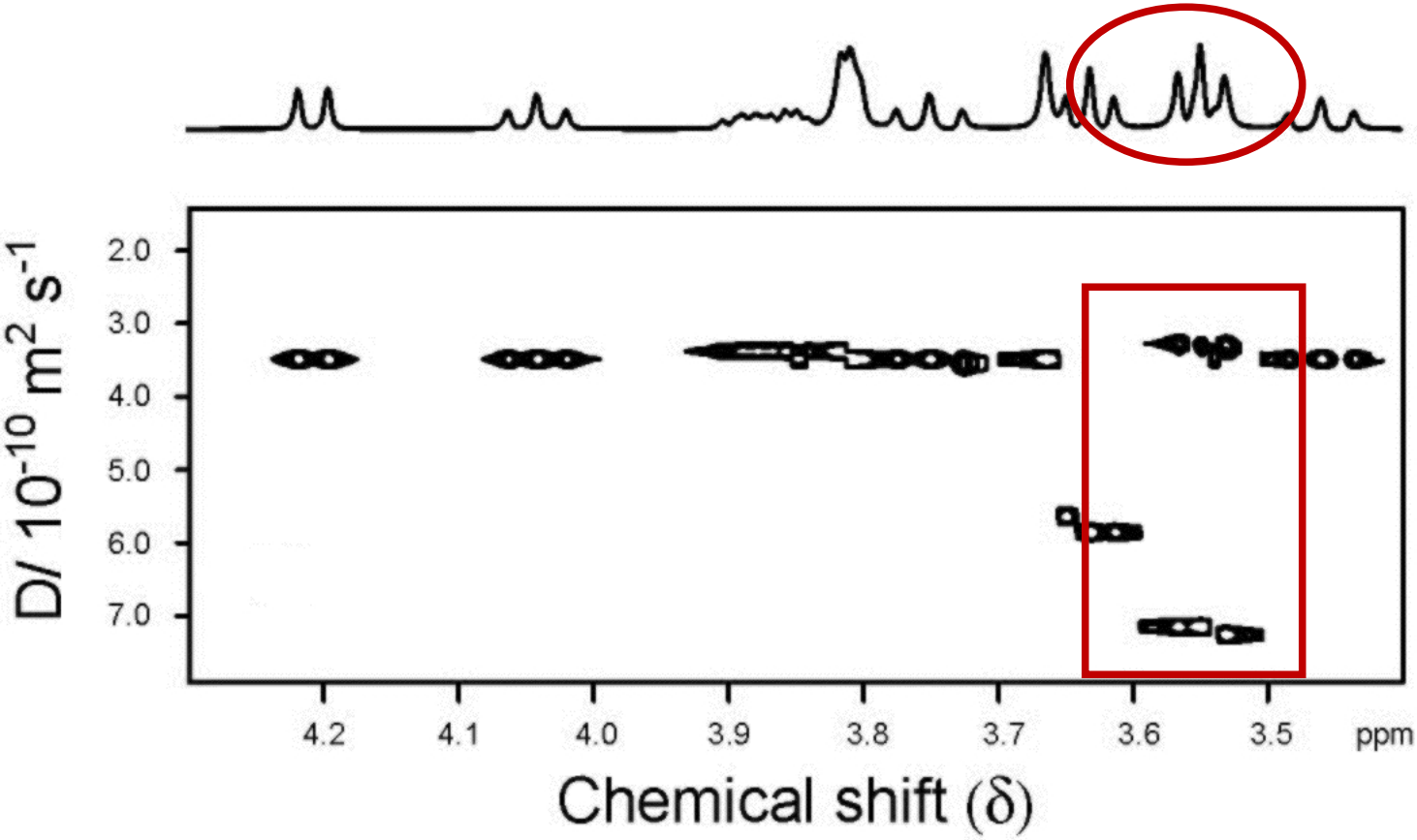


Overlapping peaks give
compromise diffusion coefficient

Anal. Chem. **2006**, 78, 3040-3045

overlap in 2D DOSY: biexponential fitting

400 MHz oneshot spectrum of sucrose, isopentanol and propan-1-ol in D_2O

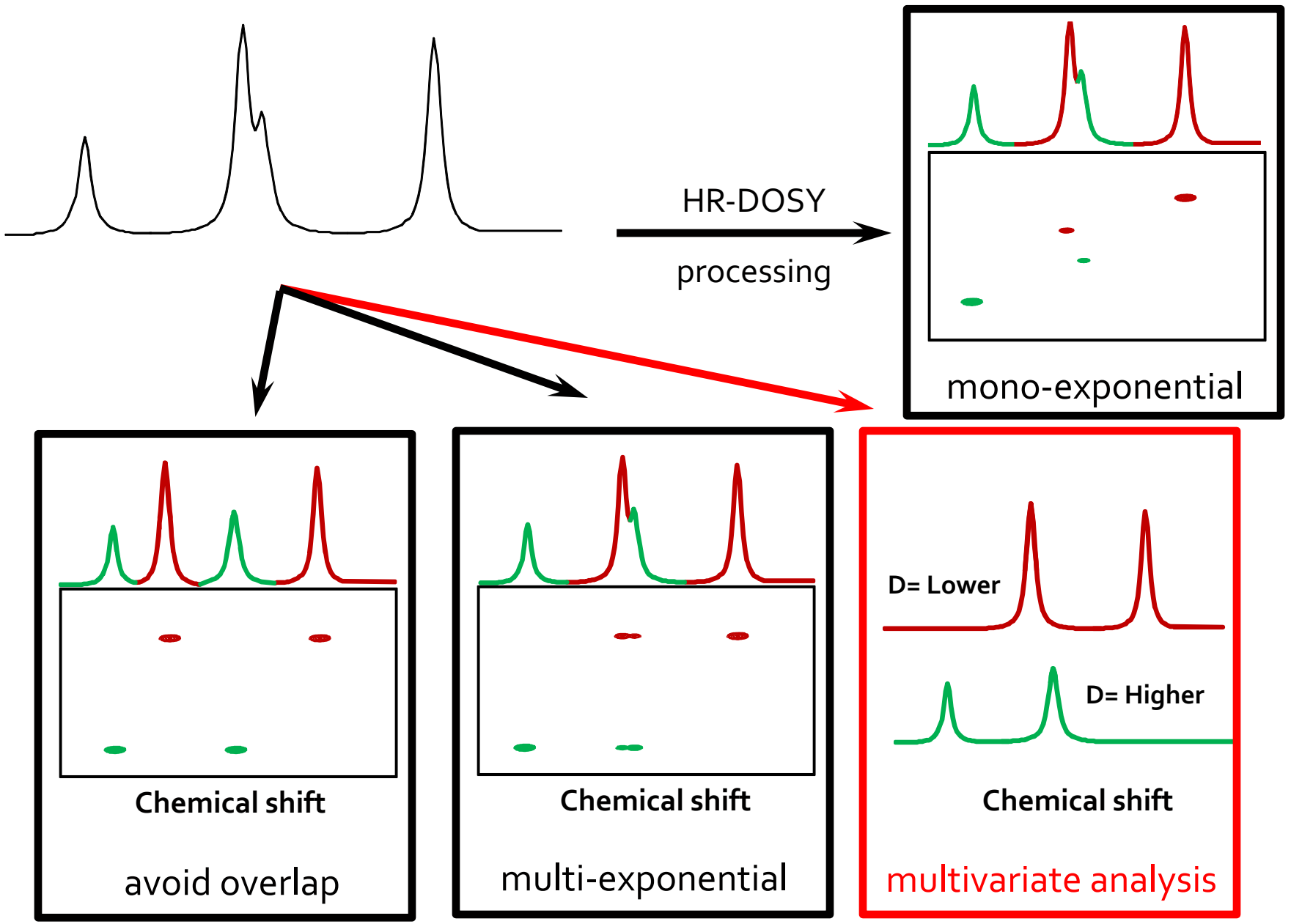


D_A and D_B must differ by at least 30%

Very dependent on the quality and S/N of data

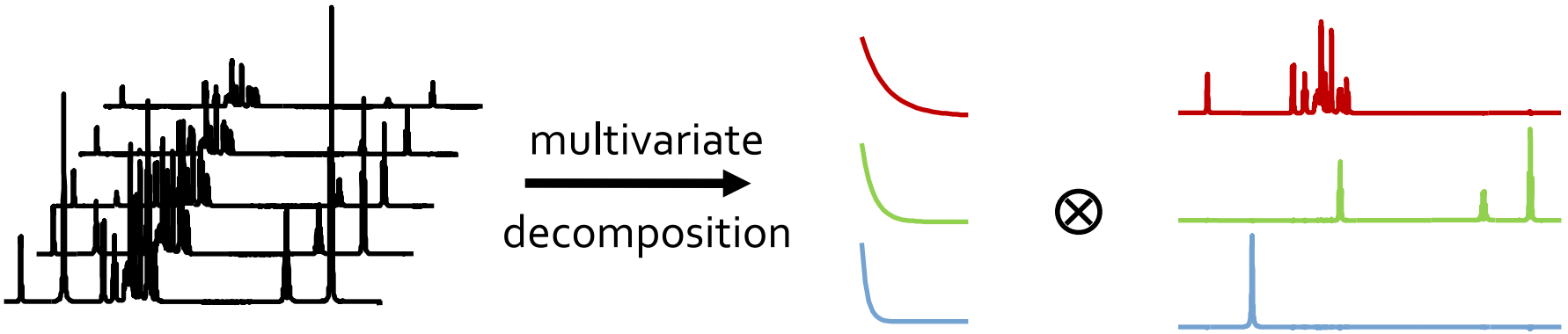
Anal. Chem. **2006**, 78, 3040-3045

signal overlap in DOSY processing



Processing “DOSY” data: SCORE/OUTSCORE

Here we fit the whole spectrum at once rather than each peak at the time as in HR-DOSY. This makes sense as all peaks in a component spectrum decays in the same way



DOSY data (**X**) decays (**C**) spectra (**S**)

$$\mathbf{X} = \mathbf{C}\,\mathbf{S}^T + \mathbf{E}$$

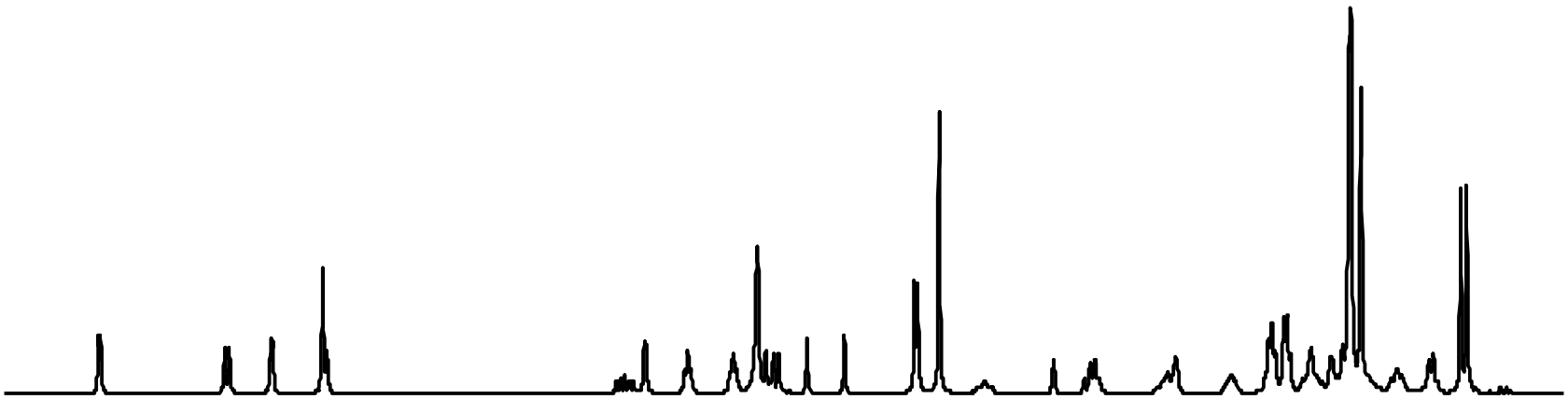
Minimize **E** assuming a known decay form

SCORE: $\mathbf{E} = \mathbf{X} - \mathbf{C}\mathbf{S}^T$ (residuals)

OUTSCORE: $\mathbf{E} = |\mathbf{S}_i| \,.\, |\mathbf{S}_j|$ (spectral similarity)

Chem. Commun. **2013**, 49, 10510; Anal. Chem. **2008**, 80, 3777

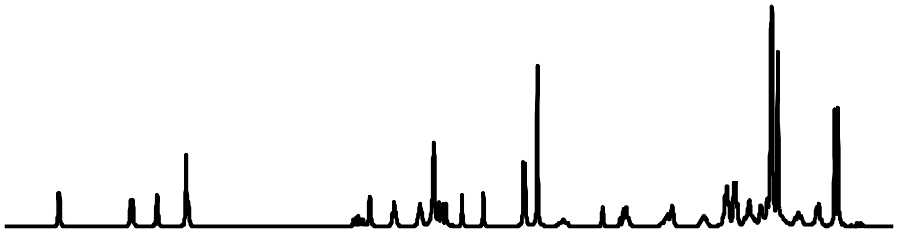
Speedy Component Resolution (SCORE)



Spectrum from mixture of quinine, camphene and geraniol in methanol-d_4

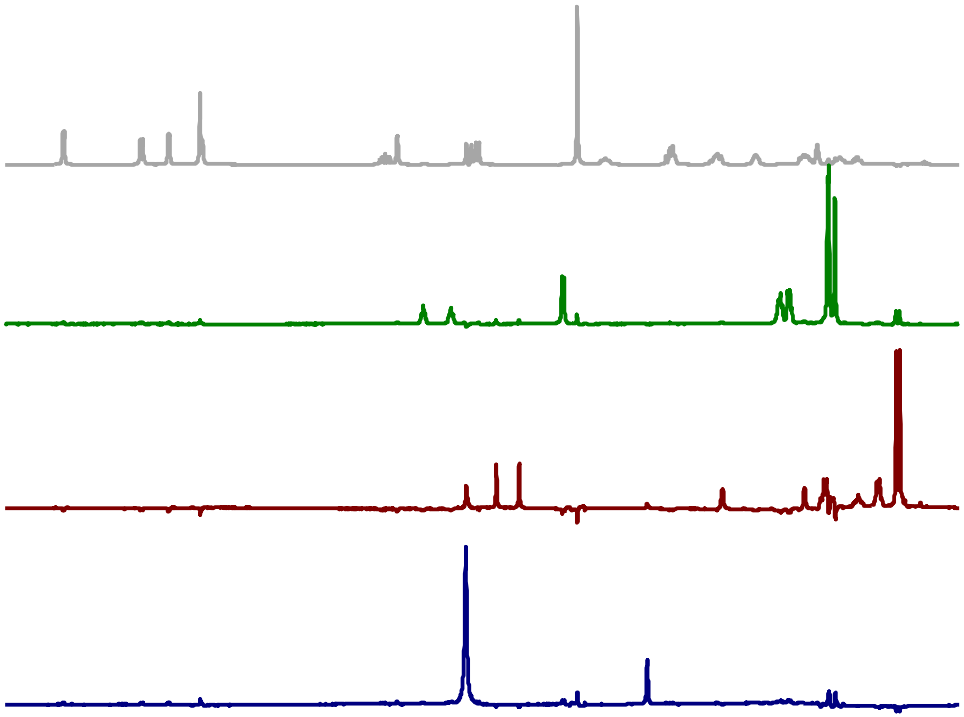
Anal. Chem. **2008**, 80, 3777

Speedy Component Resolution (SCORE)



Mixture

Fitted components



Quinine (5.1×10^{-10} m^2 s^{-1})

Geraniol 8.2×10^{-10} m^2 s^{-1}

Camphene (12×10^{-10} m^2 s^{-1})

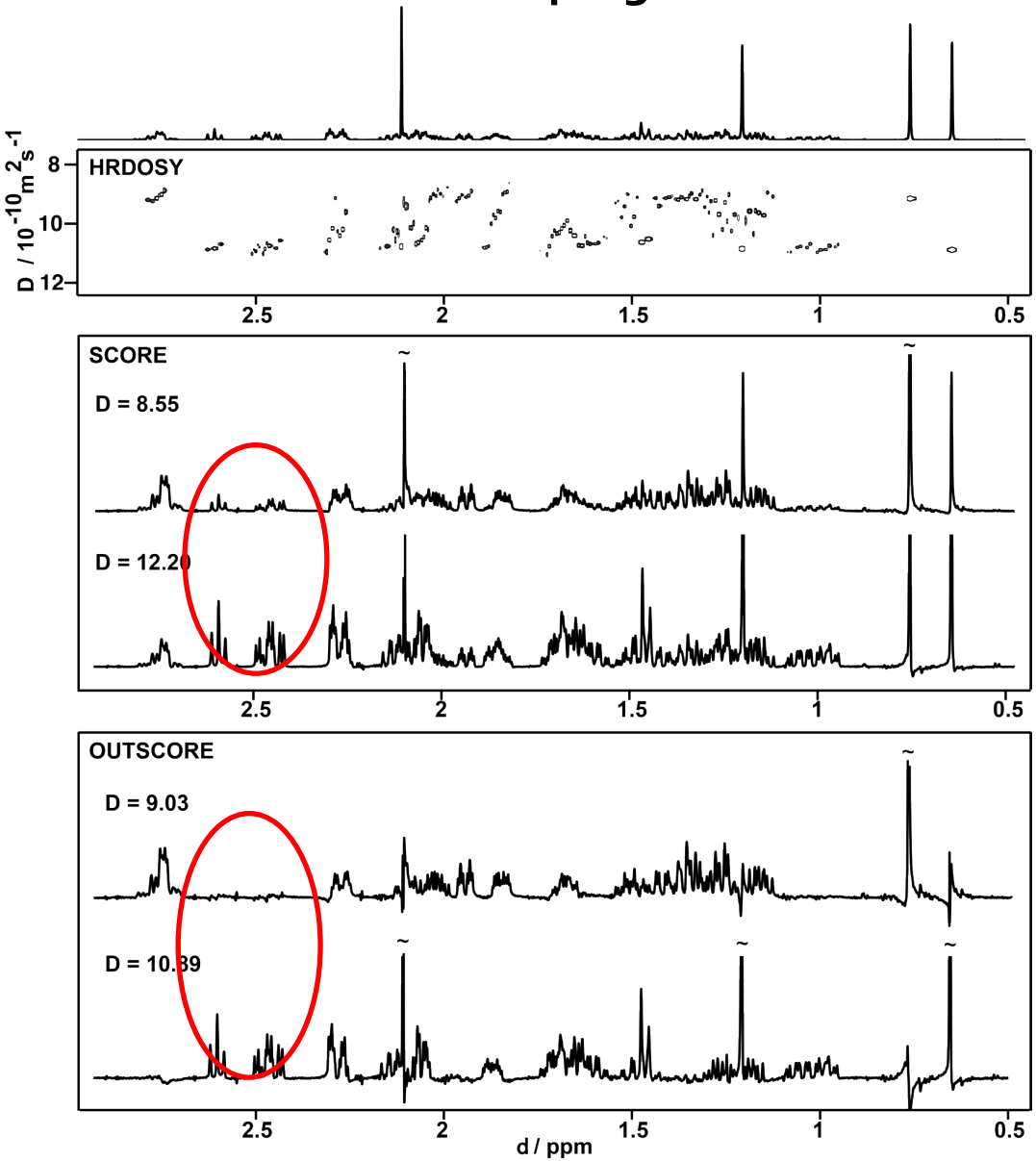
Methanol + D_2O16 $\times 10^{-10}$ m^2 s^{-1}

SCORE works well when differences in diffusion coefficient is more than 25%

Anal. Chem. **2008**, 80, 3777

Optimized Unmixing of True Spectra for COmponent Resolution (OUTSCORE)

a mixture of progesterone and estradiol in DMSO-d_6



HRDOSY

monoexponential fitting

<1% difference in D

suffers from overlap

SCORE

minimizes residuals

>30% difference in D

OUTSCORE

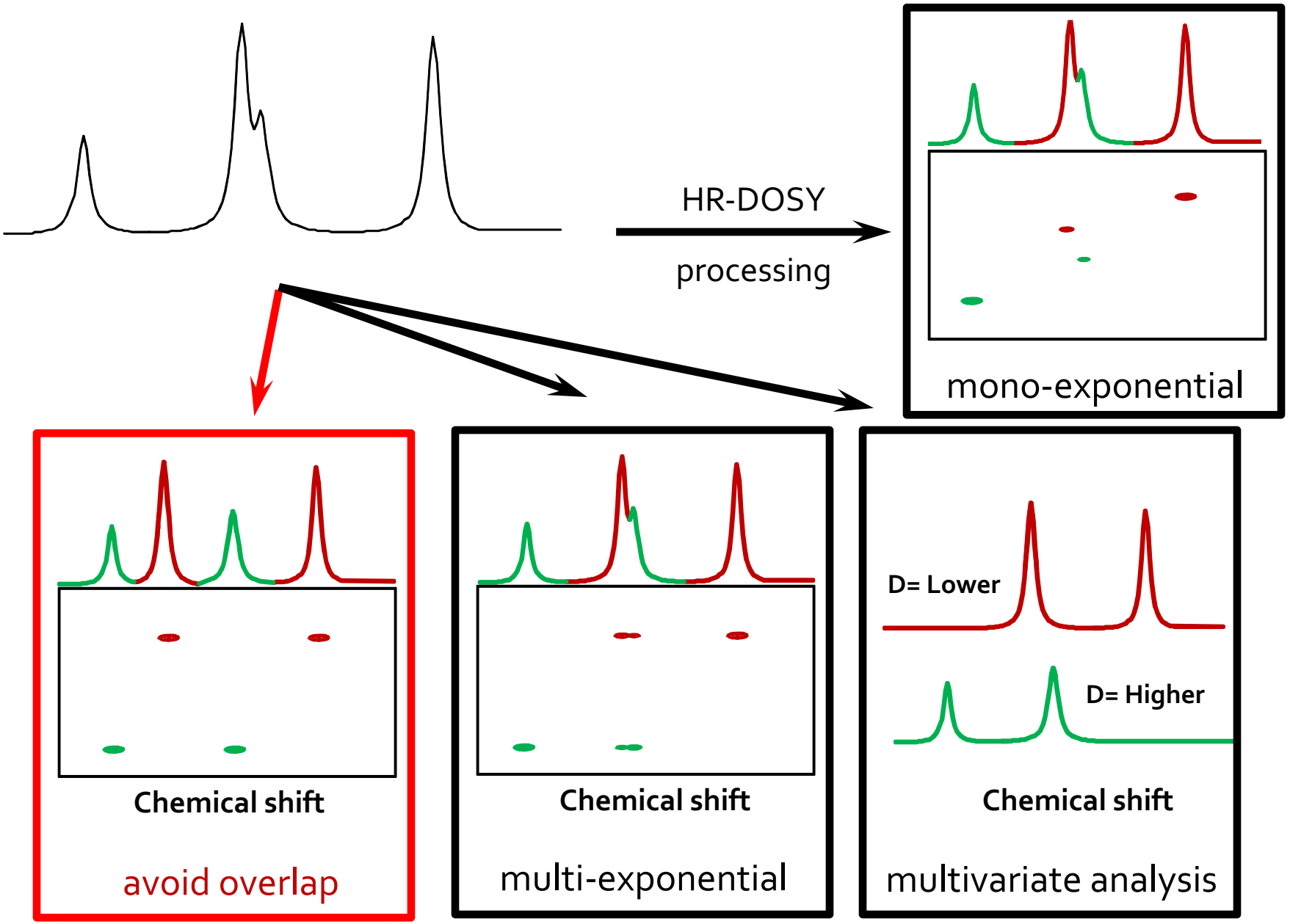
minimizes cross-talk

<5% difference in D

fewer components

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

signal overlap in DOSY processing



Advanced DOSY experiments to avoid spectral overlap

Using experiments with sparse signal gives less overlap

2D DOSY experiments

- INEPT-DOSY
- DEPT-DOSY
- Pure shift DOSY
- Different nuclei
- etc.

Spreading out the peaks in two dimensions gives less overlap

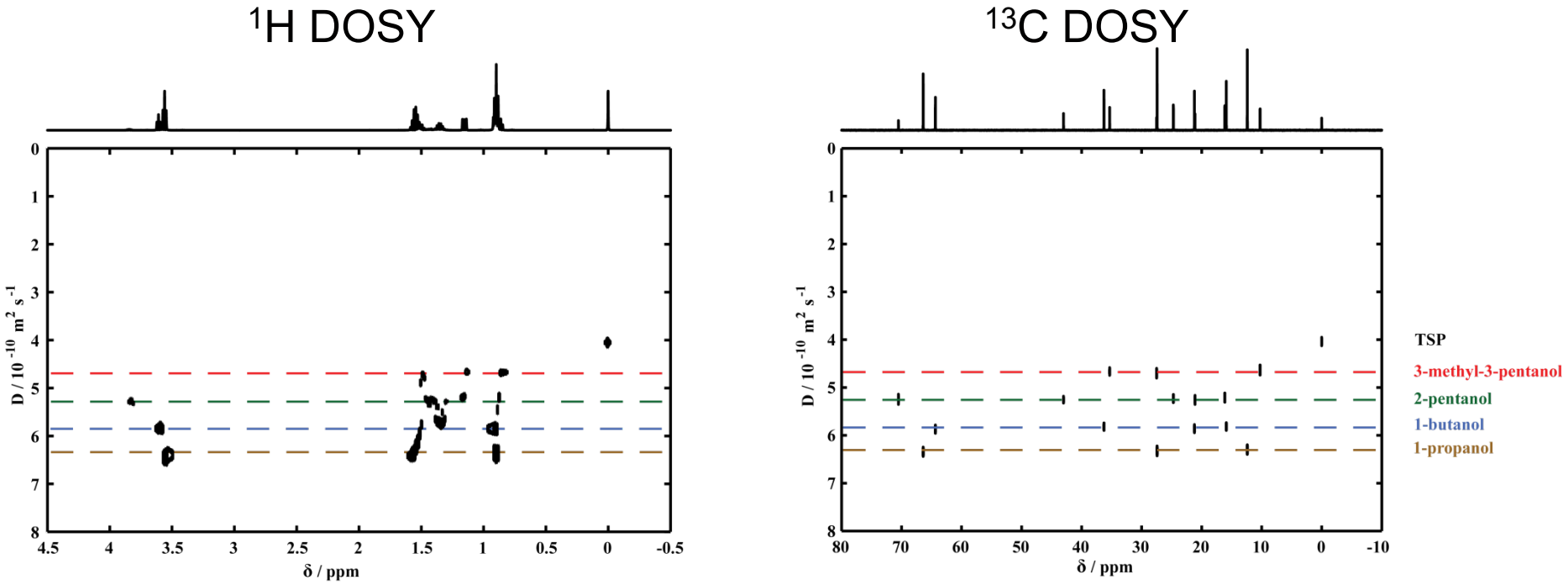
3D DOSY experiments

- COSY-DOSY
- TOCSY-DOSY
- HSQC-DOSY
- 2DJ-DOSY
- etc

DOSY can be added to any nD experiment (n+1)D DOSY

overlap in 2D DOSY: ^{13}C DOSY

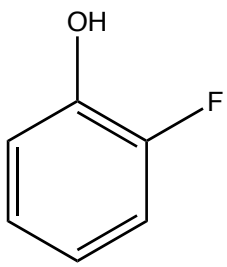
500 MHz ^{1}H and ^{13}C DOSY spectra of mixture of alcohols in D_2O



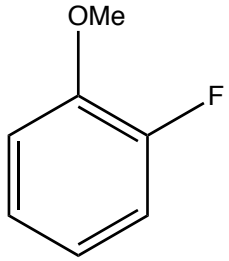
No overlap in the ^{13}C spectrum greatly facilitates interpretation

overlap in 2D DOSY: ^{19}F DOSY

600 MHz ^{1}H and ^{19}F DOSY spectra of fluorinated compounds in DMSO-d_6

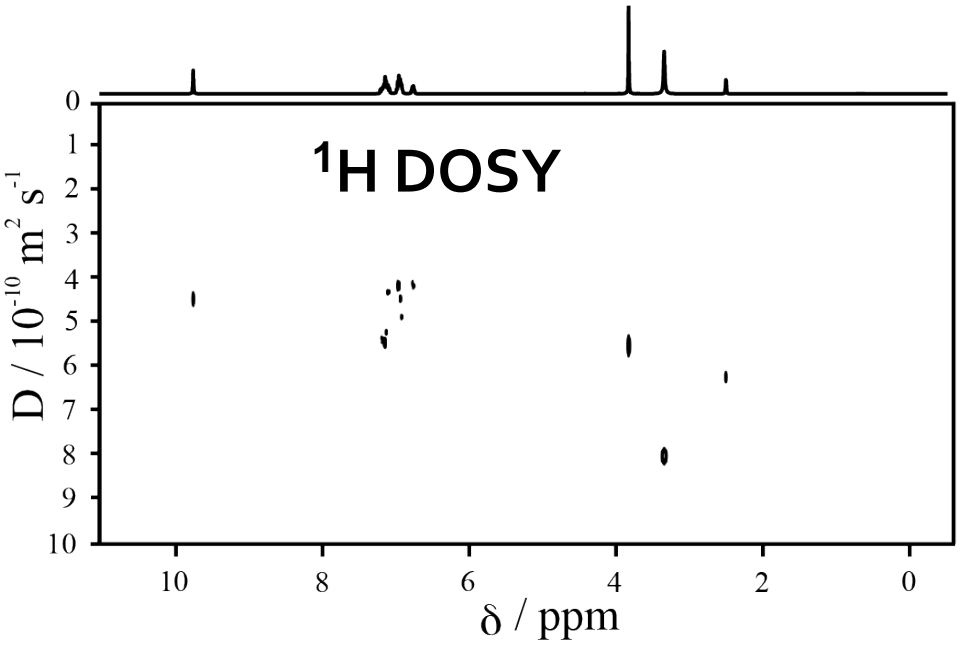


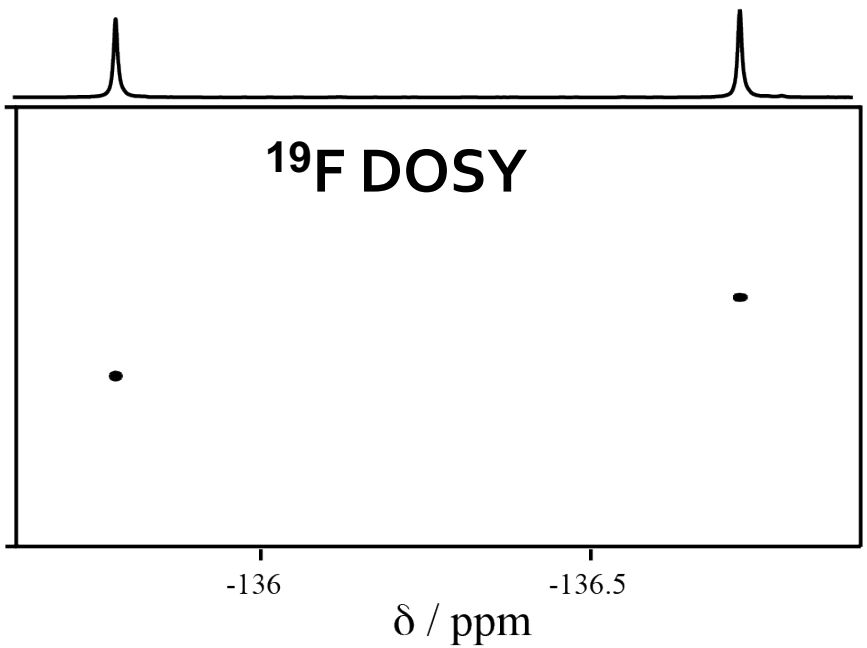
2-flourophenol



2-flouroanisol

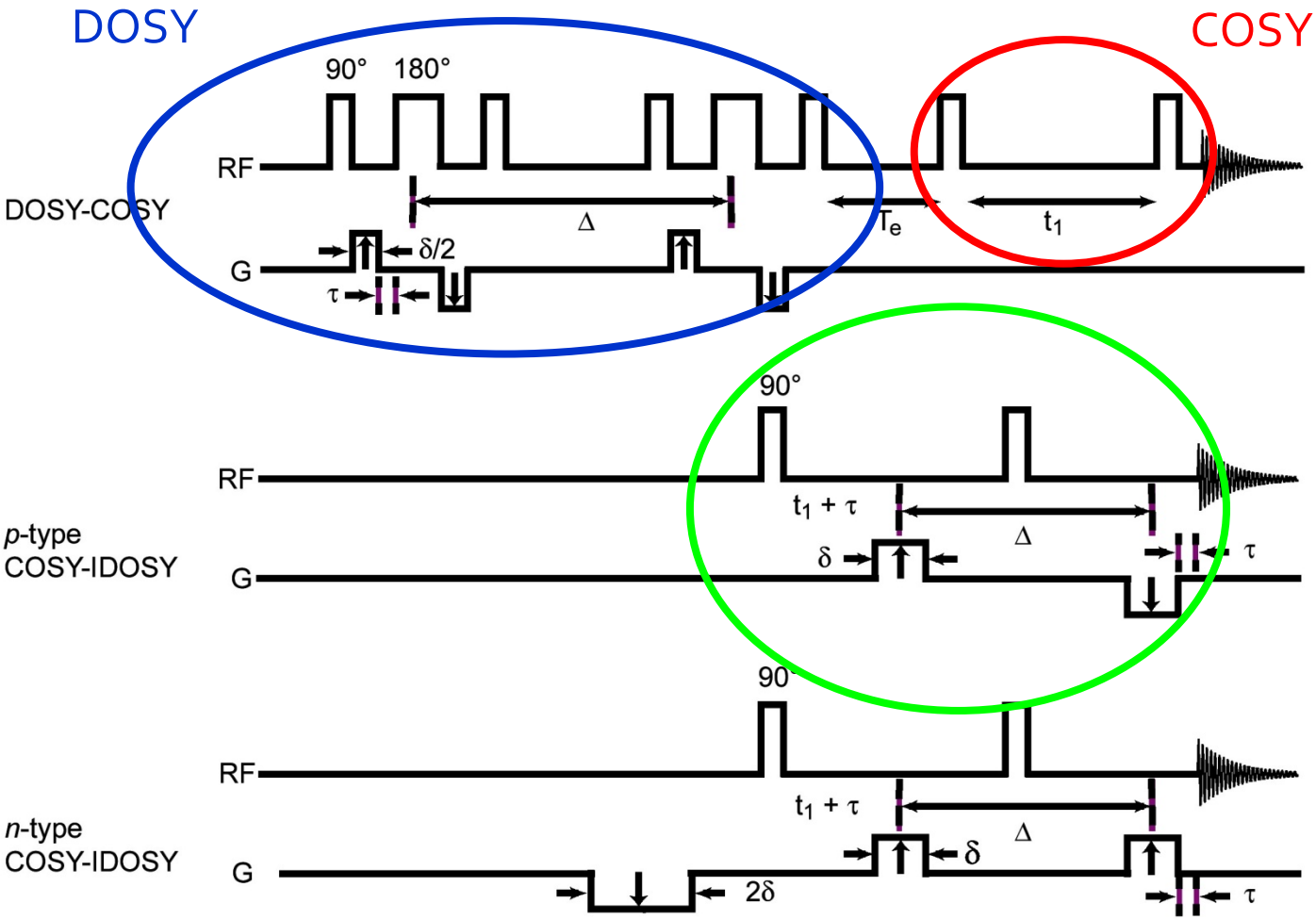
the simple and well dispersed ^{19}F spectrum allows unambiguous assignment





Magn. Reson. Chem. **2014** 52, 172

3D DOSY: COSY-DOSY



Concatenated

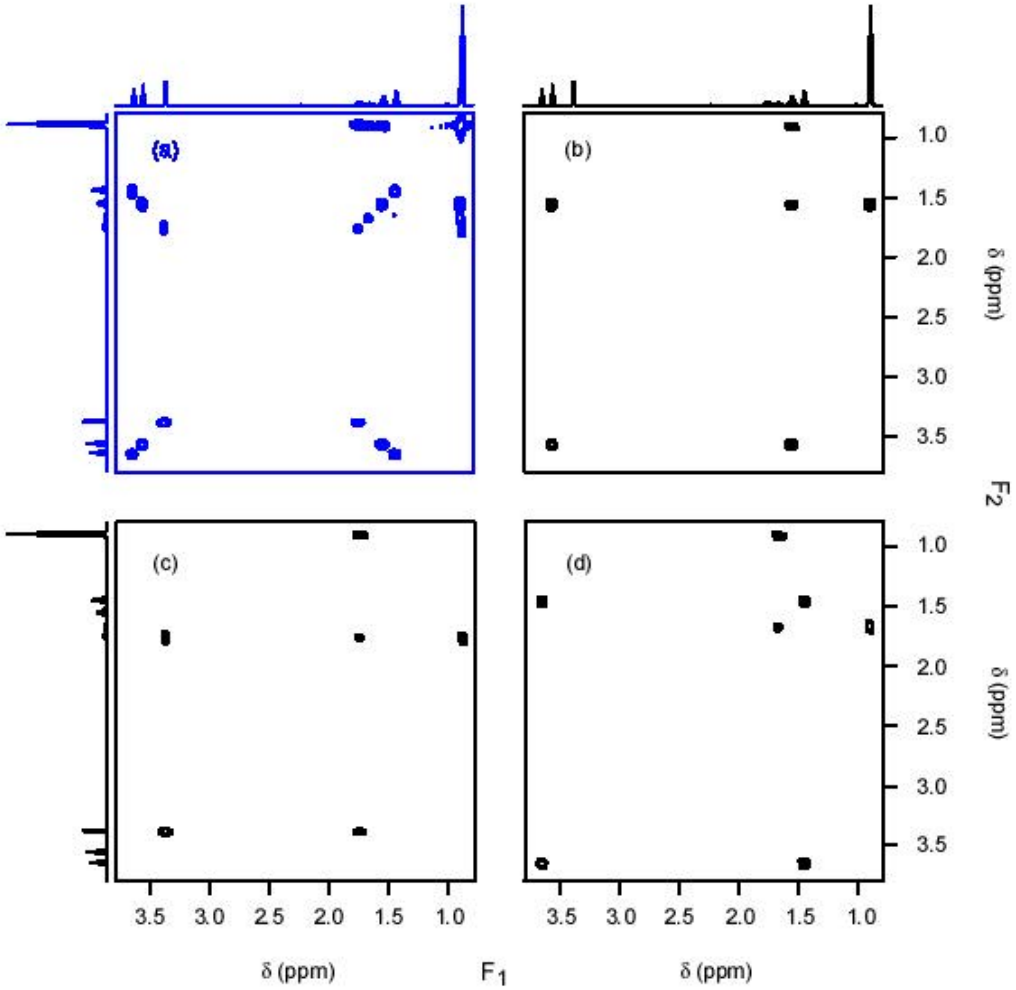
Existing gradient pulses (gCOSY) incremented for diffusion encoding

Integrated

An nD DOSY sequence can be constructed either by concatenating the mother experiment or integrated by use of existing delay(s) and/or gradient pulses for diffusion encoding

3D DOSY: COSY-DOSY

full COSY spectrum



Propanol
8.3-8.6 x 10^{-10} m^2 s^{-1}

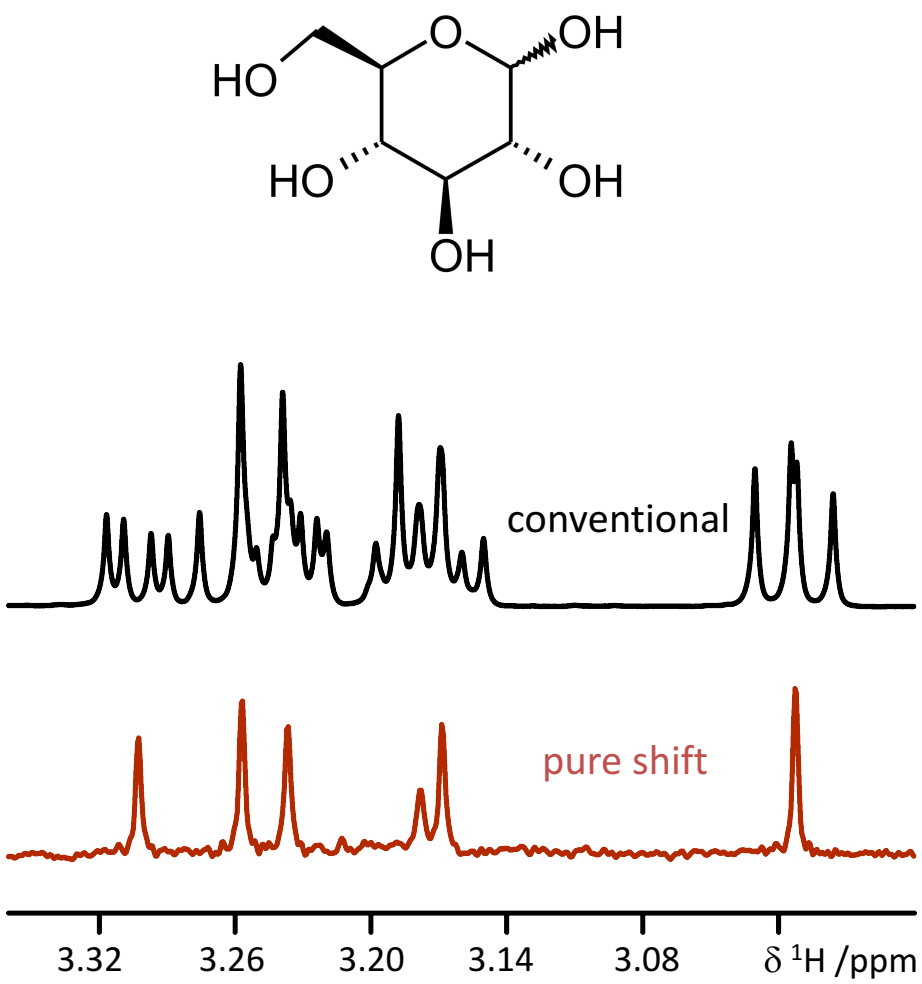
Isopentanol
7.3-7.8 x 10^{-10} m^2 s^{-1}

Isobutanol
6.8-7.2 x 10^{-10} m^2 s^{-1}

2D planes from a COSY-IDOSY 3D spectrum of a mixture of propanol, isobutanol and isopentanol.

Chem. Commun. 2005, 1737-1739

pure shift NMR

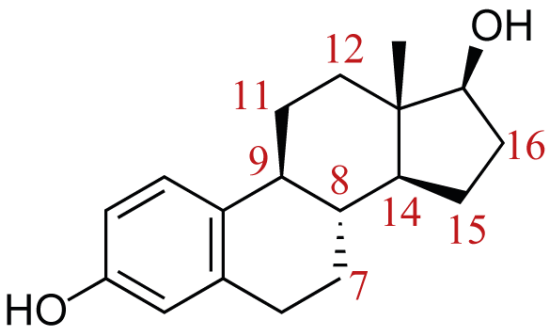


^{1}H NMR of glucose in D_2O

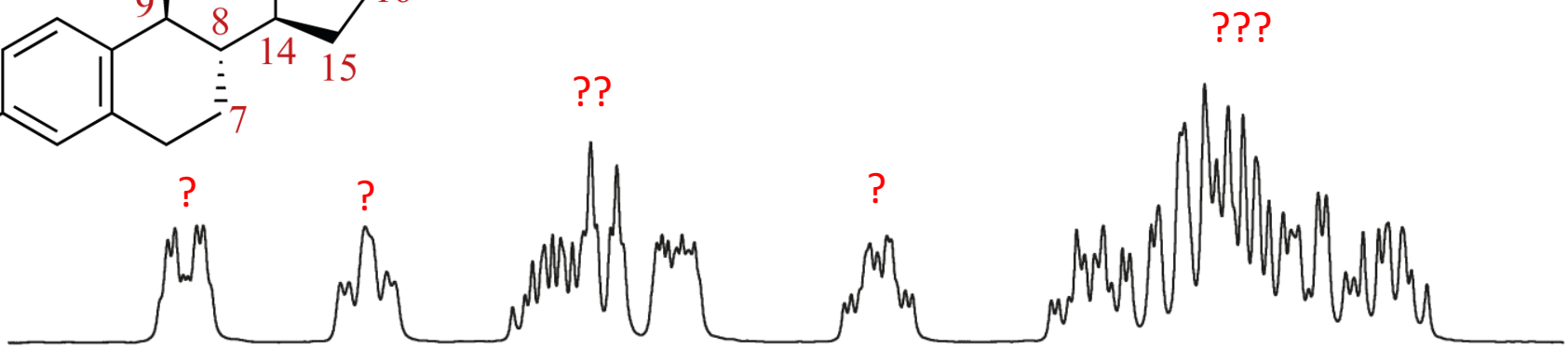
In a pure shift experiment the effect of the j-couplings are supressed. The result is a spectrum with a singlet for each chemical site (i.e. how we commonly expect ^{13}C spectra to look like).

The increase in spectral resolution can be exploited in DOSY experiments.

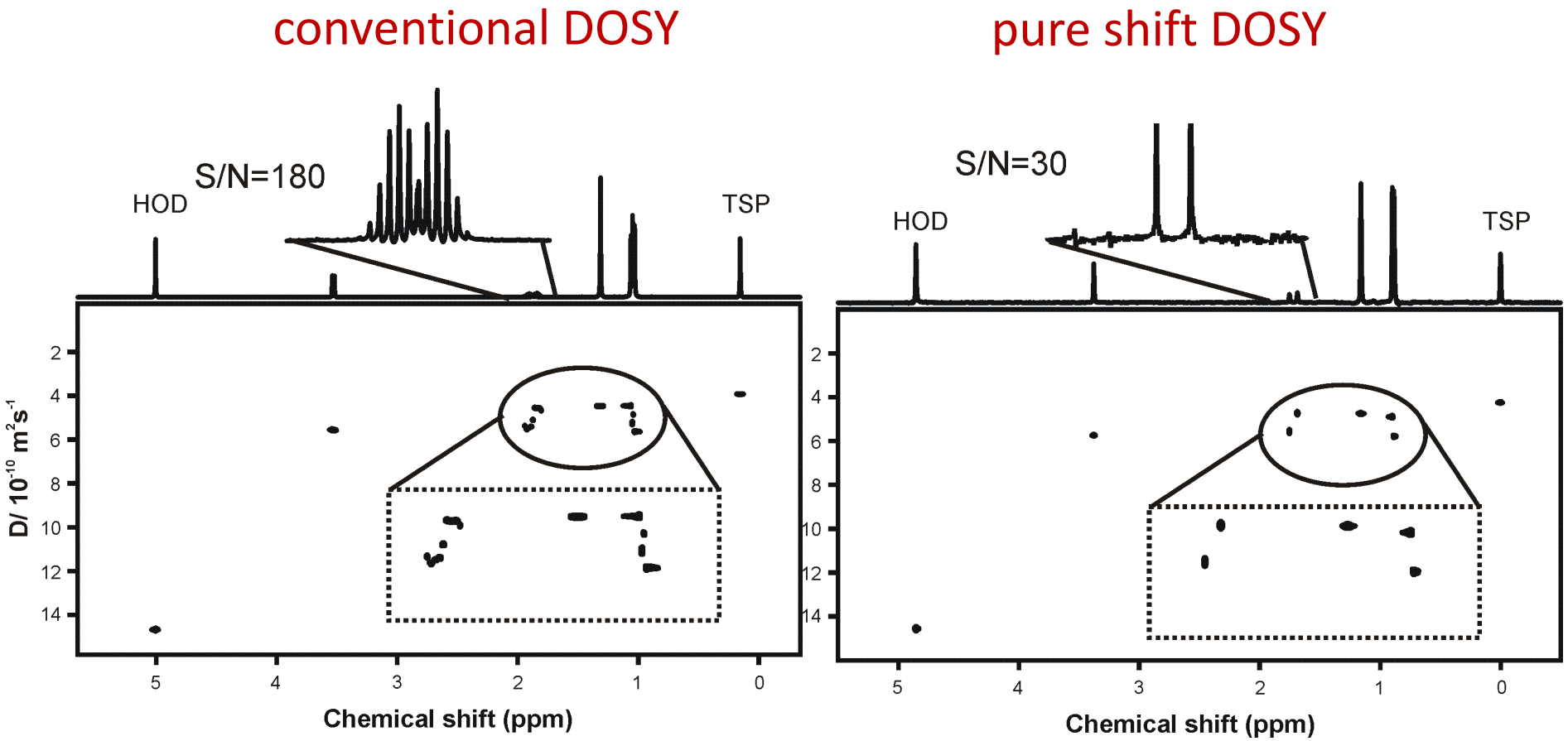
Pureshift NMR: Simplifying spectra



Estradiol: 500 MHz ^{1}H spectrum



pure shift: DOSY

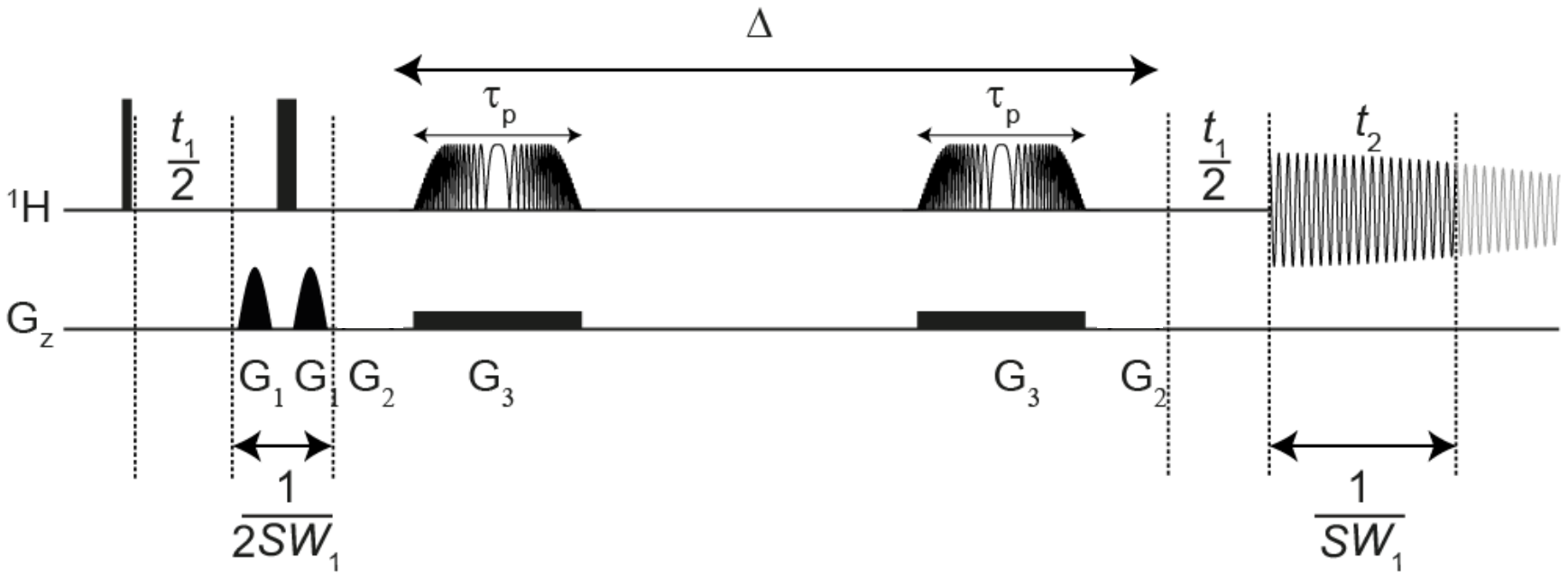


400 MHz DOSY spectra of 2-methyl-1-propanol and 2,3-dimethyl-2-butanol

Chem. Commun. **2007**, 933

PSYCHEiDOSY: an example of a pure shift DOSY sequence

The PSYCHE pure shift experiment

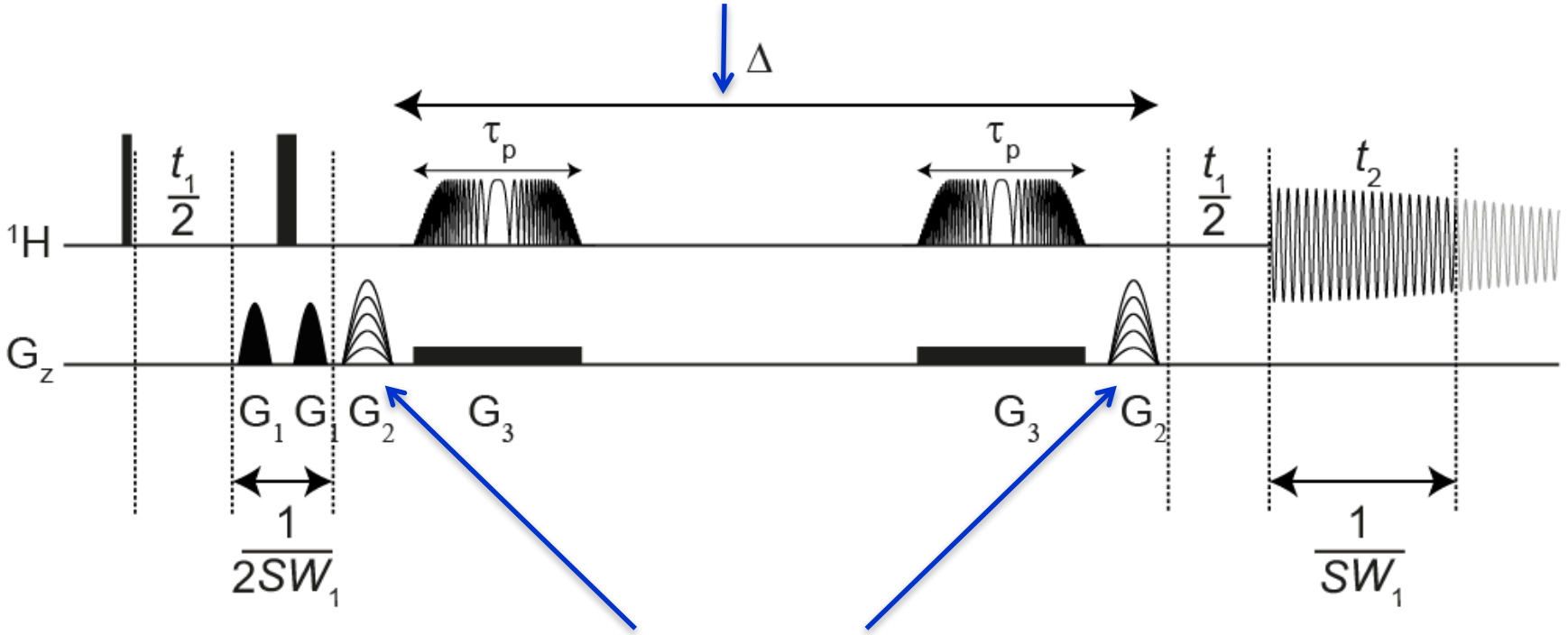


Angew. Chem. Int. Ed. **55**, 15579 (2016)

PSYCHEiDOSY: an example of a pure shift DOSY sequence

The PSYCHE pure shift experiment
can easily be adapted for diffusion encoding

Variable diffusion delay

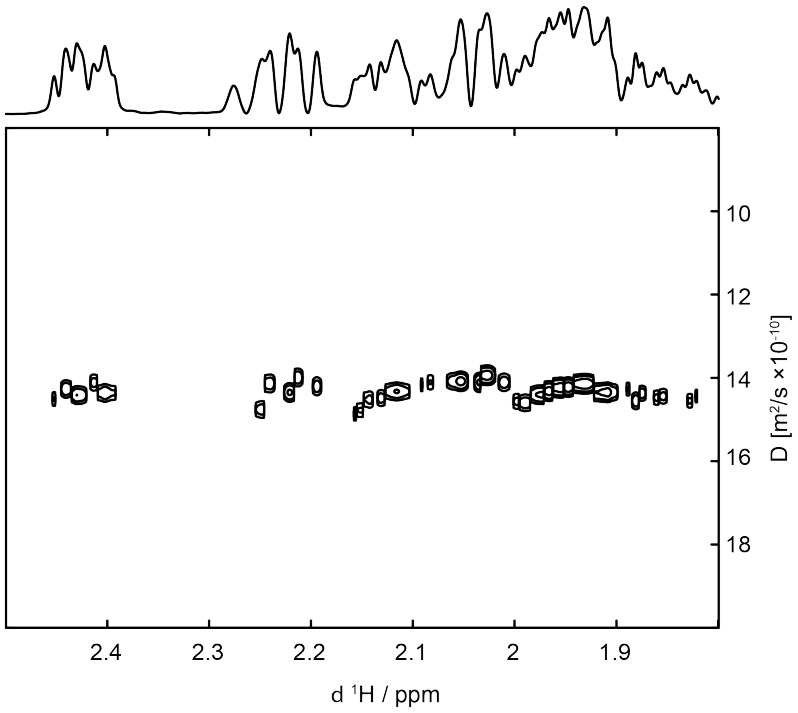


Diffusion encoding gradients

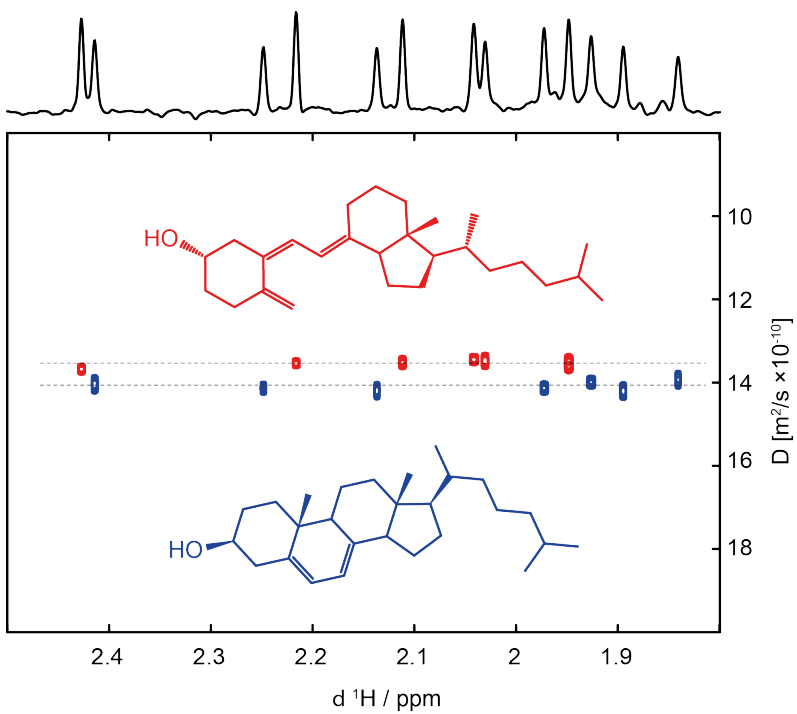
Angew. Chem. Int. Ed. **55**, 15579 (2016)

PSYCHE-IDOSY

Conventional



Pure shift

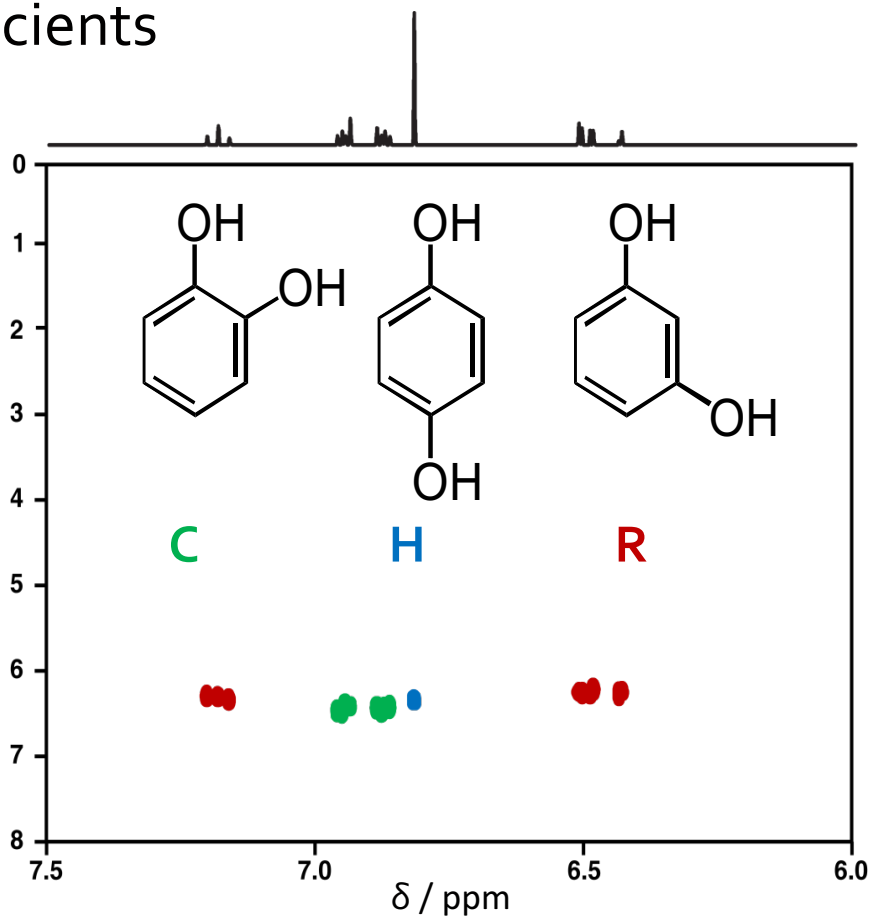


The conventional spectra of vitamin D_3 and provitamin D_3 are almost completely overlapped. Pure shift allows resolution in both spectral and diffusion dimensions.

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

Matrix-Assisted DOSY (MAD)

DOSY can only separate signals from species that have different diffusion coefficients



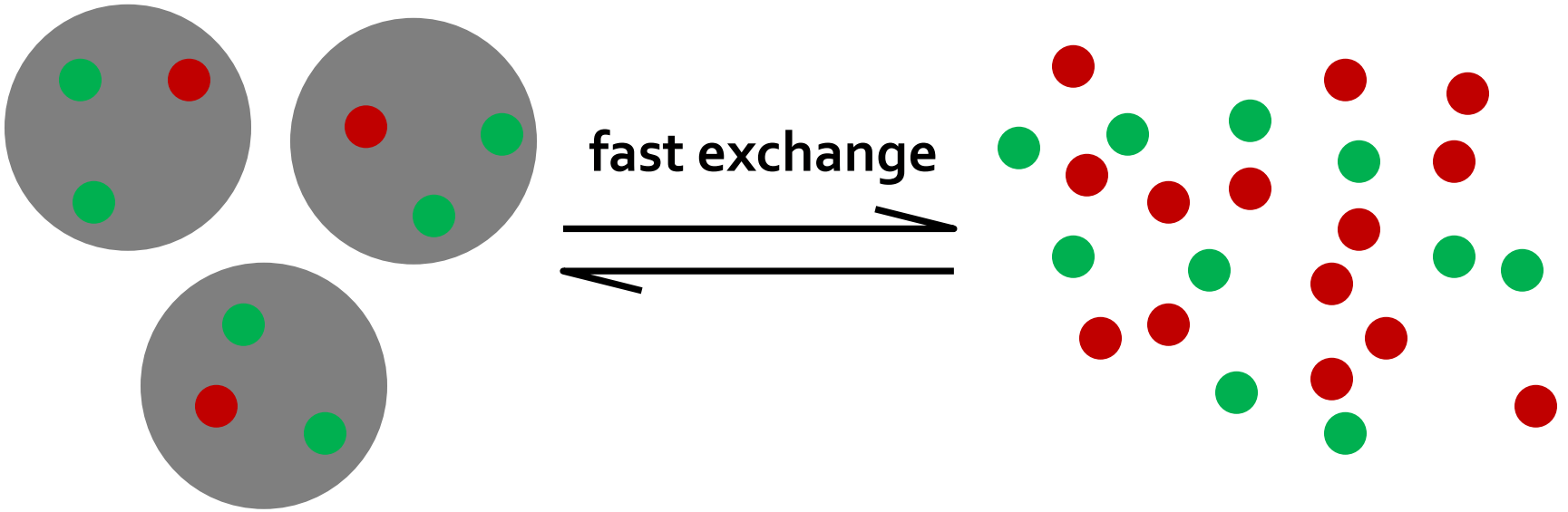
Can we manipulate the way different species diffuse?

Interaction with a matrix

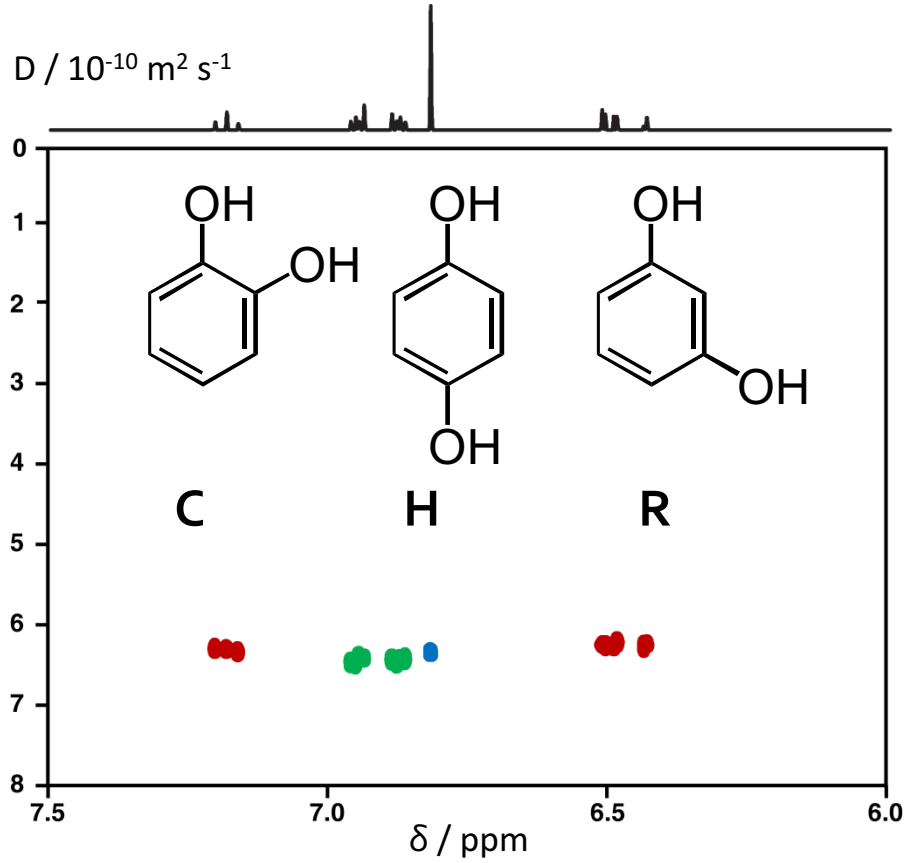
interaction of a solute with a [more slowly diffusing] matrix reduces its apparent diffusion in proportion to the strength of interaction.

a simple two-site model

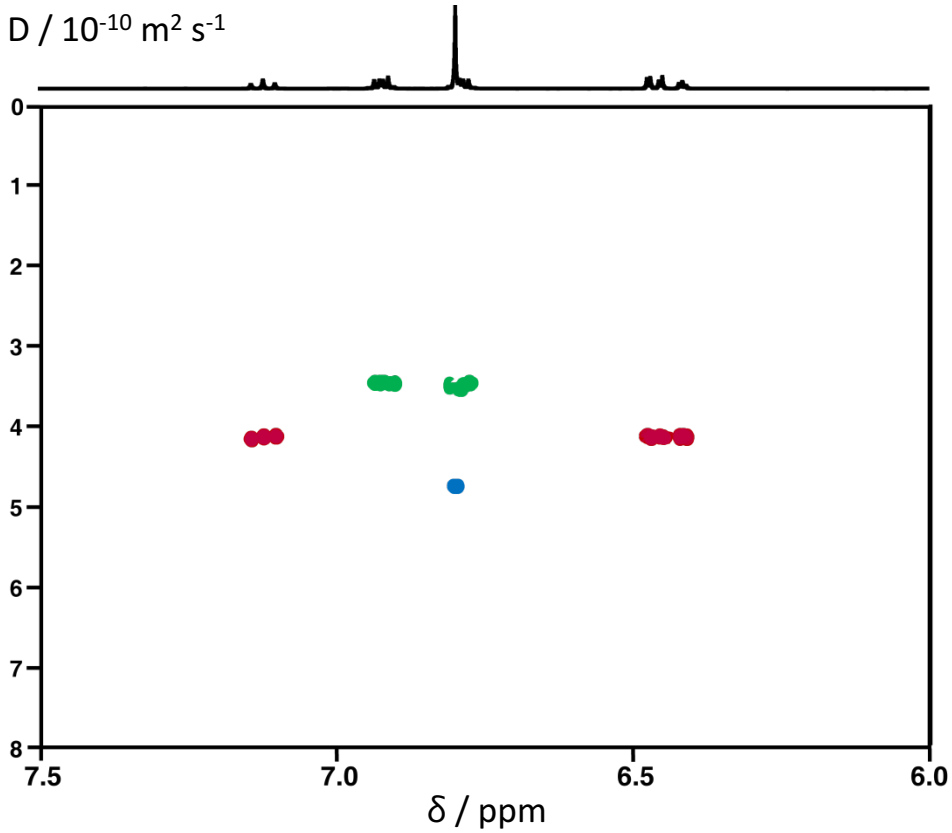
$$D_{apparent} = f_{bound} D_{matrix} + (1 - f_{bound}) D_{free}$$



isomers resolved using micelles



D_2O

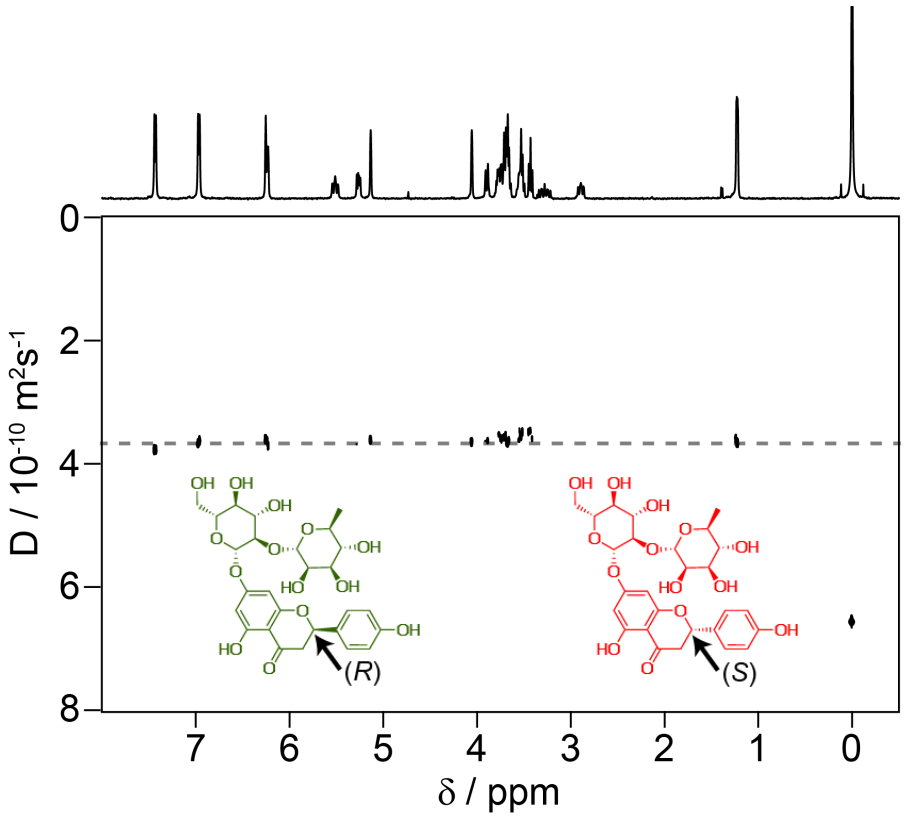


D_2O with 150 mM SDS

using a micellar matrix exploits differences in binding to separate the signals of species with similar or identical diffusion coefficients

Anal. Chem. **2009**, 81, 4548

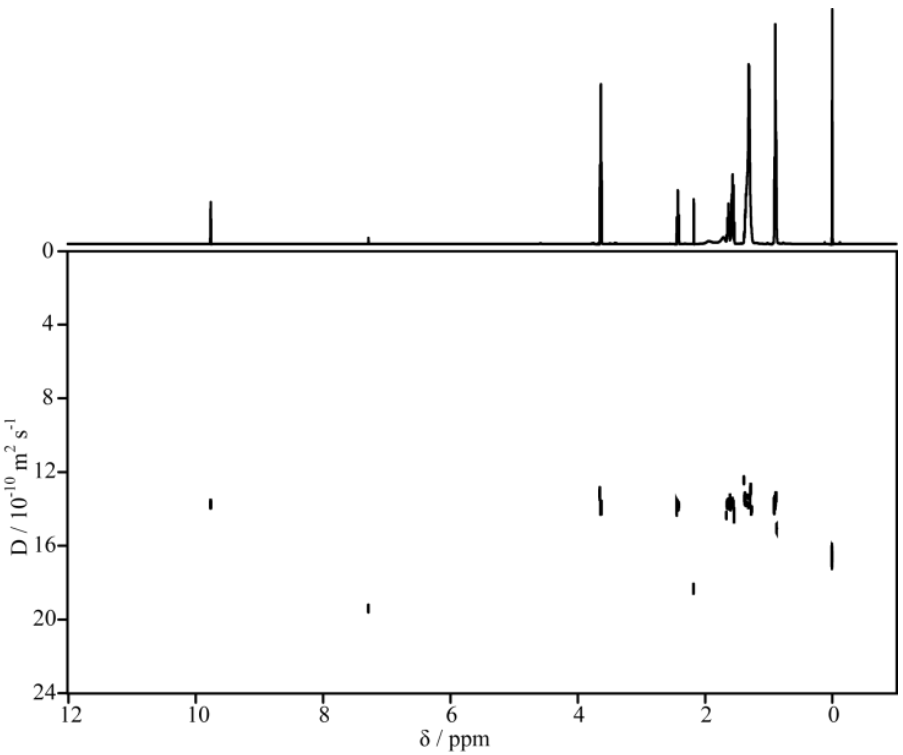
Chiral MAD: epimers resolved using cyclodextrins



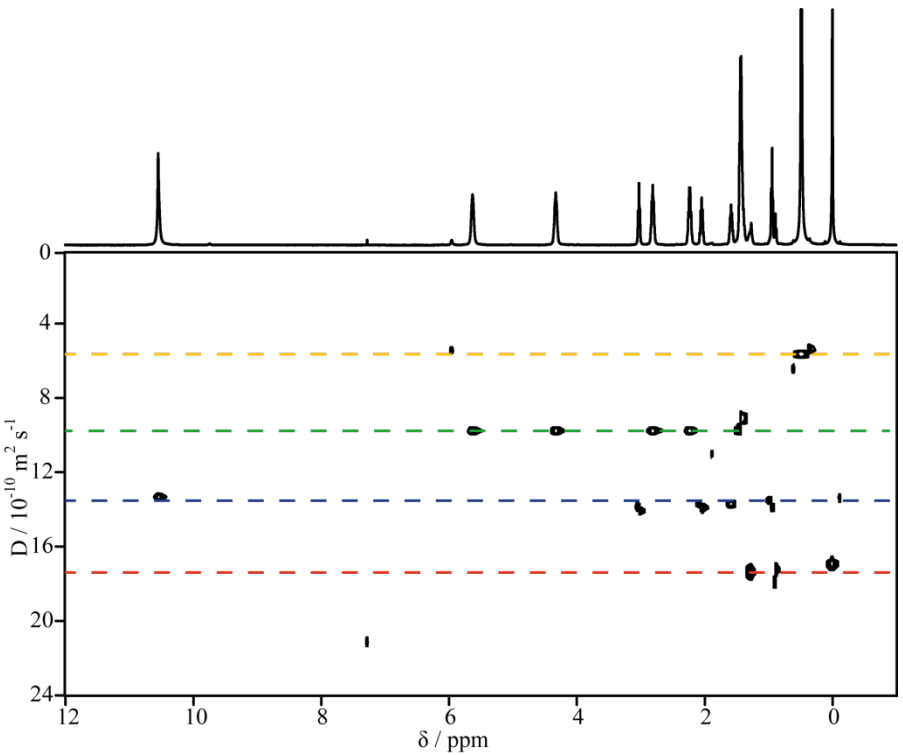
differential inclusion by β-cyclodextrin of the epimers of the natural product naringin is exploited, allowing separation of the naringin epimer signals by high resolution DOSY.

Org. Biomol. Chem. **2011**, 9, 7062

Lanthanide shift reagents



an "impossible" mixture of hexane, hexanol and hexanal.



adding **Eu(fod)$_3$** resolves the signals in both dimensions. The signals from **hexane**, **hexanal** and **hexanol** can now be identified

Chem. Commun. **2011**, 47, 7063

Diffusion NMR: today's programme

9.00-9.50	Introduction and Theory
9.50-10.10	Break
10:10-11.00	Acquisition, Analysis and Practicalities
11.00-11.30	Questions and Answers
11.30-14.00	Lunch
14.00-14.50	Advanced experiments
14.50-15.00	Break
15.00-15.50	Introduction to the GNAT Processing software
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16.00-17.00	Hands on analysis using GNAT, with your own (or provided) data.
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General NMR Analysis Toolbox (GNAT)

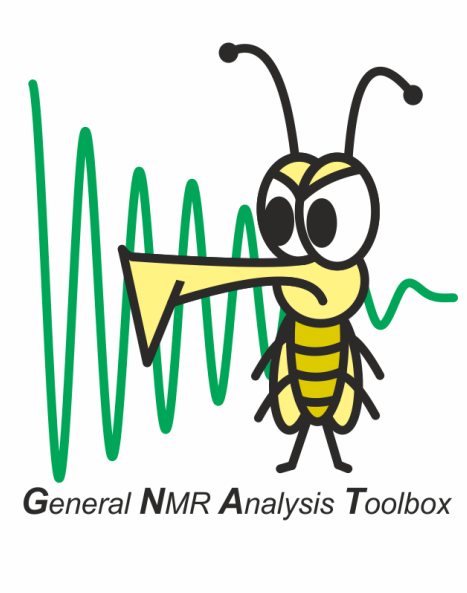
Supersedes the DOSY Toolbox as a more general software package.

Focused on arrayed experiments:

- diffusion
- relaxation
- time series
- …

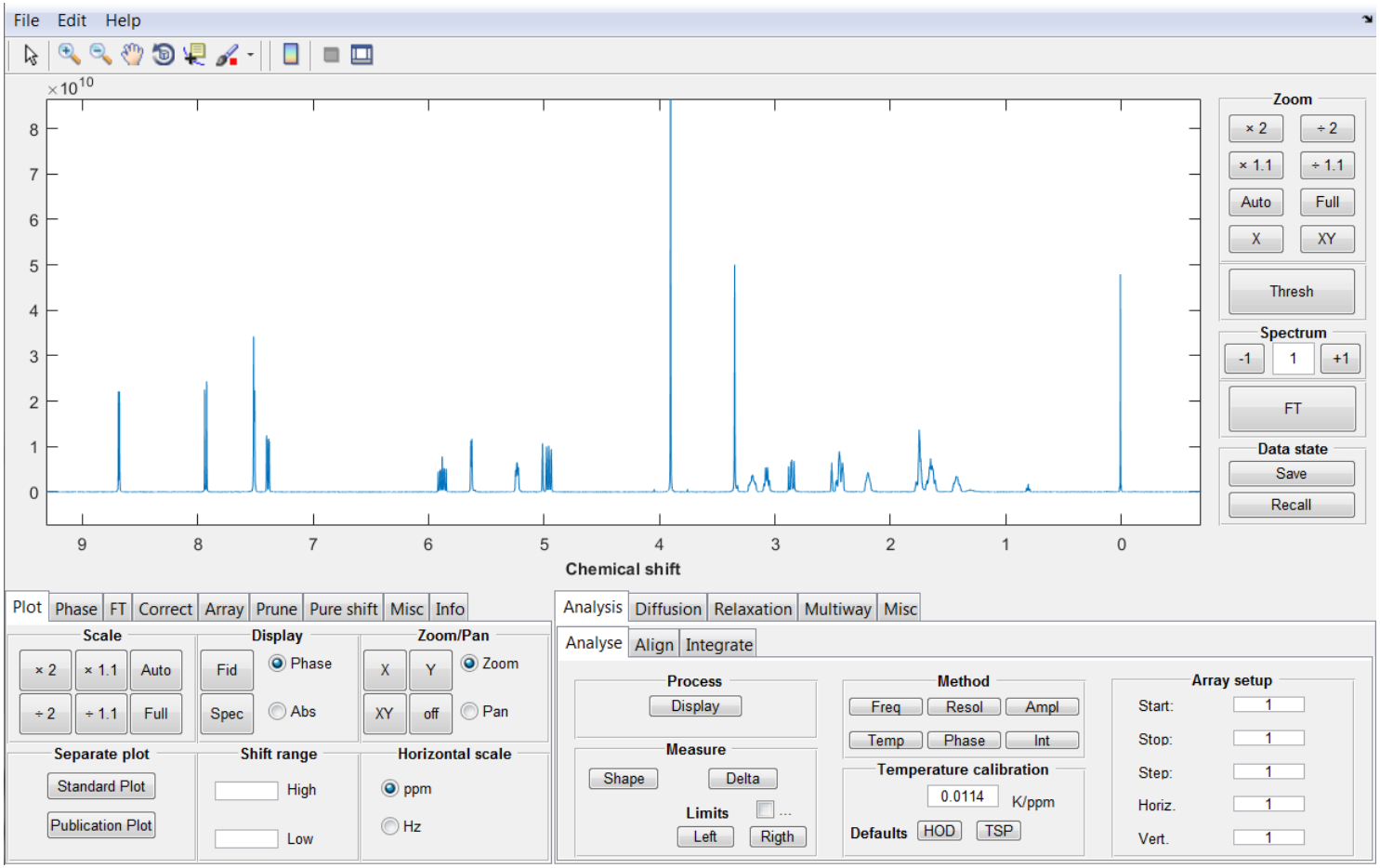
Runs under Matlab 2017a or higher
Compiled versions for Windows, Mac and Linux

Licensed under the GPL licence, i.e. free and open-source



General NMR Analysis Toolbox (GNAT)

Main Window of the Graphical User Interface



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Live demonstration

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If you don't have your own data we will provide example data sets

Download from our website:
http://nmr.chemistry.manchester.ac.uk/

How far does a molecule move by diffusion

The root-mean-square displacement, x, due to diffusion is given by:

$$x^2 = \alpha D t$$

Where *D* is the diffusion coefficient, *t* is time and α is a constant depending on dimensionality; α is 2, 4 or 6 for 1, 2 or 3 dimensional diffusion.

In water at 25°C, D is approximately 2×10^{-9} m^2 s^{-1}. In 1 minute the average displacement of a water molecule is:

$$x = \sqrt{6\times2\times10^{-9}\times60}$$

x = 0.6 mm

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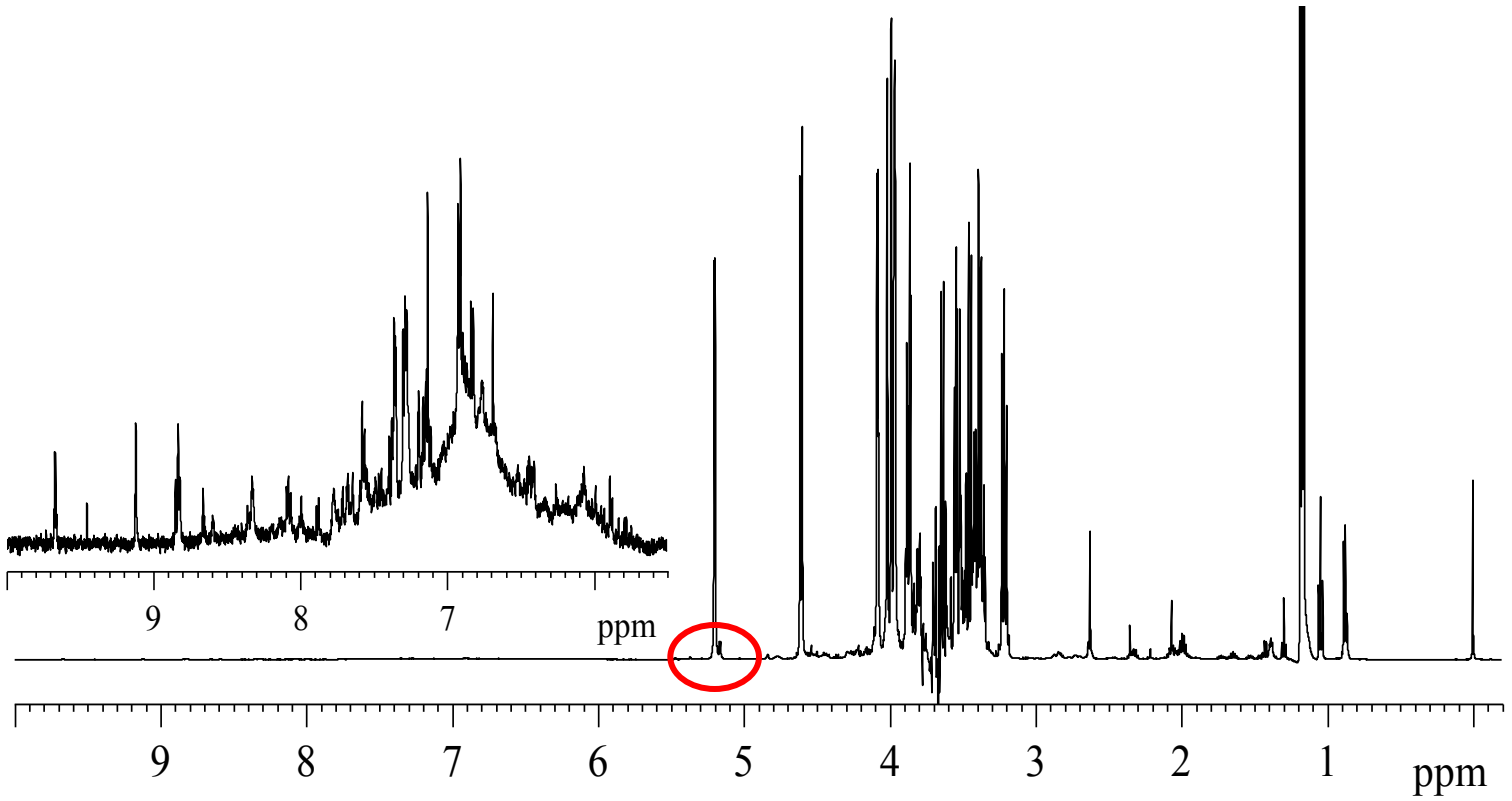
Port Wine: 500 MHz proton spectrum



(with triple presaturation of water and ethanol)

J. Agric. Food Chem. **52**, 3736 (2004)