DOSY (Diffusion Ordered SpectroscopY)

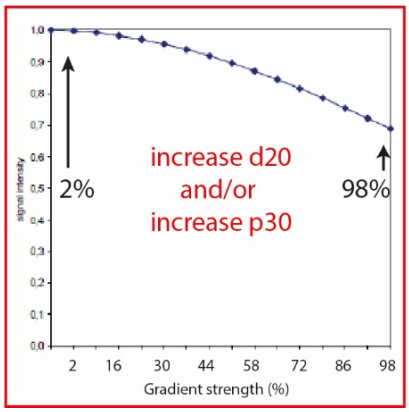
This protocol is a reminder of the commands. Contact us if it is the first time you run a DOSY

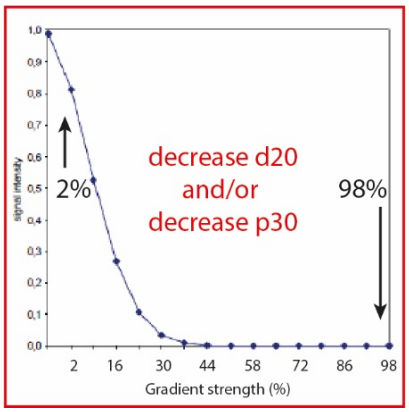
A. Acquire a 1d 1H spectrum

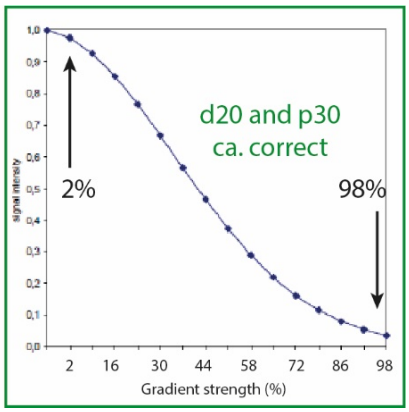
expno = 1
Cf. Procedure "Commands for routine experiment on TOPSPIN" (edc; rpar refe*; atma; topshim; rga)

B. Optimize DOSY parameters d20 (Δ) and p30 (δ)

The goal here is to optimize the diffusion delay d20 and the field gradient pulse length p30 in order to detect the whole DOSY decay function properly and to, in fine, have a good fit of the diffusion constant.

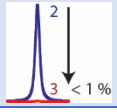
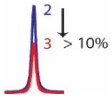






expno = 2	
edc	create a new experiment (increment expno)
rpar ref*	choose refe_DOSY-1d
RG	same as you optimise for the 1d 1H spectrum
gpz6	**2%**
Acquire and process the spectrum (zg, efp, apk	

expno = 3	
edc	create a new experiment, increment expno and choose "*Use current parameters*"
gpz6	**98%**
Acquire and process the spectrum (zg, efp, apk)	

compare expno 2 and 3	
.md or	compare the two spectra with 2% and 98% gradient strength (2 and 3) → the latter should be 10% lower in intensity than the former
	If the intensity of 3 is smaller than 1% of the intensity of 2 → decrease d20 in both expno 2 and 3 (limit: d20=0.01s) → decrease p30 in both expno 2 and 3 (limit: p30=500µs)
	If the intensity of 3 is higher than 10% of the intensity of 2 → increase d20 in both expno 2 and 3 (limit: d20=0.5s) → increase p30 in both expno 2 and 3 (limit: p30=3000µs; **DO NOT** go higher)
acquire again the spectra 2 and 3 (zg, efp, apk)	
optimise d20 and p30 until the peaks of interest in spectrum with 98% gradient (3) are about 10% of the ones in with 2% gradient intensity (2) **It is important to keep p30 lower than 3000 µs. A higher value can potentially damage the probe**	

C. Acquire the DOSY 2D

expno = 4	
edc	create a new experiment (increment expno)
rpar ref*	choose refe_DOSY-2d
set the same RG you optimise for the 1d 1H spectrum set the same p30 and d20 as the one optimise in dosy-1d spectra	
1 td, 1 si	set the number of gradient increment td1=si1=16 for a quick dosy, td1=si1=32 or 64 for more defined peaks in the diffusion dimension
dosy	setup the gradient array - acknowledge the first message (gradient shape) - lower gradient amplitude : 2% - higher gradient amplitude: 98% - increment of gradient amplitude : l (linear), **q (square rooted)**, e (exponential) - start the acquisition

D. DOSY processing in MestreNova

Import your Dosy 2D data in MestreNova (expno 4)		
Advanced DOSY transform	Method:	Peak Height Fit
	DOSY Spectrum :	Minimum 1e-8 (D min in cm^2/s; can be refined) Maximum 1e-3 (D max in cm^2/s; can be refined) Points in diffusion dimension 128
Ok	Fit DOSY spectrum	

D'. DOSY processing in Topspin

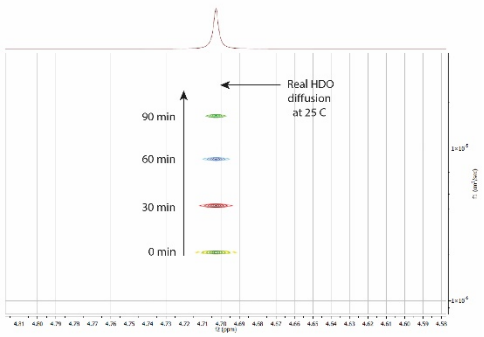
expno = 4	
efp	transform the first raw in proc 999
.ph	phase your spectrum
	save the phase parameter to the second dimension; go back to proc 1 (DOSY spectrum)
xf2, xf2p	transform and phase all fid of the DOSY (depending on Topspin version)
eddosy	open DOSY processing panel
	moves d20 and p30 into processing modules
	estimate diffusion coefficient D range
	perform DOSY transform
In the panel "spectrum" you should see the 2D DOSY spectrum with chemical shift along the F2 axis and diffusion coefficient along the F1 axis	

E. Remarks

Temperature stability

It is extremely important when running DOSY measurement to have a good **temperature stability**. As the gas flow of the temperature unit come from the bottom of the tube, a gradient of temperature can be created if the sample temperature is not sufficiently equilibrated, inducing **convection**, and thus a **misestimating of the diffusion constant**. Moreover, the temperature is measured outside the tube. Depending on the initial temperature of the solvent, of the tube thickness, etc. it can take time before the temperature measured is the same as the temperature in the tube.

A standard can be the residual peak of water in D_2O. The **self-diffusion constant of HDO at 25 °C is 1.9 10^{-5} cm^2/s** (1.9 10^{-9} m^2/s). In the example below, diffusion constant of HDO (initially stored in the fridge at 5°C) was measured at different time interval. Even if the measured temperature was stable, the temperature in the tube was still changing, and thus the diffusion constant.



DOSY resolution

Small molecules with a **difference in molecular weight (MW) smaller than 10 Da, will hardly be separated**. Moreover, there is no simple relationship between diffusion coefficient and molecular weight. Some methods have been developed to estimate the MW but still with a significant error of ca. 30%.
In the example below, one can easily separate citrate ion (191 g/mol) from acetate ion (59 g/mol) and ethanol (46g/mol) and from HDO (19 g/mol); but depending on the optimization, the gradient sampling, the temperature and the processing it can be difficult to separate the acetate ion peak from the ones of ethanol.