
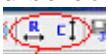
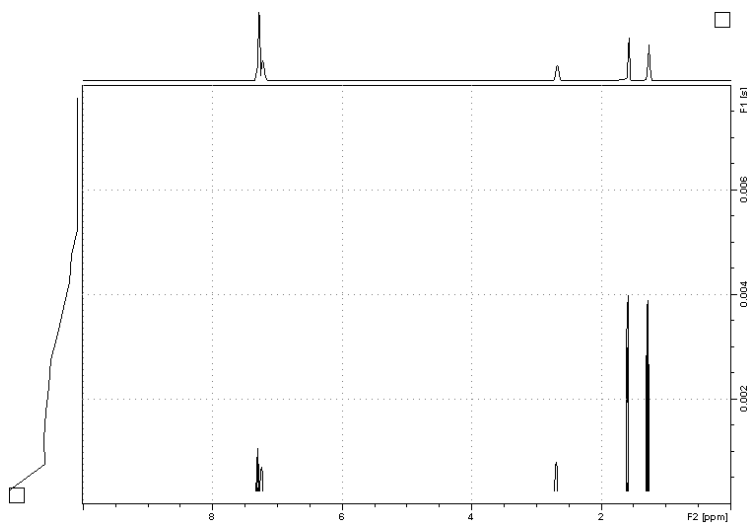



Diffusion (DOSY) Guide

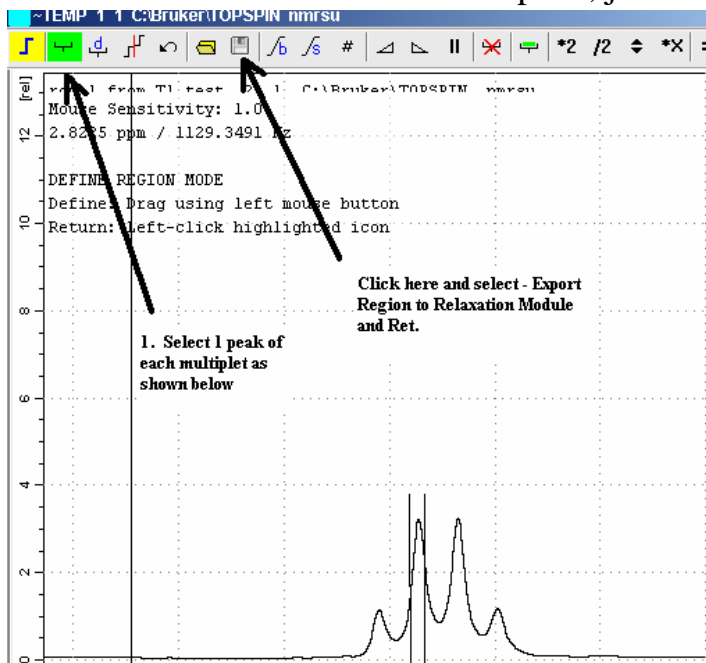
1. Setup and obtain a 1-D proton spectrum. Optimize your SW and o1p according to the directions on the COSY Guide. Rerun your experiment with the new values. Record the values.
2. To obtain a quick T1 estimate, type '**iexpno**' [enter]. Type '**eda**' [enter] and change the pulse program to **t1ir1d**. Type '**d7**' [enter] and type **15ms** [enter]. Type '**ns**' [enter] and type '**1**' [enter]. Type '**phmod**' [enter] and set it to **pk**. Type '**zz**' [enter]. Phase your spectrum so all the peaks are negative. Now, type '**iexpno**' [enter]. Type '**d7**' [enter] and type '**1s**' [enter]. Type '**zz**' [enter]. Some of your peaks may still be negative, some may be positive, some may be null. Your goal is find the resonance with the shortest T1 by adjusting d7 and rerunning the experiment (which peak reaches a null point the fastest?). Record the value of d7 that provides the first null point for a resonance in your molecule and multiply it by 1.44 (this is the T1 estimate of the fastest relaxing nucleus in your molecule).
3. Type '**iexpno**' [enter]. Type '**rpar**' [enter] and select '**DOSY_2D**' and '**copy all**'. Change **SW** and **o1p** to the values determined in step 1. Make sure the solvent is correct in the acquisition parameters. It is best to run a fast DOSY experiment to optimize the parameters to get the best separation prior to running your actual DOSY experiment. The default parameters are set to ns = 1, aq = 1.5s, d1 = 4, d20 = diffusion time = 100ms, p30 = gradient length = 1ms, td2 = 8. NOTE: d20 should be no longer than the T1 estimate obtained in step 2. Type '**rga**' [enter] and '**dosy**' [enter] when rga is finished. You will be prompted to accept the ramp from 2 to 95%, click 'ok' for each, accept the # of points, and the ramp mode as 1 = exponential. Finally, select yes to start the acquisition.
4. When the acquisition is finished, type '**xf2**' [enter]. Increase the intensity until you see intensity form at the bottom of the screen at the chemical shifts of your spectrum (see figure). Type **ph_mod** [enter] and select '**pk**' in the first box and then '**ok**'. Click the phase button . Right mouse click on one of the signals in your spectrum and select '**add**'. Next click on the  horizontal arrow with the R over it. Your spectrum should appear. Phase it and save it. Type '**abs2**' [enter]. Type '**setdiffparm**' [enter]. Select '**T1/T2 Relaxation**' from the '**Analysis**' pull down menu at the top of the screen.



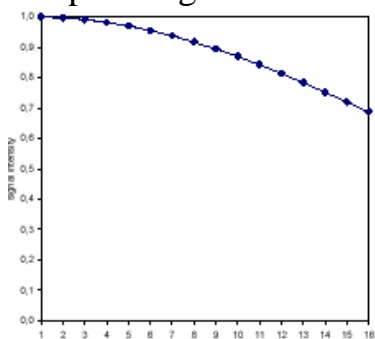
Select **'Extract Slice'** [enter], **'Spectrum'** [enter], **'Slice #1'** [OK]. Your spectrum should appear and it should be phased. Select **'Define Ranges'** in the relaxation module and click [OK] to the pop-up box. Use the normal expansion tool to expand around each of the peaks you wish to analyze. Next, click the little bracket tool to select a region in each of your resonances of interest. NOTE: For multiplets, just select one peak as shown in the figure. This process avoids baseline artifacts. When all of your resonances are selected, click the disc button and choose **'Export ...'** (See Figure).

5. Click the Relaxation window icon.

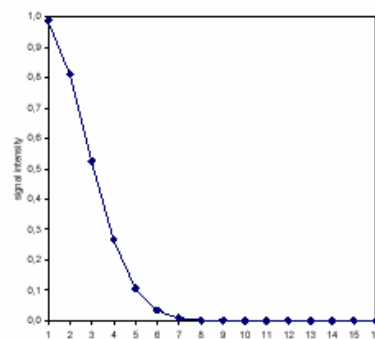
Click **'OK'**. Click the  icon to calculate the T1 of all the resonances you selected. The default mode is **'area'**. You can try **'intensity'** as well. The data should be close either way. Click the + or - to scroll through the different curves. You want the curves to look like a smooth decay. Click the **'Display Report'** icon and you will see a tabulated data table for each of your resonances that you can print if you wish. You may not be able to achieve a perfect curve for all the peaks in your spectrum (optimize D20/P30 to achieve the best curves for the most resonances). Repeat the experiment with your new values until you obtain the best curve.



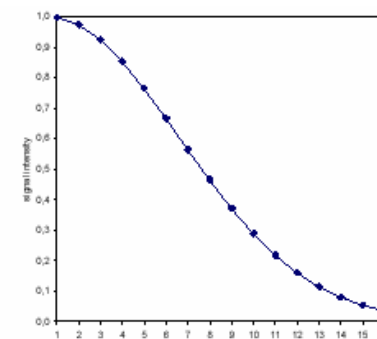
Examples of good curves and bad curves.



A






B

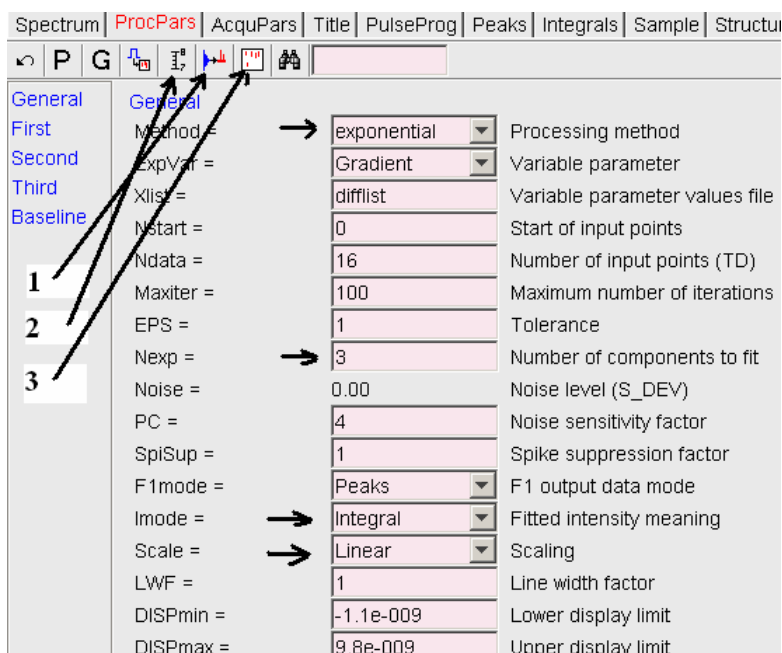



C

- A – D20 and/or P30 are not long enough.
- B – D20 and/or P30 are too long.
- C – D20 and/or P30 are ideal.

6. Rerun your experiment with the optimized D20 and P30 with an ‘ns’ of 16 (depending on your concentration), and a ‘ds’ of 8 and a td in F1 of 12 or 16.

7. When the experiment is finished, type **xf2** and phase it as you did during the optimization process (don’t forget the **phmod** setting) and type **‘setdiffparm’**, and **‘abs2’** [enter]. Type **‘eddosy’** and a new window will appear (see figure). I have obtained the best results with the values shown in the boxes. Also, please change **I2vary**, **I3vary**, **D2vary**, and **D3vary** to **yes**. When you are finished checking all the boxes, click the  button, type **‘abs2’** [enter], then click , and finally  as shown in the figure. Feel free to change the Imode and Scale but each time you make a change you need to click the buttons and perform ‘abs2’ in the aforementioned manner. Once you click #3, click the spectrum button and when it is finished processing, you should see a diffusion spectrum as shown below.



8. Type **‘slice’** [enter] and select **‘interactive row/column display’**. Next, click the little vertical arrow  and a horizontal line will appear in your diffusion spectrum. As you scroll over the vertical lines of your spectrum you will see the individual traces for each different diffusion coefficient. Right mouse click and select **‘extract row/column’**. A row number and process number box will appear. The easiest thing to do is to change the process # to the row # (do not change the row number). You just saved that trace and it will automatically launch in a new window. Just close it or minimize it to return to the slice selection interactive window.

