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:

obs 90_x τ 180_y τ

Δ

G G_1 δ G_2 δ

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 GPNAM6 and GPNAM7 both to "SMSQ10.100". 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 $_1{}^{\downarrow}_{2}{}_3$ icon under the AcquPars 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)

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* On the new TOPSPIN, this generates a "GPNAM6= Gradient funtion file name is empty" error. Please let me know if the things work with the "SMSQ10.100" change.

10. Set PhMod(F2,F1) to (pk, no). Type *setdiffparm* to transfer key parameters, andXF2 to transform the data in F2.

11. If necessary, phase the data:
    a. Enter phase mode by typing *.ph* .
    b. Right-click on peaks of interest and "add" them.
    c. Click the "Phase Rows button ($\xleftarrow{R}$)
    d. Phase, then save-and-return (⌨↵ button) as usual.
    e. check by viewing with the $\wedge^\wedge$ 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 top buttons, choose Analyze : Dynamics : T1/T2. Follow the top row of buttons:
    a. Use the FID button to extract a slice from your data to phase. Either the FID or the spectrum is fine. The first slice (default) is fine. Phase the data ("apk").
    b. Use the Peaks/Ranges button to define the ranges of the spectrum to regress. Integrate the spectrum by dragging across the peaks, then export the data back to the T1/T2 routine (⌨ₐ-button; export to Relaxation Module).
    c. Use the Relaxation button to regress the peak intensity *vs* τ data. Click the check-box icon, and verify that the Function Type is "VARGRAD". Press ⊘ to calculate T1 for all peaks. The + and – buttons will allow you to change which peak the plot displays data for.

       You may need to delete the last data point or two (↓-button, then right-click on the peak). In this case, use the ⊘ or ⊙ button to recalculate the fit.

       If only a single point shows up for a spectrum, try switching between "FID" and "Area"