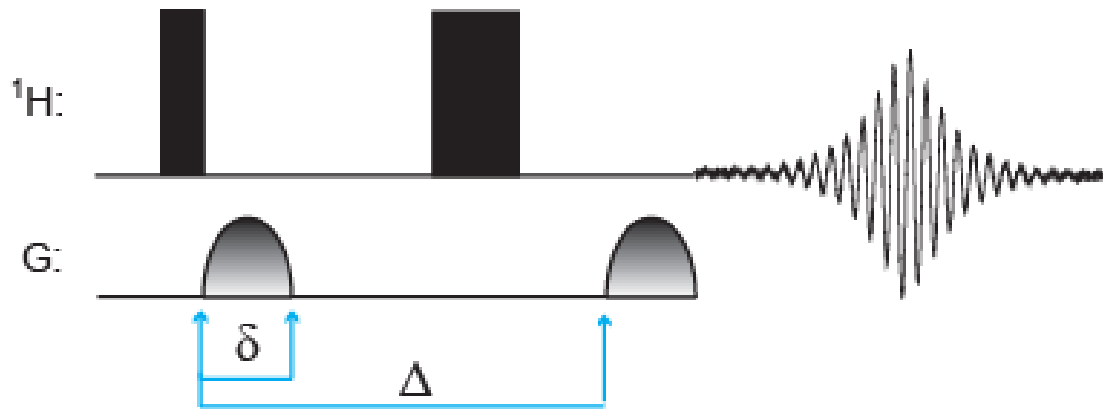


Diffusion Ordered Spectroscopy (DOSY)

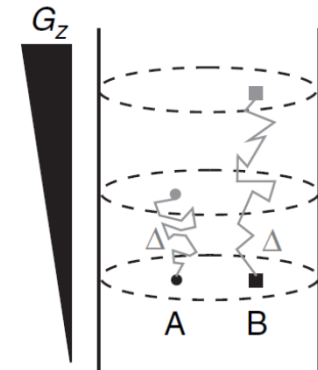


Measuring Diffusion Coefficients by NMR

- Diffusion Coefficient is defined by the Stokes-Einstein equation: $D = \frac{kT}{6\pi\eta r_s}$



- Using gradients, molecules can be spatially labeled, based on their position in the tube. If they move after the encoding, or 'labeling', during the diffusion time (Δ) that follows in the pulse sequence, their new position can be decoded with a second gradient.

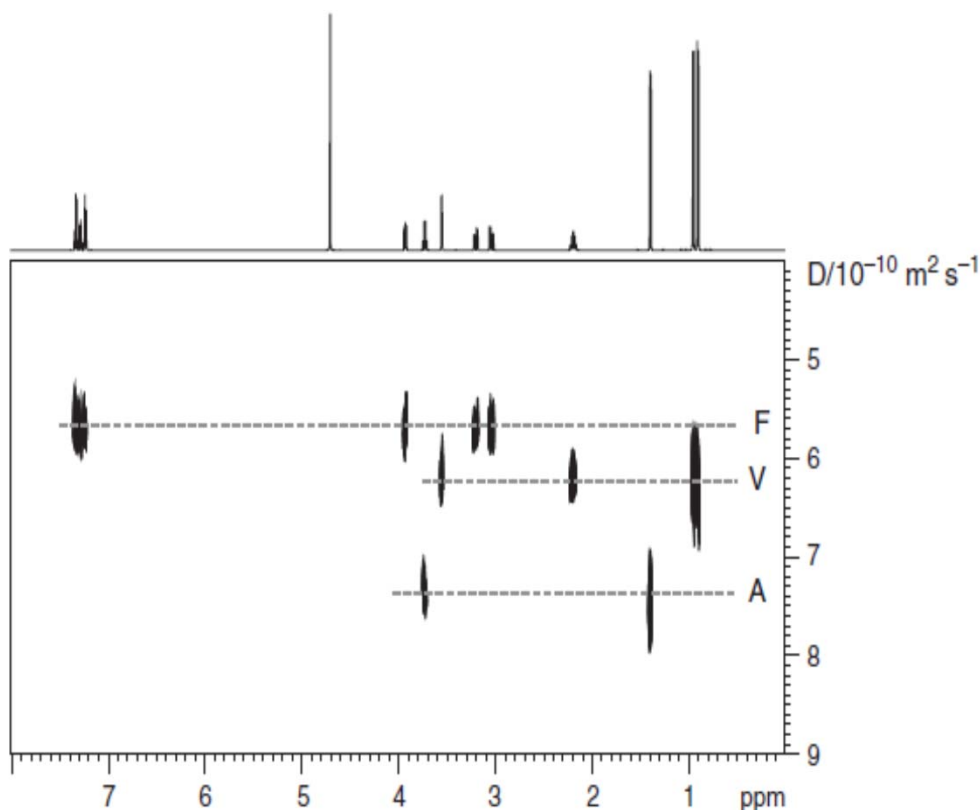


- The NMR signal intensity is attenuated depending on the diffusion time, Δ , and the gradient parameter, δ , as described by $I = I_0 e^{-D\gamma^2 g^2 \delta^2 (\Delta - \delta/3)}$



Diffusion Ordered Spectroscopy (DOSY)

- DOSY spectrum example of an equimolar mixture of three L-amino acids alanine (A), valine (V), and phenylalanine (F) in aqueous solution at 298K.



- Bottom axis represents chemical shifts and the right axis shows the diffusion constant of the corresponding site.
- The rate of diffusion is inversely related to the molecular weight/size. As a loose estimate, the Diffusion rate is inversely proportional to the cube root of the molecular weight, assuming a spherical shape and the particle density does not change.



Convection Issues and DOSY

- Convection occurs within the sample when there are temperature gradients along the axis of the sample tube which will skew the diffusion rate constant. The influence of convection can be seen in distortions in the decay profiles.
- To minimize the problem of convection the follow should be considered:
 - Use a smaller NMR Tube diameter: By reducing the tube size a larger temperature gradient is required to create a convection within the sample.

Solvent	Viscosity (mPa·s)
Acetone	0.31
Acetonitrile	0.35
THF	0.46
Chloroform	0.54
Benzene	0.60
D ₂ O	0.89
DMSO	1.99

- Select a solvent with a high viscosity: More viscous material will be less likely to create a convection. Use solvents such as DMSO or D₂O when possible.

- Spin your sample: Spinning can help stabilize forces that may create the convective flow.

- Alternatively, specialized sequences can be used to compensate for convection influences.



Setting up the DOSY Experiment

1. Setup and acquire a standard proton experiment.
 - Set the sw and center your data in the spectrum so that there is approximately 10% of clear baseline (no signal) on both sides signal region.
2. Measure the T_1 of your sample (see T_1 measurement notes) and set the $d1 = 5 \times T_1$ with respect to the longest relaxation measurement.
3. Read in the DOSY-ledbpgp2s experiment
4. Spin your sample at 20 Hz
5. In the command line enter the following to start the experiment:







xau dosy 5 80 32 l y y

The above command will execute a dosy script that will generate a gradient table consisting of 32 points between 5 to 80 % gradient power linearly. It will then automatically determine the receiver gain setting (rga), then begin acquiring the data.

NOTE: To attain accurate diffusion coefficients, need to ensure that the residual signal will decay to approximately 5% of the original intensity. To do a quick check, instead of 64 points in the gradient table, use 3 or 4 then compare the first and last spectra. If the signal is still too strong, increase the value of p30 by 200 until the you reach the desired signal intensity.



Processing DOSY Data

1. Bring up the DOSY processing parameters using the command **eddosy**.
2. Read in the diffusion parameters used in the experiment with the command **setdiffparm** or pressing on the  icon.
3. The icons  and  are used to toggle between the experimental parameters and DOSY parameters, respectively.
4. In the DOSY parameters set the scale to “Logarithmic” or “Linear”.
5. Fourier transform the F2 dimension only using the command **xf2** or the icon .
6. Determine the proper diffusion axis limits by calling the command **dosy2d setup** or using the icon .
7. Process the data by pressing the .
8. The center of the cross peaks is the Diffusion coefficients with the breadth of the peak in the F1 dimension as the fitting error.
 - If the scale is set to “Logarithmic” then the scale is read as logD in m^2/s and should be translated accordingly.
 - If the scale is set to “Linear” then the cross peak is directly the diffusion constant (just make note of the scale)

