DOSY

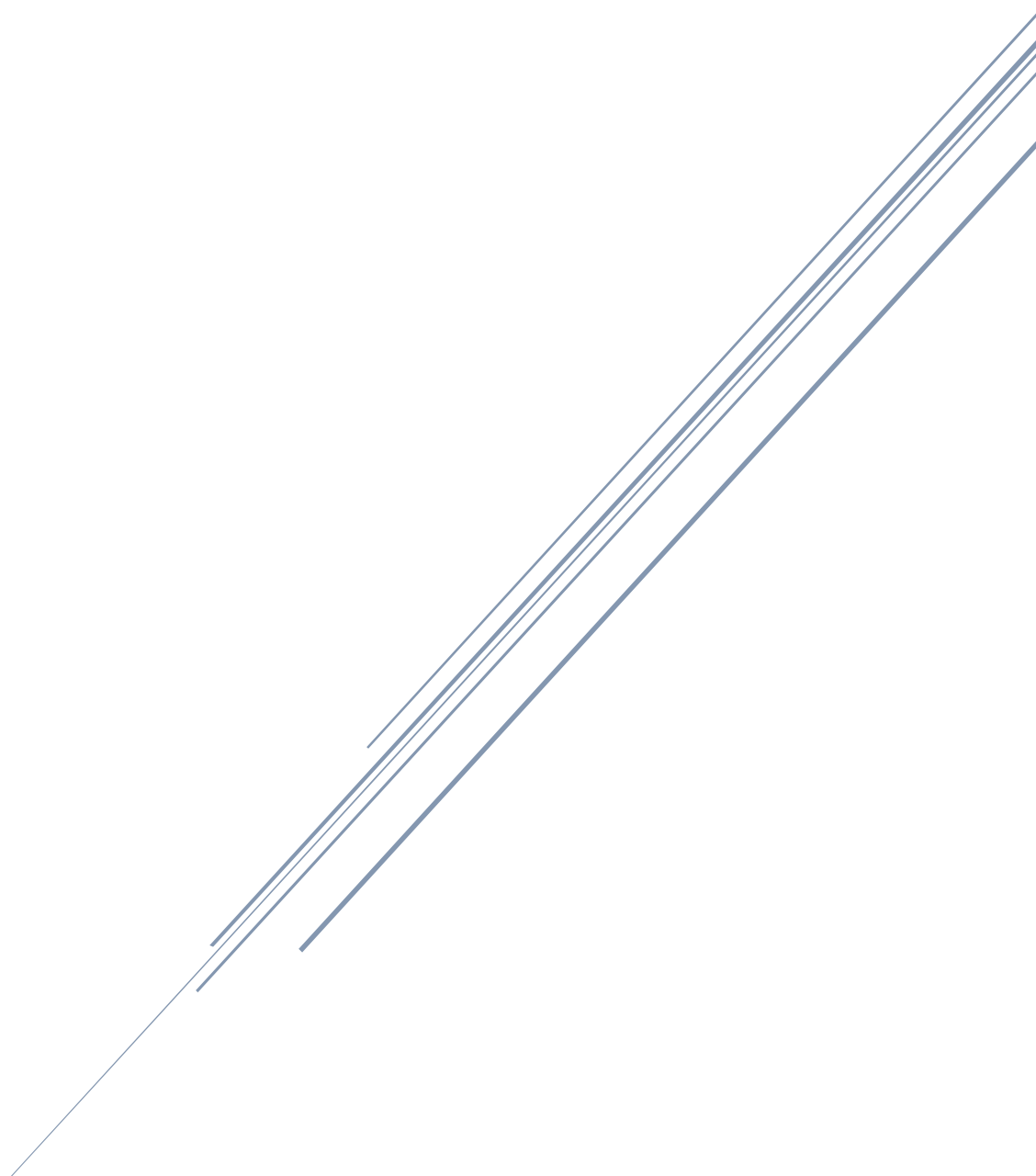## Diffusion Ordered Spectroscopy
- a manual for AVII600 and AVIIIHD800

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Theory

The DOSY experiment allows to see how fast molecules diffuse in your NMR sample. Diffusion only creates a net change if there is a concentration difference. Therefore, you would expect a diffusion measurement to require a concentration gradient. As you have learned, NMR samples must be homogeneous, and we cannot employ a real concentration gradient. Instead, NMR uses a trick, by having a gradient pulse (for which a special gradient coil is needed, not all instruments have one). A gradient pulse allows to give the magnetisation vector a spatial 'label'. Two such pulses are employed, and the time span in between the two gradient pulses is called Δ (not to be mistaken for δ, the duration of the gradient pulse). The farther the molecule has travelled during Δ and the stronger the gradient (δ), the bigger the mismatch between the defocussing and refocussing pulse. This mismatch causes an attenuation (becoming less) of the peaks. For the DOSY spectrum, we need to collect a series of 1D proton spectra with increasing gradient strength (so-called transients). The DOSY spectrum itself is created from these spectra by fitting either the intensities or the integrals of the peaks. Thereby, the diffusion constant can be determined and you obtain a pseudo 2D spectrum which, instead of cross peaks like you have encountered them in 2D NMR, shows the measured diffusion coefficient of each peak. If you have a mixture of molecules, they are separated by diffusion coefficient (all peaks of one molecule should have the same), earning DOSY the nickname 'NMR chromatography'. A word of caution in the end: This technique, while powerful, is easily disturbed by other phenomena that make the observed proton travel: chemical exchange and convection, for example due to a temperature gradient in your solvent, create false results. The more viscous your solvent is, the lesser the convection issue. Therefore, DMSO and water are the most popular DOSY solvent.

The DOSY experiment is used in all kind of subfields of chemistry:

- Aggregation
- Hydrogen bonding, Ion Pairing, etc.
- Host-guest complexes and supramolecular assemblies
- Identification of mixtures, especially in food chemistry
- Estimate the molecular weight of polymers

For the theory behind it, consult the respective chapter in 'High Resolution NMR Techniques in Organic Chemistry' by Claridge.

A shorter summary can be found on

http://chem.ch.huji.ac.il/nmr/techniques/other/diff/diff.html

A more pharmaceutical perspective on

https://www.sciencedirect.com/science/article/pii/S0079656599000035

Experiment

A. Acquisition

Start by running a normal ¹H experiment (you need to edc, rpar, getprosol, lock the solvent, wobb, topshim gui, pulsecal, rga & zg). Make sure the spin is off. Phase and calibrate the spectrum. Use wrpa to make a second experiment in the same folder.

- Change the pulse program in the acquisition parameters to **stebpgp1s1d**
- Type **d20** (this is Δ) and set it to a value between 50 and 100 ms (beware, the entry is in seconds, so you should write something between 0.05 and 0.1)
- Type **p30** (this is ½ δ) and set it to a value between 1 and 3 ms (beware, it is given in μs, so you should write something between 1000 and 3000)
- Set **gpz6** (this is the gradient strength) to 2%
- Type **gpnam**. Set **GPNAM** for **6,7**, and **8** to **SINE.32**.
- **getprosol**
- set **ds** to 8
- **zg**
- do **NOT** rga or rpar

Look at your spectrum. Ideally, it looks similar to your previously collected 'normal' proton.

Use **wrp 2** to copy everything to procnum 2.

Go back to procnum 1 and change **gpz6** to 95%. Use getprosol and zg to obtain a second spectrum that you **wrp 3** to have a copy in procnum 3.

Use the overlay function to compare the spectra with 2 and 95% gradient strength. No signals should have disappeared completely, but the ones that are most affected should only have 5% of their initial intensity in the 95% spectrum.

If the spectra do not fulfil the requirements, you need to change **p30** and **d20** and rerun both spectra (one with 2 % and one with 95 % gradient strength). Increasing either will increase the attenuation, while decreasing them will reduce the attenuation (If any signals were gone, you need to decrease them, while if you want a stronger effect, you need to increase them). Changing p30 has a more pronounced effect than changing d20. **NEVER** set them outside of the range given above, you can irreversibly damage the instrument.

When you have found a satisfactory setting, **wrpa 3** one of the experiments (and note down your settings, you will need them for your report). This creates a new experiment in the same folder. This experiment will be the one running the series of 1D experiments. Open the Acquisition Parameters tab.

- Change the pulse program to **stebpgp1s**
- Change the acquisition dimension to 2D:

- Set **FnMODE** to **QF**
- Set the **TD** in **F1** to 16

- Check if d20 and p30 are set correctly (the values you used before on the 1D)
- **getprosol**, (Pulsecal??),**rga**,
- **dosy** (this opens a command window. The red cross in it is decorative and doesn't mean you created an error. Just press close and the settings window will open.). Set the ramp from 2-95 % and choose a linear ramp (l). Agree to start the experiment.

B. Data Treatment

Once the experiment is completed, use **wra** to copy the data into a new proc number. Do all the processing on the copied version.

Go into the processing parameters. In **Window Function**, Set LB of F2 to 2. In **Baseline Correction**, set ABSG for F2 to 0. Set ABSF1 for both dimensions to 1000 and ABSF2 for both dimensions to -1000.

Execute **xf2** (the peaks are only in the bottom of the 2D at this point, see below). Phase the spectrum manually, but only the rows. Execute **abs2**. Execute **setdiffparm**.
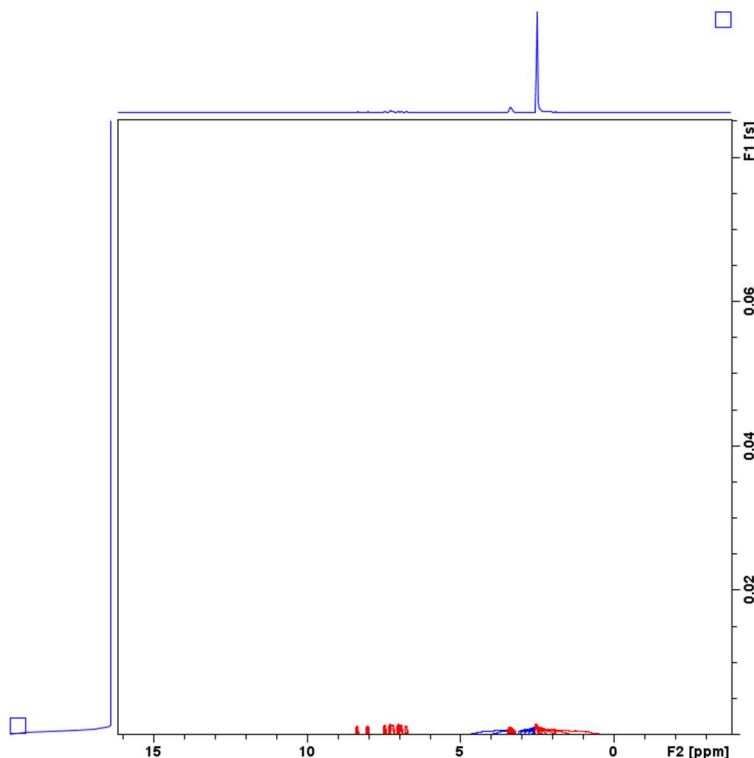
Open the 'Dynamics Centre' (can be found on some NMR machines, in the remote desktop, but not through VM Horizon). Either you write **dync** into the command line in topspin, or you find it on your desktop. A general word of advice: The Dynamics Centre is prone to crashing, especially if you are impatiently pressing buttons (we did that a lot, you can trust us :D).
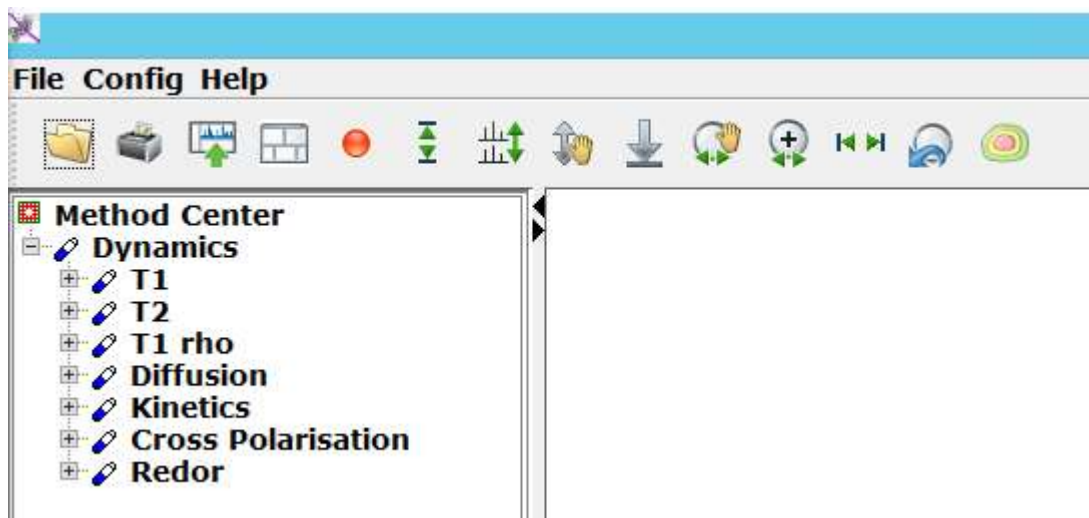
Double click the diffusion option. This opens six subchapters. You will go through them in the order they are displayed.

Sample

Double click 'sample'. Give it an appropriate name. Also set the date to todays date.

Data

A window pops up with multiple tabs. In the 'spectra' tab, browse for your experiment in the **pseudo spectrum** line. >This PC >NMR (D:) > uio > the instrument you took the experiment on > data > your user > nmr > experiment name > experiment number > pdata > proc number > 2rr

The path can differ between computers, essentially you need to find your spectrum.

Then go to the **integral** tab. Choose 'use peak area (user defined) integrals)'.

Press ok. Wait patiently, until the red square before data becomes green. The screen now shows something like below.
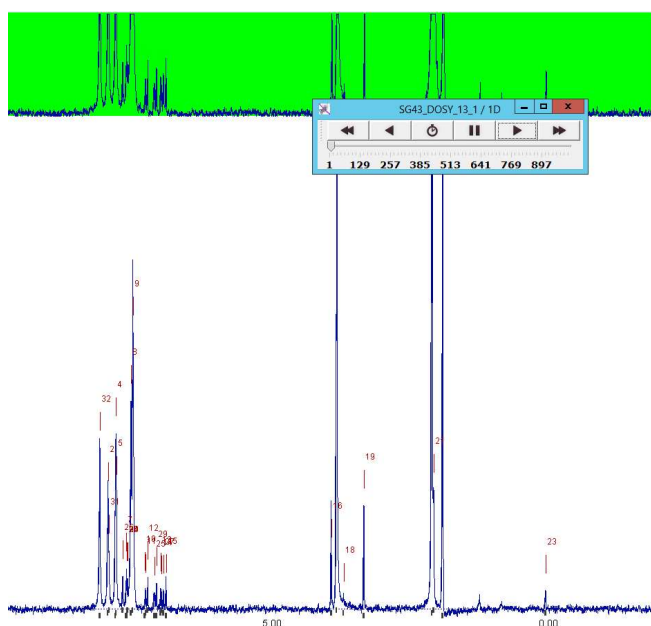
You can press the play button in the pop-up window to see the signal attenuation progress. It's prone to cause freezing also, so we leave it up to you, if you are willing to take that risk. When you're done, we suggest you close the pop-up window for the next steps. Zoom in on your peaks. With right click, you can choose to delete peaks or pick new ones. Remove any overlapping peaks, their analysis is misleading. For multiplets, we advise to only keep one peak.

Set the integrals the way you would normally integrate. By dragging the black part, you affect the placement and you can stretch them by dragging the white borders. The integral adjustment can be a bit slow… just give it time.

Analysis

Double click the 'analysis' button. Again, a pop-up window is opened. From here on, your data treatment skills are important. The results you obtain from a DOSY are dependent on the inputs you choose in this section. This demands a bit of chemical finger tip feeling, trial and error and patience. Therefore, you should try out to do the analysis step a couple of times, changing

- **number of function components** (remember Occam's razor: if you have a good fit with 1 component, only use one).
- **Error estimation method** (we found both 'error estimation by fit' and 'error estimation by weighted fit' reasonable choices. It really depends on the measurement.)
- **Integral areas** (this is not in the pop-up, but done as in **data** you will have to re-click **analysis** after adjustment)

Press ok. A window stating that the fit curves have been calculated. If there are failed fits, you should change parameters mentioned above.

View

Double click **view** and press ok. (If issues are encountered, you can try to change the **display x variable** to the second option. Furthermore you can adjust the DOSY plot range in the DOSY plot tab, if you want to. For that, it is important to know where your diffusion coefficients are, so we only recommend doing that on later iterations.)

Wait until the square is green. By hovering over the peaks, their respective fits are shown. If the fits are not good, we recommend going back to the above steps and adjusting the bullet points addressed in **analysis**. If only some fits are off, you should try to change your peak picking and integrals.

The fits are displayed in a pseudo 2D spectrum – your DOSY! If you were successful with the fitting, you should see that each component's peaks (this can be species originating from different

compounds or from the same, e.g. aggregates) have 'cross peaks' in a horizontal line (more or less). This line corresponds to the diffusion coefficient for your component.

Once you are satisfied with your DOSY spectrum, save it as 2rr spectrum (option when you right-click on the pseudo-2D spectrum). Pick a procnumber, that you don't have yet. This will transfer the spectrum back to topspin.

Report

Double click 'report'. Press the 'browse' button and find your user folder for the instrument (the same as the one where we got the data from earlier). Save the pdf under an appropriate name.

(Other processing methods that don't use the dynamics centre can be found under: http://nmrweb.chem.ox.ac.uk/Data/Sites/70/userfiles/pdfs/diffusion-nmr.pdf)

Report

Note down the diffusion coefficients of your sample. How many components are there?

If the 2rr spectrum you transferred into TopSpin is functional (sometimes the file is corrupted for reasons unknown), make a DOSY plot in the plot editor. If the file does not work, use the plot in your pdf. Specify the parameters you used to acquire the DOSY (δ, Δ, number of transients, NS, pulse sequence).

Calculate the Mw of the components in your sample using the SEGWE calculator from the Manchester NMR Methodology Group. There you can also find their scientific publication on the calculator.

https://nmr.chemistry.manchester.ac.uk/?q=node/432