# DOSY Experiments with VNMRJ 3.2A

## Introduction

### *Stejskal-Tanner (S-T) equation*

The DOSY (Difussion-Ordered SpectrocopY) experiment provides accurate, noninvasive, molecular diffusion measurements on biofluids, complex chemical mixtures and multicomponent solutions. In DOSY spectra, chemical shift is along the detected F2 axis and diffusion coefficient is along the other F1 axis.

Self-diffusion coefficients (D) are measured by recording a series of NMR experiments in which the overall gradient strength ($g$) is progressively incremented in each experiment. The attenuated NMR signal ($I$) follows this equation:

$$I = I_0 \, e^{-D \gamma^2 g^2 \delta^2 (\Delta - \delta / 3)}$$

where $I$ and $I_0$ are the signal intensities obtained with gradients strengths of $g$ and 0, respectively; $D$ is the diffusion coefficient; $\gamma$ is the gyromagnetic ratio; $g$ is the gradient strength, $\delta$ is the gradient pulse duration, and $\Delta$ is the effective diffusion delay. Our setup is to keep $\delta$ and $\Delta$ at default values and array gzlvl1 to change $g$.

### *Possible Application:*

Diffusion coefficients are directly related to molecular size and, therefore, we can study any topic involving changes in molecular size:

a. Determination of hydrodynamic radii and molecular weights
b. Aggregation states
c. Chemical structures composition
d. Intra-molecular interactions
e. Multi-component NMR separation

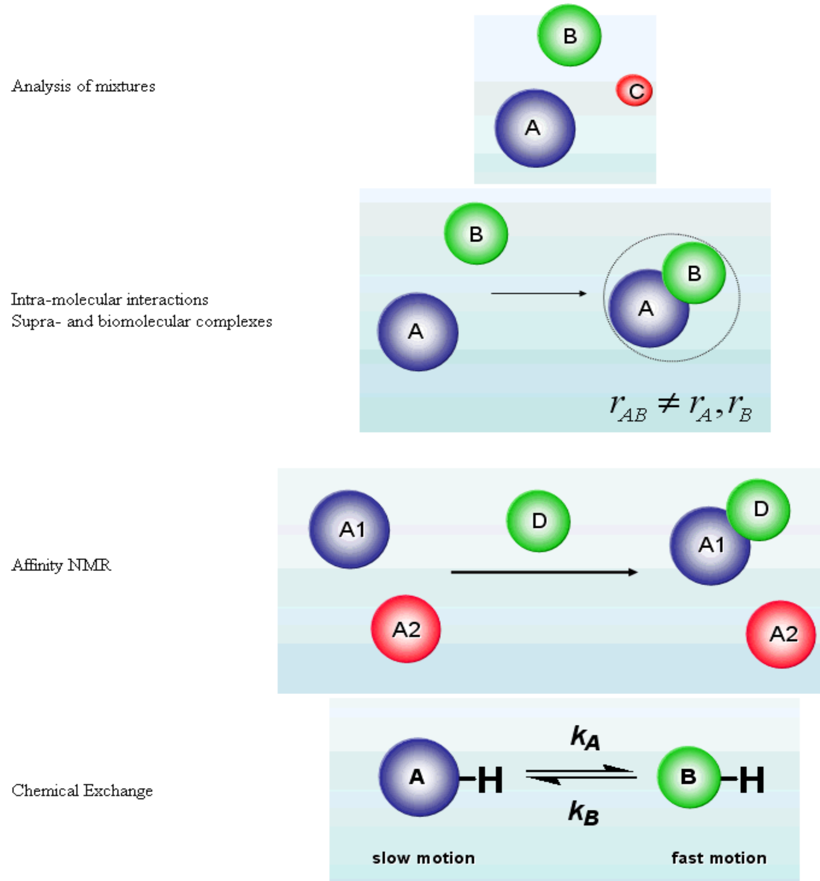


Figure 2 Possible applications

## Experiment Setup

It is advised to have TMS in your sample as reference that you will use later to do reference deconvolution during processing if you use organic solvents. You can use residual HDO signal for that purpose if you use D2O as solvent. The Dbppste_cc is recommended for most samples.

### *Select right sequences*

Samples with organic solvents and enough concentration should go with Dbppste_cc sequence. If sensitivity is a problem and you are doing the experiment close to room temperature, the Dbppste should be used. Every pulse sequence with convection compensation (Dbppste_cc) contains an extra stimulated echo step and therefore has an inherent 50% signal attenuation with respect to its equivalent without convection compensation (Dbppste). For samples dissolved in H2O/D2O mixture, simple solvent presaturation is typically not sufficient to reduce the water amplitude to a level where signal intensities from the sample are not affected by the residual solvent signal or by its dispersive component. Therefore, solvent presaturation needs to be combined with efficient extra post-sequence solvent suppression scheme like Watergate 3-9-19 or excitation sculpting (Double Pulsed Field Gradient Spin Echo = DPFGSE). For best results, especially in sub-millimolar concentrations, using a digital solvent suppression filter may also be recommended during processing.

## *Select right delays and gradient strength*

All sequences use a common set of parameters to define the duration of the diffusion gradient length (gt1, the total defocusing time), the diffusion gradient level (gzlvl1), and the diffusion delay (del). Choosing the values of DOSY parameters for a given sample involves determining the proper relationship among these three parameters. The best setting primarily depends on the sample itself (solvent, viscosity, molecular size and shape, the isotope to be detected) and on the experimental conditions (temperature, etc.). It is, therefore, recommended that the experimental parameters be optimized using the DOSY sample to be measured and the pulse sequence to be used.

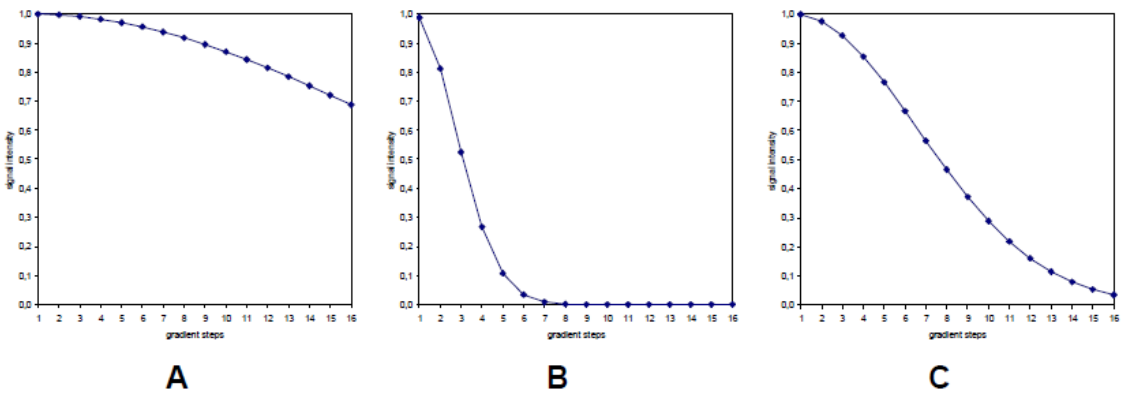


Figure 2 Simulated diffusion decay curves by varying the gradient strength from 2 to 95% in 16 steps for the same diffusion constant, but with different selection for Δ and **δ.** They are chosen too small (A), too big (B), and properly (C) to sample data points along the whole decay curve.

## *Do I need Convection-Compensation in DOSY Experiment?*

Convection within the sample tube, such as, moving liquid columns along the sample axis (primarily due to temperature gradients), can seriously affect diffusion experiments, in particular, at elevated temperatures. Convection currents are caused by small temperature gradients in the sample and result in additional signal decay that can be mistaken for faster diffusion.

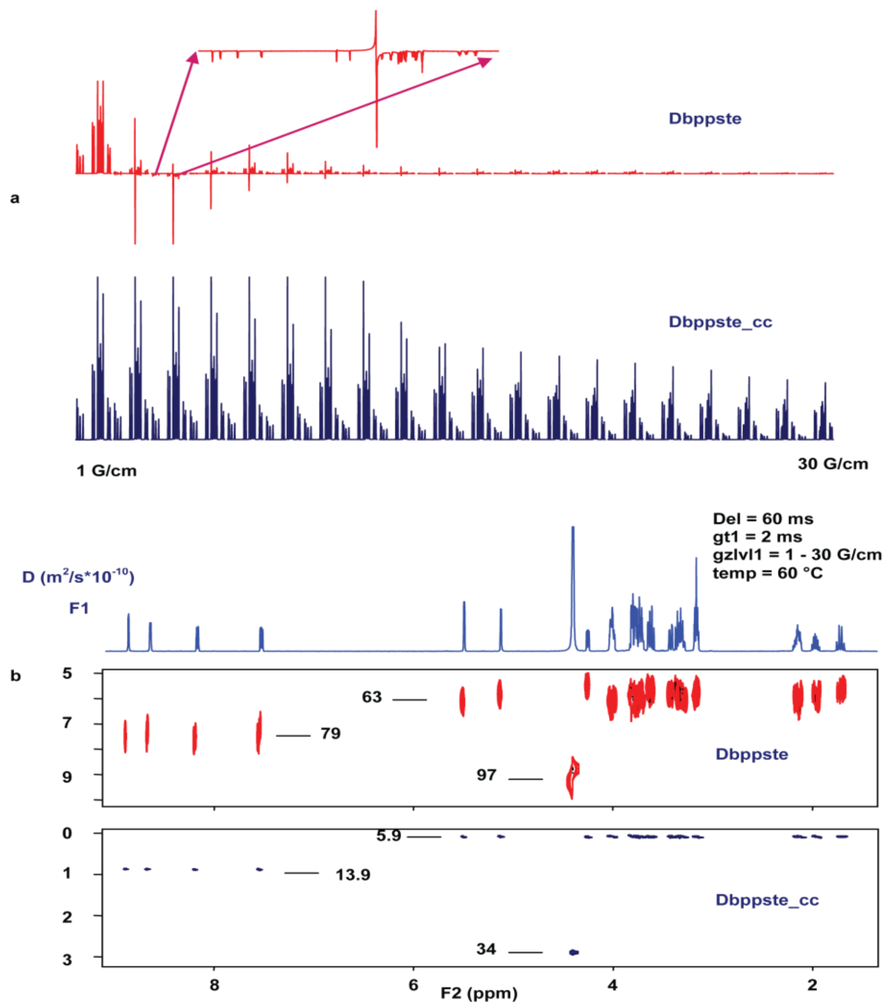


Figure 3 Signal intensities as a function of gradient power (a) and the 2D DOSY plots (b) of the nicotinic acid amide - amikacin mixture at 60° C