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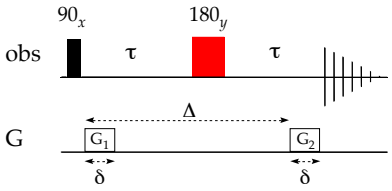
DOSY (Diffusion Ordered Spectroscopy)

Background

In DOSY, the spins are gradient-encoded by their z-position in the NMR tube. After a brief diffusion time (Δ) the spins are decoded. If a molecule remains within the same slice of the NMR tube, it will appear at full intensity, but if it diffuses to another slice, its signal will be attenuated as a function of the distance it has diffused. A simplified pulse sequence is shown below:



	typ value
Δ = diffusion delay	50-100 ms
δ = gradient length	2-4 ms
$G_1 = G_2$	

The peak attenuation is controlled by three variables. Typically, the gradient power (G) is varied over the course of the 2D experiment. The diffusion delay (Δ) and gradient length (δ), however, must be optimized.

Reducing the Spectral Window

1. Insert a sample, lock, shim, and record a normal ^{1}H spectrum.
2. Type IEXPNO to move to a new EXPNO. Expand the spectrum so that all the peaks are on the screen, and click the icon to adjust SW and O1. Reduce TD to 16K and SI to 8K.
3. Acquire another spectrum to check that the parameters are good.

Optimizing the DOSY Parameters:

4. In the ASED window, set PULPRG = StebPgp1s1D, GPz6 = 2 (the percentage of the full gradient to use for this spectrum), GPz7 = -17.13 (homospoil), D20 = 0.1 s (Δ-value), P30 = 1800 μs (δ-value). Turn the spinner off ("ro off").
5. Obtain a spectrum (rga / zg / efp / apk).
6. Move to a new expno (IEXPNO), change GPz6 to 95 percent of the full gradient, and acquire another spectrum.
7. Compare the two spectra: MDISP then RE *n*, where *n* is the expno from step 5 (eg, "re 3") If the intensity of the second spectrum is roughly 1/50 that of the first, all is good. If not, adjust D20 (Δ) or P30 (δ) until they are.

Running the DOSY

8. Change the pulse program to StebPgp1s. Click the 1 2 3 icon under the Pulse Sequence tab to change the data-set to 2D. Increase NS and DS (typically 32 + 4). Set TD(F2,F1) = (16k, *n*), where *n* is the number of F1 slices desired (ie, number of G values to use, typically 16). Set SI(F2,F1) to (8k, *n*), and FnMOD = QF .
9. Type DOSY, which will ask about additional parameters. Set the Gradient Ramp using the minimum and maximum values you used in steps 4 and 6 (2% and 95%). Set the number of points to the same *n* value used above (16). A linear ramp is most common. Press OK and step back.

Processing the DOSY Data

(see below if you want numerical diffusion coefficients, rather than a 2-D spectrum)

10. Set PhMod(F2,F1) to (pk, no). Type SetDiffParam to transfer key parameters, andXF2 to transform the data in F2.
11. If necessary, phase the data (check by viewing with the button).

13. Process the frequency *vs* time data using DOSY2D, to obtain a frequency *vs* diffusion-coefficient plot.
14. View slices across F1 through the "Slice" /"interactive" command: click the  icon. The slices may be saved to a procno by right-clicking and choosing "extract".

Obtaining Numerical Diffusion Coefficients

The data may be explicitly regressed and printed as a report, rather than graphically. This follows the same principle used for obtaining T1 and T2 values.

10′. Set PhMod(F2,F1) to (pk, no).

11′. XF2 to transform the data.

12′. From the menus, choose Analysis : T1/T2 Relaxation. Follow the flowchart down the left-column of buttons:

a. Extract a slice from your data to phase. Choose "spectrum" and use the first slice (default). Phase the data ( -button on top row), then save-and-return ( button within phase window).

b. Use the flow-chart's Peaks/Ranges button to define the ranges of the spectrum to regress. Integrate the spectrum (∫-button) by dragging across the peaks, then export the data back to the T1/T2 routine ( -button; export to Relaxation Module).

c. Use the flow-chart's Relaxation Window button to regress the peak intensity *vs* τ data. On Fitting Functions step, ( icon), set the Fitting Function to VARGRAD and the List File name to DIFFLIST. The + and – buttons will allow you to change which peak the plot displays data for. The -button will allow you to delete any bad data-points; if you do, use the and buttons (left edge of bar) to recalculate the fit.