NMR Training Course

9th September 2021

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JEOL UK Demo Lab

Diffusion-Ordered SpectroscopY

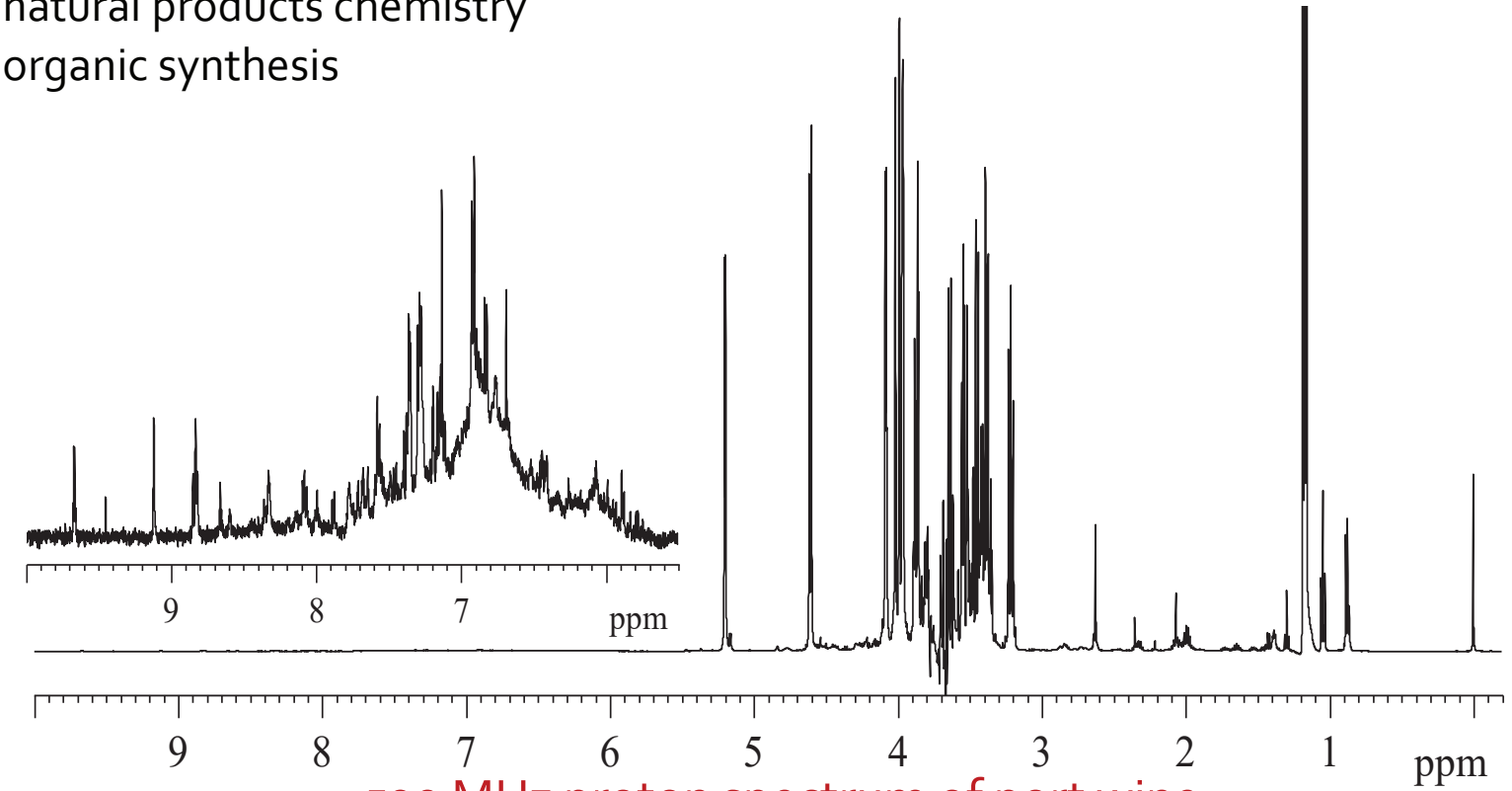
Mixture analysis by NMR

applications

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

pros/cons

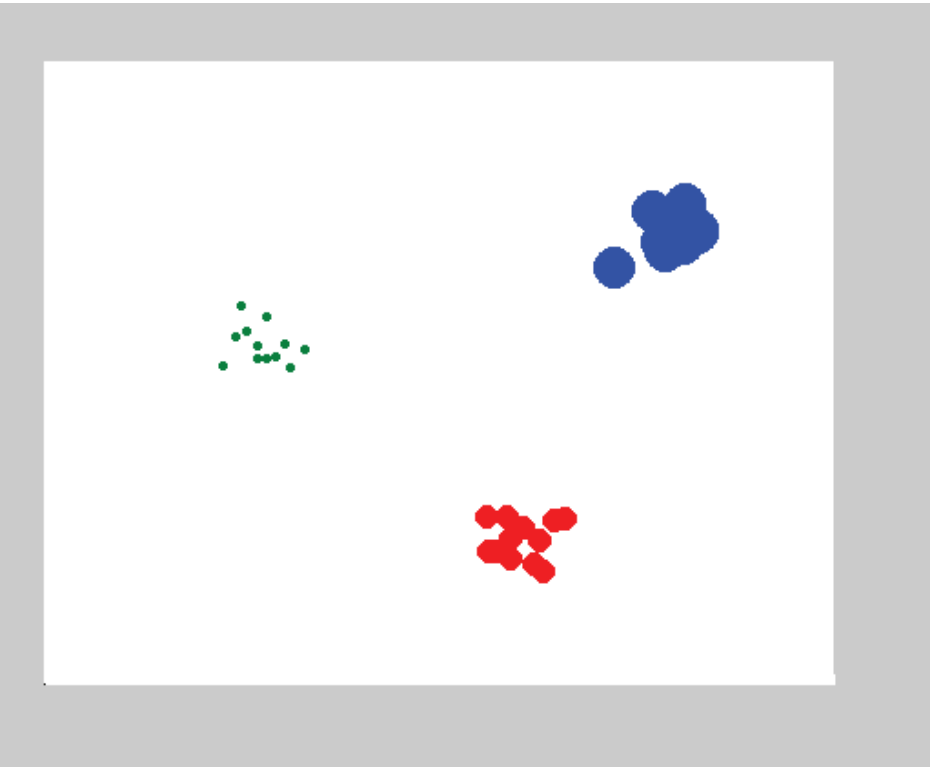
+ structural information
+ nondestructive
– low(ish) sensitivity
– usually needs separation (e.g. LC-NMR)



500 MHz proton spectrum of port wine

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

Self-Diffusion



- Molecules experience both rotational and translational Brownian motion
- Stokes-Einstein equation (spherical molecules) can be used to determine the mobility of the molecules

$$D = {k_B T}/{6\pi\eta r_H}$$

k_B : Boltzmann constant

T: temperature

η: viscosity

r_H: hydrodynamic radius

- 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

$$D = {k_{\mathrm{B}}T}/{6\pi\eta r_{\mathrm{H}}}$$

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.

Self-Diffusion and molecular weight

- Morris approximation for the correlation between molecular weight and self-diffusion coefficient
- More advanced than previous estimation of $r_H \propto \sqrt[3]{MW}$
- Takes into account the molecular interactions of solvent and solutes
- **Calculator available: Temperature, solvent and MW or D**

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

k_B : Boltzmann constant

T: temperature

η: viscosity

ρ_{eff}: effective density of the molecule (packing effects, geometry, solvation and flexibility)

MW : molecular weight of the molecule

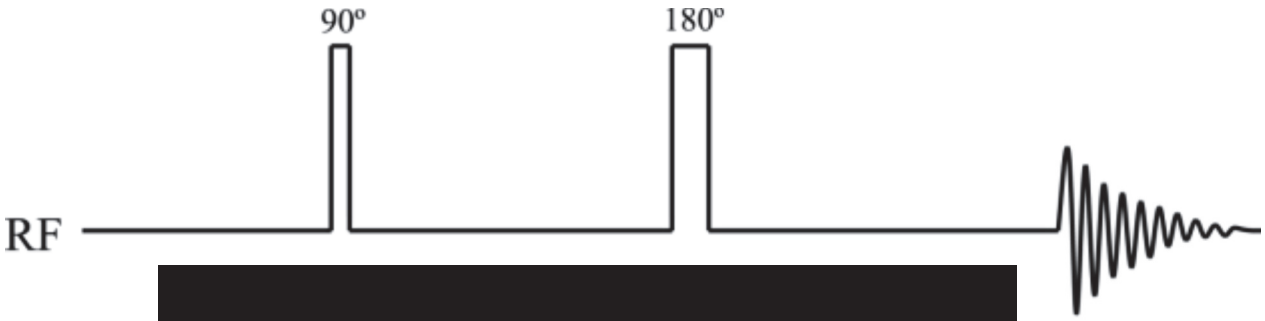
MW_S: molecular weight of the solvent

N_A: Avogadro number

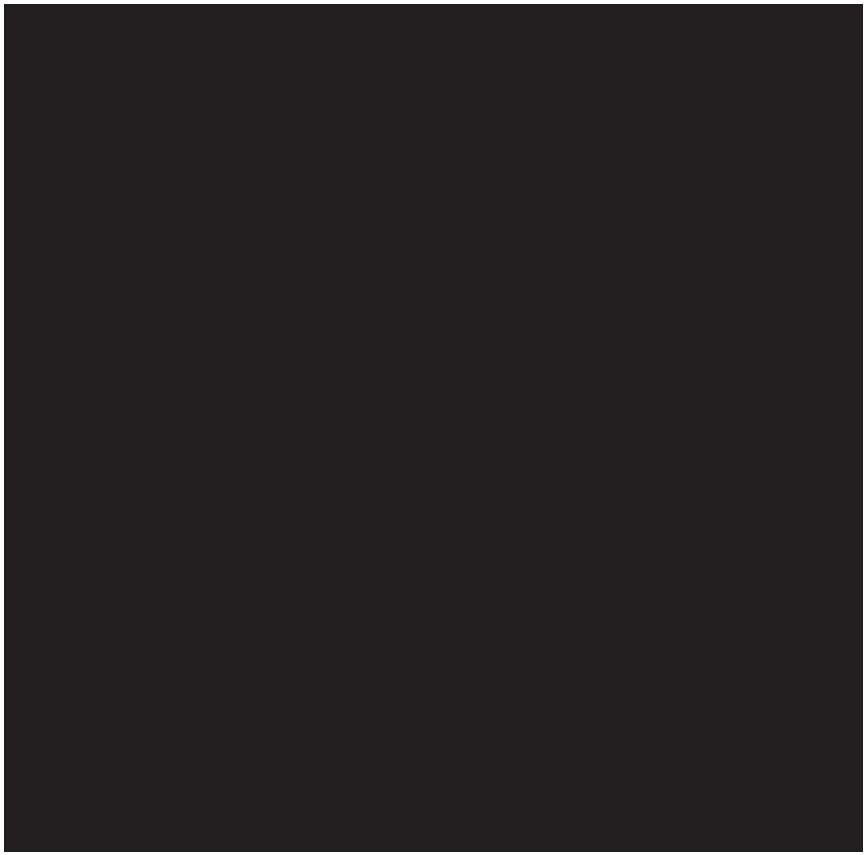
R. Evans et al, *Angew. Chem. Int. Ed.* (2013), 52: 3199–3202
R. Evans et al, *Anal. Chem.* (2018), *90* (6), 3987–3994
R. Evans, *Prog Nucl Magn Reson Spectrosc* (2020), 117, 33-69
https://www.nmr.chemistry.manchester.ac.uk/?q=node/432

Spin echo

Signal is refocused…



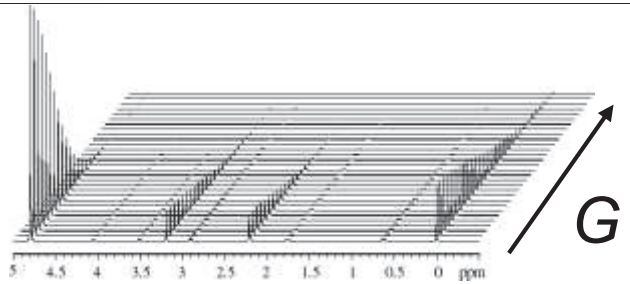
as droplets in corn syrup



https://twitter.com/wonderofscience/status/1320692387925032961

Pulsed field gradient spin echo

- Application of PFG will result in a diffusivity dependent attenuation



$$S = S_0 \, e^{-D\gamma^2\delta^2G^2\Delta'}$$

S : signal amplitude

S_0: signal amplitude without diffusion

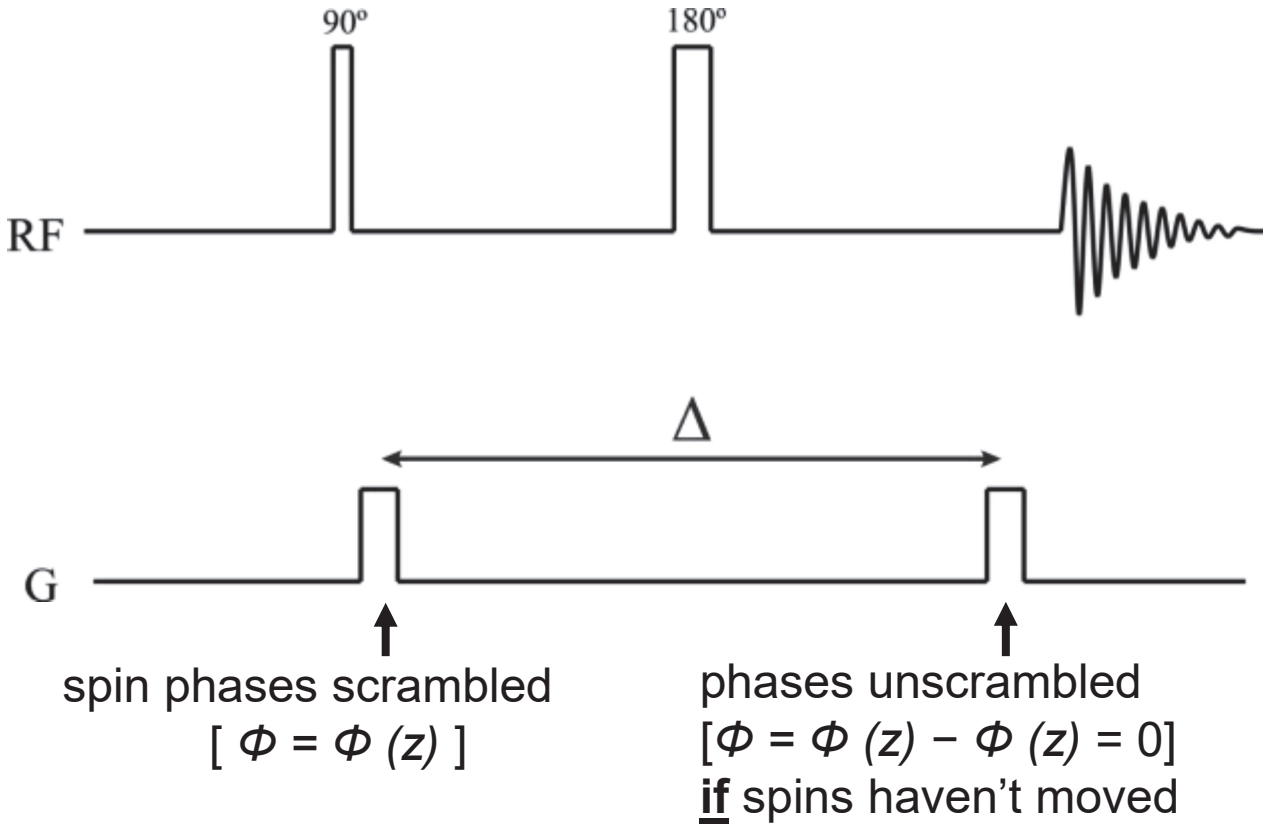
D : diffusion coefficient

γ : gyromagnetic ratio

δ : gradient pulse width

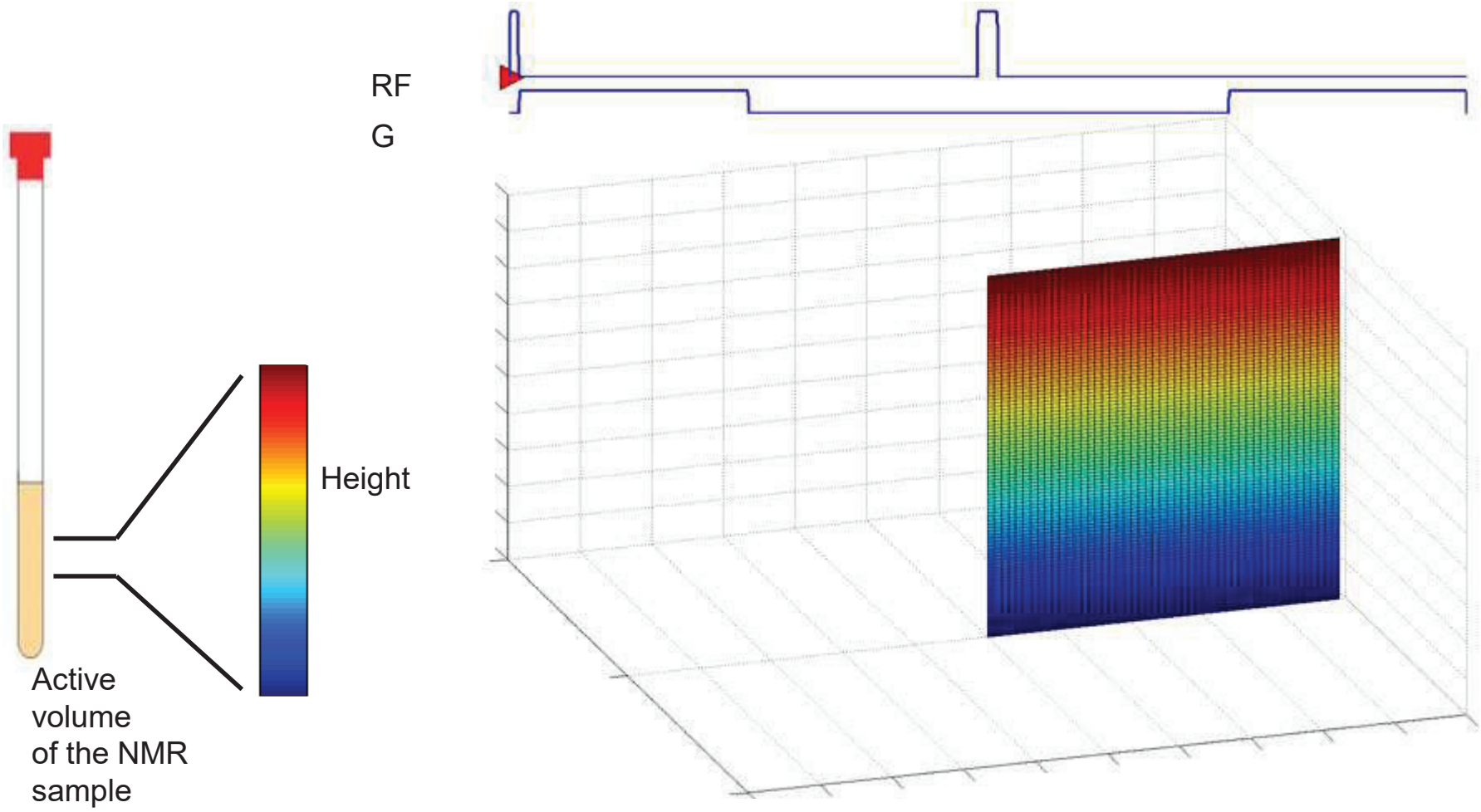
G : gradient amplitude

Δ' : corrected diffusion time



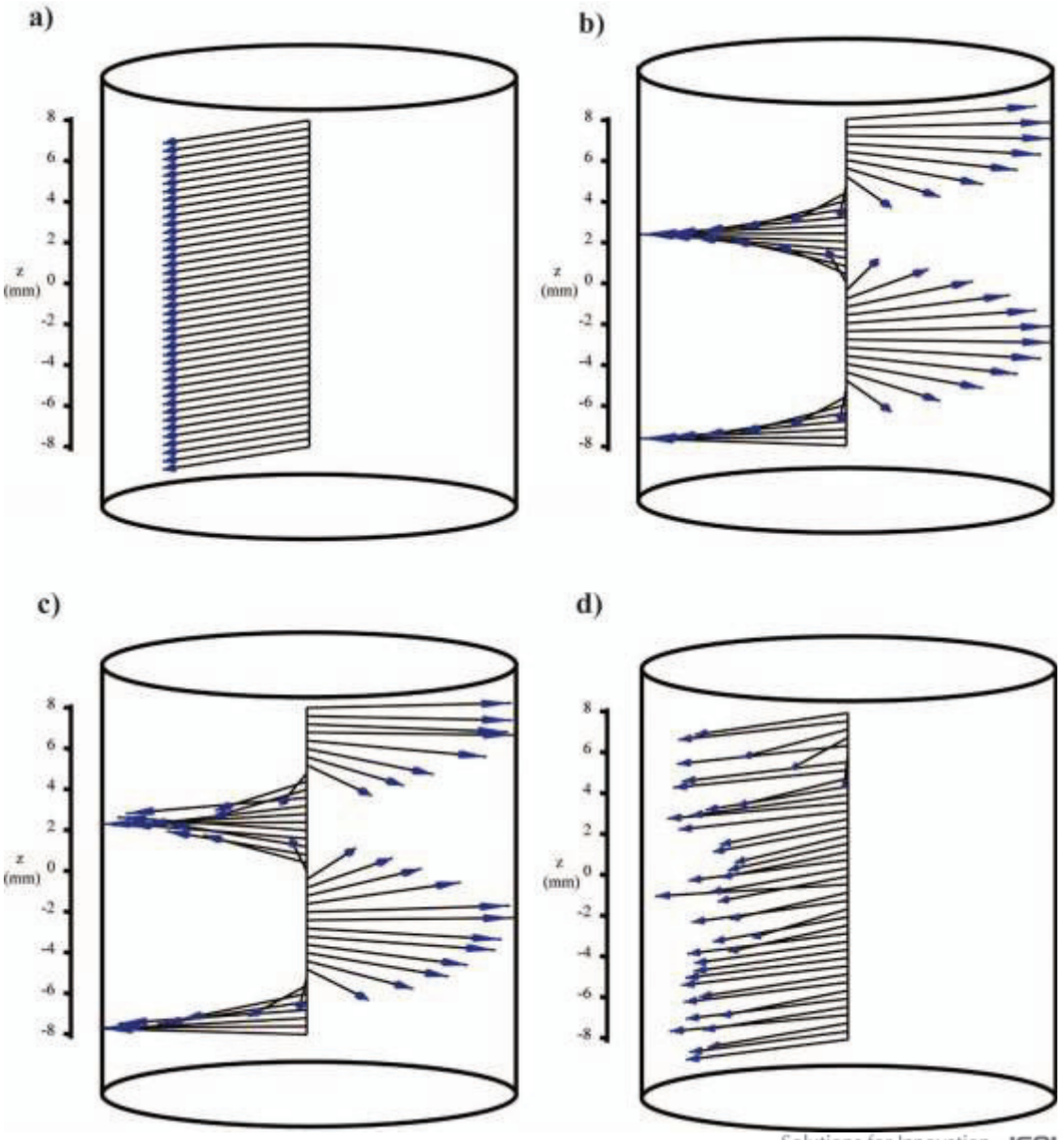
Pulsed field gradient spin echo

- Magnetization evolution without diffusion

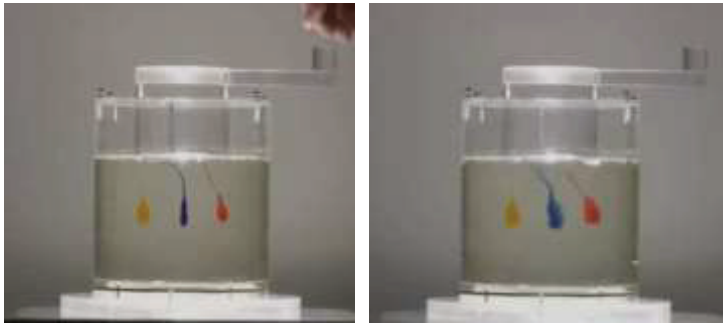


Pulsed field gradient spin echo

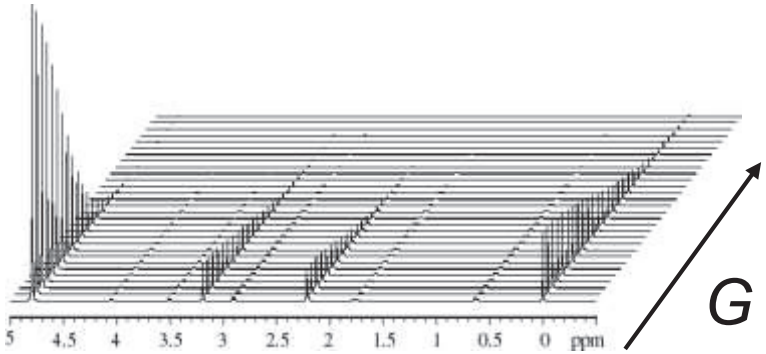
- Magnetization evolution with diffusion
- Diffusion results in imperfect refocusing of magnetization.
- More diffusion leads to worse refocusing, and thus more signal attenuation

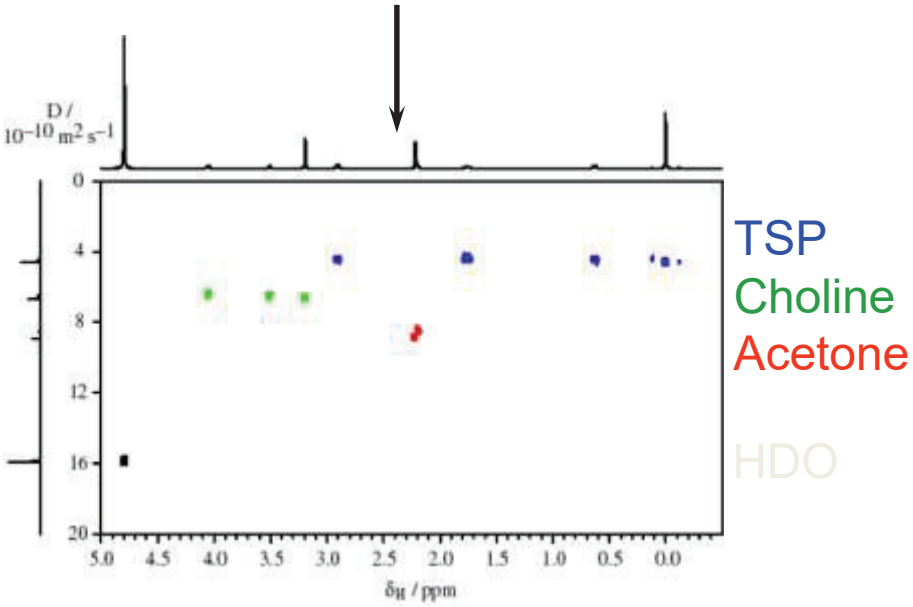


No diffusion vs diffusion



Diffusion-Ordered SpectroscopY (DOSY)





- Measure spectra as a function of *G*
- Fit peak heights to get diffusion coefficients *D*

$$S = S_0 \, e^{-D\gamma^2\delta^2G^2\Delta'}$$

- Extend 1D peaks into a second dimension, with Gaussian shapes centred on the *D*'s
- Widths determined by the standard errors σ_D

Virtual chromatography

Despite the name, DOSY is not like COSY/NOESY: spectra are statistical constructs from, not transforms of, experimental data

Practical consequences of the Stejskal-Tanner formula

Stejskal-Tanner formula for signal intensity:

$$S = S_0 \, e^{-D\gamma^2\delta^2G^2\Delta'}$$

- (γ δ G_{zi})^2 - gradient area

nuclei with high γ values are more sensitive for diffusion (^{1}H, ^{19}F, ^{31}P)
(i.e. ^{1}H is 16 times more sensitive than ^{13}C)

- δ should be kept short

during δ the magnetization is transverse, homonuclear J-couplings evolve

- G the more, the better

provided the gradient hardware allows it

- Δ should be kept short:

to minimize convection effects

Why do we lose signal intensity?

1. Instrumental imperfections
   - gradient system
     - recovery - Eddy currents with multiple time constants
       - phase and lineshape distortions
     - gradient linearity and symmetry
     - gradient reproducibility
     - noise produced by the gradient amplifier
       - Use suitable pulse sequence
2. T_1 or T_2 relaxation – Minimize pulse sequence delays
3. Diffusion - the physical basis of DOSY
4. Convection - the enemy of every diffusion experiment!
   - Minimize sample volume
   - Increase sample viscosity
   - Minimize temperature gradients
   - Use convection compensated sequences

PFG Spin echo

Field gradient pulses result in signal attenuation

$$S = S_0 \, e^{-D\gamma^2\delta^2G^2\Delta'}$$

S : signal amplitude

S_0: signal amplitude without diffusion

D : diffusion coefficient

γ : gyromagnetic ratio

δ : gradient pulse width

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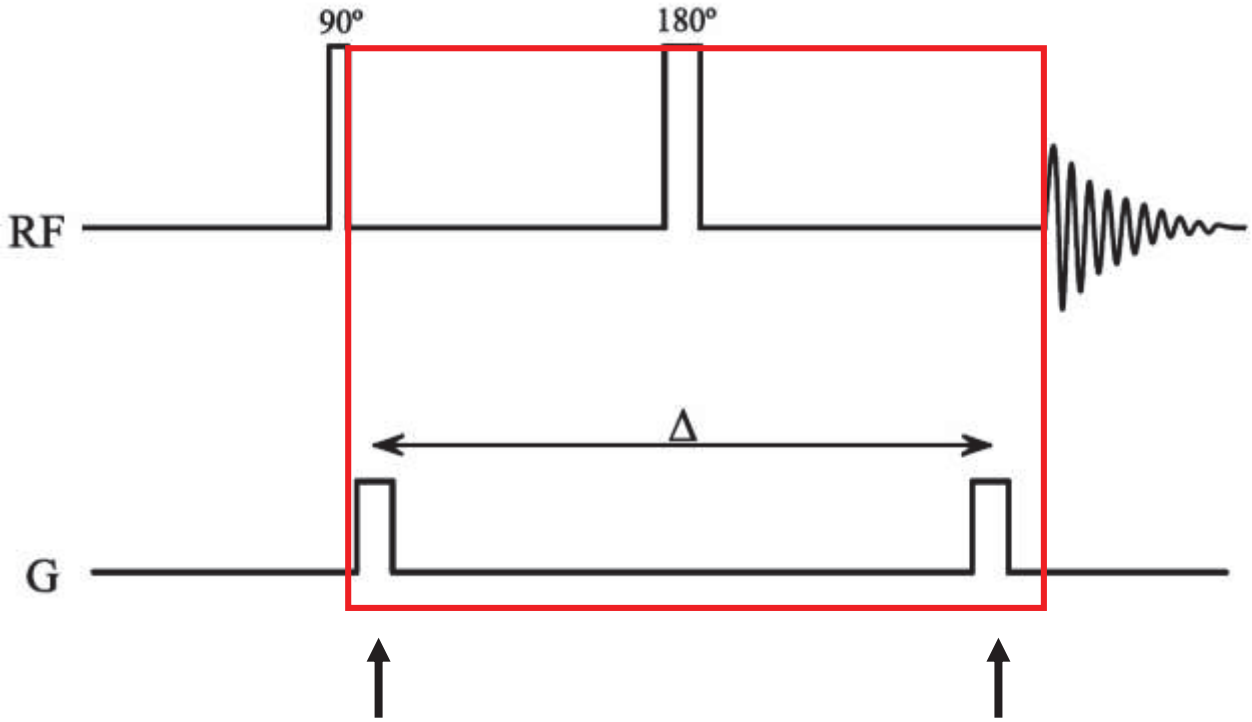
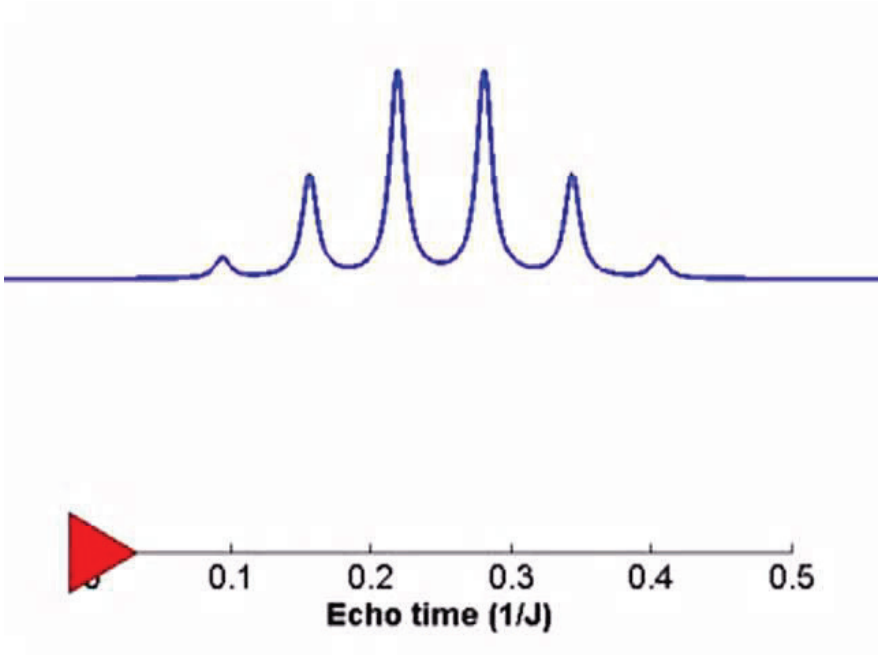
Δ' : corrected diffusion time



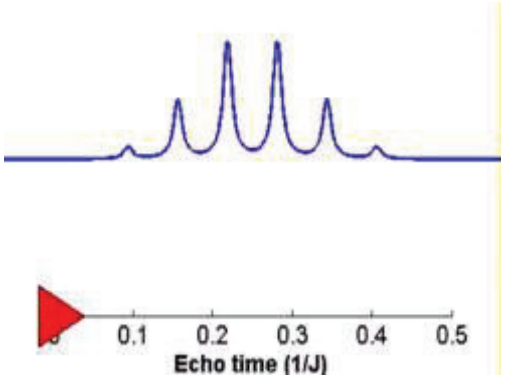
spin phases scrambled
[$\Phi = \Phi(z)$]

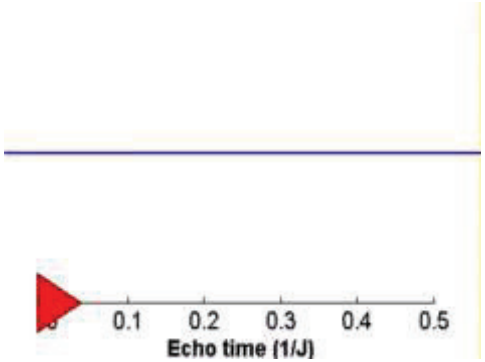
phases unscrambled
[$\Phi = \Phi(z) - \Phi(z) = 0$]
if spins haven't moved

J-modulated sexted



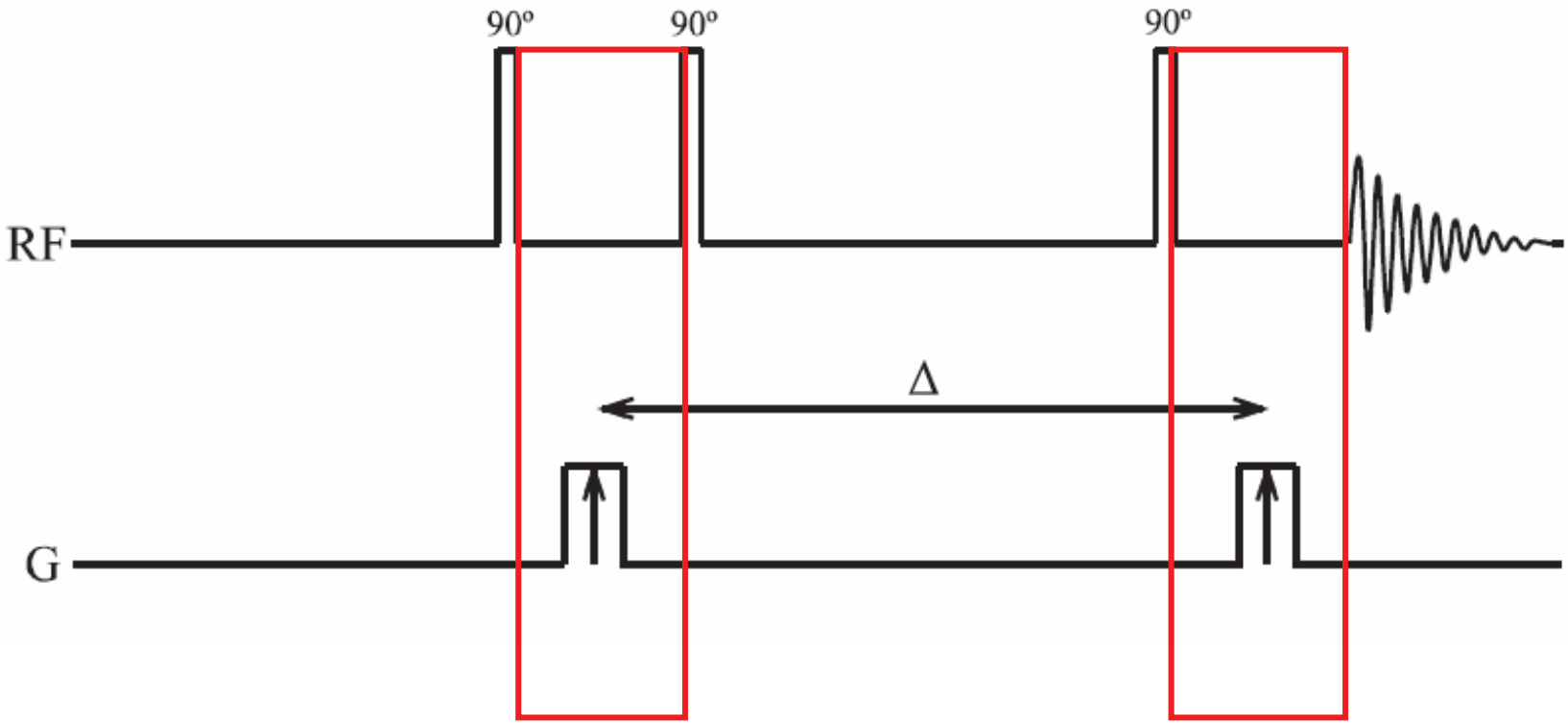
In-phase magnetization + Anti-phase magnetization



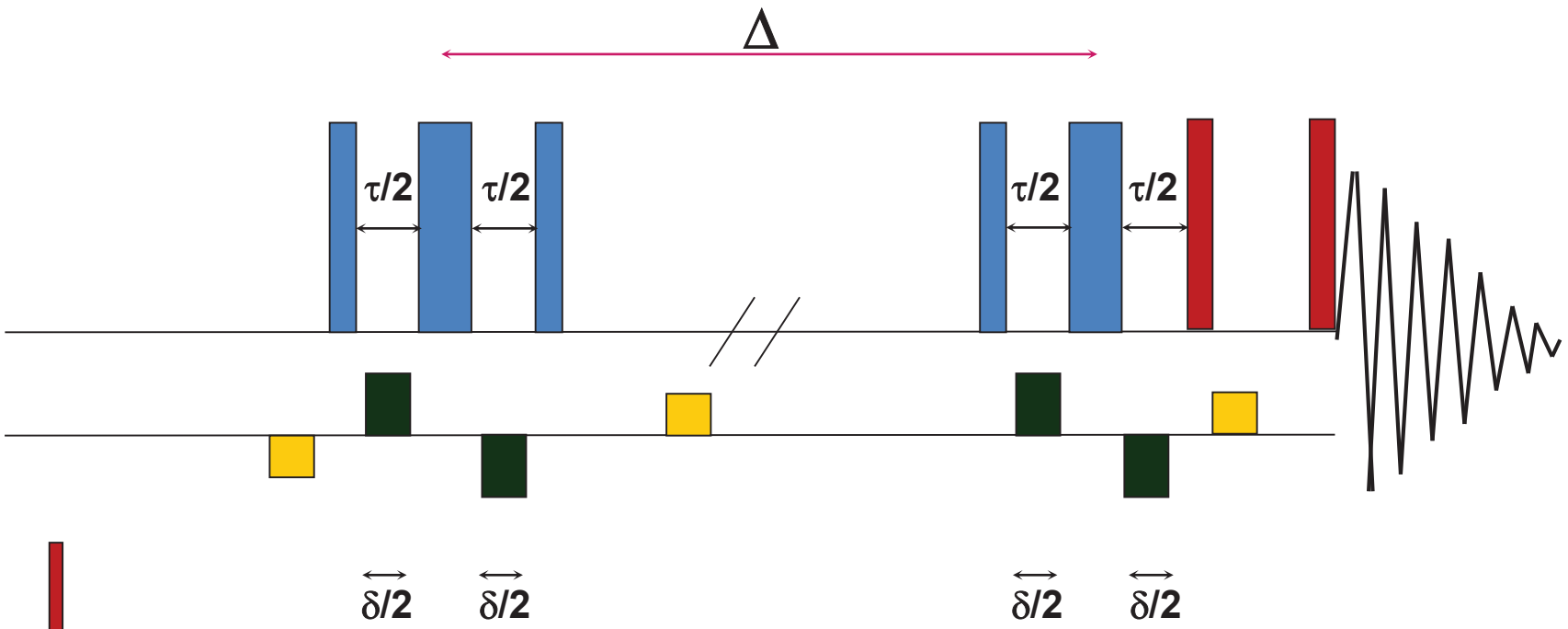


PFG Stimulated echo

- Magnetization is stored along the z-axis for most of Δ
- Reduced J-modulation
- Lose 50% of magnetization



In practice: Bpp_led_dosy_pfg.jxp



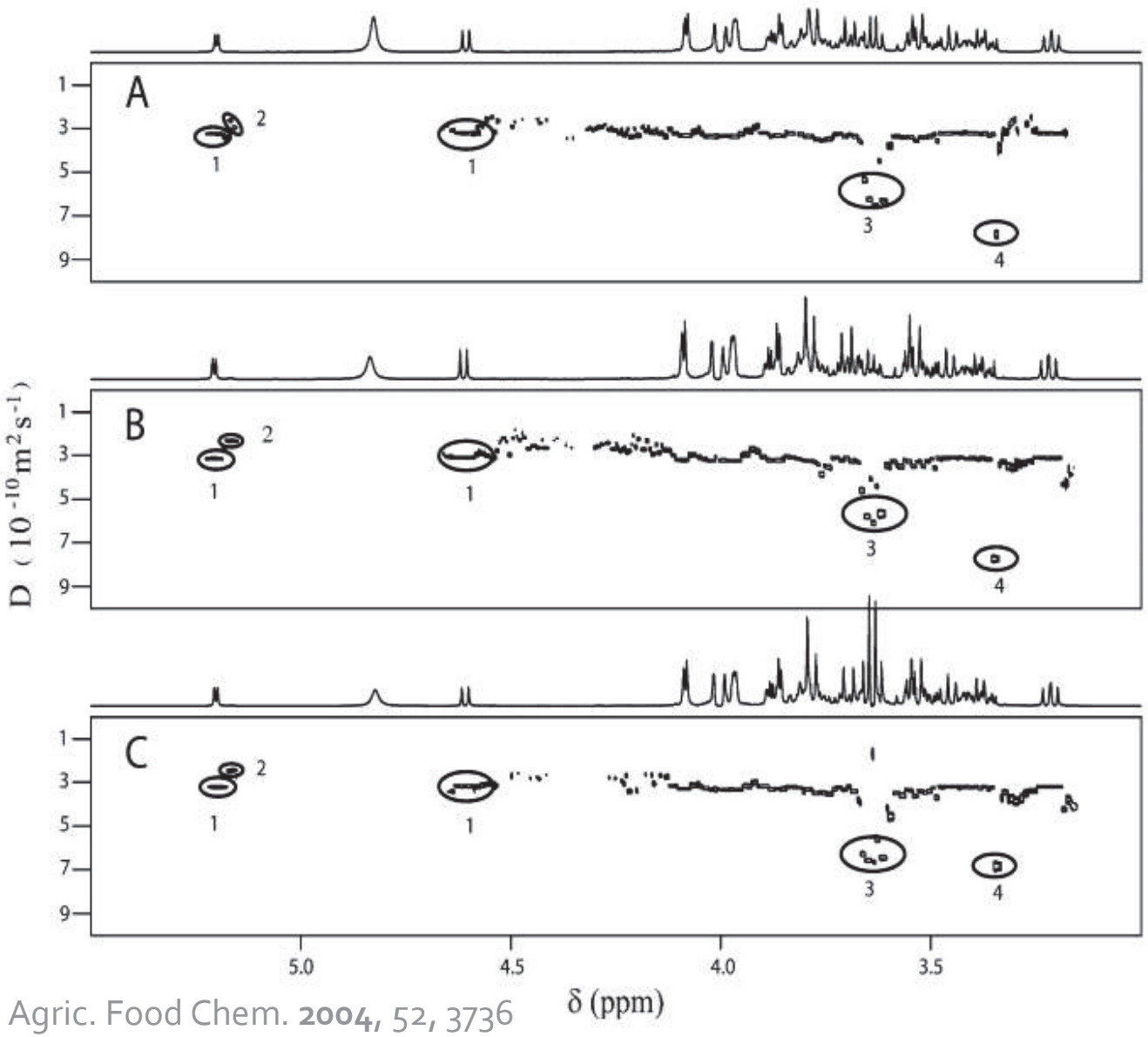
LED element

Spoiler gradient

Phase encoding/decoding gradient

Lock refocusing gradient

DOSY of 3 port wines



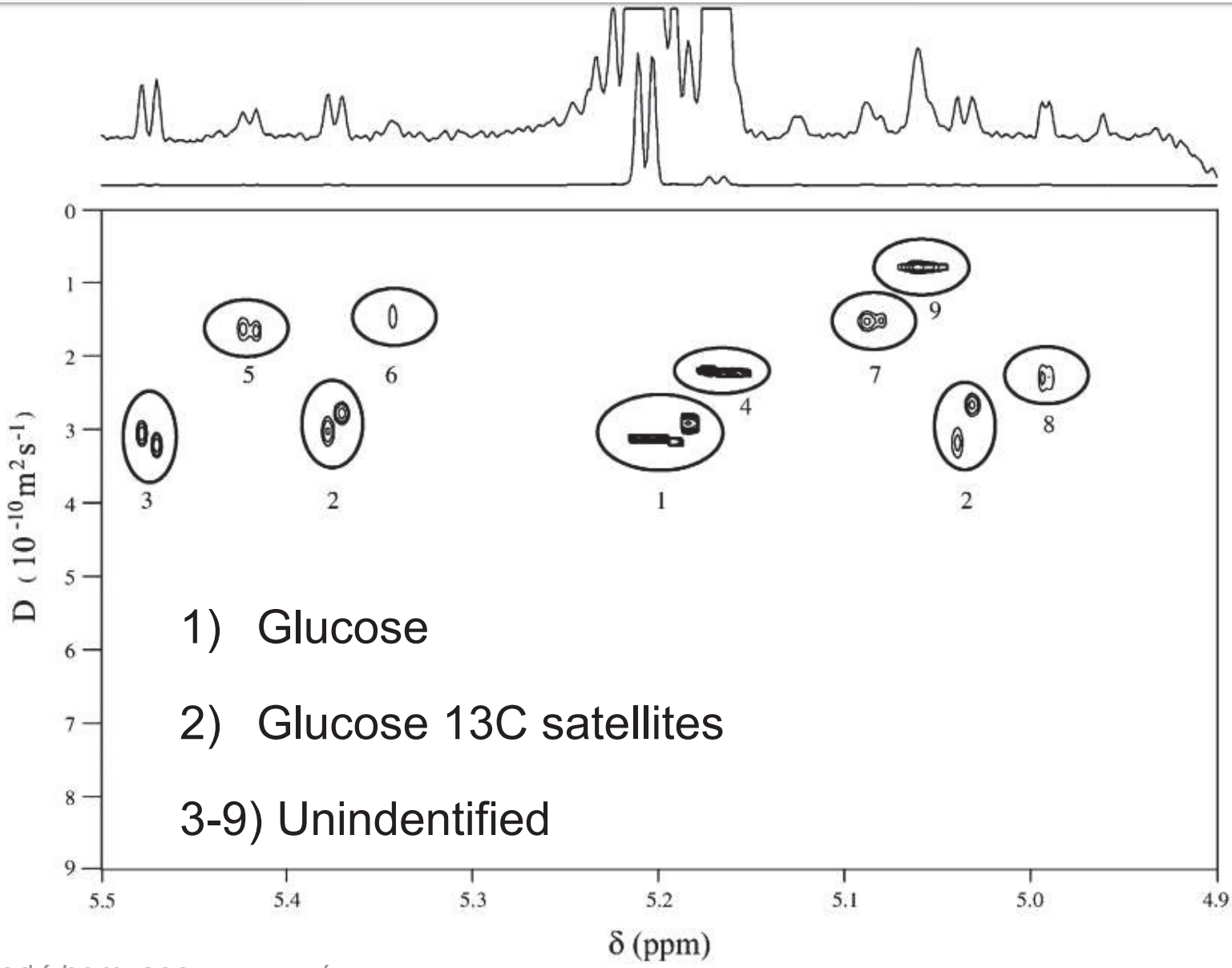
20 years old

3 years old

1 year old

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

DOSY expansion of 3 years old port wine



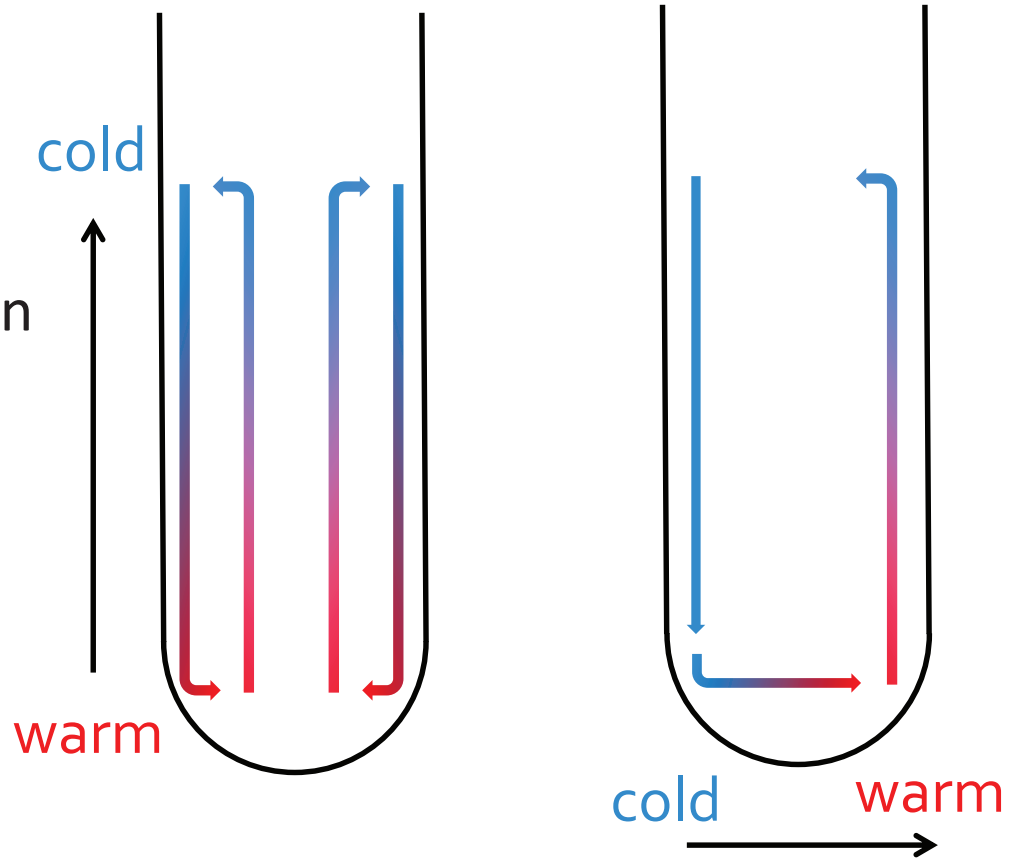
J. Agric. Food Chem. **2004,** 52, 3736

Convection

- Once a critical Raleigh number is reached (1700 for a Benard configuration, i.e., two parallel horizontal boundaries separated by a distance *d*), natural convection appears.

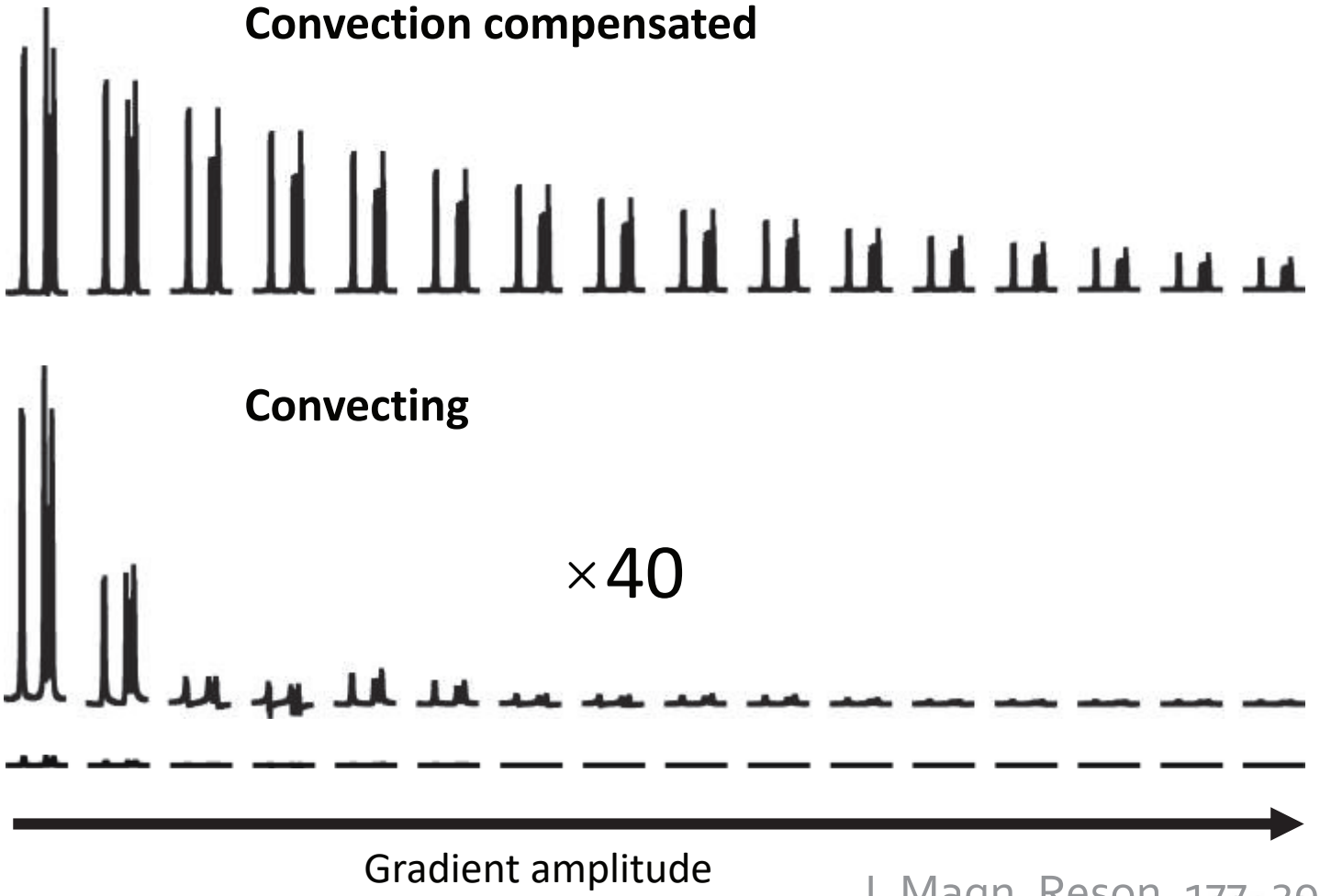
$$\mathrm{Ra} = \frac{\rho\beta\Delta T l^3 g}{\eta\alpha}$$

g: gravity,
β: coefficient of thermal expansion
ΔT: temperature difference
l: length
η: **viscosity**
α:thermal diffusivity



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.



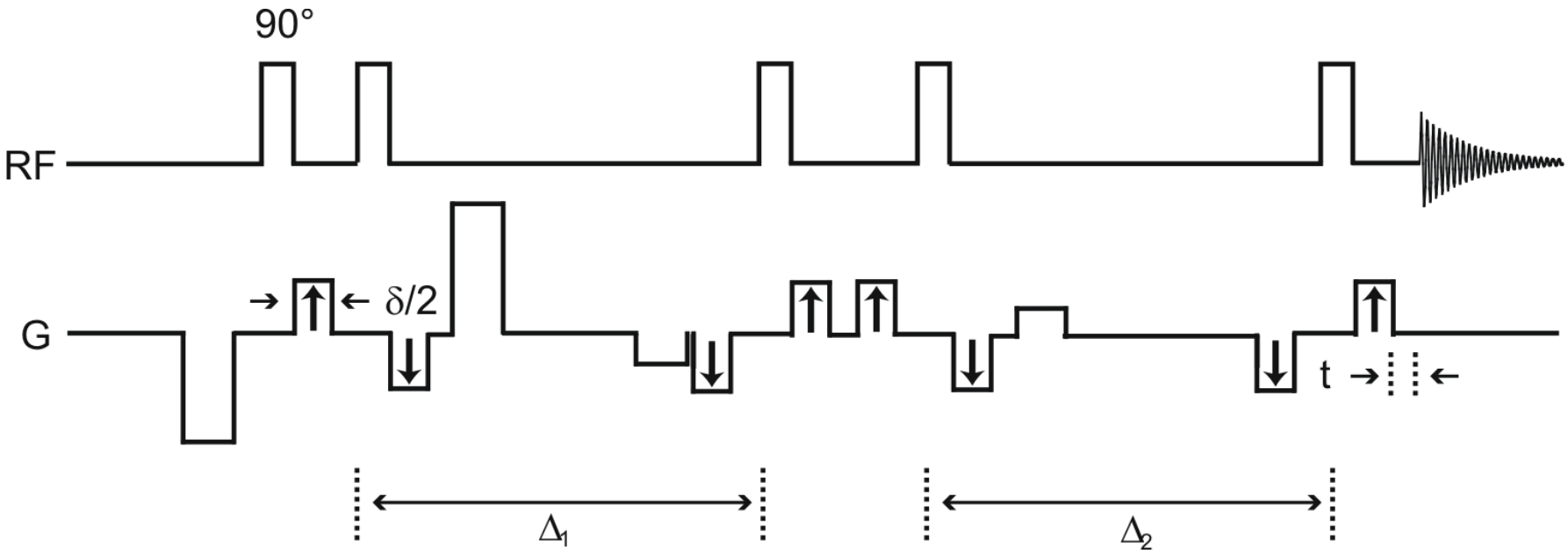
J. Magn. Reson. 177, 203 (2005)

Convection in NMR

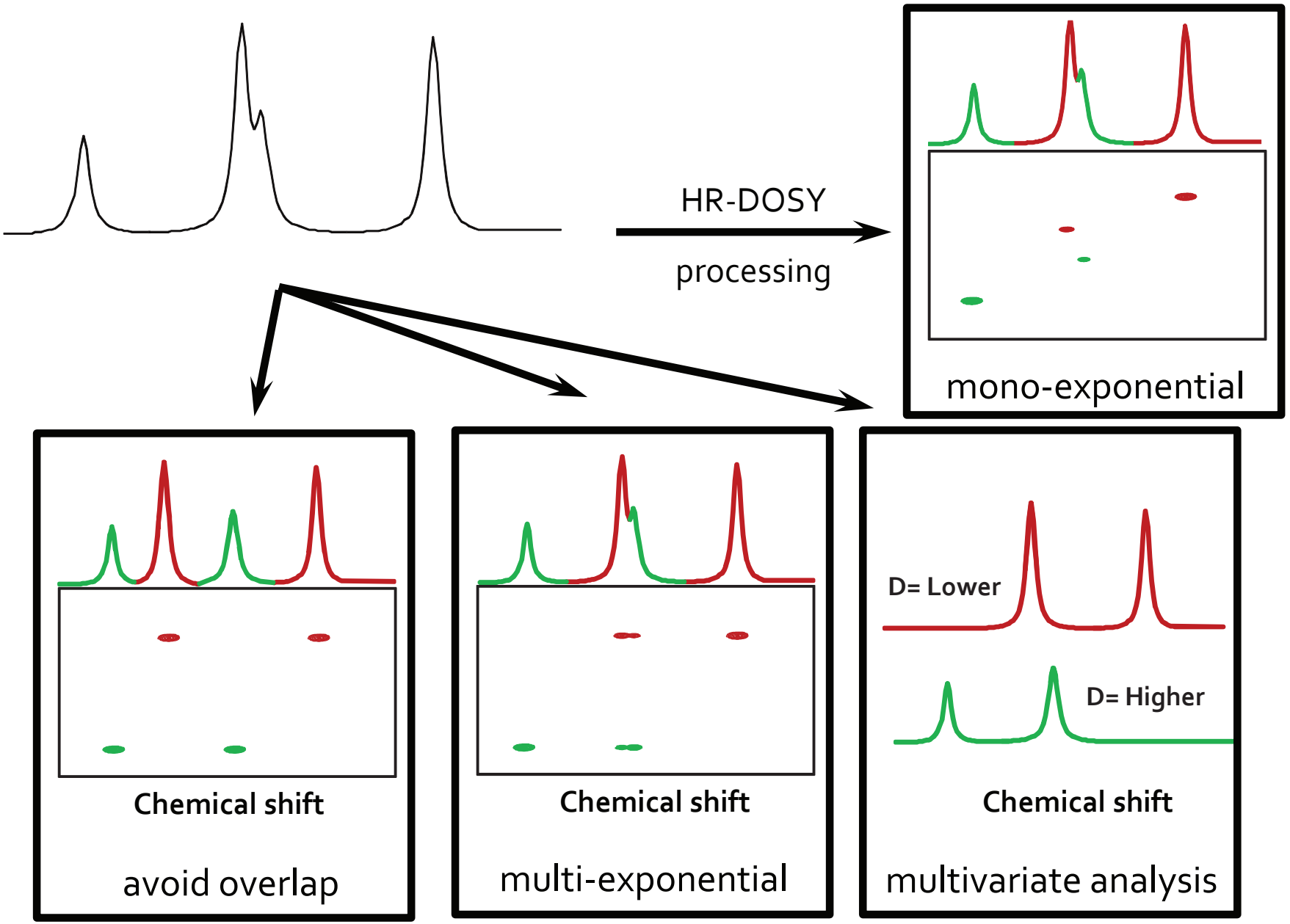
- Any pulse sequence with encoding-decoding gradient pairs suffers from convection
  - Gradient shimming (ask for alternative)
  - Gradient selective 1D
  - g….jxp
  - …

Convection compensated diffusion measurement: bpp_dste_led_dosy_pfg.jxp

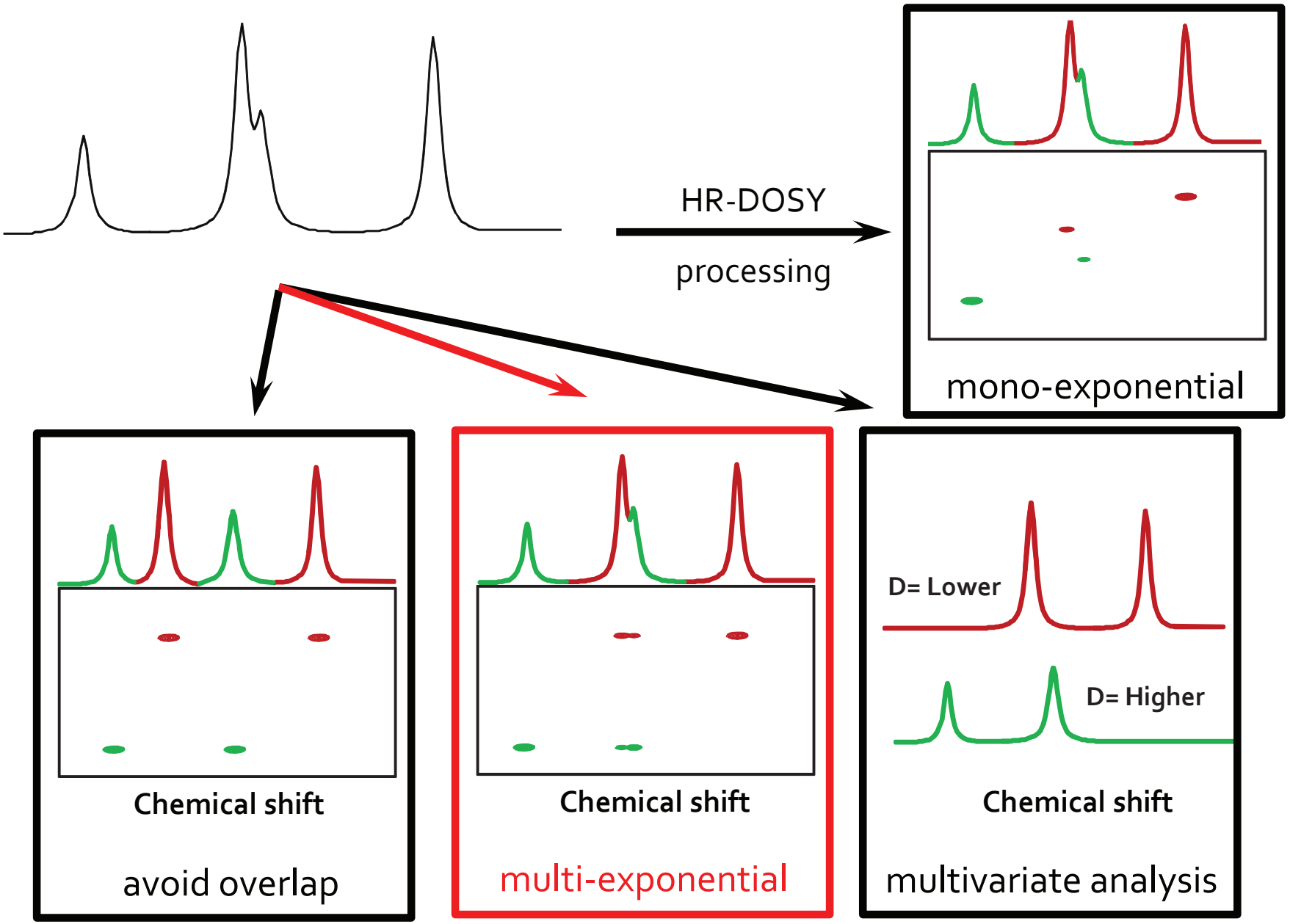
Double stimulated echo: lose 75% of signal and longer phase cycling



signal overlap in DOSY processing

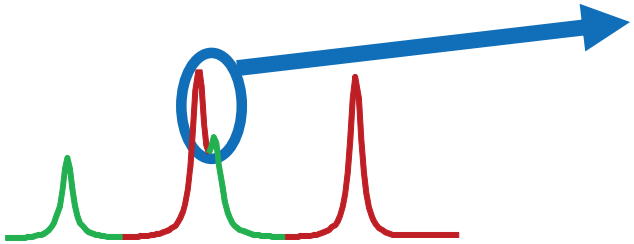


signal overlap in DOSY processing

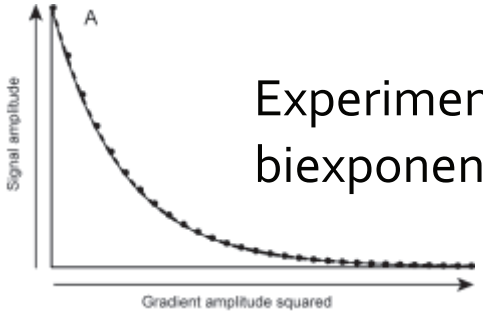


Resolving superimposed exponentials

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

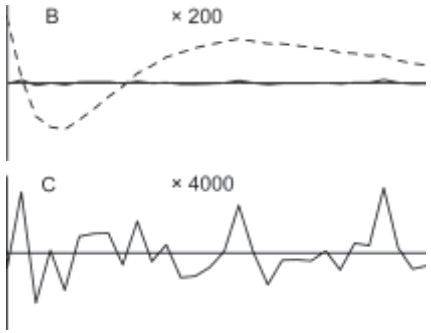


Experimental (noisy) biexponential decay

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

R = X-F

Residuals:

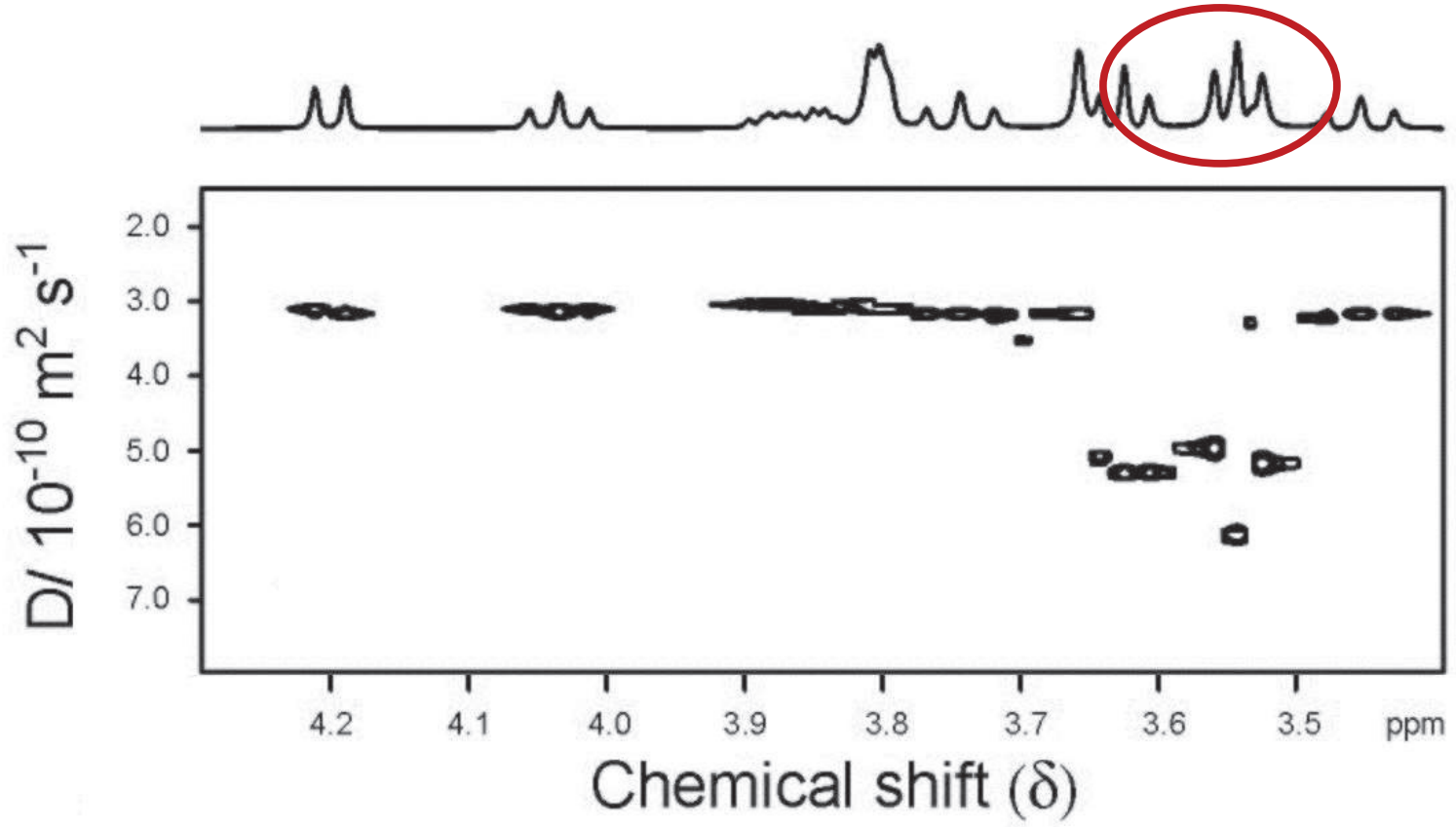


monoexponential fit

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

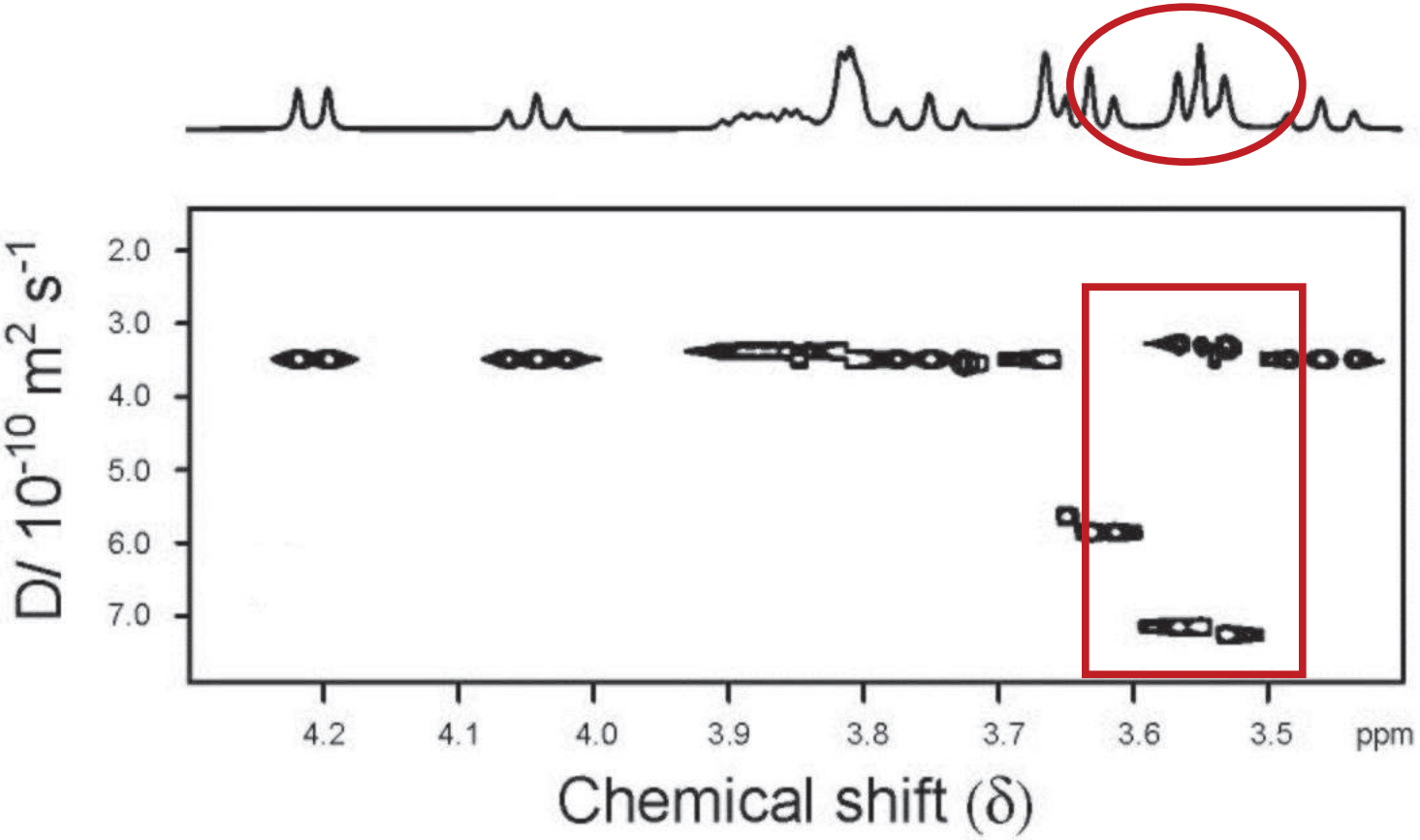


Overlapping peaks give
compromise diffusion coefficient

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

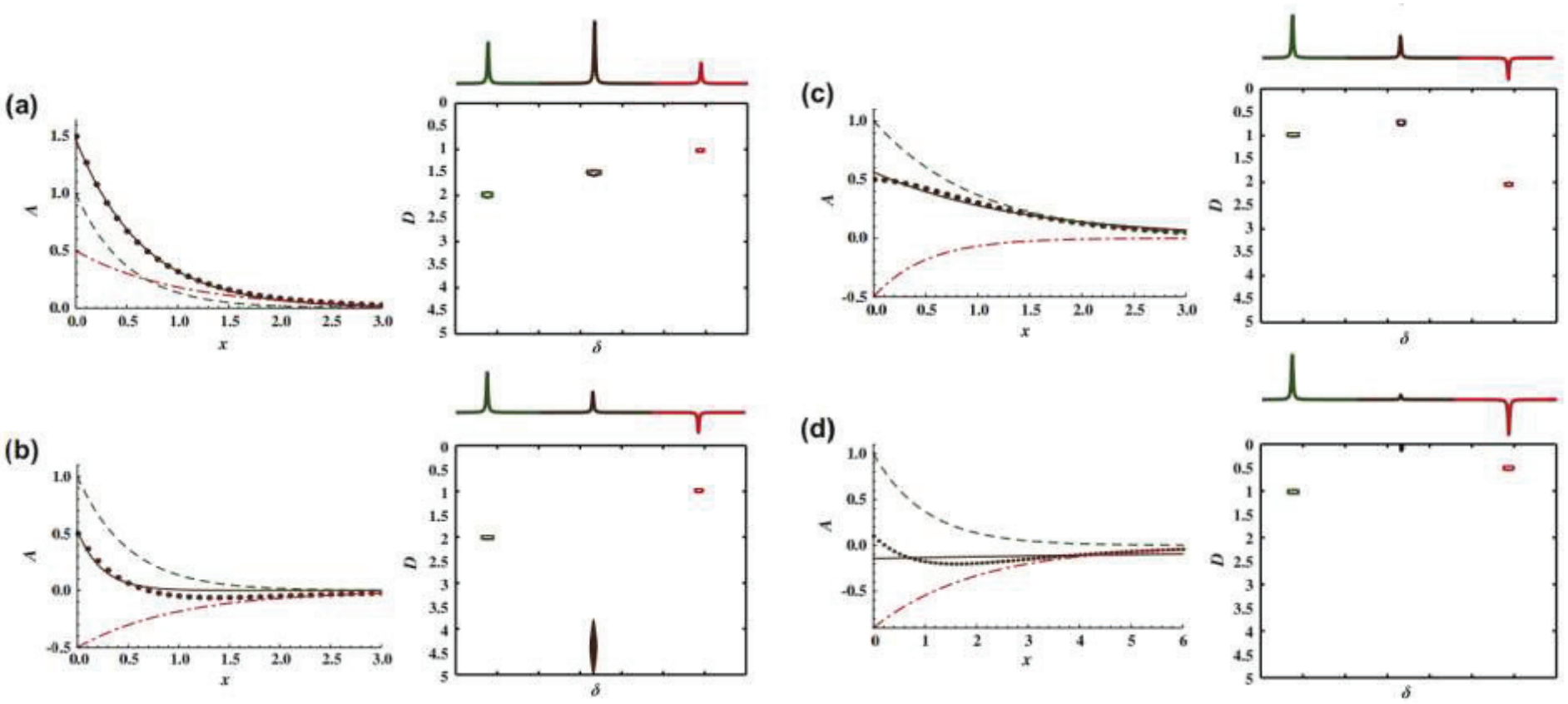


D_A and D_B must differ by at least 30%

Very dependent on the quality and S/N of data

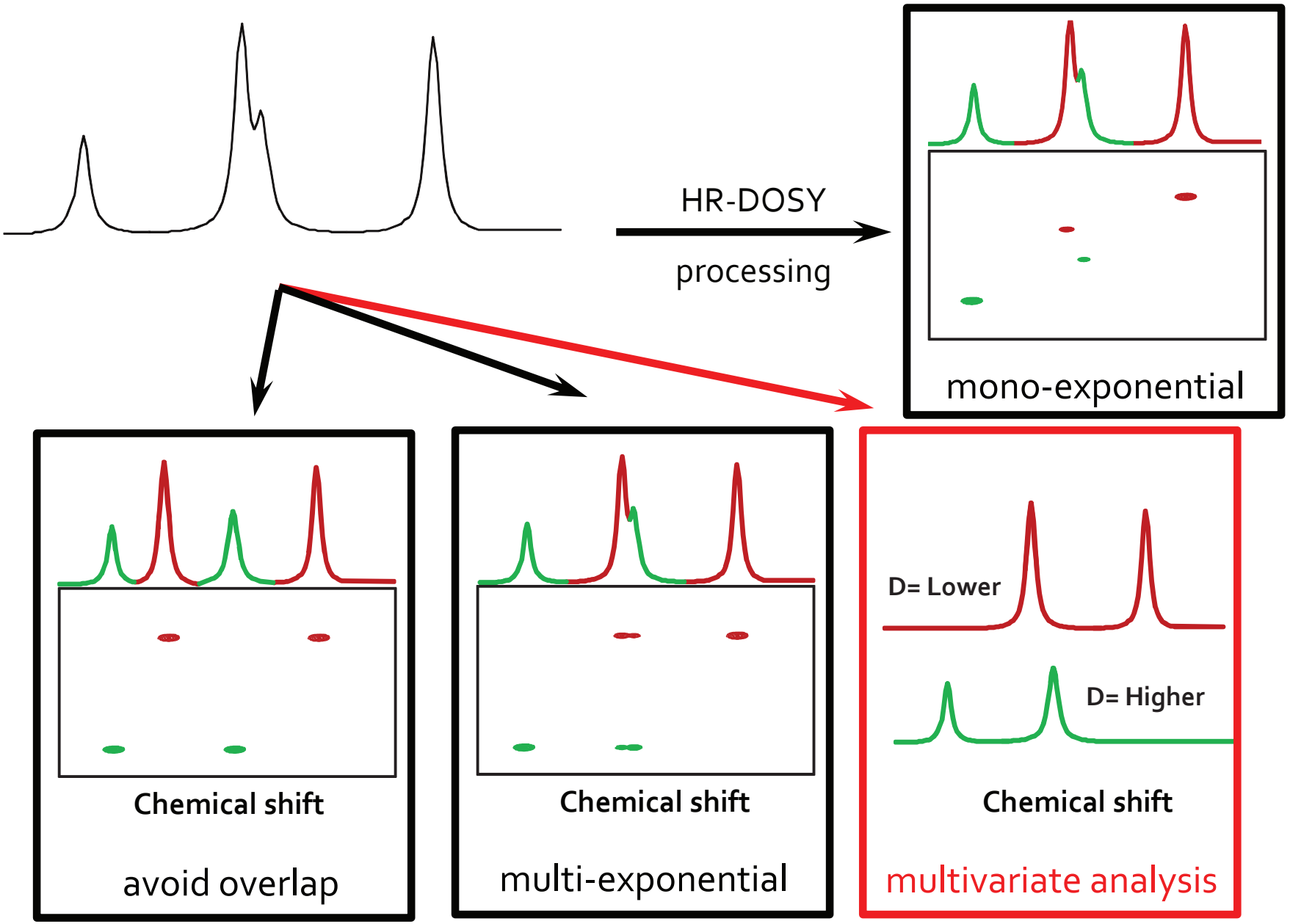
Anal. Chem. **2006**, 78, 3040-3045

Effects of overlap with J-modulation



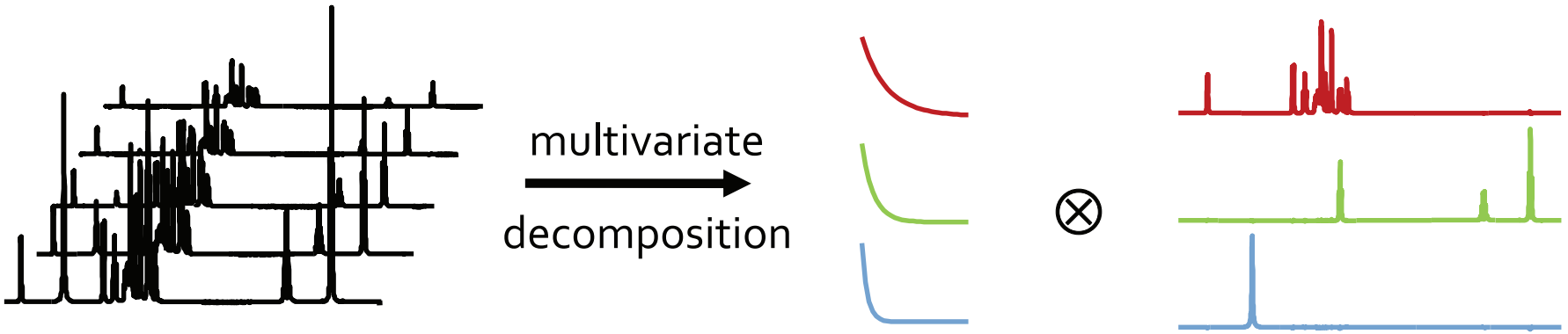
https://doi.org/10.1016/j.jmr.2010.11.012

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

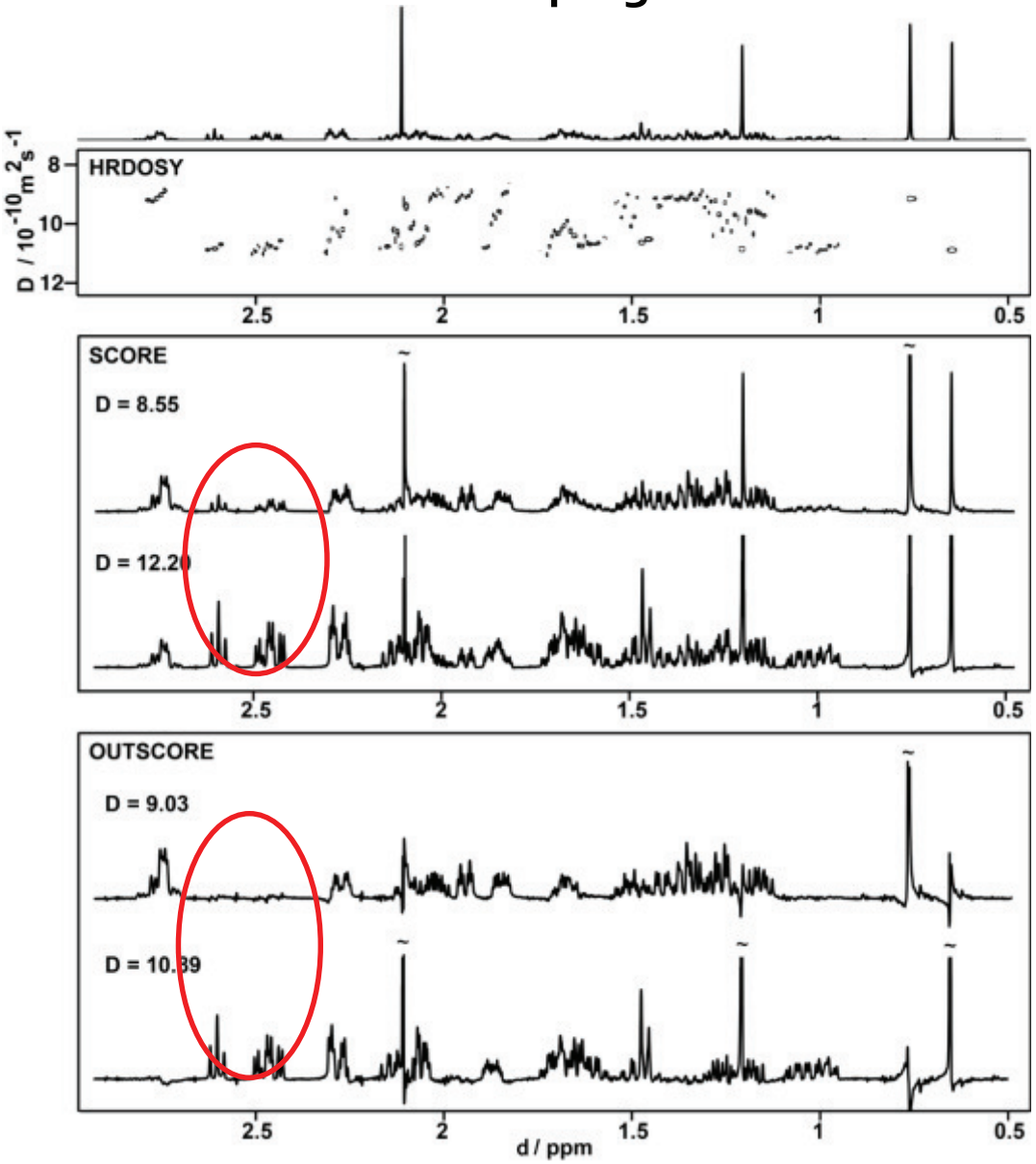
SCORE: $\mathbf{E} = \mathbf{X} - \mathbf{C}\mathbf{S}^T$ (residuals)

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

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

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

Multivariate analysis

- Reference deconvolution to improve data:

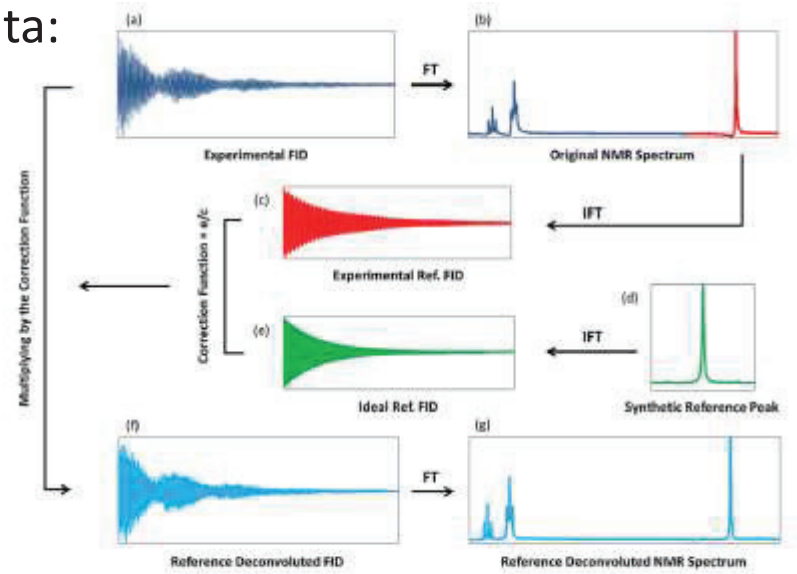
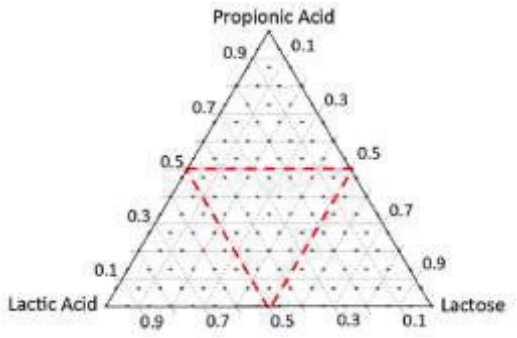
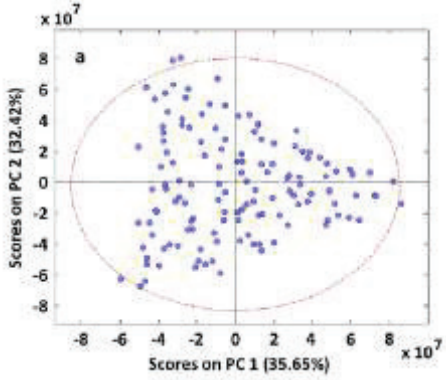


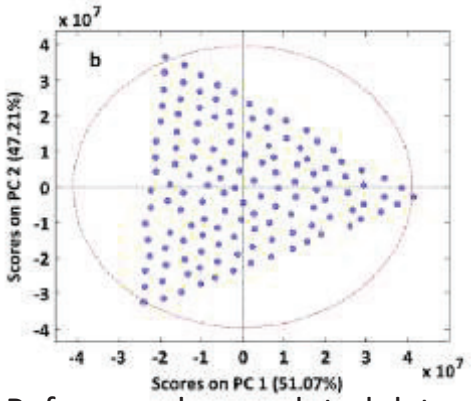
Figure 1. Schematic illustration of the FIDDLE algorithm for reference deconvolution. The reference peak is extracted from the experimental spectrum (b), and its inverse Fourier transform (c) is compared to that of "perfect" FID (e) to yield a correction function (e/c). The correction is then applied in the time domain to the entire experimental FID (a) to produce the corrected FID (f).

- Example (3 compounds with varying concentrations):





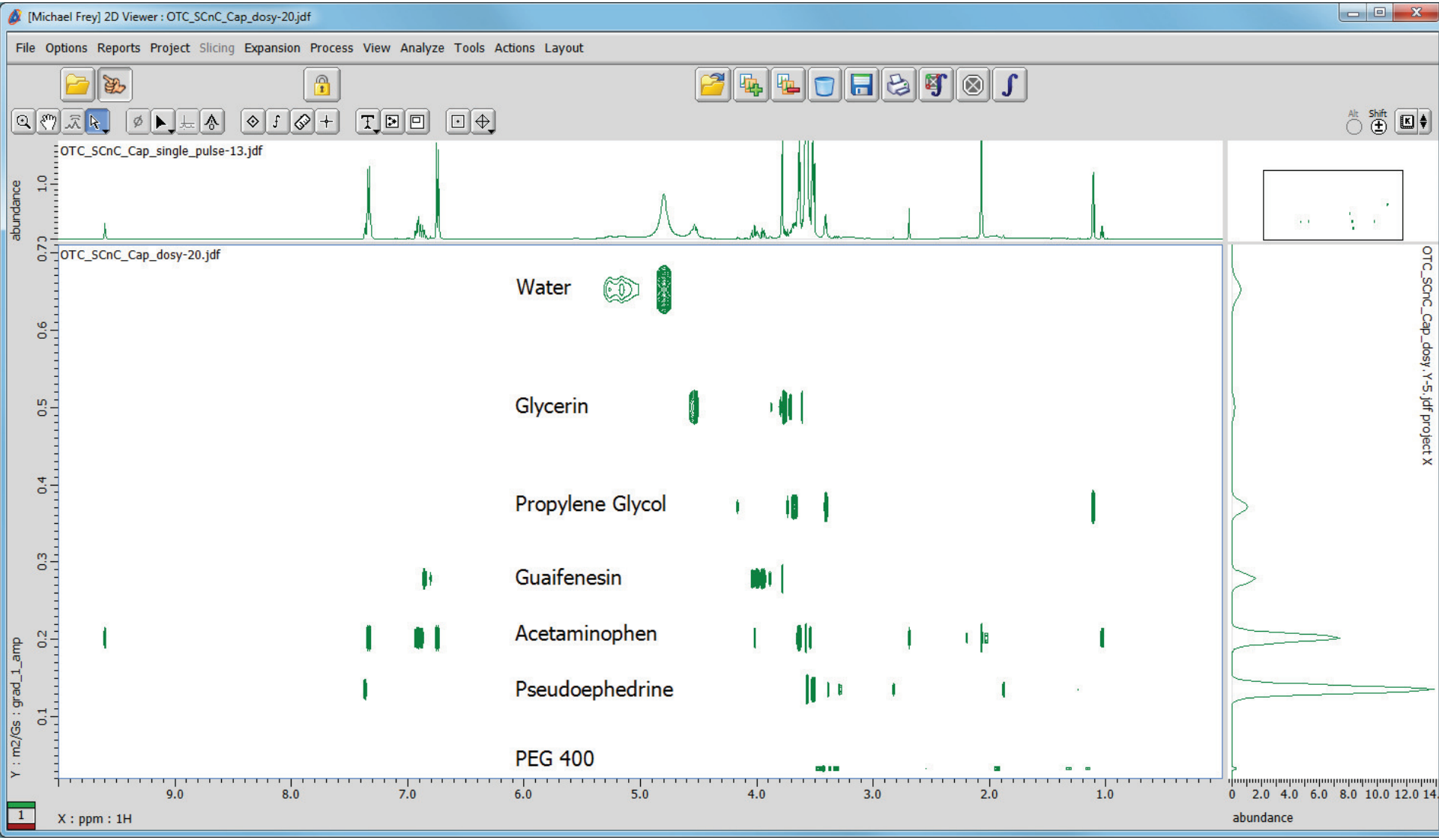
Raw data



Reference deconvoluted data

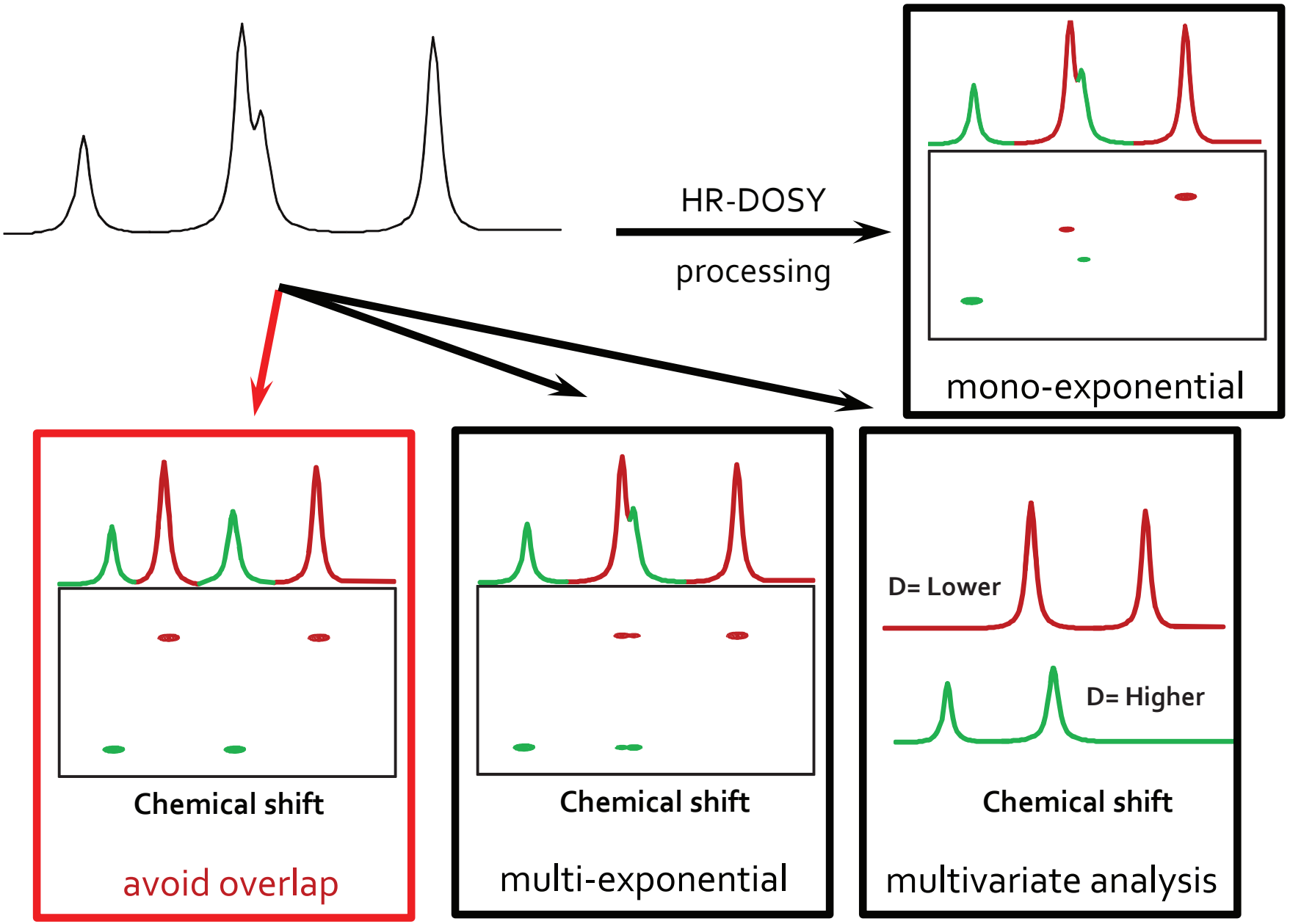
https://doi.org/10.1002/cem.2607

Multivariate DOSY NMR of paracetamol tablet



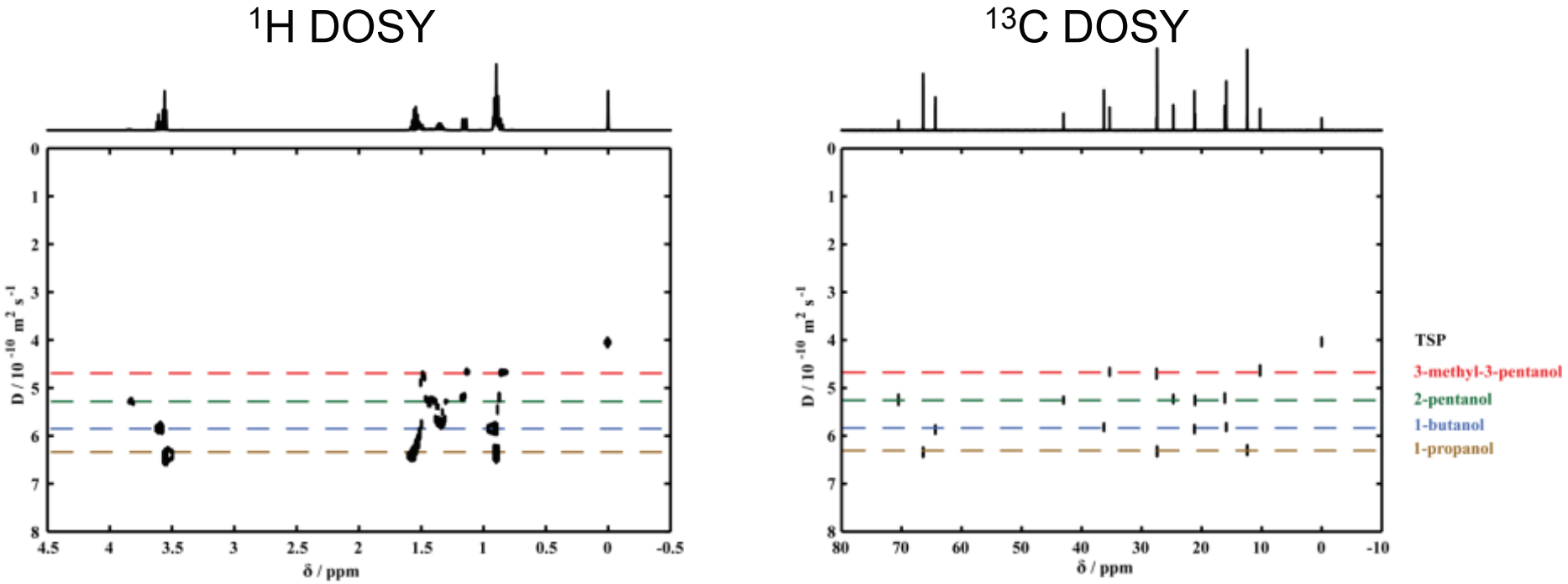
Solvent Methanol-d_4

signal overlap in DOSY processing



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

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

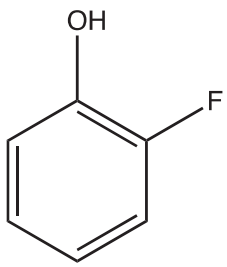


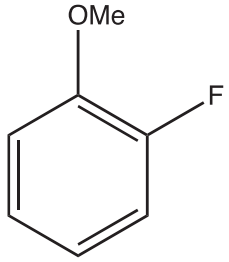
No overlap in the ^{13}C spectrum greatly facilitates interpretation

https://doi.org/10.1016/j.jmr.2011.03.016

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

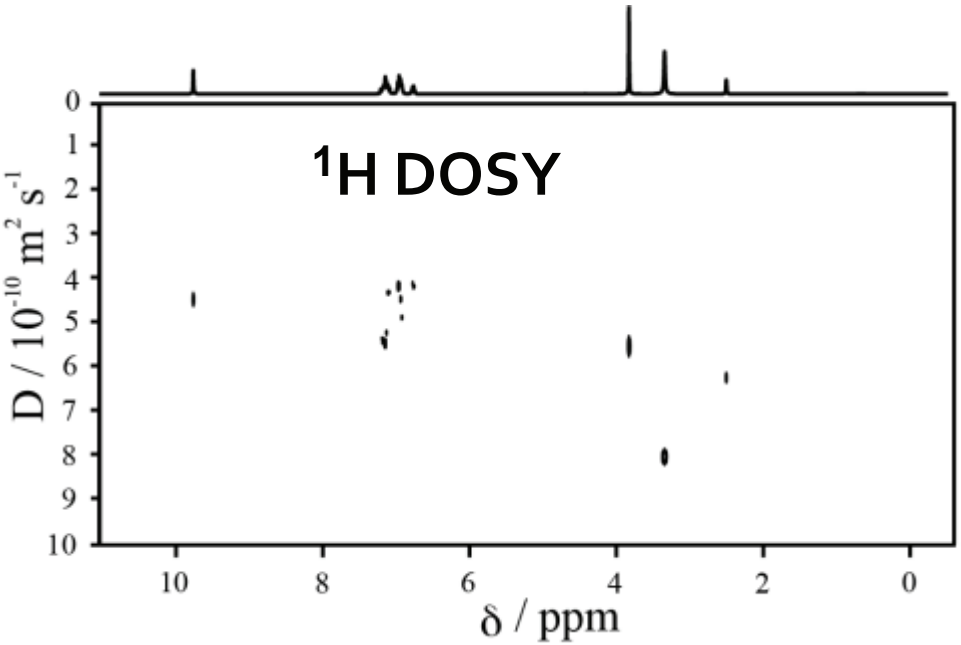
600 MHz ^{1}H and ^{19}F DOSY spectra of fluorinated compounds in DMSO-d_6

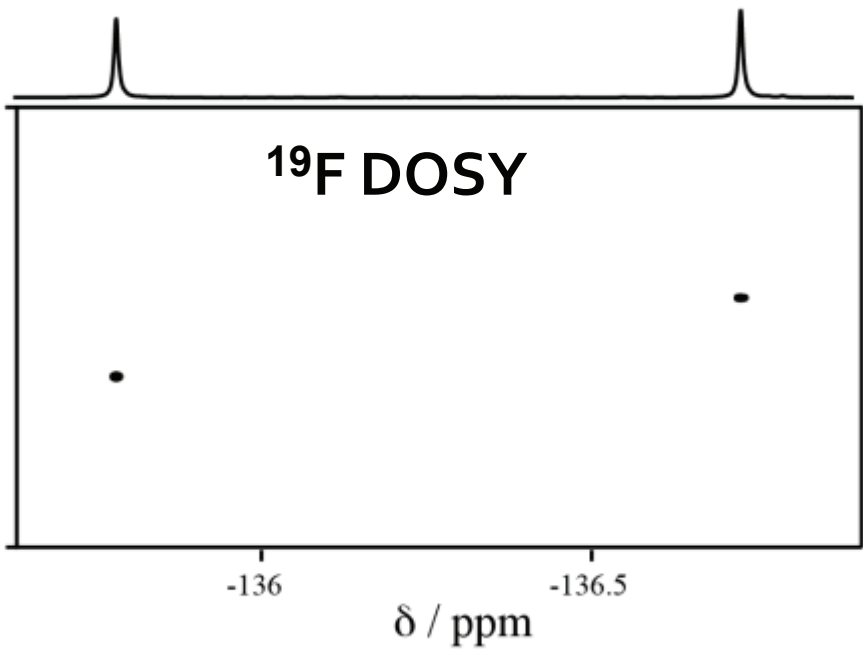




2-flourophenol 2-flouroanisol

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

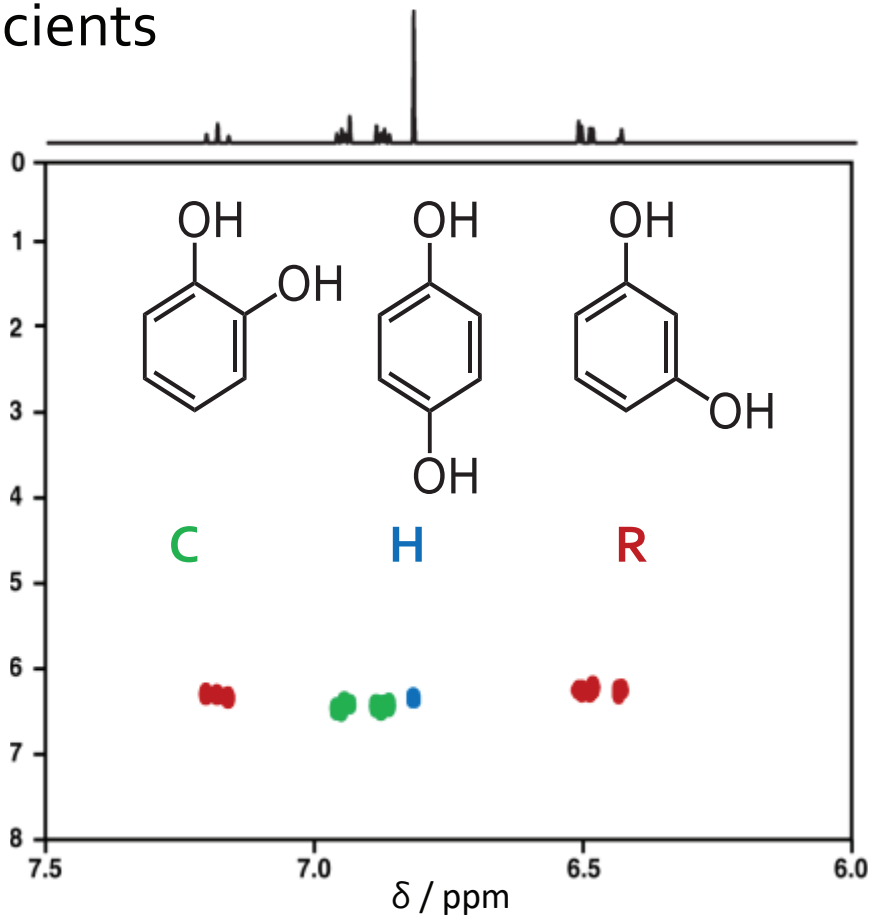




Magn. Reson. Chem. **2014** 52, 172

Matrix-Assisted DOSY (MAD)

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



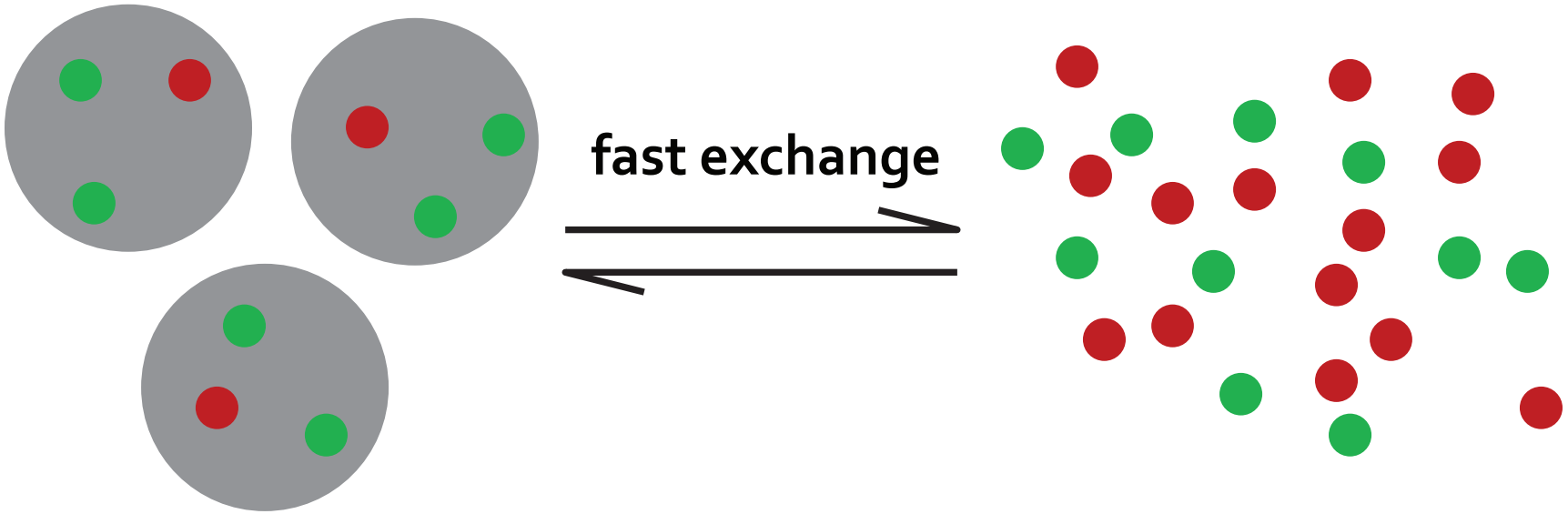
Can we manipulate the way different species diffuse?

Interaction with a matrix

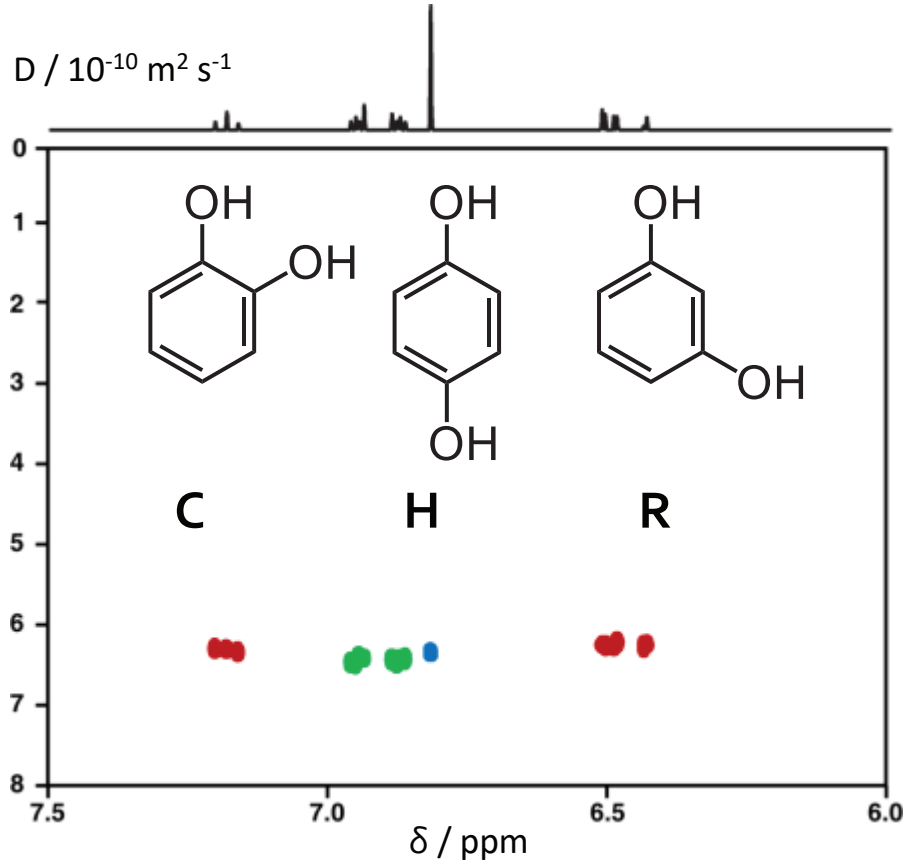
interaction of a solute with a [more slowly diffusing] matrix reduces its apparent diffusion in proportion to the strength of interaction.

a simple two-site model

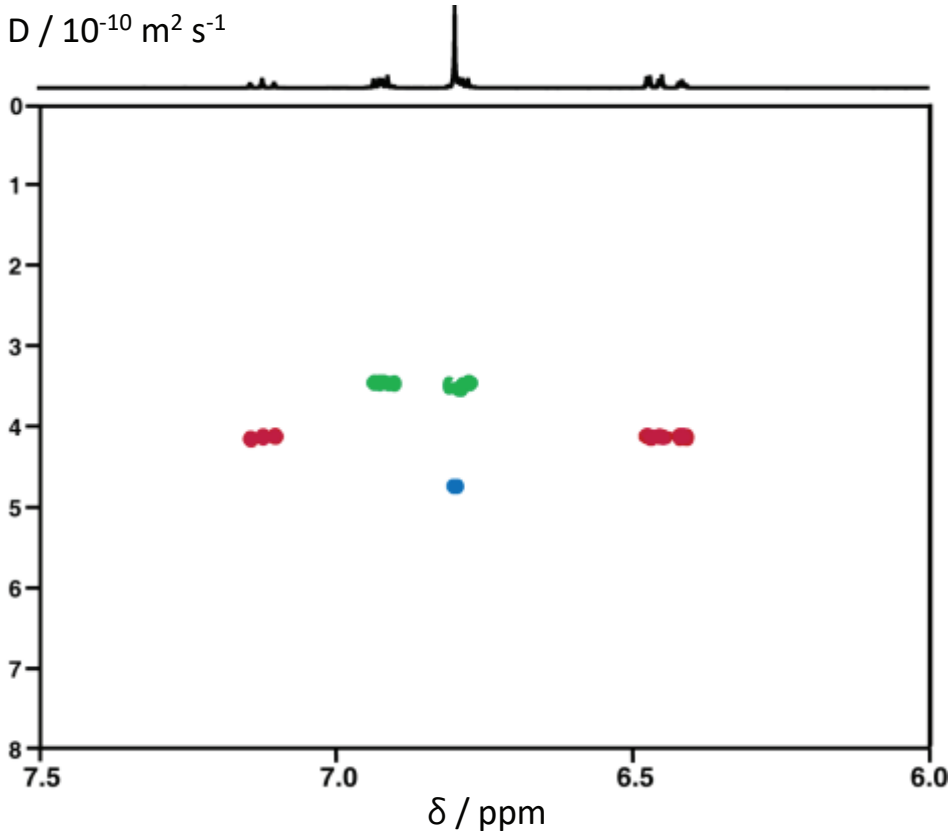
$$D_{apparent} = f_{bound} D_{matrix} + (1 - f_{bound}) D_{free}$$



isomers resolved using micelles



D_2O

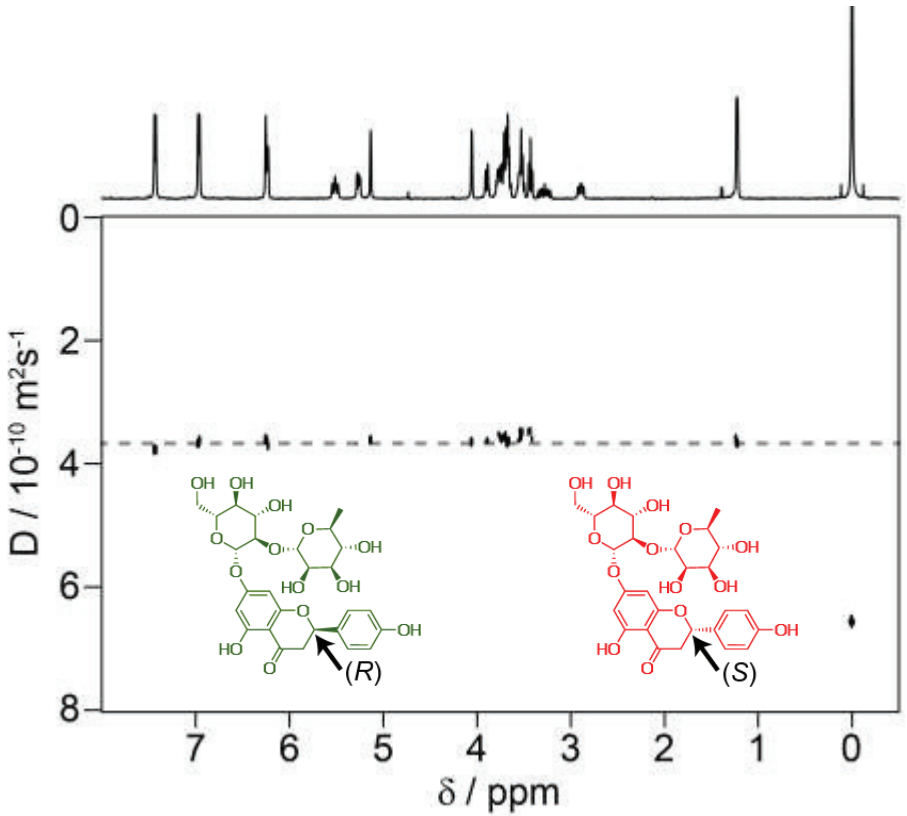


D_2O with 150 mM SDS

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

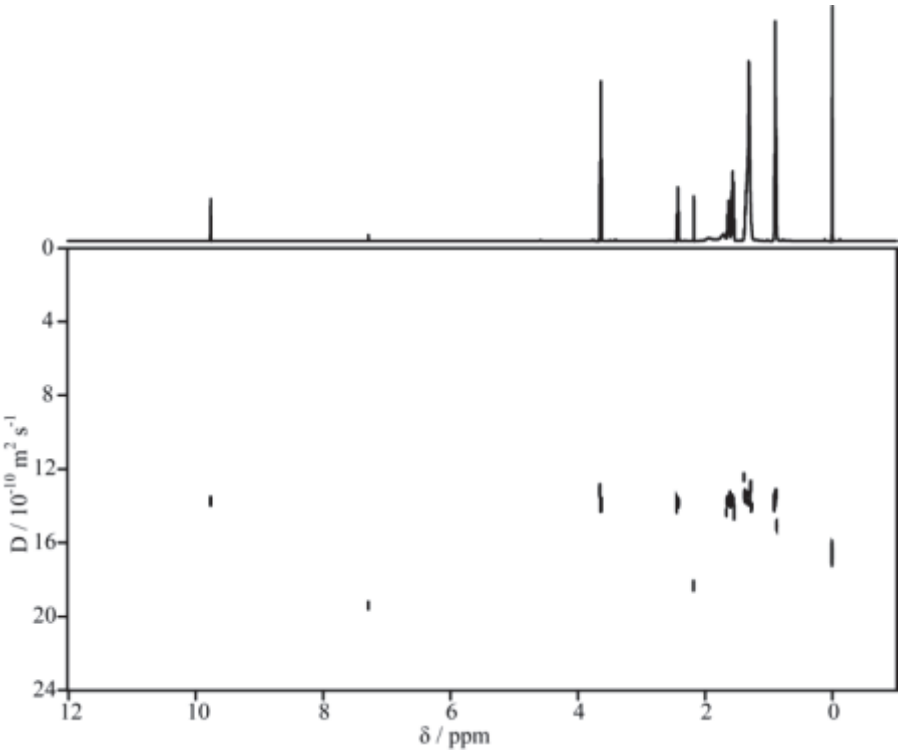
Chiral MAD: epimers resolved using cyclodextrins



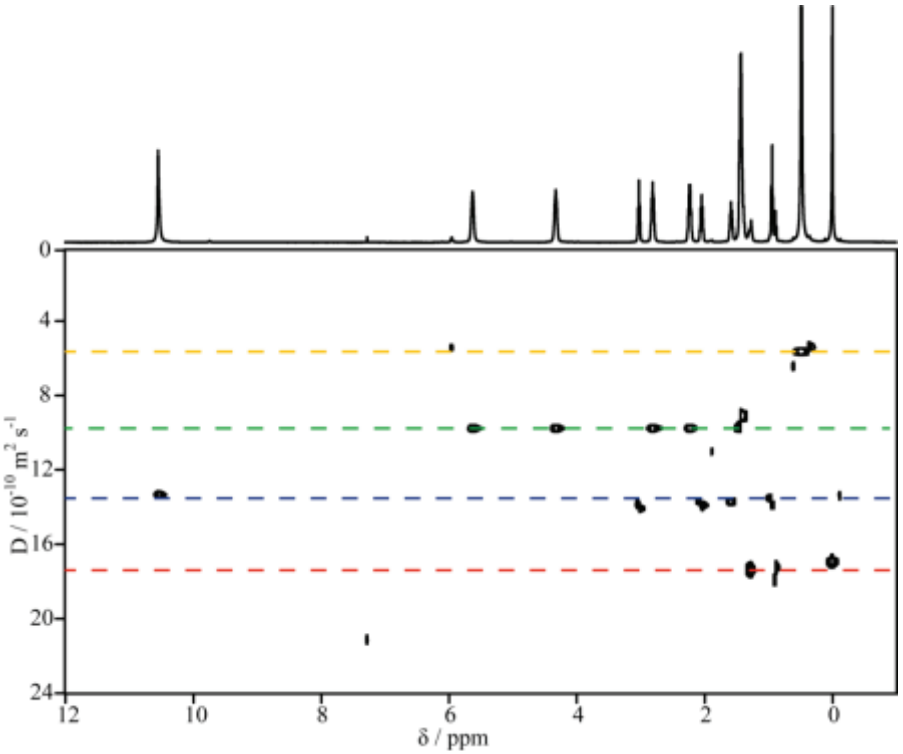
differential inclusion by β-cyclodextrin of the epimers of the natural product naringin is exploited, allowing separation of the naringin epimer signals by high resolution DOSY.

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

Thank you

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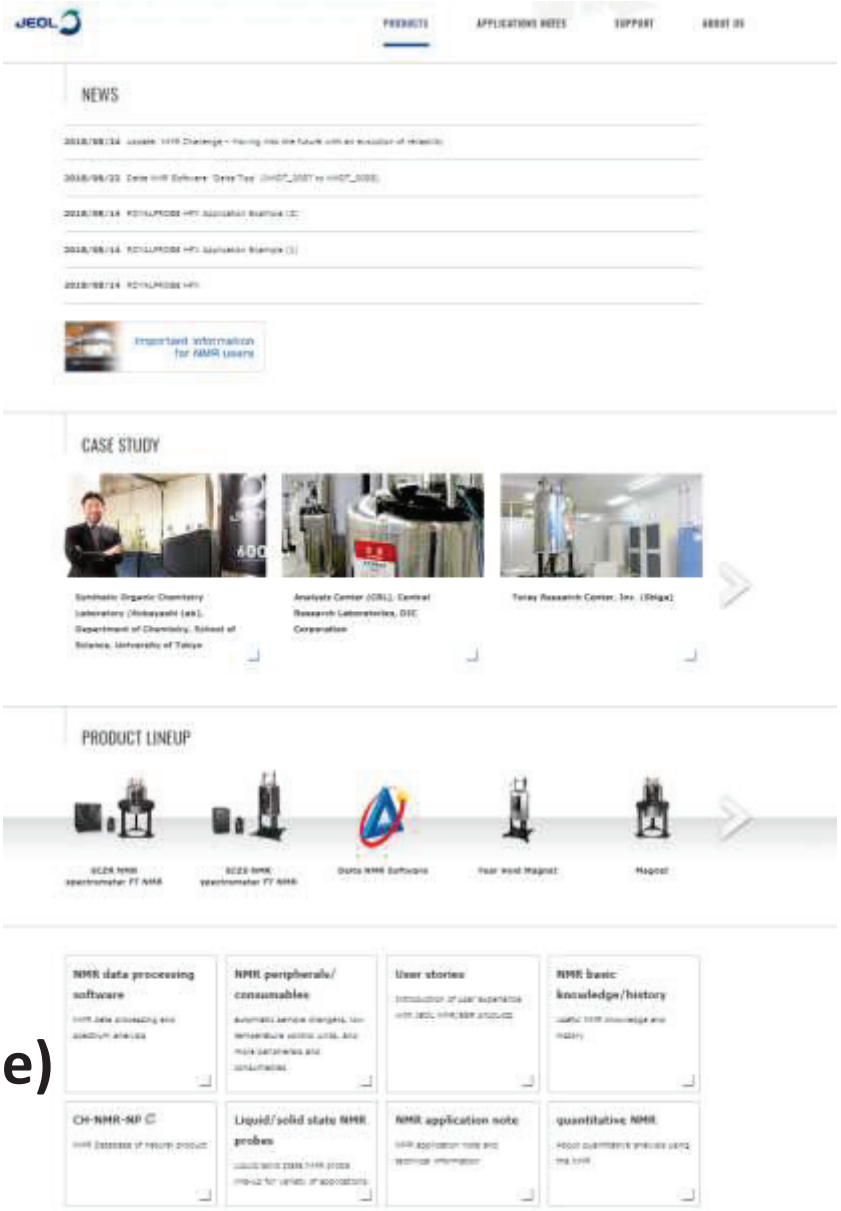

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