

DIFFUSION ORDERED SPECTROSCOPY (DOSY) OVERVIEW

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DOSY – Diffusion Ordered SpectroscopY. NMR diffusion experiments, like DOSY, are used to determine the diffusion coefficients of solute molecules of different sizes.

1D DOSY plots the signal intensity as a function of the delay. You can either vary the gradient and hold the delay (common) OR vary the delay and hold the gradient constant (rare).

2D DOSY plots the chemical shift on one axis and the diffusion coefficient on another. This allows you to separate multiple solute species.

Table 1:

Experiment	Parameter Set	Pulse Program	Minimum NS
1D DOSY	1D-DOSY-dcif	stepg1s1d	4
2D DOSY	2D-DOSY-dcif	stepg1s	4

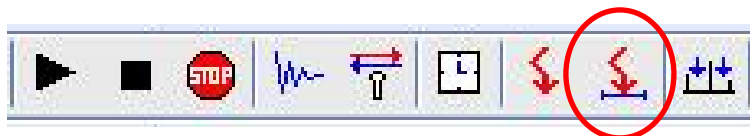
1. Lock, tune and shim.

a. Remember to tune the ^1H channel (**wobb**)

2. Collect a good 1D ^1H spectra. DO NOT use **xaua** to start your acquisition. Type **rga**, let it finish, then **zg**.

Experiment 1 (EXPNO)

a. ^1H : Acquire a 1D and reference the solvent peak. Zoom in and display all proton signals leaving 0.5 ppm of baseline on each side. Type **.setsw** (or click on the icon) to set the transmitter offset (o1p) and sweep width (sw).



b. Reacquire “reduced-sweep width spectra” with the number of scans (ns) needed to get good signal to noise and phase.

Write down the following values:

o1p: _____

sw:_____

sr:_____

ns:_____

rg:_____

*Type any parameter in the command line, hit **enter**, and TopSpin will display the value for you. You can change values here by highlighting the current value, typing in the value you want, and then hitting **enter**.*

3. Load the 1D or 2D DOSY parameter set.

Experiment 2 (or any other new experiment)

rpar 1D-DOSY-dcif OR rpar 2D-DOSY-dcif

4. Check pulse program (AcquPars tab) and make sure the correct one has been loaded (see **Table 1**).
5. Load the prosol parameters by typing **getprosol**.
6. Edit the basic parameters based on the information from the 1D ¹H experiment (the values you recorded in **step 2**).

- **ns** Number of Scans
- **o1p** Transmitter frequency (ppm)
- **sw** Sweepwidth (ppm).
- **sr** Reference (Hz).

Note: For **1D-DOSY-dcif**, ns=4 will take approximately 35 seconds
For **2D-DOSY-dcif**, ns=4 will take approximately 11 minutes

Type **expt** and TopSpin will calculate the experiment time.

IF YOU ARE RUNNING 1D-DOSY-DCIF

1. You will need to set the following parameters:

a. Gradient ratios:

gpz6 set this for a value 1 to 100. This variable depends on your diffusion coefficient and you will have to adjust this based upon the mobility of your molecule. You can start with a value of **2**. Increase the gradient strength if you have a slower moving (low diffusion coefficient) molecule.

gpz7 set this for **-17.13**

Gradient Lengths:

p30 set this for **1000 μ s**

p19 set this for **1100 μ s**

Recovery Delay:

d16 set this for **0.0001s**

Diffusion Delay:

d20 can be set for **0.150 to 0.200s**

2. Set your **rg** value (determined in **step 2** and recorded on page 2). Start the acquisition with **zg**.

3. Process your first spectra with **ft** and **apk**. It should look much like a regular 1D ^1H spectrum, but the goal here is to reduce the signal by 95%.

If you don't see any signal (the signal is totally lost), you may need to repeat the experiment, increasing the gradient strength (**gpz6**). If your signal is too large (not enough lost), increase the gradient length (**p30**). You can also change the diffusion time value (**d20**) if needed. Rerun the 1D DOSY, changing parameters as needed.

You can overlay multiple spectra using the  button.

IF YOU ARE RUNNING 2D-DOSY-DCIF

***It is recommended that you run a 1D DOSY before a 2D DOSY.
This will help you optimize parameters***

1. You will need to set the following parameters:

a. Gradient ratios:

gpz6 set this for a value 1 to 100. This variable depends on your diffusion coefficient and you will have to adjust this based upon the mobility of your molecule. Try a value in the middle and figure it out from there.

gpz7 set this for **-17.13**

Gradient Lengths:

p30 set this for **1000 μ s**

p19 set this for **1100 μ s**

Recovery Delay:

d16 set this for **0.0001s**

Diffusion Delay:

d20 can be set for **0.150 to 0.200s**

2. Set the desired number of ^1H spectra to be collected.

Type **td** and set the number of experiments to **4** or **16**, for example.

3. Type **dosy** to create the gradient ramp function:

Enter first gradient amplitude: **2**

Enter final gradient amplitude: **95**

Enter number of points: **4** or **16**

ramp type (l/q): **l** (linear)

Click OK to start the acquisition.

4. Processing:

You can move the raw data into the next PROCNO by typing **wrp** and entering a number. DO NOT do this on the spectrometer computers - you will fill up the hard drive. This way, if you make a mistake, you can easily go back and reload the raw data.

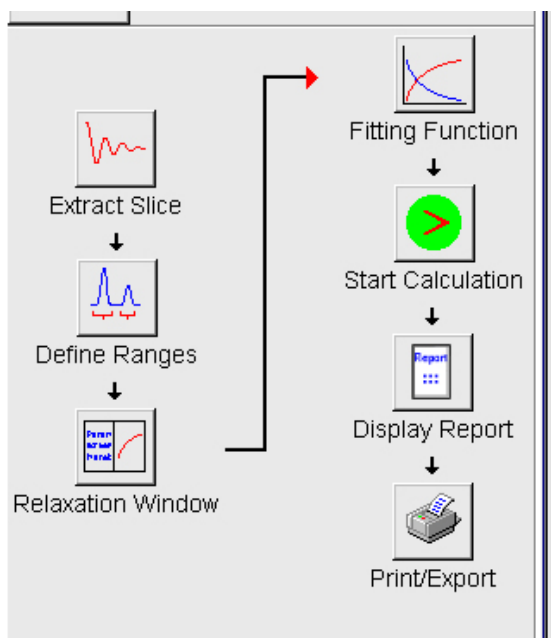
xf2 (be patient, this can take some time).
abs2
setdiffparm

Please wait for each process to finish before you begin the next one.

You can go two ways from here: The calculation way or the graphical way.

Calculation Way

- Under the *Analysis* drop down menu, select *T1/T2 Relaxation*. This will open a flowchart on the right side of the screen.



- Follow the flowchart, starting with *Extract Slice*.
- *Extract Slice*: Select *Spectrum*. You can take any slice you want, but the first one is usually fine.
- **apk** and correct phasing as needed.
- *Define Ranges*: Follow the instructions on the screen. Only integrate peaks that belong to your molecule and make sure you take enough. For example, if there are 4 pertinent peaks, integrate at least 3.

- Click the diskette button (Save region) and select *Export Regions to Relaxation Module*.



- In the *Relaxation Window*, you will see a curve. You can change the settings used to fit this curve by clicking the *Settings* button.



- It is recommended that you use the *vargrad* fitting function. Change other parameters as needed, click *Apply*, then *OK*.
- Click the *Start Calculation* icon. This should fit the function and provide you with your diffusion constant.

Graphical Way

- **eddosy**. Change parameters as needed.
- **dosy2d setup**
- **dosy2d** (what starts the actual processing. Be patient, this can take some time)



- Use the button and the crosshairs to figure out your diffusion constant.

