MANCHESTER 1824
The University of Manchester

# DOSY – Practicalities and Pitfalls

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UKMRM, Manchester, 2015

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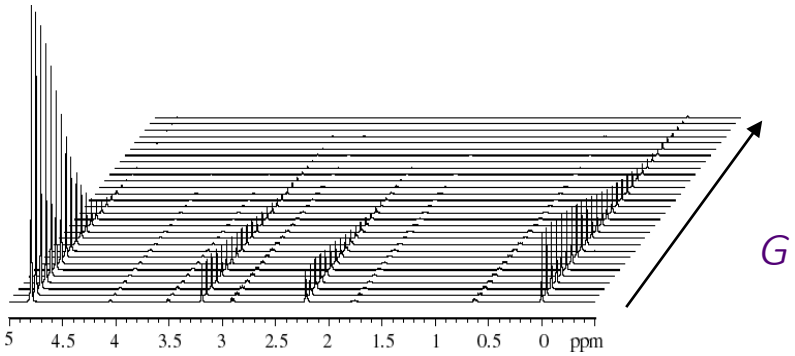
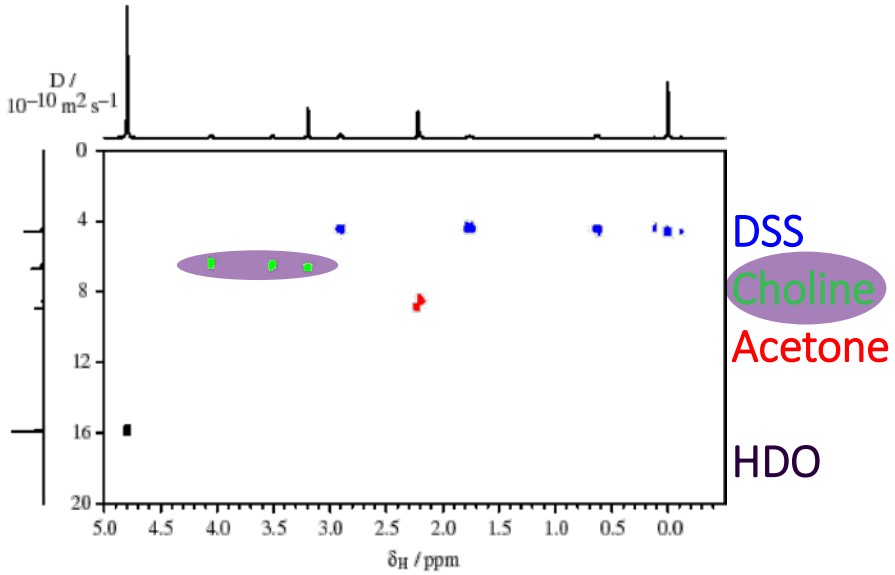
# Constructing a 2D DOSY spectrum

PFGSTE spectra are measured as a function of *G*.

By fitting peak heights to the Stejskal-Tanner equation diffusion coefficients, *D*, are obtained.

1D peaks are extended into a second dimension, with Gaussian shapes centred on the *D*'s and widths determined by the standard errors $\sigma_D$.



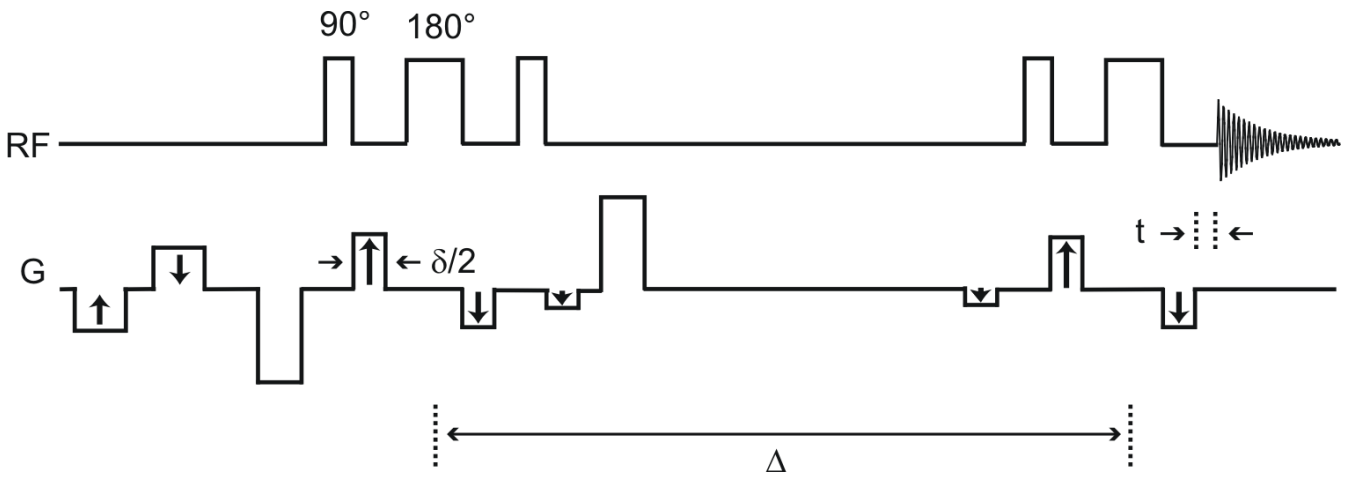




400 MHz $^1$H DOSY spectrum of choline, acetone and DSS in $D_2O$

Why might we want to acquire a DOSY spectrum?

# How do we acquire a DOSY spectrum?

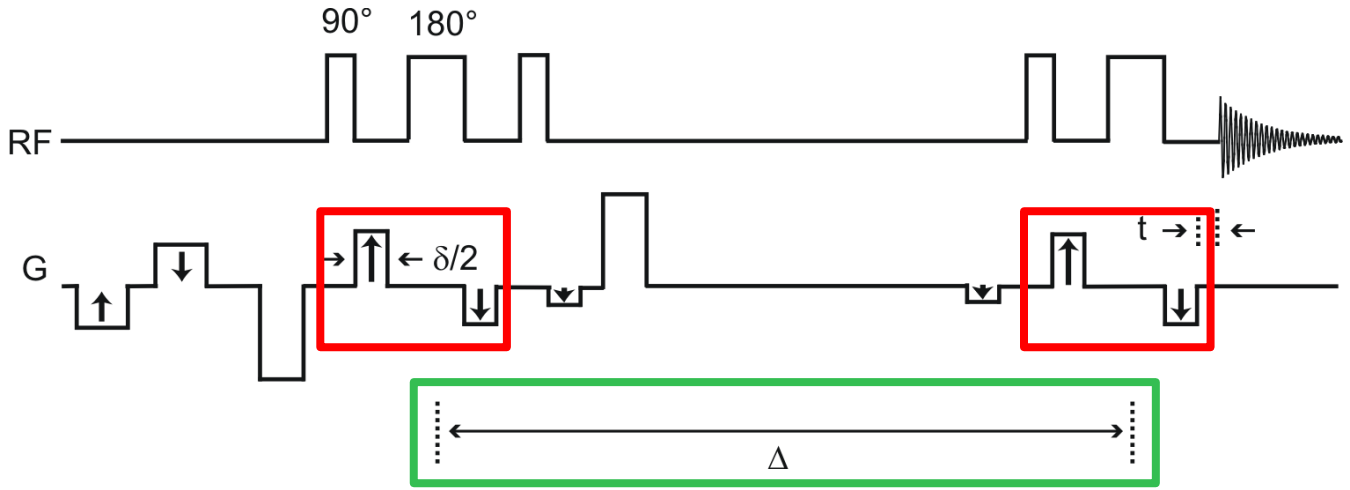
Common ‘PFGSTE’ pulse sequence




Oneshot sequence

# How do we acquire a DOSY spectrum?

## Appropriate parameter settings




Pulse calibration

Recycle time

Acquisition time, *etc*

Strengths of greatest and smallest diffusion gradient levels

Diffusion gradient pulse width

Number of gradient levels

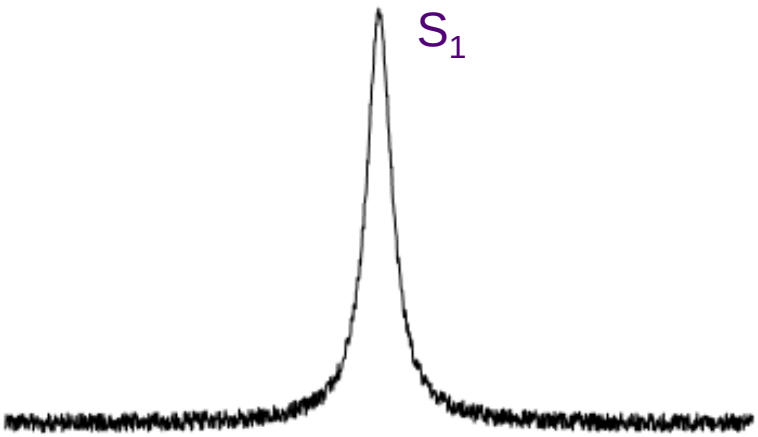
Diffusion time

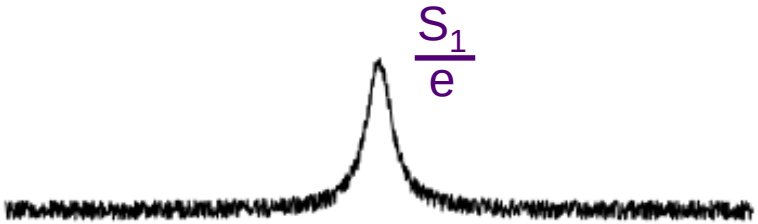
Good fitting to the Stejskal-Tanner equation can normally be achieved if the signals in the spectrum with most attenuation have intensity ≈1/e of those in the spectrum with least attenuation.

Acquiring the spectra with the greatest and smallest gradient levels from the full DOSY series, with a reduced number of transients, allows parameters to be appropriately set.

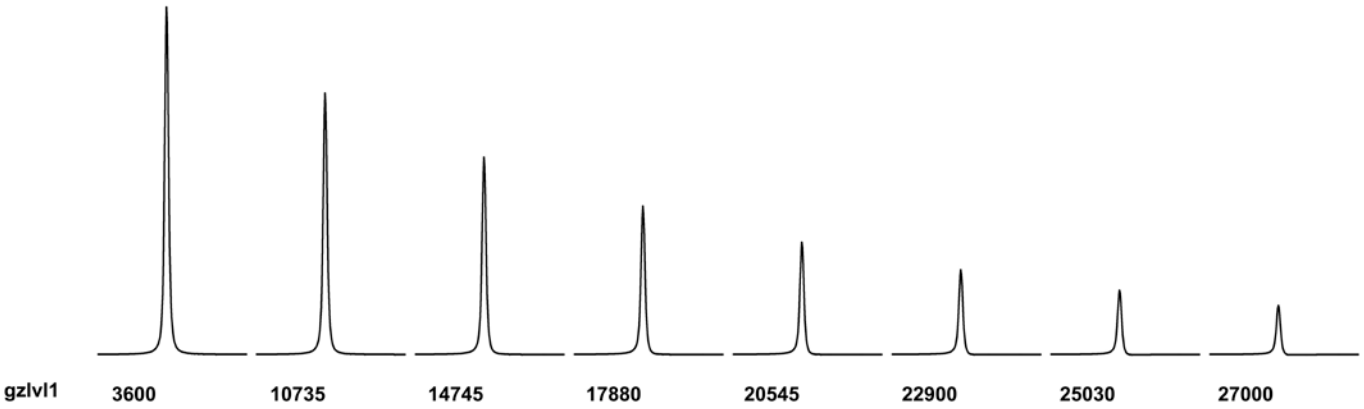
# Optimising parameter settings

| | | |
|---|---|---|
| 1. | Diffusion time | Choose Δ short compared to $T_1$. |
| 2. | Strengths of strongest and weakest diffusion gradient levels | Set to highest available for the strongest level and lowest available for weakest level (while maintaining CTP if using Oneshot type sequences). |
| 3. | Diffusion gradient pulse width | Adjust value to get desired signal attenuation. |
| 4. | Number of gradient levels | For mono-exponential fitting choose 10. |



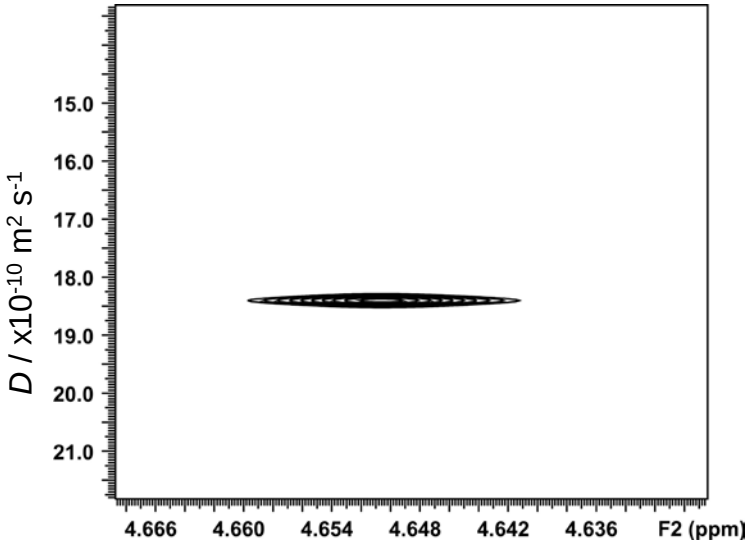
# Acquire the DOSY




DOSY spectra should be acquired with the gradient strength incremented in steps of gradient[2].

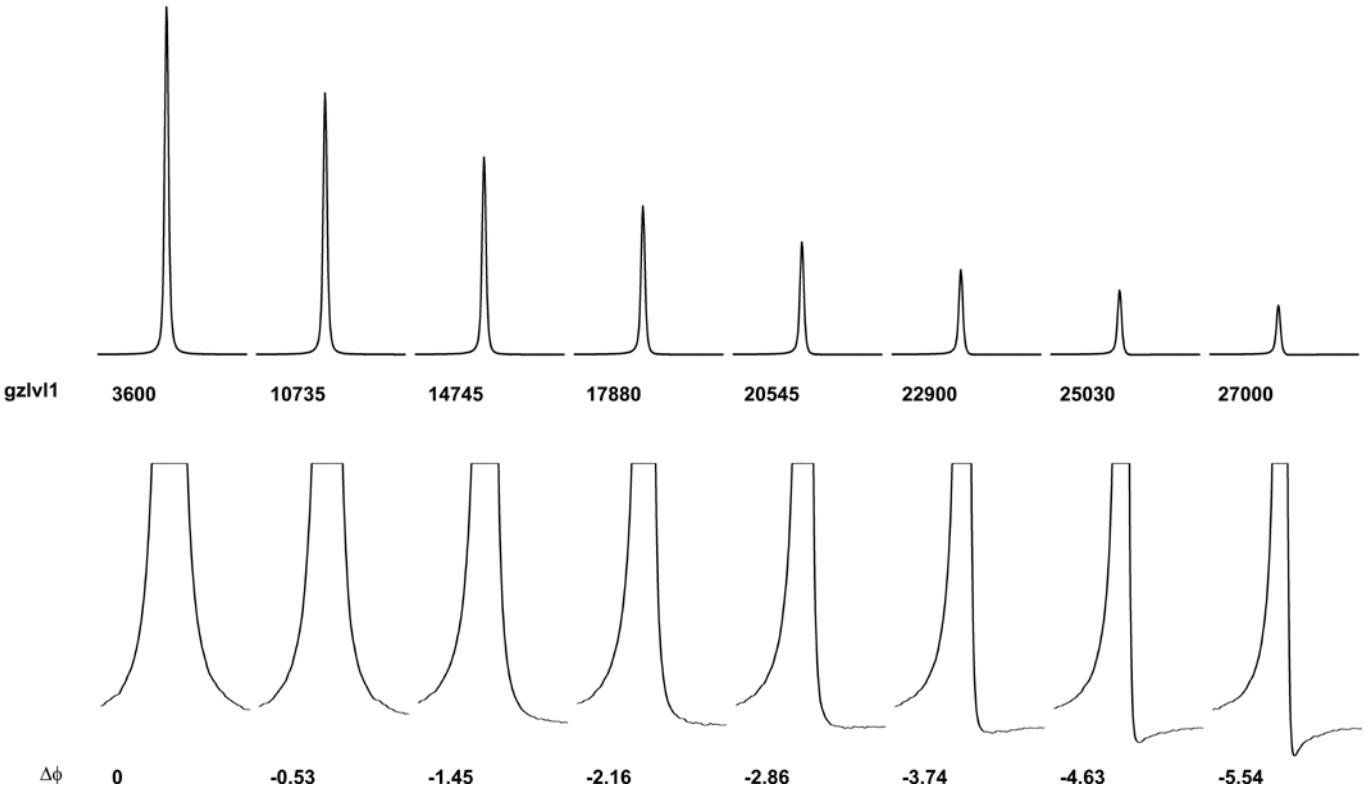
Signal attenuation in a DOSY experiment

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




DOSY spectrum

# Phase dependence on gradient strength



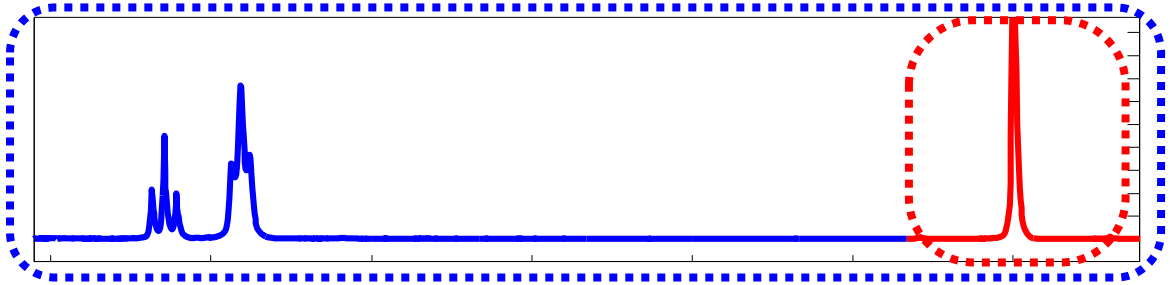

Signal phase in a DOSY experiment

In addition to the desired signal attenuation, there is often a phase change that results from the change in gradient strength.

The phase change can usually be reduced by increasing the gradient stabilisation delay (gstab/d16).

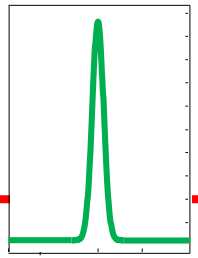
Use of reference deconvolution can completely remove the effects of phase change.

# Reference Deconvolution

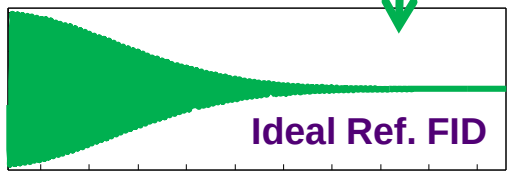


Experimental NMR Spectrum

IFT



Synthetic Reference Peak

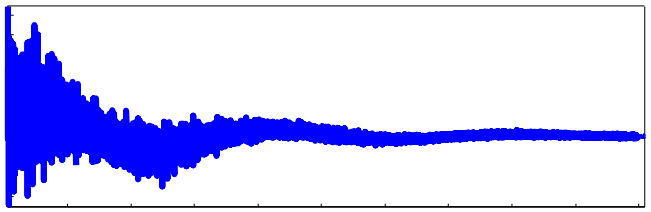


Ideal Ref. FID

C =

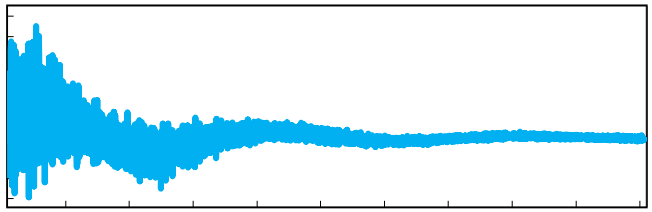
Experimental Ref. FID

IFT



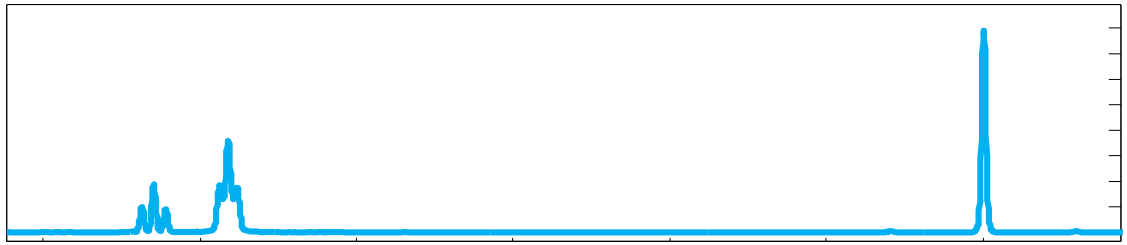
Experimental FID

× C =



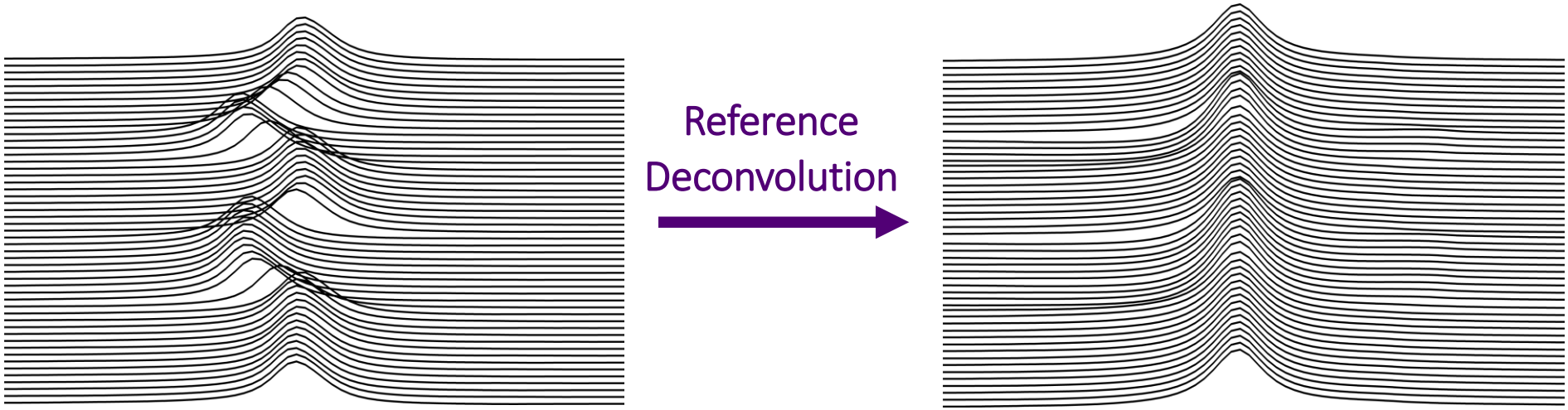
Reference Deconvoluted FID

FT



Reference Deconvoluted NMR Spectrum

# Reference Deconvolution



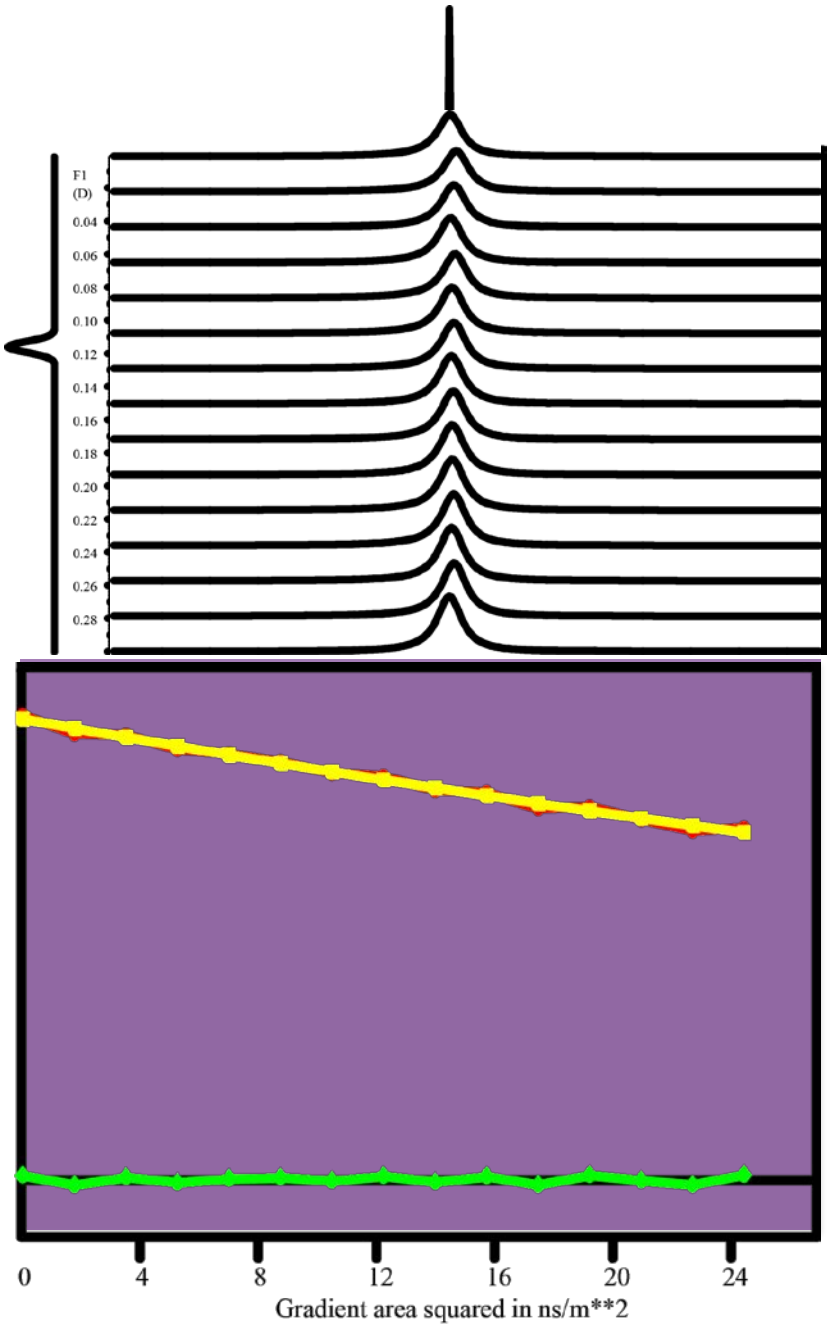

## Benefits for DOSY data

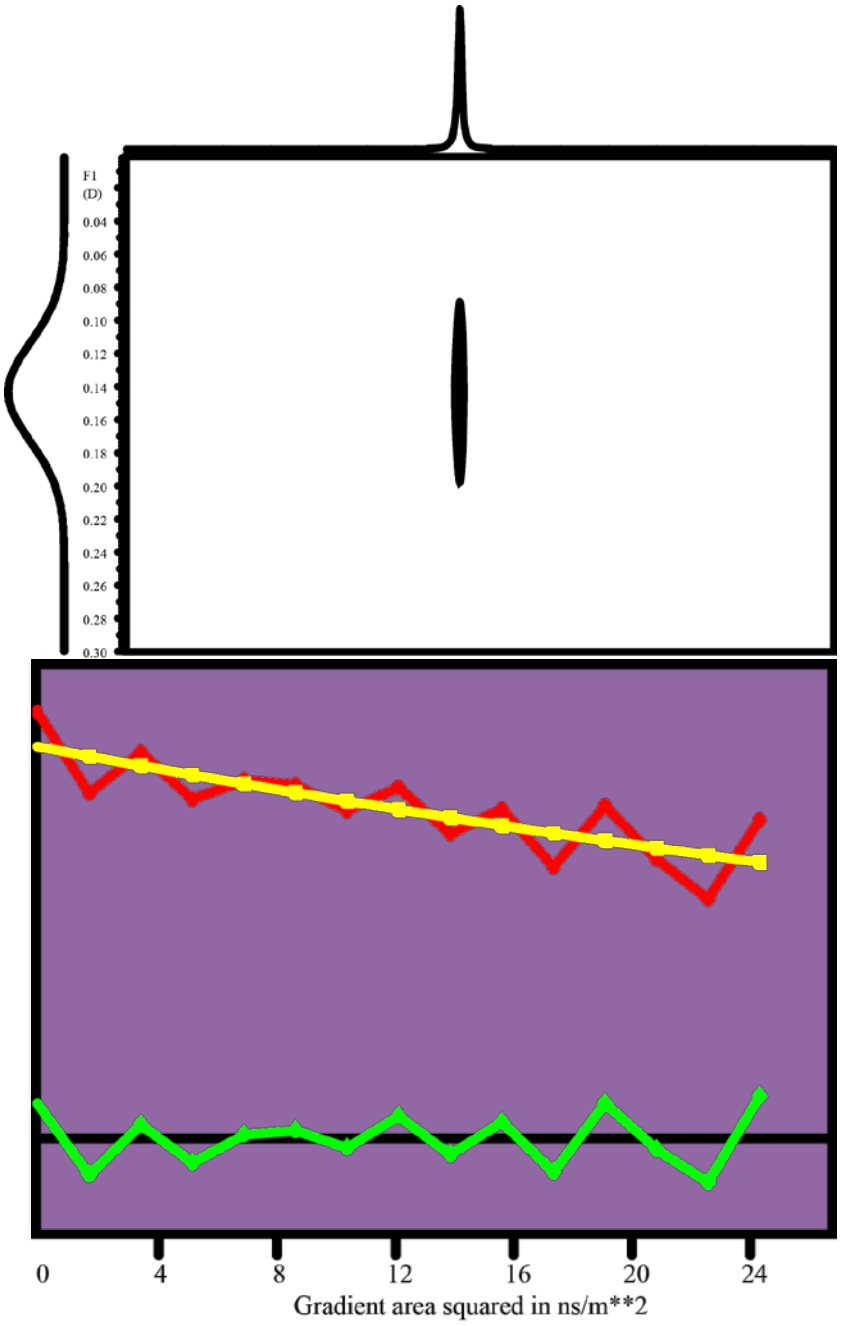
- Resolution enhancement
- Frequency correction
- Phase correction

## Requirements

- Singlet (TSP)
- Adequate amplitude in all spectra

# Reference Deconvolution



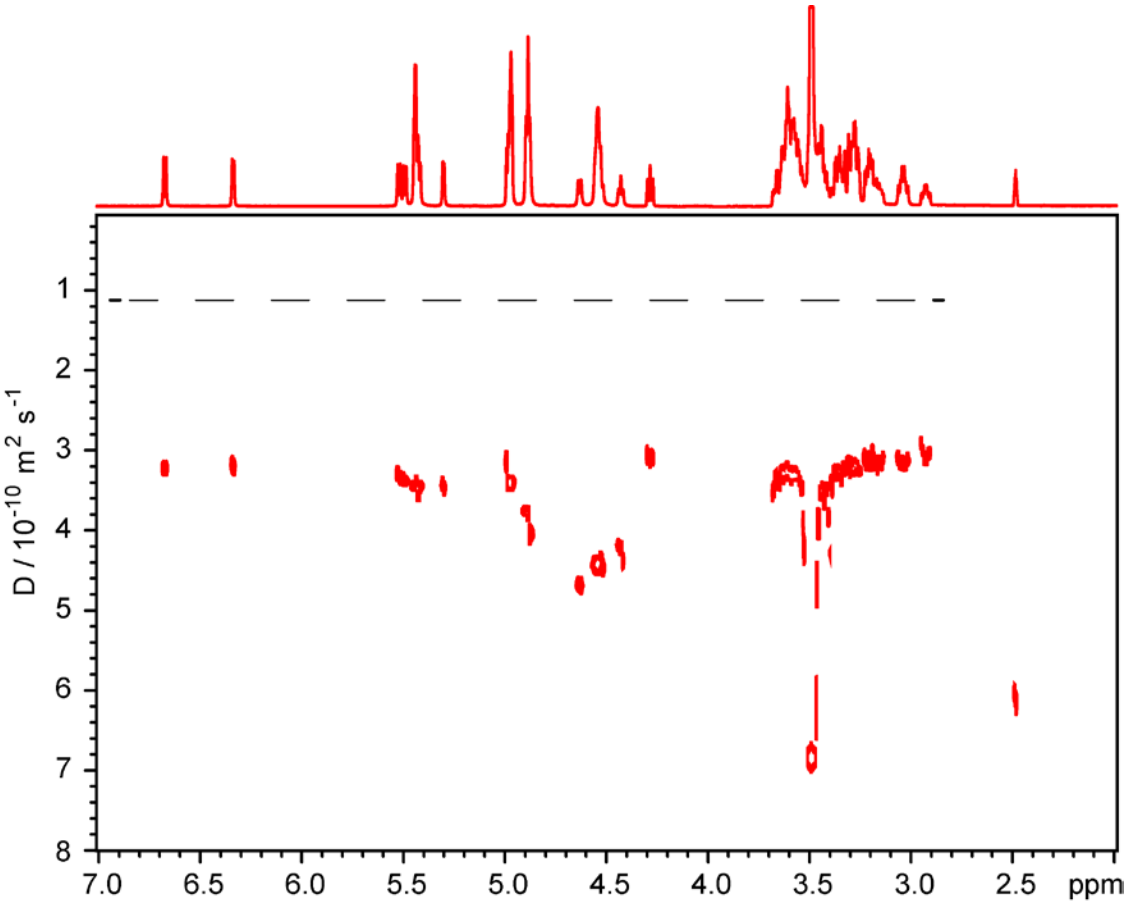



# Convection

Uncompensated convection during the diffusion period of the Oneshot stimulated echo leads to an apparent increase in *D*.

Convection compensated PFGSTE sequences can be used at a cost in sensitivity.

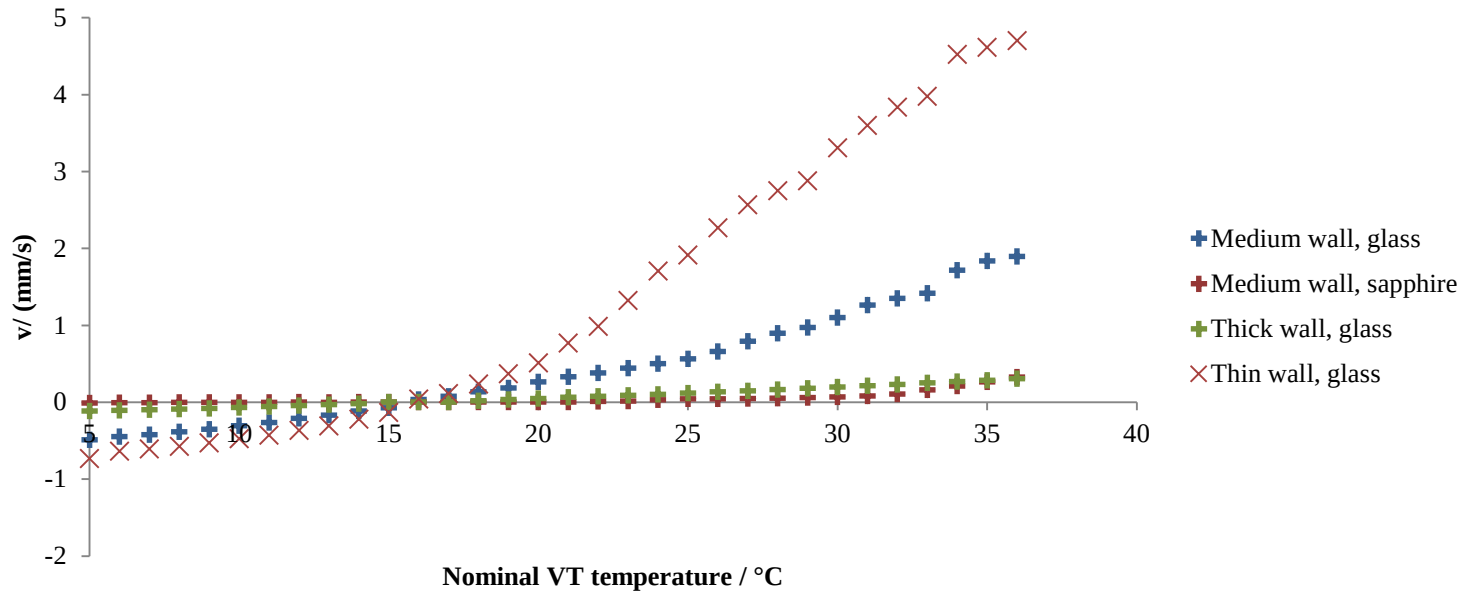
Exchange during the diffusion period leads to averaging of diffusion coefficients.




OH/$H_2O$ exchange in a convecting 30 °C solution of maltotriose in dmso-$d_6$

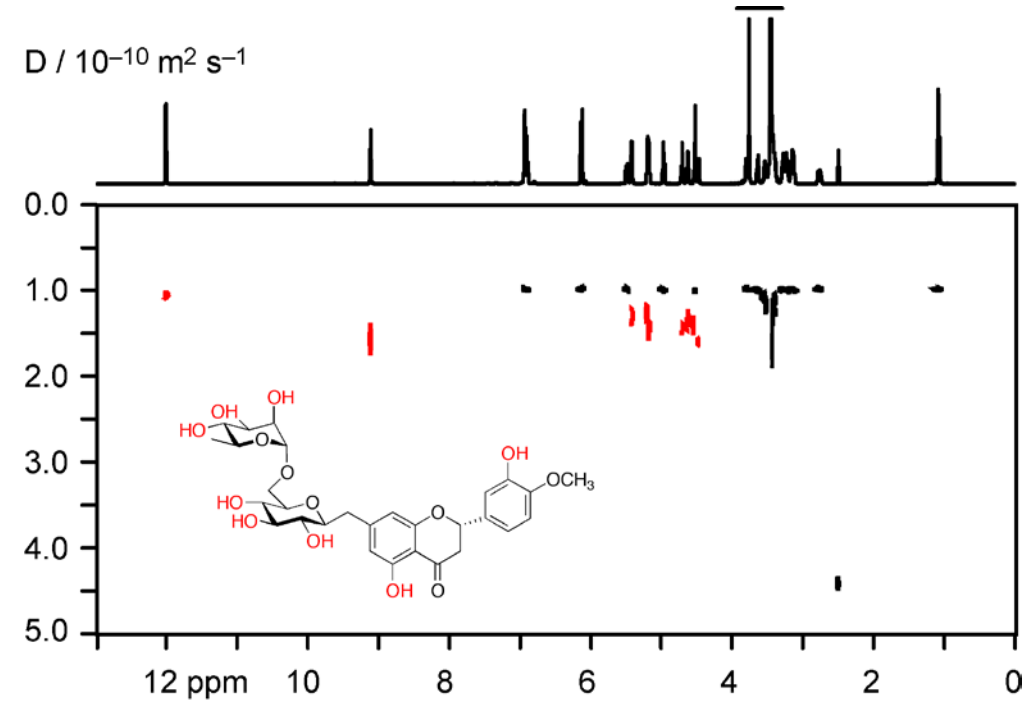
# Convection

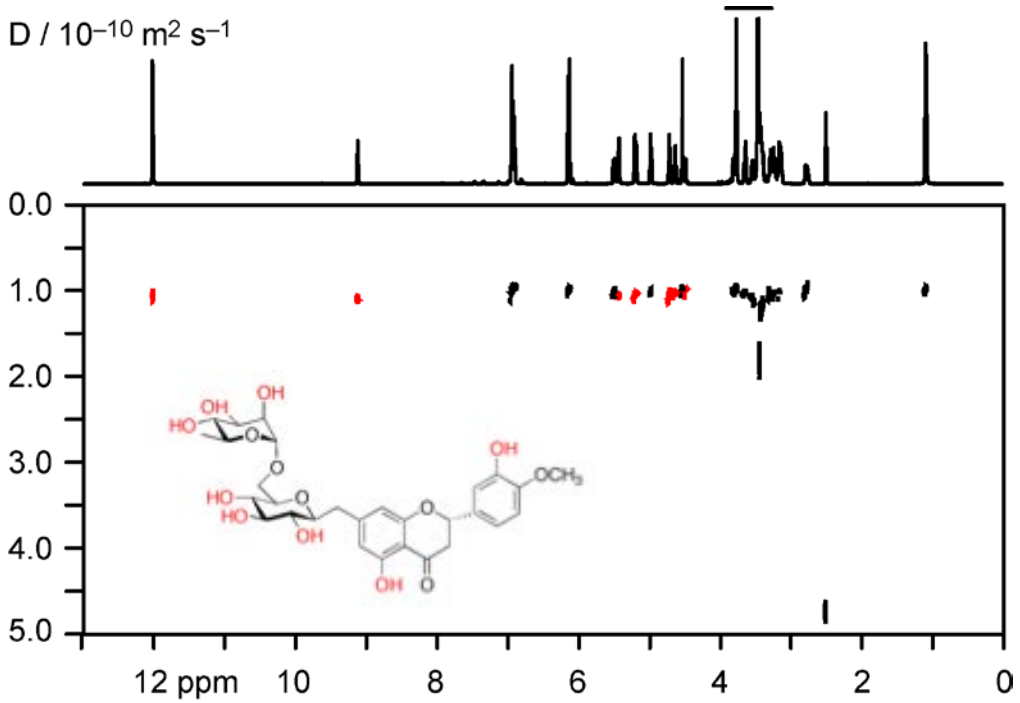
Common sense suggests that convection should only happen where the bottom of the sample is warmer than the top – a negative temperature gradient. Experiment shows that this is not the case: convection in a chloroform sample occurs both above and below the quiescent sample temperature.




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

# Chemical Exchange



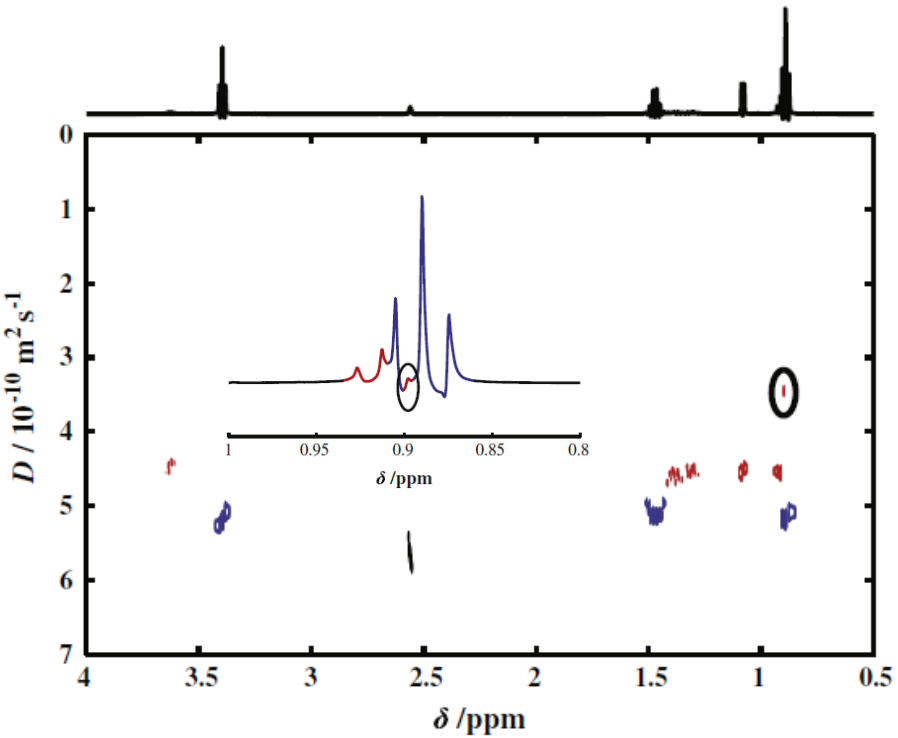




OH ⟷ $H_2O$ exchange in hesperidin in dmso-$d_6$

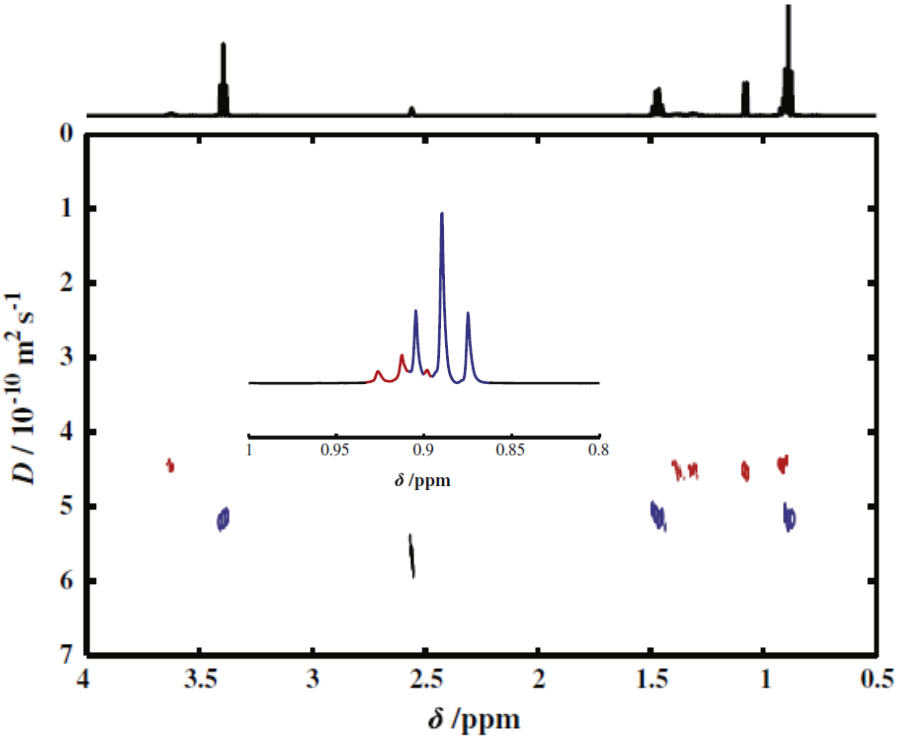
Exchange during the diffusion delay of the Oneshot45 stimulated echo leads to averaging of diffusion coefficients.

Exchange in PROJECTED (PROJECT Extended for DOSY) leads only to signal loss, not to diffusion averaging, because the magnetization remains transverse during the diffusion delay.

# Overlap due to J-modulation




Oneshot DOSY spectrum of 1-propanol and 2-pentanol




Oneshot45 DOSY spectrum of 1-propanol and 2-pentanol

Highlighted signal has an apparent diffusion coefficient outside the range spanned by the two components.

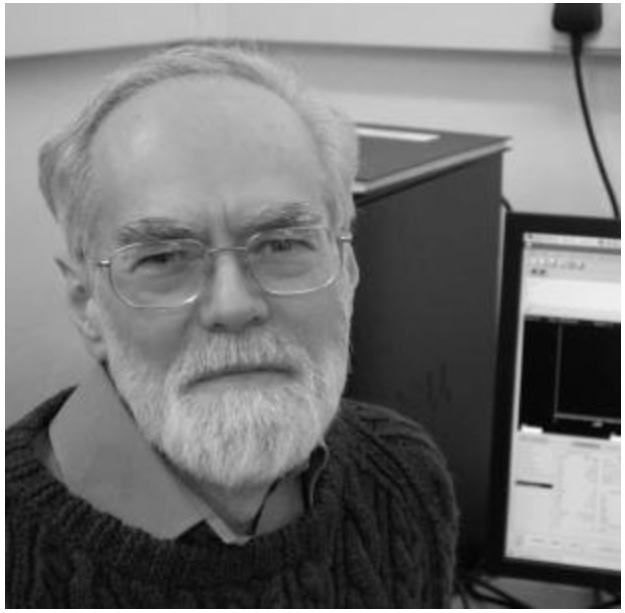
The effects of J-modulation are suppressed in the Oneshot45 sequence and the signals appear in the correct place. PROJECTED also suppresses the effects of J-modulation.

# Take Home Message

Many experiments are available for many applications but none for all.

Generally, Oneshot45 is a good starting point but be wary of convection and chemical exchange.

## Acknowledgments



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Dr. Mathias Nilsson

Dr. Péter Király

Iain Swan

# Thank you

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# Oneshot45 Sequence

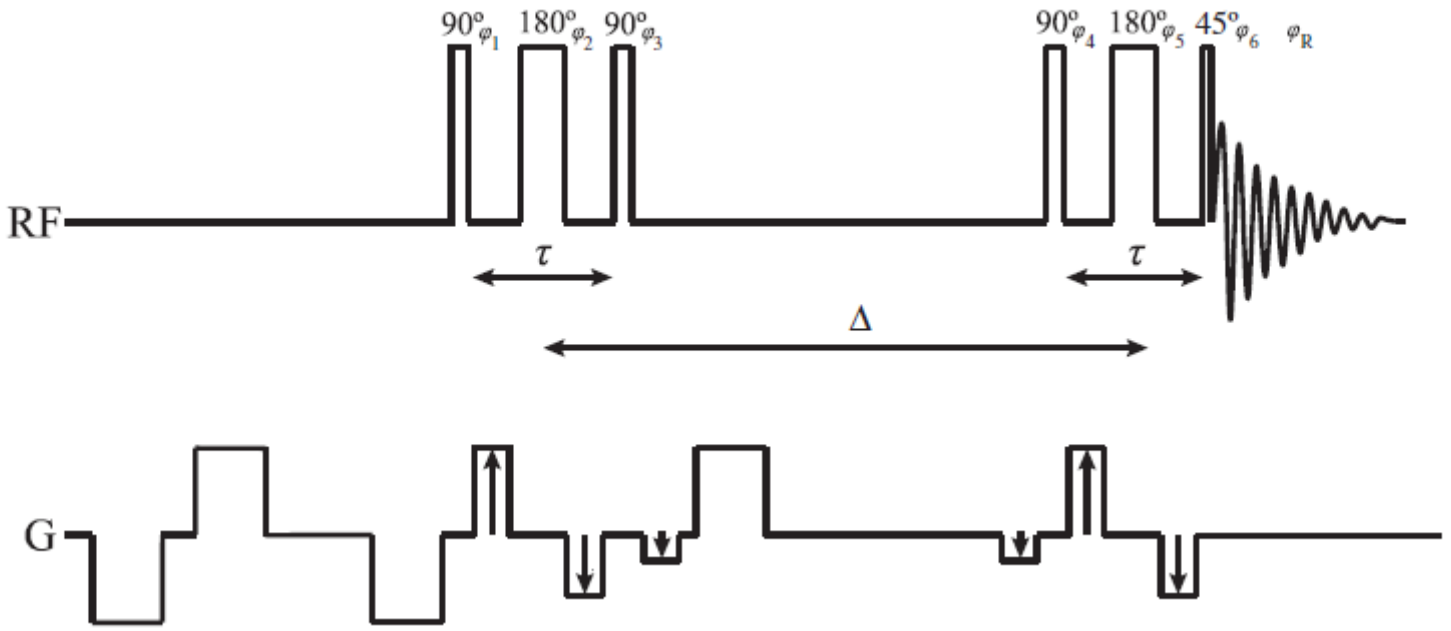
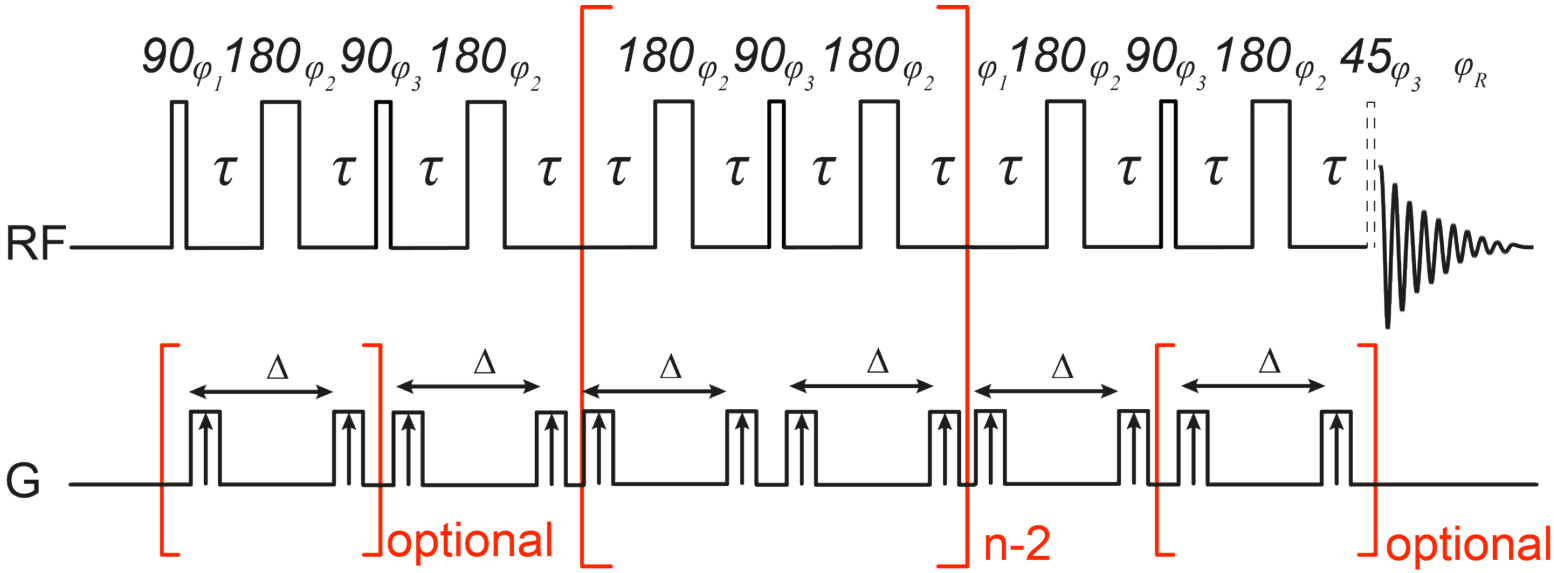



**Fig. 2.** Oneshot45 pulse sequence, which consists of Oneshot plus a 45° pulse orthogonal to the preceding 90° pulse.

# PROJECTED sequence




Adding gradient pulses to the PROJECT sequence to give diffusion weighting allows spin echo DOSY experiments free of J modulation. For small molecules this improves sensitivity, **but also, for $\Delta\nu\,\tau >> 1 >> k\tau$ it suppresses the averaging of diffusion coefficients for exchanging signals.**