Introducing 2D J Spectroscopy

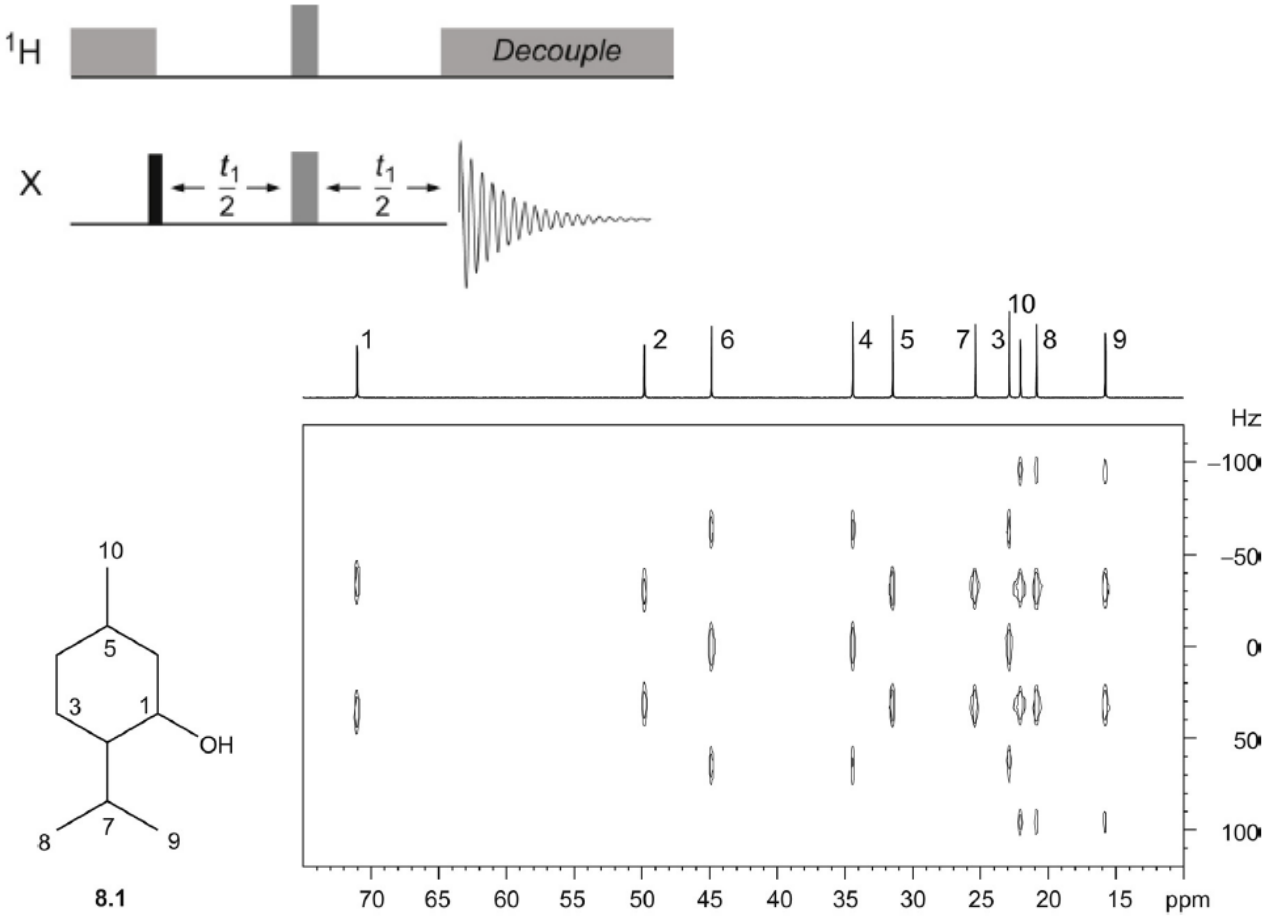
Rather than using scalar (J) couplings to correlate signals, here we aim to separate them from chemical shift.

Technique	Principal Applications
Heteronuclear J resolved	Separation of heteronuclear couplings (usually ^{1}H–X) from chemical shifts. Used to determine the multiplicity of the heteroatom or to provide direct measurement of heteronuclear coupling constants.
Homonuclear J resolved	Separation of homonuclear couplings (usually ^{1}H–^{1}H) from chemical shifts. Used to provide direct measurement of homonuclear coupling constants or to display resonance chemical shifts without homonuclear coupling fine structure ('proton-decoupled' proton spectra).
'Indirect' homonuclear J resolved	Separation of proton homonuclear couplings according to the chemical shift of an attached heteroatom centre. Used to provide direct measurement of homonuclear coupling constants.

Heteronuclear J-resolved Spectroscopy

In the heteronuclear version of the J-resolved experiment, the chemical shift of the X spin is presented in f_2 while couplings to a second nucleus, typically protons, are presented in f_1.

The f_1 dimension therefore enables an analysis of resonance multiplicity as well as measurement of heteronuclear-coupling constants (J_{XH}).



8.1

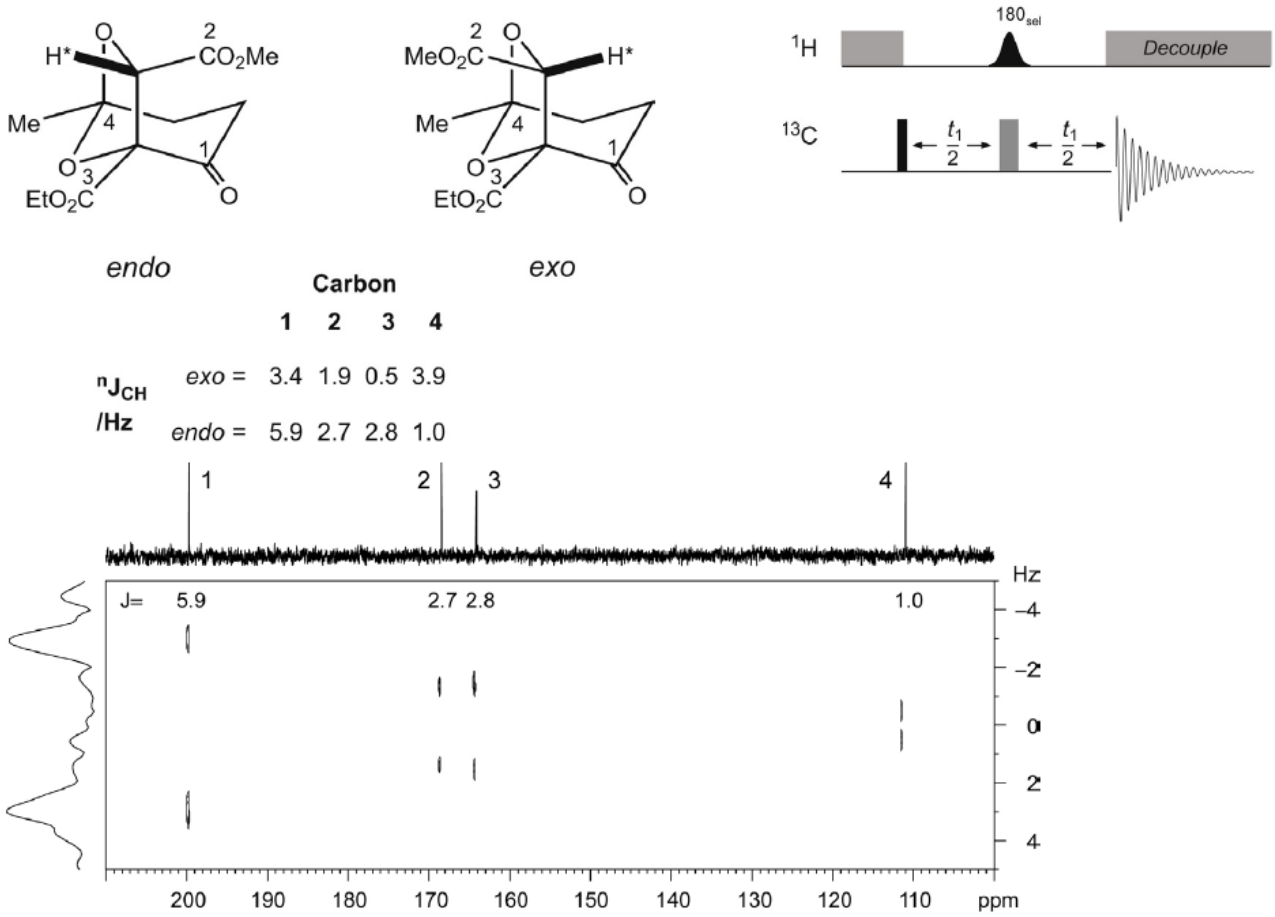
Measuring Long-Range Proton–Carbon Coupling Constants

Long-range $^{1}H–^{13}C$ coupling constants can be used to define molecular configuration or conformation

J-resolved methods are well suited to such measurements, but suffer from the presence of the far greater $^{1}J_{CH}$ couplings

If less informative one-bond $^{1}H–^{13}C$ couplings are suppressed from f_1 spectral width can be reduced and higher resolution can be achieved

$^{1}J_{CH}$ values are greater than c. 125 Hz, while long-range couplings are typically less than 10 Hz



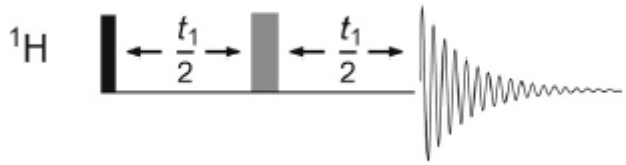
^{1}H detected - The H1' proton has been selectively inverted, and the doublet splittings in f_1 record its long-range couplings to C8 and C2'.

Homonuclear J-resolved Spectroscopy

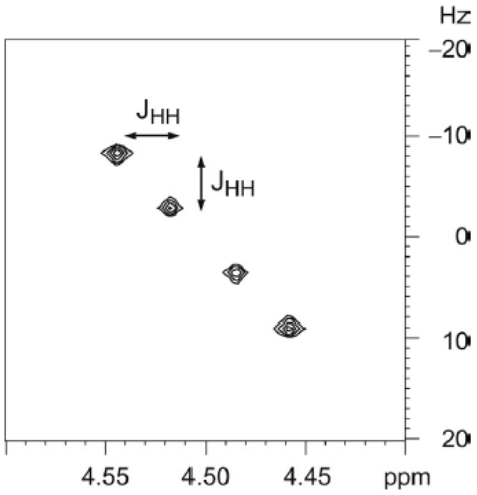
The homonuclear version of the J-resolved experiment is most frequently applied in proton spectroscopy, although it is suitable for any abundant nuclide.

The separation of δ and J should reveal proton multiplets in f1, and singlets in f2 at the corresponding chemical shifts, such that the f2 projection represents the 'broadband proton-decoupled proton spectrum'.

2D J-resolved spectroscopy generates a 'pure shift' spectrum and allows simpler measurement of homonuclear-coupling constants.



The homonuclear sequence closely resembles that of the heteronuclear methods.



The appearance of the homonuclear spectrum is different from its heteronuclear equivalent in that both *chemical shifts* and *couplings* appear in f_2. Multiplets appear at 45 degrees to either axis. Columns parallel to f_1 do not display the expected proton multiplets, and the f_2 projection displays both chemical shifts and scalar couplings.

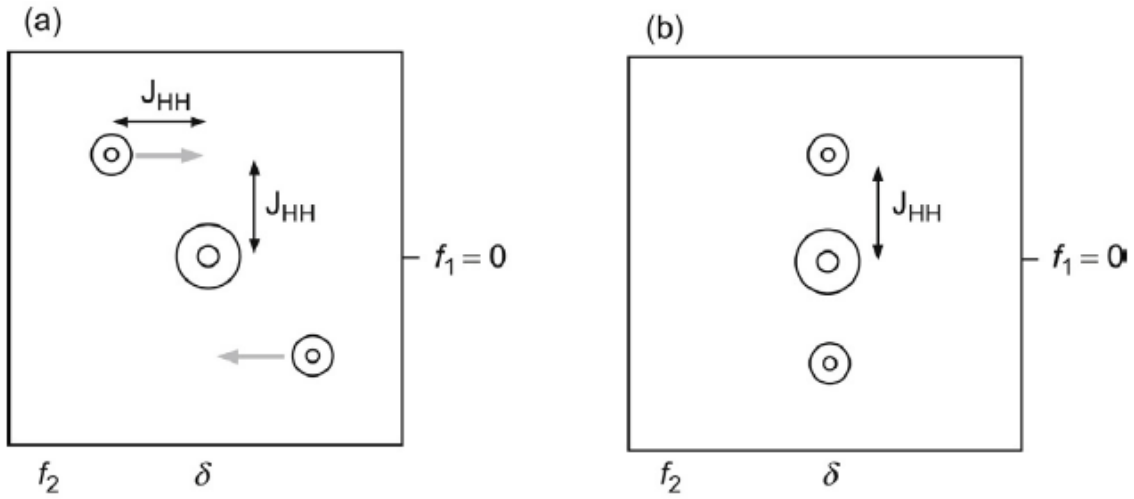
Processing

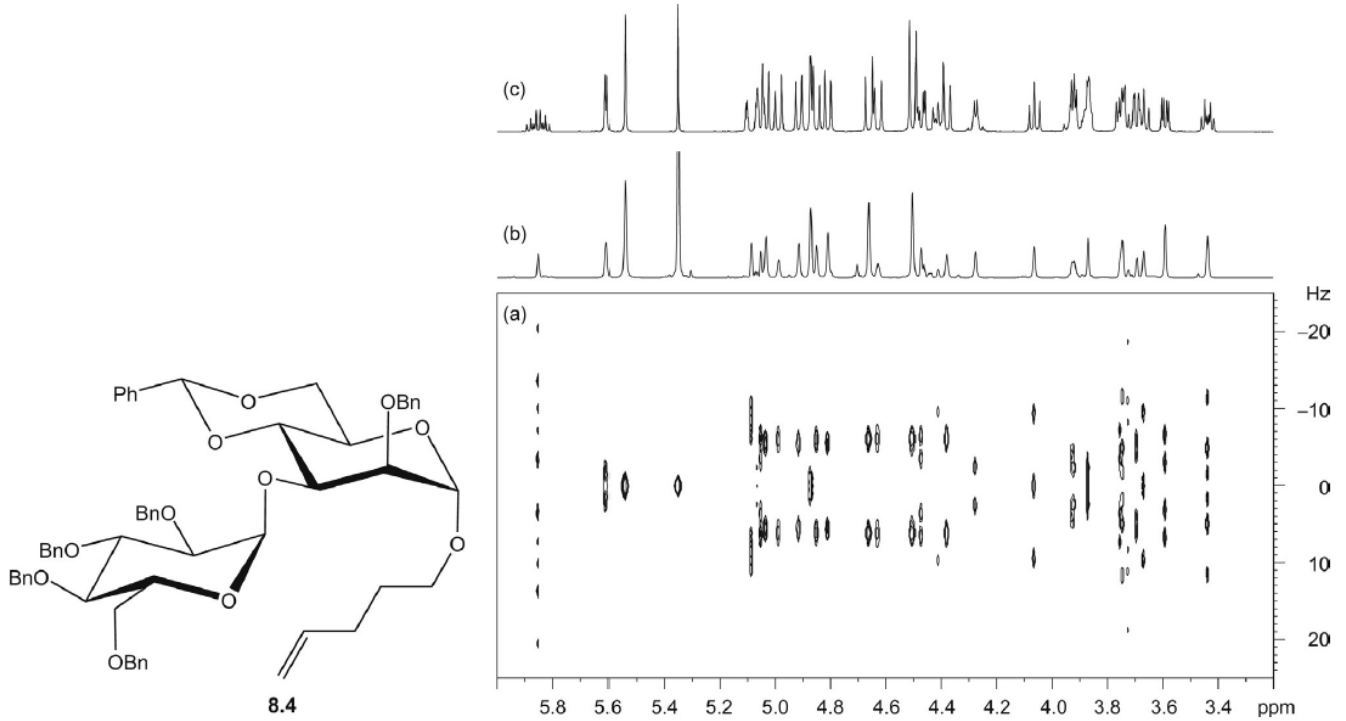
To reach the ultimate goal of retaining only chemical shifts in *f*2, it is possible to eliminate the couplings from by 'tilting' the multiplets through an angle of 45 degrees about their midpoints.

The resulting spectrum then has an appearance similar to the heteronuclear analogue, with columns parallel to *f*1 reproducing the multiplet structures.

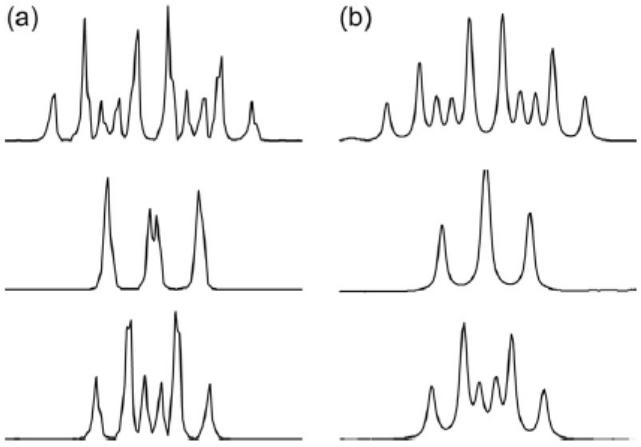
Magnitude calculation must be performed so signals will have their amplitudes and shapes distorted.

If the whole data set is symmetrised about $f_1 = 0$ the multiplets are retained whereas contributions from the sloping t_1 noise and other spectral impurities are diminished.





(a) A 2D J spectrum after tilting and symmetrisation. The *f*2 projection (b) approximates to the 'proton-decoupled proton spectrum' and is considerably less complex than the conventional 1D spectrum (c). 4K *t*2 data points were acquired for 64 *t*1 increments over spectral widths of 5 ppm and 60 Hz, respectively. The final *f*1 resolution after zero-filling was 0.5 Hz/pt. Data were processed with unshifted sine bell windows in both dimensions and are presented in magnitude mode

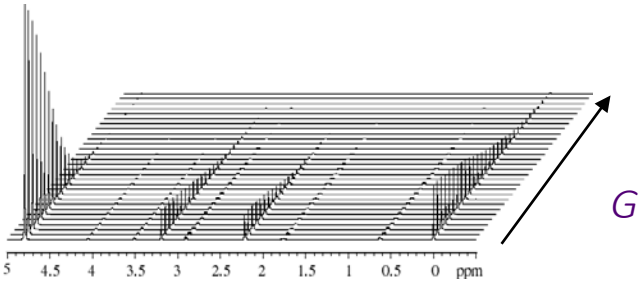


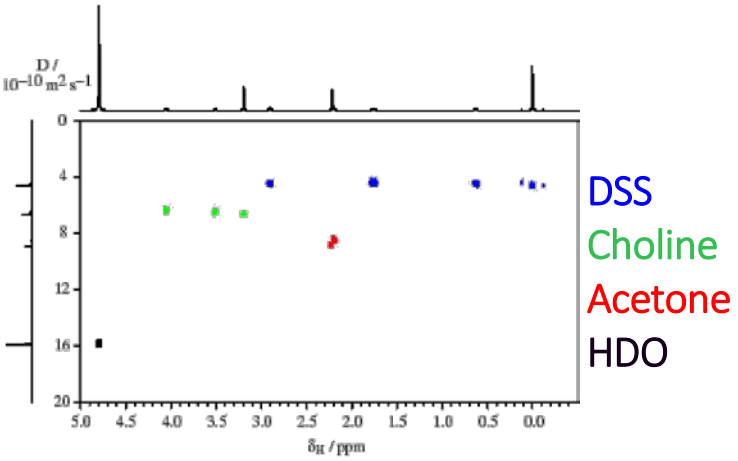
(a) Selected *f*1 traces taken from a J-resolved spectrum and (b) equivalent multiplets from the 1D proton spectrum.

DOSY

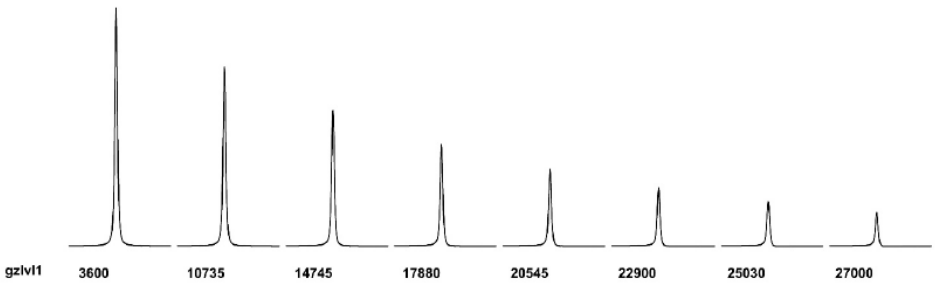
Rather than correlating spins with each other, we can measure their change in position over time to determine how the molecule to which each spin is attached diffuses in the solution. Diffusion coefficients can then be used to infer information about the molecules.

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 *σD*.





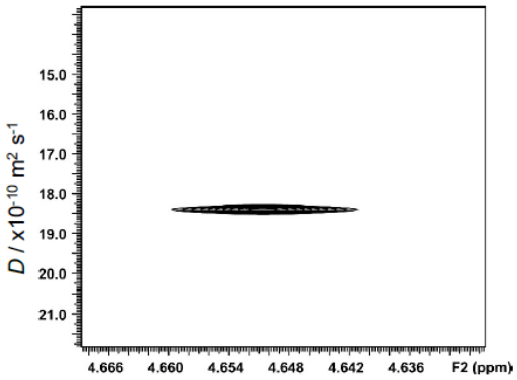
The Stejskal-Tanner Equation



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

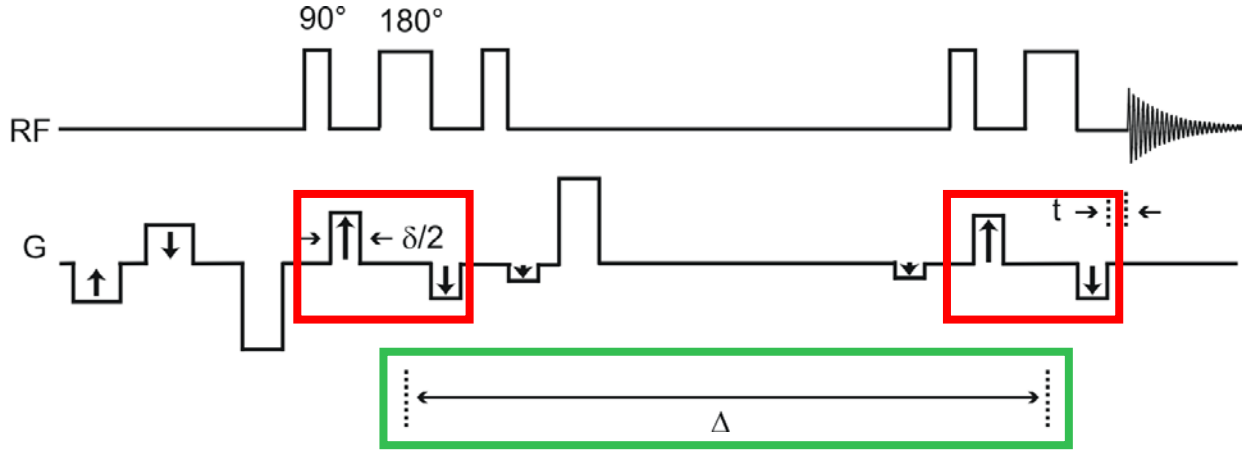
DOSY fitting

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.

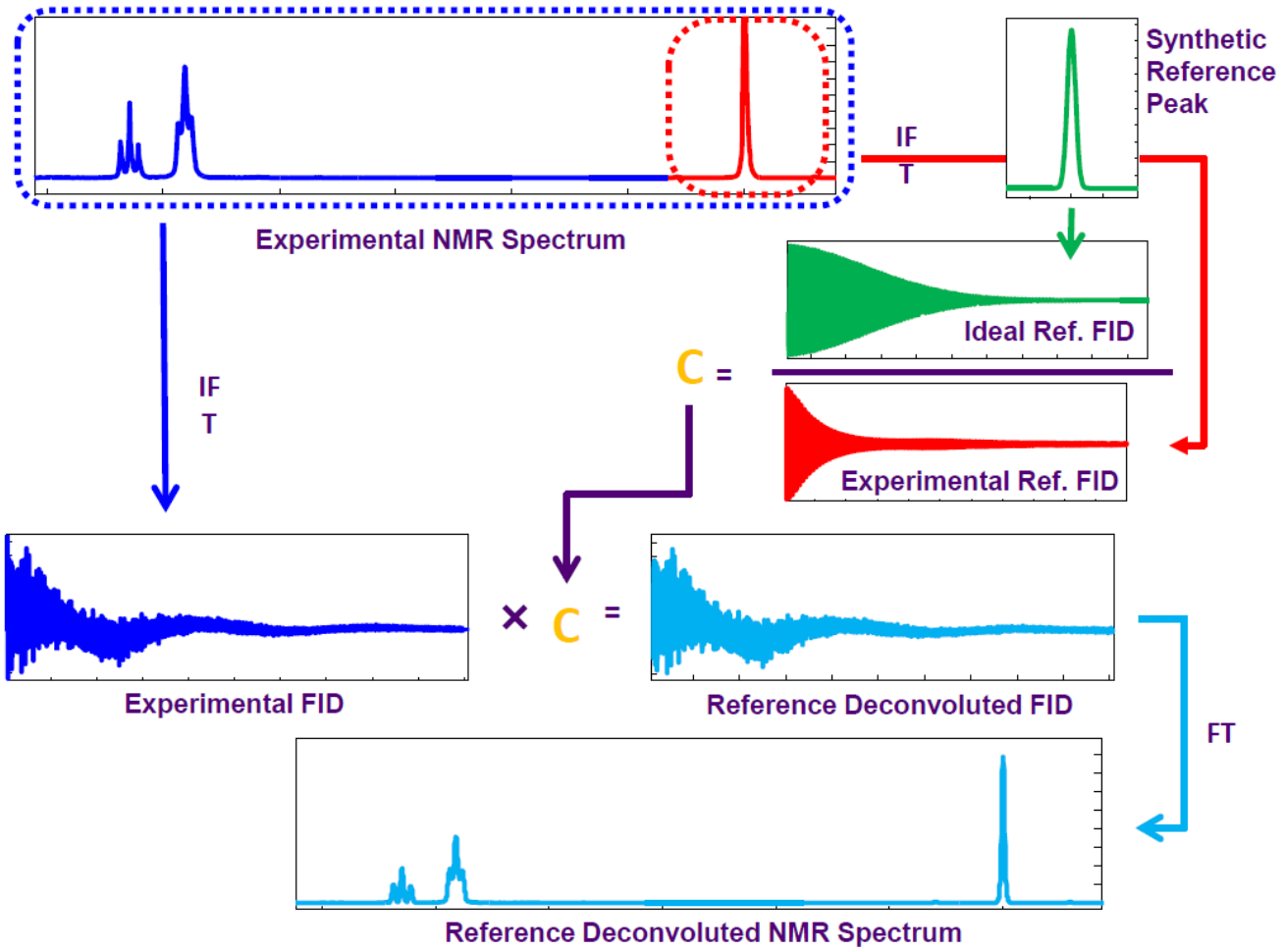
DOSY parameters

As with all NMR experiments, to run a DOSY experiment successfully you should consider the following: Pulse calibration, Recycle time, Acquisition time, *etc.* However, you should also consider: Strengths of greatest and smallest diffusion gradient levels, Diffusion gradient pulse width, Number of gradient levels, and Diffusion time



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.

Reference Deconvolution



Chemical Exchange in DOSY

Exchange during the diffusion delay of the most DOSY experiments 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.