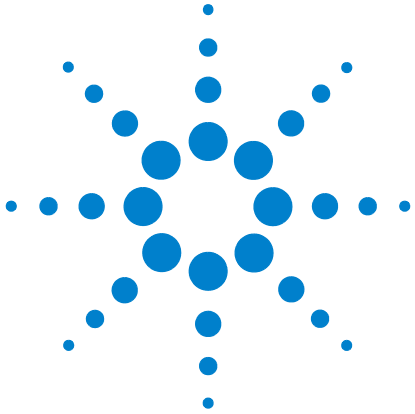

Agilent High-Resolution Diffusion-Ordered Spectroscopy (DOSY)

User Guide

Agilent Technologies

Notices

Manual Part Number

91001990

Edition

First edition, February 2011

Printed in USA

Agilent Technologies, Inc.
5301 Stevens Creek Boulevard
Santa Clara, CA 95051 USA

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Contents

1 DOSY VnmrJ 3 Release Notes

New Features

The new features of DOSY 3 are primarily associated with data processing.

New functionalities

- Non-uniform gradient (NUG) calibration
- Mono-exponential fitting with NUG correction
- Bi-exponential fitting, with and without NUG correction (uses a modified SPLMOD)
- Multi-exponential fitting, with and without NUG correction (uses a modified SPLMOD)
- Fitting of distributions of diffusion coefficients with CONTIN

Performance enhancements

- Improved support for 3D DOSY (including N- and P-type absolute value processing)
- User-friendly, phase-sensitive 3D DOSY acquisition and processing
- Display of residuals
- Optional point-by-point, instead of peak-segmented, 2D DOSY fitting and display
- Removal of peak number limitations in 2D DOSY
- Full panel support for every experiment in the package
- Full Chempack/VnmrJ 3 compatibility

The current DOSY package contains overall 17 diffusion pulse sequences as well as a sequence for NUG (Non Linear Gradient) calibration. Although most of the sequences were developed for the VnmrJ 2.2C software release, the current versions, due to the introduction of several new parameters, are NOT back compatible with previous DOSY releases. Data run with older VnmrJ versions, though, are still expected to be compatible with the current processing tools. The new package provides completely redesigned VnmrJ-type acquisition and processing panels. The Tcl-Tk panels used in the earlier VNMR interface are not supported anymore. However, the "dg" and "ap" tables are updated and are still applicable.

Some pulse sequence features that had earlier been present only for individual sequences are now universally available. These features include:

- Gradient-pw90-gradient sandwich prior to `d1` to set up steady-state conditions (`sspul` flag)
- Solvent presaturation option during the relaxation and/or diffusion delay (`satmode` flag)
- Wet solvent suppression option during the relaxation delay (`wet` flag)
- Option for gradient sign alternation on subsequent scans to occasionally minimize line shape distortions (`alt_grd` flag)
- Option to switch off the lock feedback loop during the diffusion sequence (`lkgate_flg` flag)

Pulse sequences have been added to support experiments on biological samples in H_2O/D_2O solvent at limited concentrations. They use either the well known watergate 3-9-19 (`Dbppste_wg`) or the excitation sculpting (`DgcsteSL_dpfgse`) schemes for solvent suppression. For best results, they may be combined with solvent presaturation, as well as with digital solvent filtering during data processing.

There are pulse sequences that allow convection compensation (`Dbppste_cc, DgsteSL_cc, DgcsteSL_cc and Dpfgdste_cc`) or can be used to experimentally verify whether convection is present and might distort the diffusion data.

The Doneshot sequence has been modified to allow diffusion experiments in concentrated samples or neat liquids. The flip angle of the first pulse has been made user-enterable to overcome problems associated with radiation damping.

The package contains pulse sequences that allow running phase-sensitive 3D-DOSY experiments (`Dgcstehmqc_ps, Dbppste_ghsqcse, Dhmqcidosy`). The first two sequences were

developed and tested on ^{15}N-labeled peptide/protein samples. The `Dbppste_ghsqcse` sequence was taken over from the BioPack package and has been made VnmrJ 3 compatible.

A new approach of pulse sequence programming of diffusion sequences, called inclusive-DOSY or I-DOSY, has recently been published by Gareth Morris and Matthias Nilsson. Instead of concatenating the NMR and the diffusion pulse sequence, they share delays for magnetization transfer and diffusion. They have higher inherent sensitivity than conventional sequences and allow optional convection compensation, with no sensitivity penalty. The `Dcosyidosy` and the `Dhom2djidosy` are absolute value sequences, while the `Dhmqcidosy` sequence allows the acquiring of phase-sensitive data.

The `Doneshot_nugmap` pulse sequence is provided to accurately calibrate the gradient strength of the probes used for diffusion experiments, as well as to map the spatial non-linearity of the gradient coil. The results of this calibration are stored in the corresponding probe file and are activated at any consequent diffusion setup and may be taken into account at data processing.

Two macros `showdosyfit` and `showdosyresidual` provide graphical display of the quality of the fit for each individual peak in DOSY processing. That allows identifying systematic errors or may help to exclude erroneous data points from the analysis.

1 DOSY VnmrJ 3 Release Notes

2
High-resolution Diffusion-Ordered Spectroscopy (DOSY)

The DOSY (Diffusion Ordered Spectroscopy) application separates the NMR signals of mixture components based on different diffusion coefficients. Generally speaking, DOSY increases the dimensionality of an NMR experiment by one. In 2D DOSY, the initial diffusion weighted NMR spectra are one-dimensional; adding diffusion weighting to a 2D NMR experiment such as COSY, HMQC, etc. gives 3D DOSY spectra.

Macros and Commands in the DOSY Package

The DOSY analysis involves the following two steps. Each of these steps is described in more detail in the following sections.

1 Set up and acquire a series of diffusion-weighted spectra.

2 Determine the diffusion coefficients for each line (or cross-peak) in the spectrum. Take line (or cross-peak) positions and diffusion coefficients and display the results in a DOSY plot. All of these steps are executed by the **Calculate Full DOSY** button in the **Process/DOSY Process** panel (or the `dosy` macro).

Table 1 lists the tools available for DOSY.

Table 1 Tools (Commands) for DOSY experiments

`Dbppste`	Set up parameters for the Dbppste.c pulse sequence	
`Dbppste_cc`	Set up parameters for the Dbppste_cc.c pulse sequence	
`Dbppste_ghsqcse`	Set up parameters for the Dbppste_ghsqcse.c pulse sequence	
`Dbppsteinept`	Set up parameters for the Dbppsteinept.c pulse sequence	
`Dbppste_wg`	Set up parameters for the Dbppste_wg.c pulse sequence	
`Dcosyidosy`	Set up parameters for the Dcosyidosy.c pulse sequence	
`Dgcstecosy`	Set up parameters for the Dgcstecosy.c pulse sequence	
`Dgcstehmqc`	Set up parameters for the Dgcstehmqc.c pulse sequence	
`Dgcstehmqc_ps`	Set up parameters for the Dgcstehmqc_ps.c pulse sequence	
`DgcsteSL`	Set up parameters for the DgcsteSL.c pulse sequence	
`DgcsteSL_cc`	Set up parameters for the DgcsteSL_cc.c pulse sequence	
`DgcsteSL_dpfgse`	Set up parameters for the DgcsteSL_dpfgse.c pulse sequence	
`Dghmqcidosy`	Set up parameters for the Dghmqcidosy.c pulse sequence	
`DgsteSL_cc`	Set up parameters for the DgsteSL_cc.c pulse sequence	
`Dhom2djidosy`	Set up parameters for the Dhom2djidosy.c pulse sequence	
`Doneshot`	Set up parameters for the Doneshot.c pulse sequence	
`Doneshot_nugmap`	Set Up parameter for NUG calibration	new
`Dpfgdste`	Set up parameters for the Dpfgdste.c pulse sequence	
`makedosyparams`	Creates DOSY-related parameters (called by DOSY sequences)	modified
`cleardosy`	Delete any temporarily saved data in the current (sub) experiment	
`dosy`	Start the 2D-DOSY or 3D AV-DOSY analyses	modified

Table 1 Tools (Commands) for DOSY experiments (continued)

undosy	Restore the original 1D NMR data from the subexperiment	modified
redosy	Restore the previous 2D DOSY display from the subexperiment	modified
dosy2D	Execute protocol actions of apptype dosy2d	modified
homodosy3D	Execute protocol actions of apptype homodosy3D	new
heterodosy3D	Execute protocol actions of apptype heterodosy3D	new
process_dosy2D	Auto-process 2D DOSY spectra	new
process_dosy3D	Auto-process 3D DOSY spectra	new
sdp	Show diffusion projection	
fbc	Apply baseline correction for each spectrum in the array	
makeslice	Synthesize 2D projection of a 3D DOSY spectrum in diffusion limits	
showoriginal	Restore the 1st 2D spectrum in a 3D DOSY experiment	
showdosyfit	Display the fit of the DOSY analyses for a given line	
gradfit	Macro to calculate NUG coefficients	new
showgradfit	Display the gradient strength variation with position	new
showdosyresidual	Display the difference between experimental data and the fit for a given line or crosspeak	
reorder3D	Reorder FIDs to exchange order of gzlvl1 and phase (for phase-sensitive 2Ds)	new
update_wrefshape	Create solvent suppression selective shape for DgcsteSL_dpfgse sequence	
ddif	Synthesize and display DOSY plot	
dofiddle	Does fiddle via the FIDDLE panel	
fiddle*	Perform reference deconvolution	

*fiddle(option<,file><,option<,file>><,start><,finish><,increment>

NOTE The following commands have become obsolete in the new version and are not used any more: `setup_dosyVJ, dosy3Dps, dosy_grad_calib, unpack_DOSY3Dps.`

Every DOSY pulse sequence belongs to either of three application types (`apptype` parameter): `dosy2D, homodosy3D` or `heterodosy3D`. The individual pulse sequences are set up by macros that share the same names as the pulse sequences themselves. In addition, each pulse sequence has a `sequencename_setup` macro for individual customization. This is, however, not DOSY, but VnmrJ 3 specific.

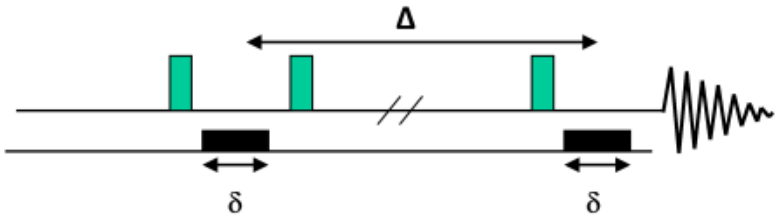
Auto-processing (via the **Autoprocess** button, process macro or during automation) is done via the macros `process_dosy2D` and `process_dosy3d` - these are set by the `execprocess` parameter. Similarly, the macros `sequencename_process`, `sequencename_plot` and `sequencename_display` are also executed (in case they exist) during automatic processing, plotting and display.

The pulse sequences (always starting with "D") supplied with this version of the DOSY software calculate the time portion of the exponent governing diffusional attenuation as well as the Larmor-frequency of the diffusing spins, and store them in the parameters `dosytimecubed` and `dosyfrq`, respectively.

General Considerations

The DOSY experiments are among the most demanding gradient sequences in NMR spectroscopy. In conventional coherence pathway selected experiments, one can optimize the experimental conditions for a given gradient setting. In DOSY, however, very often, the whole scale of available gradient power is used and high-resolution NMR conditions must still be maintained. Convection, such as, moving liquid columns along the sample axis (primarily due to temperature gradients), does not hurt the coherence pathway selected experiments seriously (apart from the obvious intensity losses). It can, on the other hand, make the DOSY analysis of the diffusion data completely useless.

DOSY pulse sequences use the gradient-stimulated echo element (or one of its modifications):



In the DOSY experiments, the strength of the diffusion-encoding gradient is arrayed and the diffusion coefficients are calculated according to the Stejskal-Tanner formula:

$$S\left(G_{zi}\right) = S(0)\exp(-D_i\gamma^2\delta^2(G_{zi})^2(\Delta - \delta/3))$$

where $S(G_{zi})$ and $S(0)$ are the signal intensities obtained with gradients strengths of G_{zi} and 0, respectively; D is the diffusion coefficient; γ is the gyromagnetic constant; δ is the gradient pulse duration; and Δ is the diffusion delay.

From the formula, one can get valuable hints on how to set DOSY-related parameters in different pulse sequences.

$(\gamma\delta G_{zi})^2$ - gradient pulse area squared

a nuclei with higher γ are more sensitive to diffusion than the low-γ nuclei. (If possible, observe ^1H or ^{19}F, or at least do the diffusion-encoding step on the high-γ nucleus (see `Dbppsteinept`).

b shaping a gradient dramatically reduces its phase encoding efficiency. Although the VnmrJ software enables the shaping of gradients on VNMRS or 400-MR spectrometers, it is not really recommended.

δ - gradient pulse duration

during δ (and the subsequent gradient stabilization delay, `gstab`), the magnetization is transverse and subject to T_2 relaxation and homonuclear J-evolution. Do not use long δ values in the presence of large homonuclear couplings or short T_2 relaxation times ($\delta << T_2$ or $1/J$).

G_z - gradient strength

use the highest values possible, provided high-resolution NMR conditions are still maintained (no phase, amplitude and line shape distortions).

Δ - diffusion delay

convection can always be an undesired competitor to diffusion, and T_1 relaxation attenuates the signal intensities. Do not use unnecessarily long diffusion delays ($\Delta < T_1$).

Some of the recommendations above may seem contradictory. Of course, in real cases, one needs to find an acceptable compromise between them.

The separation efficiency in the diffusion domain is determined by the accuracy of the measured diffusion coefficients. DOSY does not necessarily intend to get absolute diffusion coefficients (in mixtures, it is difficult to speak about "absolute" numbers anyway). The relative differences in the D values may be adequate for separation.

NOTE Changing the solvent of a DOSY mixture may change the diffusion coefficients and hence the separation power of the method. The solvent can play a similar role in DOSY as the different columns in HPLC chromatograph.

Errors in the diffusion coefficients can either be of statistical or systematic nature. The most obvious source of statistical errors is inappropriate signal-to-noise (S/N) ratio. Therefore, in DOSY experiments, relatively high S/N values must be reached even with the strongest phase-encoding gradients. Systematic errors are primarily caused by instrumental imperfections like gradient non-linearity over the active sample volume, phase distortions, changes in experimental lineshape as a function of gradient amplitude and so on. The systematic errors can be minimized by:

- careful pulse sequence design (see Magn. Reson. Chem. 1998, 36, 706.)
- by adding a suitable internal reference compound to the sample (a component producing a strong, well isolated singlet peak in the spectrum) suitable for reference deconvolution (FIDDLE) when processing DOSY.

Gradient nonlinearity can be calibrated and corrected during data processing (see "Gradient Calibration and Correction for Gradient Non-Uniformity" on page 23).

When setting up DOSY experiments, the following recommendations should be taken into consideration:

- Be sure that the `probe` parameter is set to the probe you intend to use and `Probegcal` has the right value in the probe file. The setup macros extract the gradient strength (`gcal`) from the probe file and store it in the local parameter `gcal_`. Pulse power levels and `pw90` values are also read from the probe calibration file. If the probe gradient non-linearity has been mapped, then the `nugcal_[1-5]` values are also retrieved and may be used during processing.
- Set `z0` precisely on resonance, and adjust the lock phase carefully (misadjustment may cause progressive phase errors with increasing gradient power)
- Do not spin the sample
- Use an adequate number of data points for proper spectral digitization
- When running long experiments, use interleaved acquisition (`il='y'`)
- In order to minimize temperature gradients (and hence convection), avoid using extreme (low and high) temperatures. For solutions with very low viscosity, it may be preferable to switch off the VT controller completely.

- In case you can find a substance suitable for reference deconvolution, add it to the mixture before running DOSY. For proton spectra in small molecule mixtures, TMS (organic solvents) or DSS (water) might be the ideal candidates.

Nano Probe Compatibility of DOSY Experiments

For optimum performance of pulsed field gradient experiments on a Nano Probe, the encoding and decoding gradient durations need to be fine adjusted to ensure that the duration of each gradient pulse corresponds to an integer number of rotations.

The current VnmrJ 3 release provides a general solution for the problem, covering both automatic and manual spin control.

Once the probe file is set up properly, the user hardly needs to do anything to run Nano Probe compliant experiments. For systems with software spin control, everything is automatic. However, for systems with manual spin control, prior to starting the acquisition, the parameter `srate` needs to be set to the actual spinning frequency, either in the command line or in the **Start/Standard** parameter panel.

This software setup relies upon some new probe file entries: there may be up to three Nano Probe related lines / definitions in the probe file:

```
Probeprobetype          nano
Probespintype           tach / mas*
Probespinmax            3000 / 10000*
```

(* mas and 10000 1/s will be the options for the newly released FastNano™ probe)

corresponding to VnmrJ parameters `probetype, spintype`, and `spinmax`. The first one, probetype, is a new global parameter. While the `addprobe` macro adds this new parameter to the probe file (with a default value of `liquids`), the `updateprobe` macro will add that definition to an existing probe file if it is not present yet. Alternatively, an existing probe file may be edited, adding the first of these three lines exactly as shown above. The other two parameters, `spintype` and `spinmax`, have existed since VnmrJ 2.2C, and are relevant only for systems with software spin control. Upon changing to a Nano Probe, all three parameters are activated (via the `_probe` macro) and the system is ready to do the extra, Nano Probe-related tasks automatically.

User-created DOSY pulse sequences

This software setup requires a few changes to existing pulse sequences: the actual gradient adjustment takes place within the pulse sequence itself, that is, it is performed at `"go"` time (through the **Acquire** button or the `cpgo` macro). It is, therefore, the pulse sequence programmer's privilege and responsibility to ensure that the pulse sequence is Nano Probe compliant.

All DOSY pulse sequences in VnmrJ 3 have been adjusted to be compliant with Nano Probes. A user-created diffusion pulse sequence could be made Nano Probe-compatible by following the steps listed below:

1. Be sure that the probe file has all relevant parameters defined and set, as outlined above. Include the necessary changes in your pulse sequence code as described below and recompile the pulse sequence.
2. Making an existing gradient pulse sequence compatible with Nano Probes involves several changes in the pulse sequence code itself:

   Near the top of the pulse sequence, just below the line

   ```
   #include <standard.h>
   ```

   an extra `include` line must be added for the header file `chempack.h`:

   ```
   #include <chempack.h>
   ```

   Define which parameter(s) need(s) adjustment by inserting expression "A" (or "A" and "B" together) from below. Note that a homospoil gradient pulse (for example, a gradient that simply destroys residual transverse magnetization) does *not* need this type of adjustment. Let's assume that the relevant gradient pulse has duration of gtE and amplitude of gzlvlE. In this case, expression "A":

`gtE = syncGradTime("gtE","gzlvlE",1.0)` trims the gradient pulse length `gtE` and leaves the amplitude `gzlvlE` unchanged. Each Nano Probe compatible pulse sequence must contain at *least* this definition. The third argument is a multiplier that is typically set to 1.0 in sequences with single gradient pulse duration. If a pulse sequence uses gradient pulses with lengths of both `gtE` and `gtE/2` (as many heteronuclear Chempack-type sequences), then the multiplier must be set to 0.5 to ensure that the requirements are also met for `gtE/2`.

A second expression "B":

```
gzlvlE = syncGradLvl("gtE","gzlvlE",1.0)
```

used with a combination with expression "A" above adjusts the gradient amplitude (`gzlvlE` in this example) such that the gradient area (such as, the product `gtE*gzlvlE`) remains constant.

The use of both expressions is *required* for all sequences used to measure diffusion rates.

NOTE In order to avoid any incompatibilities between current VnmrJ DOSY pulse sequences and the DOSY processing package, expressions "A" and "B" must be inserted *after* the line starting with `putCmd("makedosyparams...)`.

(Every Agilent-supplied DOSY sequence contains such a line).

Statements "A" and "B" above actually do *not* update the parameter values in any VnmrJ parameter tree. If the gradient pulse duration (and amplitude, if applicable) are adjusted "on-the-fly", the output of `dps` shows the modified values. But, after the experiment, the VnmrJ parameters will *not* reflect the values actually used. However, this will not have any negative consequences, at least as long as both the gradient pulse duration *and* the amplitude are corrected, as the gradient areas in the "real" experiment correspond to the specified values.

3
Gradient Calibration and Correction for Gradient Non-Uniformity

Introduction

As described in "Macros and Commands in the DOSY Package" on page 12, the measurement of good quality DOSY data is highly dependent on the careful optimization of experimental parameters and the reduction, or complete elimination, where possible, of sources of systematic errors in the data. However, even when all possible steps have been taken to maximize spectral data quality, some systematic sources of error will remain that can degrade the quality of the final DOSY spectrum and reduce the accuracy of the diffusion measurements. Among these remaining sources of systematic error, one of the most significant is spatial non-uniformity of the field gradient pulses produced by the probe.

As described in "Macros and Commands in the DOSY Package" on page 12, diffusion measurements by NMR involve the fitting of the signal amplitude as a function of the square of the gradient pulse area to the Stejskal-Tanner (S-T) equation:

$$S(G) = S(0)\exp(-D_i\gamma^2\delta^2G^2\Delta) \qquad [1]$$

where $S(G)$ and $S(0)$ are the signal intensities obtained with gradients strengths of G and 0, respectively; D is the diffusion coefficient; γ is the gyromagnetic ratio; δ is the gradient pulse duration, and Δ is the effective diffusion delay.

Unfortunately, largely due to necessary compromises made in all probe designs, the field gradients produced are not perfectly uniform over the active volume of the sample. In diffusion experiments, this leads to problems with gradient calibration and to signal decays whose form deviates slightly from that of the S-T equation. Fitting such data to the S-T equation without correcting for non-uniform gradients has some undesirable consequences:

- With increasing diffusion weighting, the deviation of the signal decay from the S-T equation also increases. This means that the apparent diffusion coefficient calculated from the fit depends on the level of diffusion weighting used.

- The standard deviation of *D* estimated in the fitting process is increased because the experimental and theoretical decays do not match- a problem that gets worse as the diffusion weighting increases. As the standard error is used to define the width of a peak in the diffusion domain of a DOSY spectrum (see "Processing 2D-DOSY Experiments" on page 71), any increase in the standard error leads to a loss of diffusion resolution.

Fortunately, it is easy to correct for almost all of these effects of gradient non-uniformity by fitting the experimental data to a modified S-T equation that takes into account the actual gradient shape produced by the probe. A single experiment is used to determine the necessary correction to the S-T equation, which can then be used in the processing of all DOSY data. The steps involved in correcting for gradient non-uniformity can be summarized as follows:

- Mapping of the gradient shape
- Calculation of the actual signal decay under this gradient shape
- Parameterization of the experimental signal decay

Each of these steps is described in the sections below.

Mapping the Gradient Shape

The first step in correcting for gradient non-uniformity in diffusion measurements is to map the shape of the gradient produced by the probe. This is done using a diffusion pulse sequence that has been modified to include a weak “read” gradient. VnmrJ features a sequence “Doneshot_nugmap” that is based on the standard “oneshot” sequence (Doneshot), but which includes a read gradient during acquisition. Gradient calibration should be run by the system administrator once for each probe - therefore it is not made available from the experiment selection menu for ordinary users and operators.

The Doneshot_nugmap sequence and its parameter list:

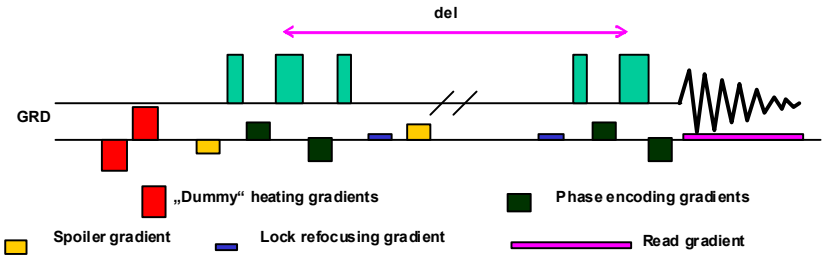


Table 2 Parameters

`delflag`	'y' runs the Doneshot_nugmap sequence
	'n' runs the normal s2pul sequence
`avflag`	'n' runs Doneshot sequence with a read gradient
	'y' as above, plus an increased gradient pulse to move the echo to middle
`del`	the actual diffusion delay
`gt1`	total diffusion-encoding pulse strength
`gzlvl1`	diffusion-encoding pulse strength
`gstab`	gradient stabilization delay ~0.0002-0.0003 s)
`gt3`	spoiling gradient duration
`gzlvl3`	spoiling gradient strength
`gzlvl_max`	maximum accepted gradient strength 32767 with Performa II or IV, 2047 with Performa I

Table 2 Parameters (continued)

`gzlvl_read`	gradient strength during acquisition, typically around 25 DAC units on a Performa II or Performa IV or about 7 DAC units on a Performa I system - the HDO signal width should be between 300–400 Hz
`kappa`	unbalancing factor between bipolar pulses as a proportion of gradient strength (~0.2)
`tweak`	correction to final gradient pulse, typically around 1 DAC point
`avflag`	'y' selects absolute value experiment n' selects phase-sensitive experiment
`nugcal_[1-5]`	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Created by the Doneshot_nugmap sequence and then copied to the corresponding probe file.
`probe_`	stores the probe name used to acquire the DOSY experiment

NOTE Select the transmitter offset `tof` to be on-resonance on the HDO signal.

The parameters for the heating gradients (`gt4, gzlvl4`) are calculated in the sequence. They cannot be set directly.

The calibration is typically done by measuring ^{1}H profiles using the standard doped 1% H_2O/99%D_2O sample. The temperature dependence of diffusion coefficient for this sample can be estimated by interpolation from values in the literature.

To set up the `Doneshot_nugmap` gradient mapping experiment, do the following:

1. Calibrate the probe temperature using one of the standard samples.
2. Insert the doped 1% H_2O/99% D_2O sample into the magnet.
3. Regulate the probe (VT) temperature and note the (calibrated) sample temperature.
4. Optimize the lock parameters, tune the probe, and shim the sample.
5. Ensure that the correct probe has been selected in VnmrJ by clicking on the **Probe** button on the Hardware Bar and selecting the appropriate probe file.

6 Call the `Doneshot_nugmap` macro from the command line.

This will set up the parameters for the Doneshot_nugmap sequence that allows the measurement of a set of diffusion-weighted profiles for characterizing the gradient non-uniformity. Select the transmitter offset `tof` to be on-resonance on the HDO signal. Adjust the `gzlvl_read` parameter to provide a HDO profile about 300–400 Hz wide (about 35 DAC units using a Performa II or Performa IV or ~7 DAC units on a Performa I system). If the phase-sensitive version is selected (`avflag='n'`), then the `tweak` parameter should be optimized for undistorted profile shapes at the baseline level.

7 Finally, set up a gzlvl1 array of 15–20 elements in the range of 1000–15000 (Performa II or IV) or 50–1700 (Performa I).

8 Review the parameters from the **Acquire-Defaults** and/or **Acquire-Pulse Sequence** panels, as shown in Figure 1 below (for Performa I gradient amplifier).

9 Click **Acquire** to start the acquisition.

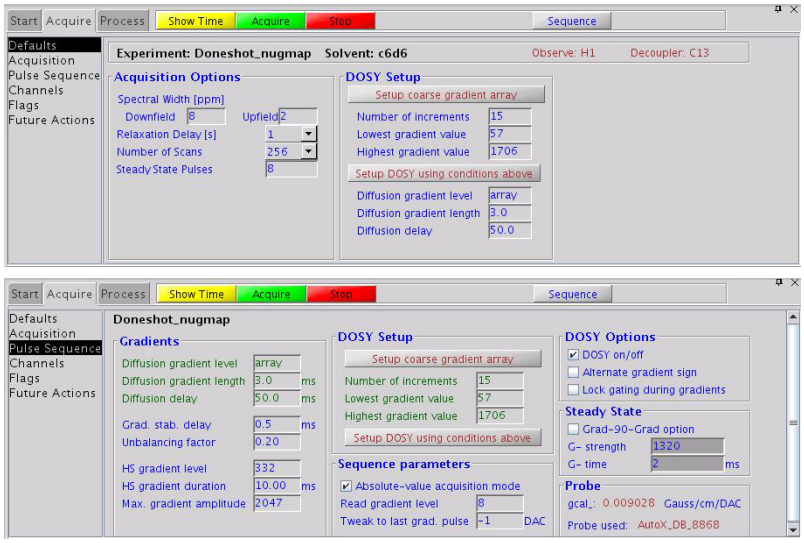


Figure 1 The Acquire-Defaults and Acquire-Pulse Sequence panels for the Doneshot_nugmap sequence

Processing the Gradient Mapping Data

Once the gradient mapping data has been acquired, the next step is to process the data. The processing involves the following steps:

1. Fourier transformation of the time-domain data to give a series of profiles.
2. Baseline correction of the profiles.
3. Fitting of corresponding points on each profile to the standard S-T equation to give an apparent variation in diffusion coefficient (D) as a function of the position along the (z) axis of the sample.
4. Fitting of the apparent variation in D to a power series to yield a gradient shape function. The coefficients of the power series are then stored in the probe file as parameters `Probegradcoeff1, Probegradcoeff2, ..., Probegradcoeff9.`
5. Calculation of the signal decay under the influence of the gradient shape function determined in 4.
6. Fitting of the signal decay to an exponential of a power series. The coefficients of the power series are then stored in the probe file as parameters `Probenugcal1, Probenugcal2, ..., Probenugcal5.`

Step-by-step description

To begin processing the gradient mapping data, first select the **Process/NUG Calib** panel.

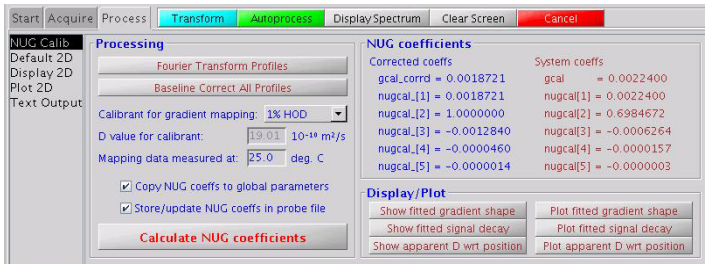


Figure 2 The Process/NUG Calib panel, used for processing gradient mapping data from the Doneshot_nugmap sequence.

To process the gradient map data, do the following:

1. Click on **Fourier Transform Profiles**. This will perform a weighted Fourier transform of the FID data and display the first profile. A typical set of profiles (shown stacked vertically) obtained from the Doneshot_nugmap sequence is shown in Figure 3 on page 31.
2. Display the first spectrum and set integral regions manually.
3. Now, click the **Baseline Correct All Profiles** button. This will automatically baseline-correct all the profiles using the integral settings defined above.
4. From the **Calibrant for gradient mapping** drop-down menu, select either **Pure H2O**, **1% HOD**, or **Other**, depending on which sample was used to obtain the profiles. If "Other" was selected, enter the expected diffusion coefficient for the calibration at the temperature the data were recorded at.
5. Enter the (calibrated) temperature that the data were recorded at into the relevant field.
6. Select or clear the options **Copy NUG coeffs to global parameters and Store/update NUG coeffs** in probe file, as appropriate. If this is the first time the calibration has been done for a particular probe, it is recommended that these options be selected.
7. Click **Calculate NUG coefficients**. A semi-logarithmic plot of the calculated versus fitted signal attenuation will be displayed, together with the calculated NUG coefficient.

The **Calculated nugcal_ array** contains the NUG coefficients calculated using the original (uncorrected) probe file value of `gcal` (stored locally as `gcal_`). The **Corrected nugcal_ array** contains the NUG coefficients calculated, instead using a corrected version of `gcal_`.

Corrected `gcal_` = Original `gcal_` * Correction factor

where the **Correction factor** is the square root of the second NUG coefficient. The corrected `gcal` is stored in the probe file as parameter `Probegcal_corrd` (see "Probe File Entries" on page 32).

NOTE

Any DOSY measurements recorded after the gradient mapping has been carried out will use the corrected value of `gcal` in place of the original `gcal`. The corrected value is the signal-weighted average of the gradient strengths across the sample, and is generally different from that obtained from the width of the signal profile. The latter method is relatively inaccurate and does not allow for gradient non-uniformity corrections.

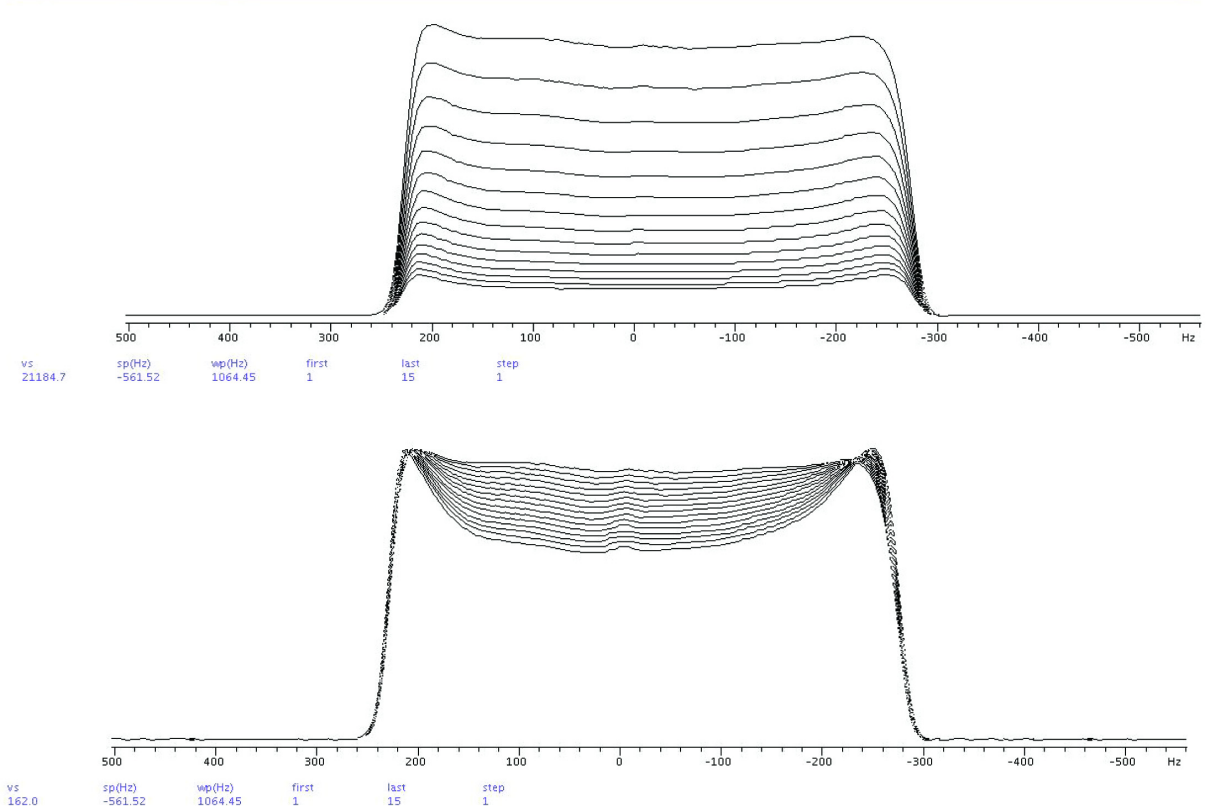


Figure 3 Set of profiles measured on a doped sample of 1% H_2O/99% D_2O, using the Doneshot_nugmap sequence. The top trace shows the profiles in "absolute intensity" mode, while the bottom trace shows the profiles normalized to the same intensity. The "smile" seen on the profiles displayed in normalized mode is due to the non-uniformity of the gradient having caused the profile to decay more quickly in the middle than at its edges.

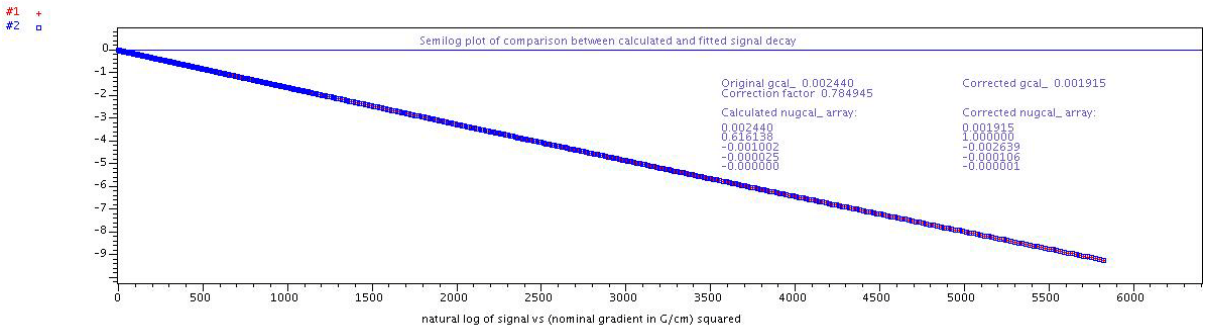


Figure 4 Typical output from non-uniform gradient (NUG) processing

Probe File Entries

If the option, Store/update NUG coeffs in probe file, is selected before the **Calculate NUG Coefficients** button was pressed, then the following parameters are written to the probe file (seeFigure 5):

Probeprobetype	liquids
Probenugcal1	0.01067000000
Probenugcal2	1.04977365653
Probenugcal3	-0.00273292896
Probenugcal4	-0.00012465238
Probenugcal5	-0.00000426686
Probegcal_corrd	0.009250
Probegradcoeff1	6.0000000000000000
Probegradcoeff2	1.0411584899999999
Probegradcoeff3	-0.0000984377422000
Probegradcoeff4	0.0000005243765730
Probegradcoeff5	-0.0000000110202274
Probegradcoeff6	-0.0000000001095542
Probegradcoeff7	-0.0000000000000368
Probegradcoeff8	-0.0000000000000011
Probegradcoeff9	-0.0000000000000000

Figure 5 Excerpt from a typical probe file, showing the parameters stored during the NUG processing.

- Probe gradient coefficients: the parameters `Probegradcoeff1, Probegradcoeff2, ..., Probegradcoeff9`, which correspond to the power series coefficients that describe the gradient shape produced by the probe. These coefficients are not used for the processing of data measured using the standard Agilent-supplied DOSY pulse sequences, but are useful for the analysis of 'pureshift' and other spatially-resolved DOSY datasets ("Pure shift Proton DOSY: Diffusion-Ordered 1H Spectra without multiplet structure." M. Nilsson and G.A. Morris, Chem. Commun. 2007, 933-935.).
- Non-uniform gradient (NUG) coefficients: the parameters `Probenugcal1, Probenugcal2, …, Probenugcal5`, which describe the actual signal decay (as opposed to the 'idealized' signal decay described by the S-T equation). During the processing of routine DOSY data, if the **Correct for non-uniform gradients** option (`nugcal='y'`) is selected on the **DOSY Process** panel, then signal decays are fitted to an equation of the form:

$$S(G) = S(0)\exp\left[-\sum_{n=1}^{5} \text{nugcal}\, n\, \eta^n G^{2n}\right] \quad [2]$$

where $\eta = D\gamma^2\delta^2 D$ and G is the nominal gradient amplitude.

- A "corrected" `gcal`: the parameter `Probegcal_corrd` corresponds to the conversion factor from gradient DAC units to the *average* gradient strength (in G/cm) across the sample:

  Average gradient strength (G/cm) = `gcal_corrd` × gradient DAC units

  `gcal_corrd` provides a more accurate estimate of the average gradient strength across the sample than `gcal`.

NOTE

When subsequent DOSY experiments are set up, the value of `gcal_corrd` is used in place of `gcal` (and is stored locally as `gcal_`). Using `gcal_corrd` results in diffusion coefficients that converge upon the same values with and without non-uniform gradient correction, as the degree of diffusion weighting is reduced (for examlpe, for low signal attenuation).

Display and Plot Options

The Process-NUG Calib panel contains a number of display and plot options, which are outlined below:

Show (plot) apparent D WRT position

Displays (plots) the variation of the apparent diffusion coefficient with respect to position. This apparent variation is due to the variation in gradient strength across the sample. Typically (though not always) the gradient is stronger in the middle of the coil and declines towards its edges (see Figure 6).

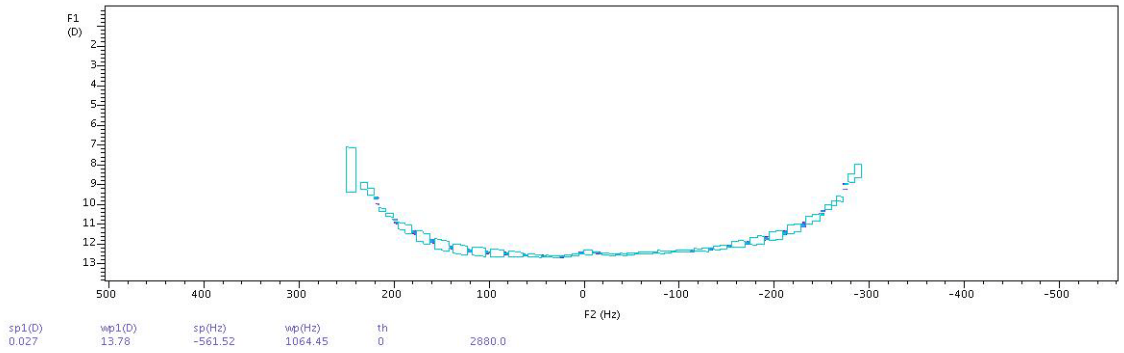


Figure 6 Typical plot of variation in diffusion coefficient version Z (position)

Show (plot) fitted gradient shape

Displays (plots) a comparison between the experimental gradient shape (shown in red), derived from the apparent variation in D across the signal profiles, and the fitted gradient shape (shown in blue) derived using the power series coefficients `Probegradcoeff1` through to `Probegradcoeff9` (see Figure 7 on page 35).

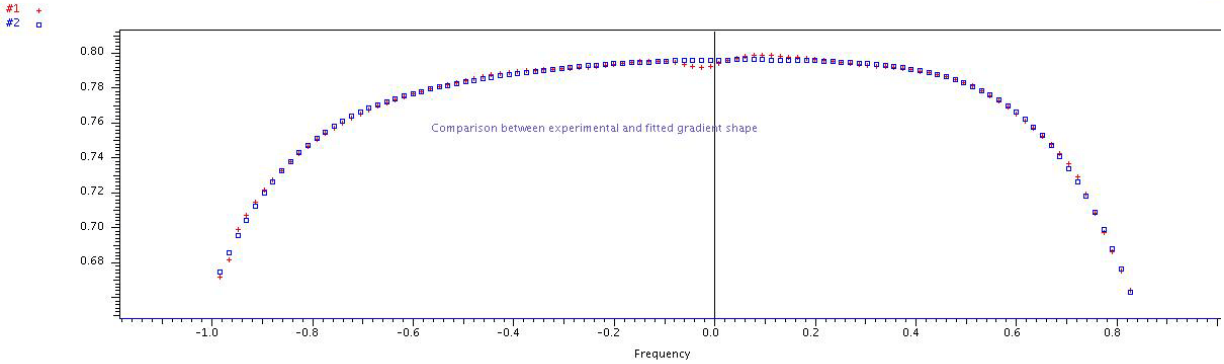


Figure 7 Typical comparison between the experimental and fitted gradient shape produced by the probe.

Show (plot) fitted signal decay

Displays (plots) a semi-logarithmic plot of the calculated (shown in red) versus fitted (shown in blue) signal decays (see Figure 8). There is normally good agreement between these two decays down to -9 (more than 1000-fold attenuation).

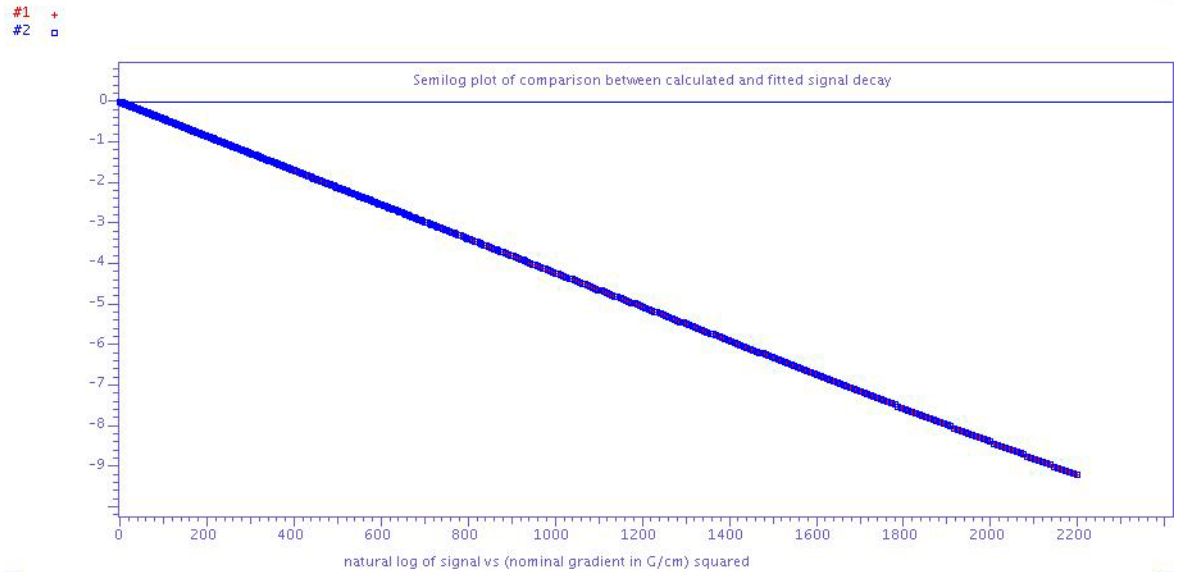


Figure 8 Typical semilog plot of calculated versus fitted signal decay

3 Gradient Calibration and Correction for Gradient Non-Uniformity

4
2D-DOSY Experiments

Setting up Basic 2D-DOSY Experiments

The current DOSY package includes four basic 2D-DOSY sequences: `Dbppste, DgcsteSL, Doneshot`, and `Dbppsteinept`. To set up any of the experiments, start with recording a normal s2pul spectrum on the nucleus to be observed, followed by calibrating (or checking) pulse widths, if necessary. It is a good idea to reduce the spectral window to the region of interest as well as define integral regions for future baseline correction before selecting the requested experiment from the menu or calling the `setup` macro from the command line (which always has the same name as the pulse sequence itself).

Each sequence has a parameter called `delflag`. By setting it to `'y'`, the actual DOSY sequence is activated (default value); the `'n'` option allows going back to the basic s2pul (`Dbppste, DgcsteSL, Doneshot`) or INEPT (`Dbppsteinept`) sequence without changing the experiment workspace or the parameter set.

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. To get an approximate idea for these parameters, use the **Setup coarse gradient array** button in the **Acquire/Pulse Sequence** VnmrJ panel. Alternatively, use the command line to set `gt1=0.002, del=0.05` s and to array the gradient strength: `gzlvl1`=500,5000,15000,20000,25000,`gzlvl1_max` for the Performa II or IV gradient amplifiers, or `gzlvl1`=50,500,1000,1500,`gzlvl1_max` for the Performa I gradient system.

For the maximum gradient power used in the DOSY experiment, select the `gzlvl1` value that attenuates the signal intensities to 5–15 % of the intensities obtained with the weakest gradient pulse. If the intensity drop is not sufficient at the end of the array, `del` or `gt1` may be increased. If no signal is detected towards the end of the array, decrease `del` or `gt1` and repeat the procedure again. Before the final setup, the best baseline

performance should be achieved. With the Agilent-supplied sequences, `alfa`, `rof2`, and `ddrtc` delays should be set to (near) optimum by default. If, however, the spectra still need first order phase correction (`lp` <> 0), use the `setlp0` macro to reach `lp=0` and good baseline performance. After having determined suitable values for `gt1`,`del` and the maximum gradient power, the number of increments, the minimum and maximum gradient power can be set and a suitable gradient array be calculated by clicking on the **Setup DOSY using conditions above** button. The `setup_dosy` macro behind this button sets up a range of `gzlvl1` values with their squares evenly spaced assuring that each gradient strength value will have the same weight when fitting the data to the S-T formula. The minimum gradient strength may be set to 0.5–2 Gauss/cm.

The number of increments to use depends on the range of diffusion coefficients to be covered and on the balance between systematic and random errors, but will typically be in the range of 15 to 30. If significantly different diffusion coefficients are expected in a mixture, more gradient strengths might be needed to have sufficient number of usable data points also for the slowest and the fastest diffusing component. As in any quantitative experiment, there is a balance to be struck between signal-to-noise and accuracy when choosing a repetition rate (`d1`). In DOSY experiments, a delay of 1-2 T_1 suffices, provided that care is taken to establish a steady state before acquiring data. It is recommended to set `ss` to `8` or `16` to have steady-state pulses at the beginning of the experiment and run the acquisition interleaved (set `il='y'`) especially for experiments covering several hours of experimental time.

Each sequence comes with a pulse sequence specific acquisition panel. It enables the operator to set parameters and setup related commands directly. Figure 9 on page 40 shows the acquisition panels of the Doneshot sequence. The **Acquire/Defaults** panels provide access to the most important parameters to set up the experiment. For users with low panel level, this is the only acquisition panel available. The **Acquire/Pulse Sequence** panel lists all relevant sequence related parameters.

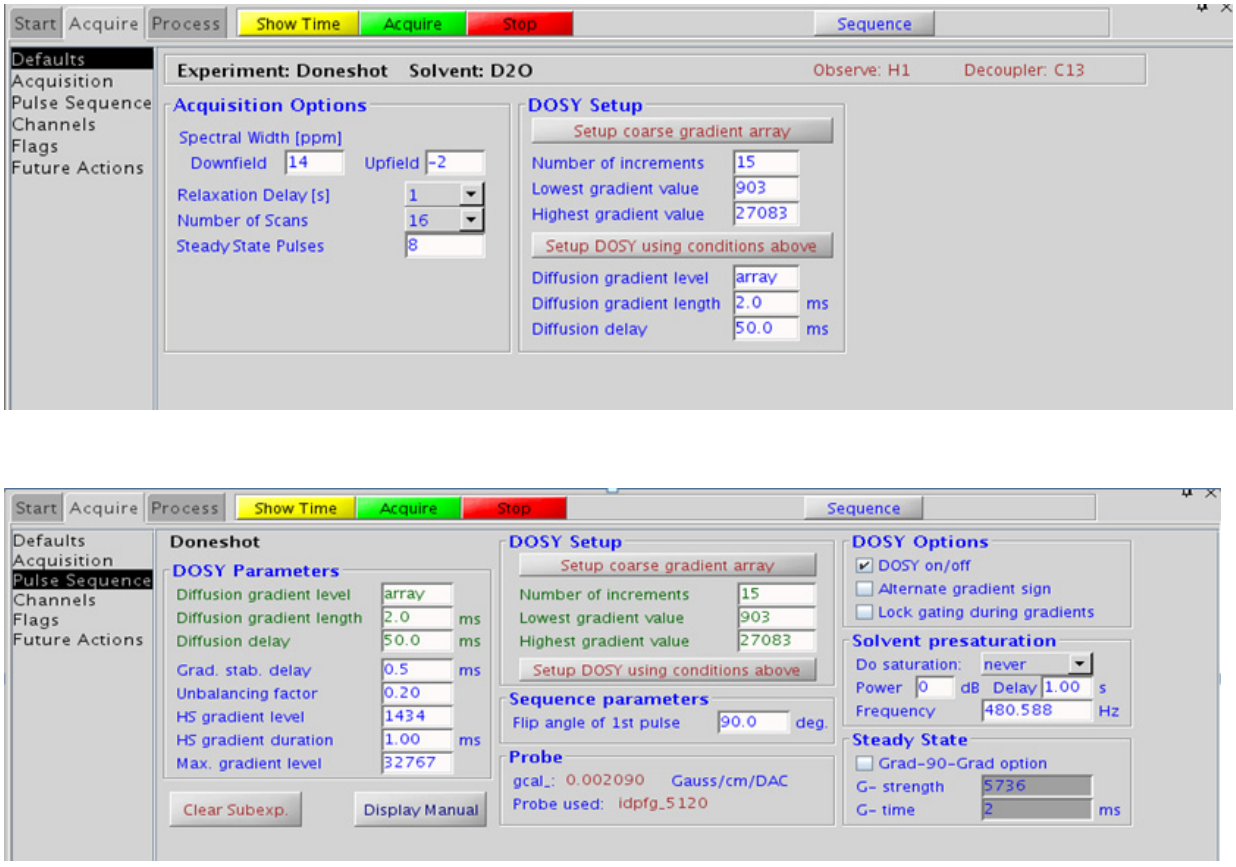
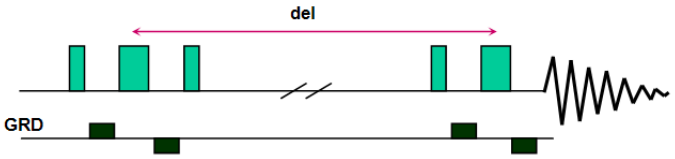


Figure 9 The Acquire/Defaults and Acquire/Pulse Sequence VnmrJ panels of the Doneshot pulse sequence

Simple 2D DOSY Pulse Sequences

Dbppste (DOSY bipolar pulse pair stimulated echo) experiment



Reference: D. Wu, A. Chen, C. S. Johnson, Jr. J. Magn. Reson. 1995, 115, Series(A), 260-264

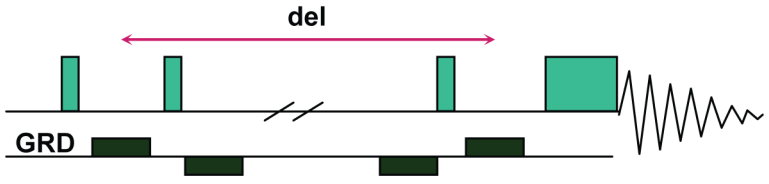
Table 3 Parameters

`delflag`	'y' runs the Dbppste sequence
	'n' runs the normal s2pul sequence
`del`	the actual diffusion delay
`gt1`	total diffusion-encoding pulse strength
`gzlvl1`	diffusion-encoding pulse strength
`gstab`	gradient stabilization delay (~0.0002-0.0003 s)
`satmode`	'y' turns on presaturation during d1 and/or during the diffusion delay
`satfrq`	presaturation frequency
`satdly`	saturation delay (part of d1)
`alt_grd`	flag to invert gradient sign on alternate scans (default='n')
`lkgate_flg`	flag to gate the lock sampling off during the diffusion period (default = 'n')
`sspul`	flag for a GRD-90-GRD homospoil block
`gzlvlhs`	gradient level for sspul
`hsgt`	gradient duration for sspul
`probe_`	stores the probe name used to acquire the DOSY experiment

Table 4 Processing parameters

`ncomp`	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
`nugflag`	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients y' uses a modified S-T equation in which the exponent is replaced by a power series
`nugcal_[1-5]`	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'. Requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition
`dosyproc`	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
`dosybypoints`	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the `selected` threshold

DgcsteSL (DOSY gradient compensated stimulated echo with Spin Lock) experiment



Reference: M. D. Pelta, H. Barjat, G. A. Morris, A .L .Davis, S. J. Hammond, Magn. Reson. Chem. 1998, 36, 706.

Table 5 Parameters

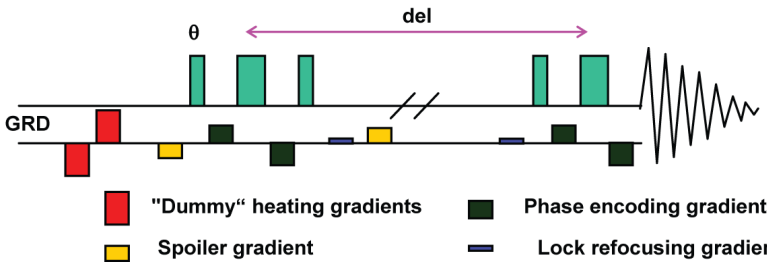
delflag	'y' runs the Dbppste sequence
	'n' runs the normal s2pul sequence
del	the actual diffusion delay
gt1	total diffusion-encoding pulse strength
gzlvl1	diffusion-encoding pulse strength
gstab	gradient stabilization delay (~0.0002-0.0005 s)
tweek	tuning factor to limit eddy currents (can be set between 0 and 1, usually set to 0.0)
prg_flg	'y' selects purging trim pulse 'n' omits purging pulse (default)
prgtime	purging pulse length (~0.002 s) used if prg_flg='y'
prgpwr	power level for the purge pulse (use 6-8 db less power than for tpwr)
lkgate_flg	flag to gate off the lock sampling during gradient pulses (default = 'n')
alt_grd	a flag to invert the gradient sign on alternate scans (default = 'n')
satmode	'y' turns on presaturation during d1 and/or during the diffusion delay
satfrq	presaturation frequency
satdly	saturation delay (part of d1)
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
probe_	stores the probe name used to acquire the DOSY experiment

Table 6 Processing parameters

`ncomp`	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
`nugflag`	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
`nugcal_[1-5]`	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'. Requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.
`dosyproc`	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
`dosybypoints`	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the `selected` threshold

The optional purging pulse (`prg_flg`) can effectively eliminate dispersion signal components. It can also be used as a T_2 relaxation filter to get rid of undesired broad signals. One should, however, be careful not to create convection in the sample through RF heating caused by the trim pulse.

The "Doneshot" experiment



Reference: M. D. Pelta, G. A. Morris, M. J. Tschedroff and S. J. Hammond: MRC 40, 147-152 (2002)

For eliminating radiation damping: M. A. Conell, A. L. Davis, A. M. Kenwright and G. A. Morris: Anal. Bioanal. Chem. 378, 1568-1573, (2004)

Table 7 Parameters

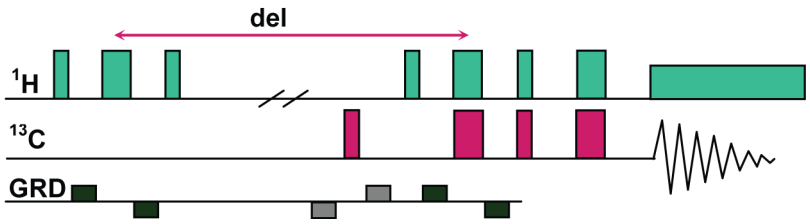
`delflag`	'y' runs the Doneshot sequence 'n' runs the normal s2pul sequence
`del`	the actual diffusion delay
`gt1`	total diffusion-encoding pulse strength
`gzlvl1`	diffusion-encoding pulse strength
`gstab`	gradient stabilization delay (~0.0002-0.0003 s)
`gt3`	spoiling gradient duration (in seconds)
`gzlvl3`	spoiling gradient strength (destroys transverse magnetization during the diffusion delay)
`gzlvl_max`	maximum accepted gradient strength 32767 with Performa II or IV, 2047 with Performa I
`kappa`	unbalancing factor between bipolar pulses as a proportion of gradient strength (~0.2)
`startflip`	flip angle of the first pulse to eliminate radiation damping for very concentrated samples labeled by θ on the pulse sequence above
`alt_grd`	flag to invert gradient sign on alternate scans (default='n')
`lkgate_flg`	flag to gate the lock sampling off during gradient pulses (default='n')
`sspul`	flag for a GRD-90-GRD homospoil block
`gzlvlhs`	gradient level for sspul
`hsgt`	gradient duration for sspul
`satmode`	flag for optional solvent presaturation 'ynn' - does presat during satdly 'yyn' - does presat during satdly and the diffusion delay
`satdly`	presaturation delay before the sequence (part of d1)
`satpwr`	saturation power level
`satfrq`	saturation frequency
`wet`	flag for optional wet solvent suppression
`probe_`	stores the probe name used to acquire the dosy experiment

Table 8 Processing parameters

ncomp	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
nugcal_[1-5]	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'. Requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.
dosyproc	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
dosybypoints	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the selected threshold.

The parameters for the heating gradients (`gt4, gzlvl4`) are calculated in the sequence; they cannot be set directly. The lock refocusing gradient is determined by `kappa` and `gzlvl1`. The dummy heating gradients are automatically adjusted by the sequence. For the maximum gradient power available in the experiment: `gzlvl_max > gzlvl1*(1+kappa)`, the total gradient power transmitted to the sample remains independent of the phase encoding gradient power.

Dbppsteinept (DOSY bipolar pulse pair stimulated echo inept) experiment



Reference : D. Wu, A. Chen and C. S. Johnson, Jr., J. Magn. Reson. Series A, 123, 222-225 (1996)

Table 9 Parameters

`delflag`	'y' runs the dosyinept 'n' runs the normal inept without dosy
`del`	the actual diffusion delay
`gt1`	total length of the phase encoding gradient
`gzlvl1`	strength of the phase encoding gradient
`pp`	90 deg. hard ^{1}H pulse
`pplvl`	decoupler power level for pp pulses
`sspul`	flag for a GRD-90-GRD homospoil block
`gzlvlhs`	gradient level for sspul
`hsgt`	gradient duration for sspul
`sspulX`	flag for a GRD-90-GRD homospoil block during del to destroy original X magnetization (using hsgt and gzlvlhs)
`j1xh`	one-bond X-H coupling
`mult`	multiplicity: 1 selects CH's (doublets) 1.5 gives CH2's down, CH's and CH3's up 0.5 enhances all protonated carbons
`alt_grd`	flag to invert gradient sign on alternate scans (default = 'n')
`lkgate_flg`	flag to gate the lock sampling off during gradient pulses
`probe_`	stores the probe name used to acquire the dosy experiment

Table 10 Processing parameters

`ncomp`	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
`nugflag`	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
`nugcal_[1-5]`	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'. Rrequires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.
`dosyproc`	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
`dosybypoints`	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the selected threshold

This sequence uses the higher "resolving power" of the wide ^{13}C chemical shift range, while the phase encoding and decoding step is done more effectively on the ^{1}H magnetization.

DOSY Pulse Sequences for H_2O Samples

For biological samples dissolved in H_2O/D_2O 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.

DgcsteSL_dpfgse - (DOSY gradient compensated stimulated echo with Spin Lock) experiment using the DPFGSE solvent suppression method

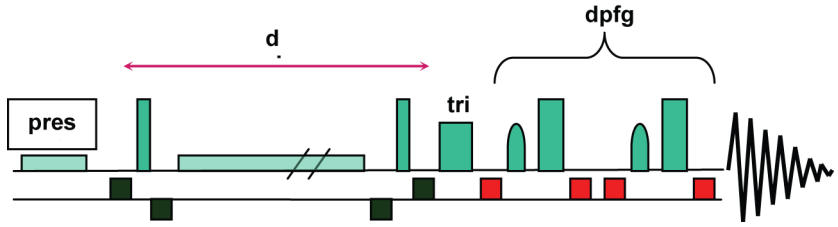


Table 11 Parameters

`delflag`	'y 'runs the DgcsteSL sequence 'n' runs the normal s2pul sequence
`del`	the actual diffusion delay
`gt1`	total diffusion-encoding pulse width
`gzlvl1`	diffusion-encoding pulse strength
`gstab`	gradient stabilization delay (~0.0002-0.0003 s)
`tweek`	tuning factor to limit eddy currents, (can be set from 0 to 0.2, usually set to 0.0)
`prg_flg`	'y' selects purging pulse (default) 'n' omits purging pulse
`prgtime`	purging pulse length (~0.002 s), used if prg_flg='y'
`prgpwr`	purging pulse power, used if prg_flg='y'
`lkgate_flg`	lock gating flag, if set to 'y', the lock is gated off during gradient pulses (default = 'n')

Table 11 Parameters (continued)

satmode	flag for optional solvent presaturation 'ynn' - does presat during satdly 'yyn' - does presat during satdly and the diffusion delay
satdly	presaturation delay before the sequence (part of d1)
satpwr	saturation power level
satfrq	saturation frequency
wrefshape	shape file of the 180 deg. selective refocusing pulse on the solvent (may be convoluted for multiple solvents)
wrefpw	pulse width for wrefshape (as given by Pbox)
wrefpwr	power level for wrefshape (as given by Pbox)
wrefpwrf	fine power for wrefshape, by default 2048, needs optimization for multiple solvent suppression with fixed wrefpw
gt2	gradient duration for the solvent suppression echo
gzlvl2	gradient power for the solvent suppression echo
alt_grd	alternate gradient sign(s) on even transients (default = 'n')
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
probe_	stores the probe name used to acquire the dosy experiment

Table 12 Processing parameters

ncomp	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
nugcal_[1-5]	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'. Requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition

Table 12 Processing parameters (continued)

dosyproc	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
dosybypoints	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the selected threshold.

The water refocusing shape can be created/updated using the **Recreate water refocusing shape** button (update_wrefshape macro).

Dbppste_wg - (DOSY bipolar pulse pair stimulated echo) experiment using watergate 3-9-19 solvent suppression

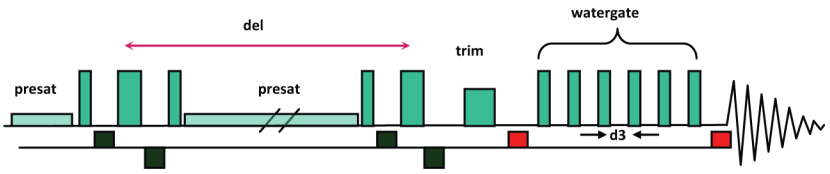


Table 13 Parameters

delflag	'y' runs the Dbppste sequence 'n' runs the normal s2pul sequence
del	the actual diffusion delay
gt1	total diffusion-encoding pulse width
gzlvl1	diffusion-encoding pulse strength
alt_grd	alternate gradient sign(s) on even transients (default = 'n')
lkgate_flg	flag to gate the lock sampling off during the diffusion sequence
d3	watergate delay, the excitation maximum is defined by 1.0/(2.0*d3)

Table 13 Parameters (continued)

ex_max	excitation maximum from the XMTR position (= 1/(2*d3)
gt2	watergate diffusion-encoding pulse width
gzlvl2	watergate encoding pulse strength
gstab	gradient stabilization delay (~0.0002-0.0003 s)
satmode	'y' turns on presaturation during d1 and/or del
satdly	presaturation delay before the sequence (part of d1)
satpwr	saturation power level
satfrq	saturation frequency
prg_flg	'y' selects purging pulse (default) 'n' omits purging pulse
prgtime	purging pulse length (~0.002 s)
prgpwr	purging pulse power
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
probe_	stores the probe name used to acquire the dosy experiment

Table 14 Processing parameters

ncomp	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
nugcal_[1-5]	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'; requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.

Table 14 Processing parameters (continued)

`dosyproc`	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 ‘continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
`dosybypoints`	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the selected threshold

Convection and Convection-Compensation in Diffusion Experiments

Convection within the sample tube 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.

The convection conditions are described by the *Rayleigh-Bénard* equation:

$$R_a = \frac{g\beta R^4}{\nu\chi}\frac{\partial T}{\partial z}$$

R_a = 67 for insulating walls
R_a = 216 for conducting walls

where g is the gravitational acceleration, v is the viscosity, χ the thermal diffusivity, β the expansion coefficient of the liquid, R the internal diameter of the NMR tube, and δT/δz the temperature gradient along the sample axis. When the critical Rayleigh number (R_a) is exceeded, convection will occur.

Convection typically causes the following anomalies in diffusion experiments:

- Anomalously large diffusion coefficients (D)
- D values that are not independent of gradient duration (δ) and the diffusion delay (`del`)
- Stejskal-Tanner plots that show periodicity
- Irregular (non-Arrhenius) temperature dependence of D

A simple calculation based on the Rayleigh-Bénard equation indicates that, for a solvent like chloroform, a temperature gradient of as little as 0.05 K/cm is sufficient to cause convection flow. In general, larger temperature gradients are needed for more viscous solvents.

In a typical DOSY experiment, a uniform sample flow velocity v introduces a phase modulation of the signal:

$$S(G_{zi}) = S(0)\exp(-D_i\gamma^2\delta^2(G_{zi})^2\Delta) * \exp(i\gamma\delta G_{zi}v\Delta))$$

Representing convection by a crude model of equal and opposite flows each of uniform velocity leads to cancellation of the imaginary part above and the result is a cosine modulation:

$$S(G_{zi}) = S(0)\exp(-D_i\gamma^2\delta^2(G_{zi})^2\Delta * \cos(\gamma\delta G_{zi} v\Delta))$$

Observing such an oscillatory behavior of the signal decay (see Figure 14 on page 70) is a clear sign that convection occurs.

With the assumption that convection is constant in time and is strictly laminar, its effect on diffusion spectra can be efficiently eliminated. Figure 10 displays the necessary modifications (orange box) on a gradient stimulated echo pulse sequence. Halfway through the diffusion delay, the magnetization is moved back to the transverse plane by a 90° pulse and gets refocused by the first (green) gradient pulse. The second green gradient, identical in sign, duration and length to the previous one, phase labels the spins in the opposite direction, then the magnetization in converted back to axial for the second half of the diffusion delay. The ordered nature of convection assures that the phase evolution due to convection is opposite during the two halves of the diffusion delay and therefore compensate each other, while diffusion - being a random (and omnidirectional) process - does not get affected. In order to detect only desired coherences, homospoil gradient pulses (shown in red) are used in both halves of the diffusion delay.

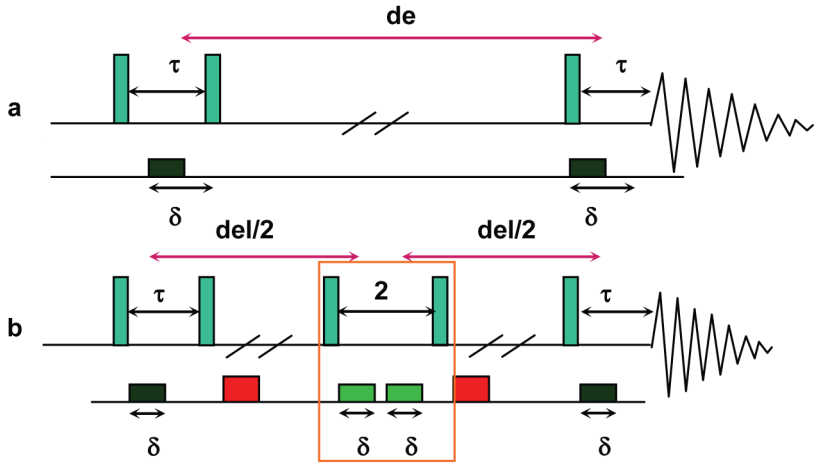


Figure 10 Modification of a gradient stimulated echo experiment with convection compensation

In the VnmrJ DOSY 3 package, four pulse sequences are provided with convection compensation:

1 **DgsteSL_cc** (Gradient STimulated Echo with Spin-Lock and Convection Compensation) has only the absolute minimum number of gradients (six) necessary for the pulse sequence to work.

2 **DgcsteSL_cc** (Gradient Compensated STimulated Echo with Spin-Lock and Convection Compensation) is a direct derivative of the DgcsteSL sequence and contains an identical number of positive and negative gradients to provide "internal" Eddy-current compensation as well. Note that of the 12 gradient pulses used in the pulse sequence, only two (the black ones) are used to measure diffusion.

3 **Dbppste_cc** (Bipolar Pulse Pair STimulated Echo with Convection Compensation) is a direct derivative of the Dbppste sequence. Apart from the "heating gradients" the pulse sequence has got all the features of the Doneshot sequence discussed earlier.

4 **Dpfgdste** (Pulse Field Gradient Double STimulated Echo) is a variant of the DgcsteSL_cc sequence with no Spin Lock and with a different phase cycle.

Important sensitivity note: Every pulse sequence with convection compensation contains an extra stimulated echo step and therefore has an inherent **50% signal attenuation** with respect to its equivalent without convection compensation. If the experimental conditions exclude the possibility of convection, then the non-compensated pulse sequences should be used, as they can provide twice the signal-to-noise. This does not apply to the I-DOSY type experiments discussed in “IDOSY (Inclusive DOSY) Experiments” on page 95.

How can one find out whether convection is present in the NMR sample and, if yes, how serious its effect can be on the diffusion measurements? The most sensitive test can be provided by the NMR pulse sequence itself used to measure diffusion. It is easy to understand that complete convection compensation can only be achieved if the compensation block (orange box in Figure 10 on page 55) is applied exactly halfway through the diffusion delay. Therefore, if the block is shifted towards either the beginning or the end of the diffusion delay (for example, time symmetry is broken), then signal attenuation and/or phase distortion will be obtained in the presence of convection. While without convection the signal amplitudes and phases must stay unaffected. Each pulse sequence below has an auxiliary delay parameter (`del2`) allowing the operator to move the convection compensation block systematically along the diffusion delay

and by doing so to record a so called "velocity profile". This can be used either for qualitative (seeFigure 11) or quantitative characterization of convection in diffusion experiments (for details see: N.M. Loening and J. Keeler, J. Magn. Reson. 139, 334-341 (1999).)

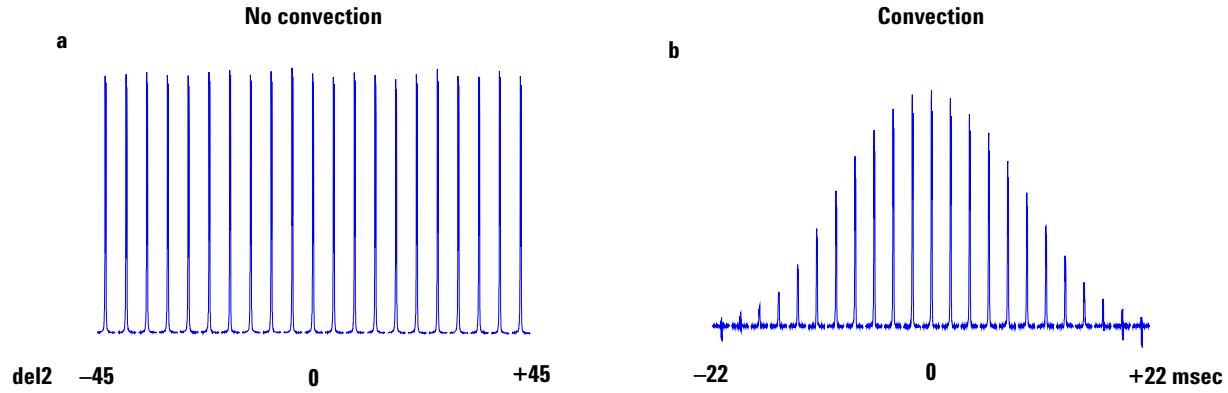
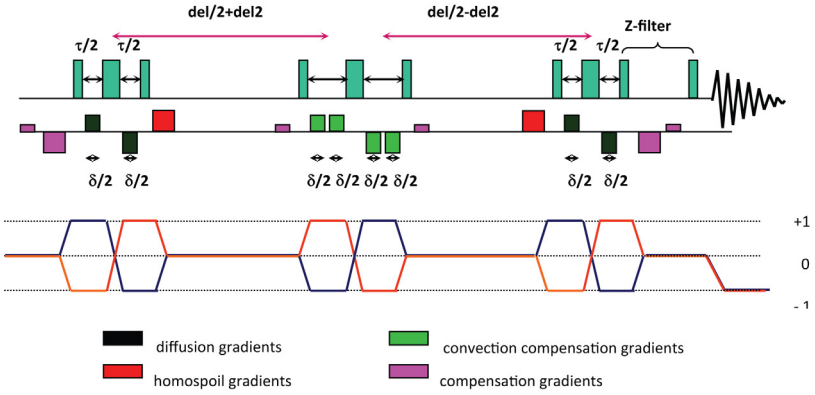


Figure 11 Velocity map (signal intensities as a function of the del2 delay) of a sample dissolved in D_2O using the Dbppste_cc pulse sequence and identical gradient conditions (6 G/cm):
a, temp = 25° C (del = 120 ms, del2 varies from –45 to + 45 ms in 5 ms steps)
b, temp = 60° C (del = 60 ms, del2 varies from –22 to + 22 ms in 2 ms steps)

Pulse sequences with convection compensation

Dbppste_cc (DOSY bipolar pulse pair stimulated echo with convection compensation)



Reference: A. Jerchow and N. Müller, J. Magn. Reson. 125, 372-375 (1997).

Table 15 Parameters

delflag	'y' runs the Dbppste_cc sequence 'n' runs the normal s2pul sequence
del	the actual diffusion delay
del2	delay parameter that can shift the convection compensation sequence elements off the center of the pulse sequence allowing to run a velocity profile can also be negative but in absolute value cannot exceed del/2 minus the gradient and gradient-stabilization delays (default value for diffusion measurements is zero)
gt1	total diffusion-encoding pulse width
gzlvl1	diffusion-encoding pulse strength
gstab	gradient stabilization delay (~0.0002-0.0003 s)
lkgate_flg	lock gating flag, if set to 'y', the lock is gated off during gradient pulses (default = 'n')
satmode	flag for optional solvent presaturation 'ynn' - does presat during satdly 'yyn' - does presat during satdly and the diffusion delay
satdly	presaturation delay before the sequence (part of d1)
satpwr	saturation power level
satfrq	saturation frequency
alt_grd	alternate gradient sign(s) on even transients (default = 'n')
triax_flg	flag for using triax gradient amplifiers and probes 'y' - homospoil gradients are applied along X- and Y- axis all the diffusion gradients are Z-gradients 'n' - all gradients in the sequence are Z-gradients
gt2	1st homospoil gradient duration
gzlvl2	1st homospoil gradient power level executed as X-gradient if triax_flg is set and triax amplifier and probe is available
gt3	2nd homospoil gradient duration
gzlvl3	2nd homospoil gradient power level executed as Y-gradient if triax_flg is set and triax amplifier and probe is available
wet	flag for optional wet solvent suppression
sspul	flag for a GRD-90-GRD homospoil block

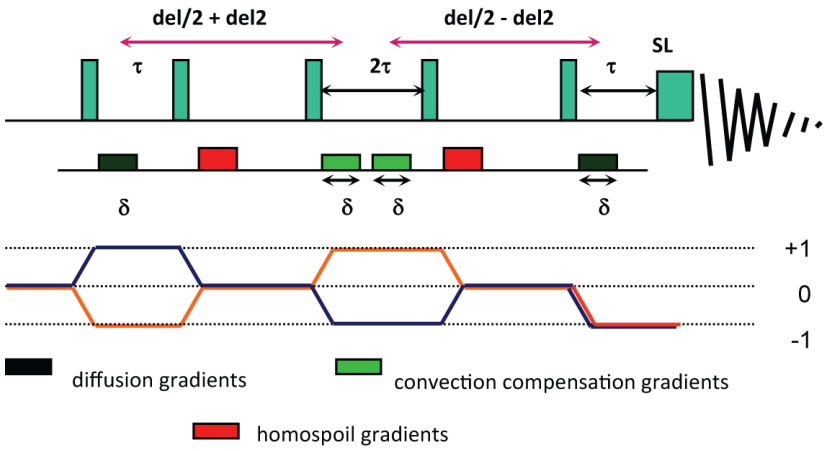
Table 15 Parameters (continued)

gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
probe_	stores the probe name used to acquire the dosy experiment

Table 16 Processing parameters

ncomp	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
nugcal_[1-5]	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'. Requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.
dosyproc	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
dosybypoints	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the selected threshold

DgsteSL_cc (DOSY gradient stimulated echo with Spin Lock and convection compensation)



Reference: A. Jerchow and N. Müller, J. Magn. Reson. 125, 372-375 (1997).

Table 17 Parameters

`delflag`	'y' runs the DgscteSL_cc sequence 'n' runs the normal s2pul sequence
`del`	the actual diffusion delay
`del2`	delay parameter that can shift the convection compensation sequence elements off the center of the pulse sequence allowing to run a velocity profile can also be negative but in absolute value cannot exceed del/2 minus the gradient and gradient-stabilization delays (default value for diffusion measurements is zero)
`gt1`	total diffusion-encoding pulse width
`gzlvl1`	diffusion-encoding pulse strength
`gstab`	gradient stabilization delay (~0.0002-0.0003 s)
`alt_grd`	flag to invert gradient sign on alternate scans (default = 'n')
`lkgate_flg`	flag to gate the lock signal during diffusion gradient pulses
`triax_flg`	flag for using triax gradient amplifiers and probes 'y' - homospoil gradients are applied along X- and Y- axis all the diffusion gradients are Z-gradients 'n' - all gradients in the sequence are Z-gradients
`gt2`	1st homospoil gradient duration

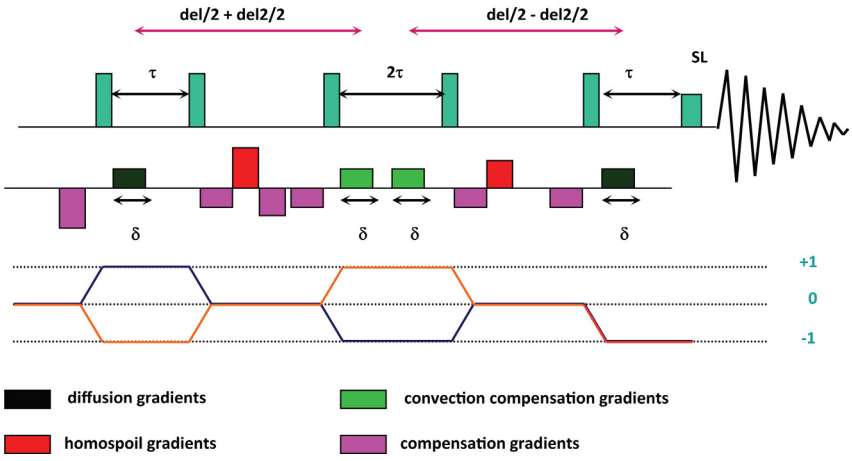
Table 17 Parameters (continued)

gzlvl2	1st homospoil gradient power level executed as X-gradient if triax_flg is set and triax amplifier and probe is available.
gt3	2nd homospoil gradient duration
gzlvl3	2nd homospoil gradient power level executed as Y-gradient if triax_flg is set and triax amplifier and probe is available
prg_flg	'y' selects purging pulse (default) 'n' omits purging pulse
prgtime	purging pulse length (~0.002 s), used if prg_flg='y'
prgpwr	purging pulse power, used if prg_flg='y'
satmode	flag for optional solvent presaturation 'ynn' - does presat during satdly 'yyn' - does presat during satdly and the diffusion delay
satdly	presaturation delay before the sequence (part of d1)
satpwr	saturation power level
satfrq	saturation frequency
wet	flag for optional wet solvent suppression
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
probe_	stores the probe name used to acquire the dosy experiment

Table 18 Processing parameters

`ncomp`	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
`nugflag`	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
`nugcal_[1-5]`	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'. Requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition
`dosyproc`	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
`dosybypoints`	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the selected threshold

DgcsteSL_cc (DOSY gradient compensated stimulated echo with Spin Lock and convection compensation



Reference: A. Jerchow and N. Müller, J. Magn. Reson. 125, 372-375 (1997).

Table 19 Parameters

delflag	'y' runs the DgcsteSL_cc sequence 'n' runs the normal s2pul sequence
del	the actual diffusion delay
del2	delay parameter that can shift the convection compensation sequence elements off the center of the pulse sequence allowing to run a velocity profile can also be negative but in absolute value cannot exceed del/2 minus the gradient and gradient-stabilization delays (default value for diffusion measurements is zero)
gt1	total diffusion-encoding pulse width
gzlvl1	diffusion-encoding pulse strength
gstab	gradient stabilization delay (~0.0002-0.0003 s)
alt_grd	flag to invert gradient sign on alternate scans (default = 'n')
lkgate_flg	flag to gate the lock signal during diffusion gradient pulses
triax_flg	flag for using triax gradient amplifiers and probes 'y' - homospoil gradients are applied along X- and Y- axis all the diffusion gradients are Z-gradients 'n' - all gradients in the sequence are Z-gradients
gt2	1st homospoil gradient duration
gzlvl2	1st homospoil gradient power level executed as X-gradient if triax_flg is set and triax amplifier and probe is available
gt3	2nd homospoil gradient duration
gzlvl3	2nd homospoil gradient power level executed as Y-gradient if triax_flg is set and triax amplifier and probe is available
prg_flg	'y' selects purging pulse (default) 'n' omits purging pulse
prgtime	purging pulse length (~0.002 s), used if prg_flg='y'
prgpwr	purging pulse power, used if prg_flg='y'
wet	flag for optional wet solvent suppression
satmode	flag for optional solvent presaturation 'ynn' - does presat during satdly 'yyn' - does presat during satdly and the diffusion delay

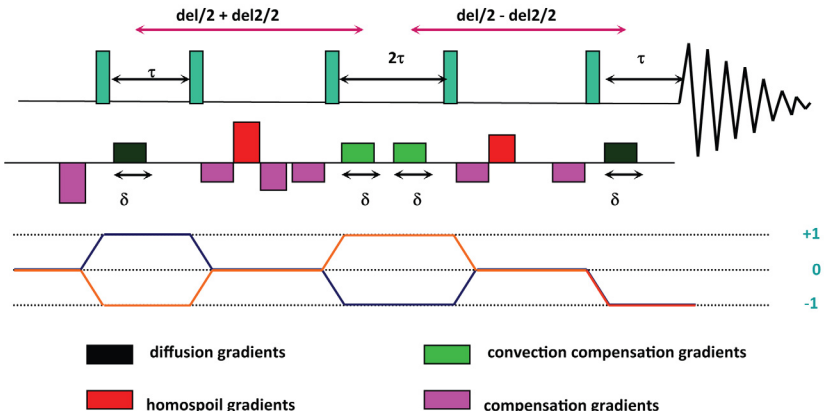
Table 19 Parameters (continued)

satdly	presaturation delay before the sequence (part of d1)
satpwr	saturation power level
satfrq	saturation frequency
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
probe_	stores the probe name usedto acquire the dosy experiment

Table 20 Processing parameters

ncomp	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
nugcal_[1-5]	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'; requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition
dosyproc	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
dosybypoints	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the selected threshold

Dpfgdste (DOSY pulsed field gradient double stimulated echo)



Reference: M. Nilsson, A. M. Gil, I. Delgadillo, G. A. Morris, Anal Chem 2004.76:5418-5422

Table 21 Parameters

`delflag`	'y' runs the Dpfgdste_cc sequence 'n' runs the normal s2pul sequence
`del`	the actual diffusion delay
`del2`	delay parameter that can shift the convection compensation sequence elements off the center of the pulse sequence allowing to run a velocity profile. Can also be negative but in absolute value cannot exceed del minus the gradient and gradient-stabilization delays (default value for diffusion measurements is zero)
`gt1`	total diffusion-encoding pulse width
`gzlvl1`	diffusion-encoding pulse strength
`gzlvl3`	2nd homospoil gradient power level executed as Y-gradient if triax_flg is set and triax amplifier and probe is available
`gt3`	2nd homospoil gradient duration
`gzlvl2`	1st homospoil gradient power level executed as X-gradient if triax_flg is set and triax amplifier and probe is available.
`gstab`	gradient stabilization delay (~0.0002-0.0003 s)
`satmode`	'y' turns on presaturation during d1and/ or during the diffusion delay
`satfrq`	saturation frequency
`satdly`	presaturation delay before the sequence (part of d1)
`satpwr`	saturation power level

Table 21 Parameters (continued)

wet	flag for optional wet solvent suppression
alt_grd	flag to invert gradient sign on alternate scans (default = 'n')
lkgate_flg	flag to gate the lock signal during diffusion gradient pulses
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
probe_	stores the probe name used to acquire the dosy experiment

Table 22 Processing parameters

ncomp	determines the number of components to be used in fitting the signal decay in DOSY when dosyproc='discrete'
nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
nugcal_[1-5]	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y', requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.
dosyproc	'discrete' - invokes monoexponential fitting with dosyfit if ncomp=1, and multiexponential fitting with the external program SPLMOD if ncomp>1 'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain
dosybypoints	'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum 'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the selected threshold.

Comparison of diffusion results obtained with and without convection compensation

This chapter compares experimental results of an aqueous solution of a mixture of nicotinic acid amide and amikacin (see Figure 12 on page 67 for the structural formulas). The diffusion experiments were performed without (sequence: Dbppste) and with convection compensation (sequence: `Dbppste_cc`) at two different temperatures: 30° C and 60° C, respectively, in a 5 mm sample tube. One may think that this sample in not particularly challenging because the components differ significantly in size (or molecular weight) and there is no signal overlap between the aromatic and the sugar protons in the 500 MHz spectrum of the mixture. Both components, however, contain numerous proton lines (remember the DOSY analysis handles multiplet components individually) and therefore the sample is particularly suitable to provide information about the accuracy of the diffusion data.

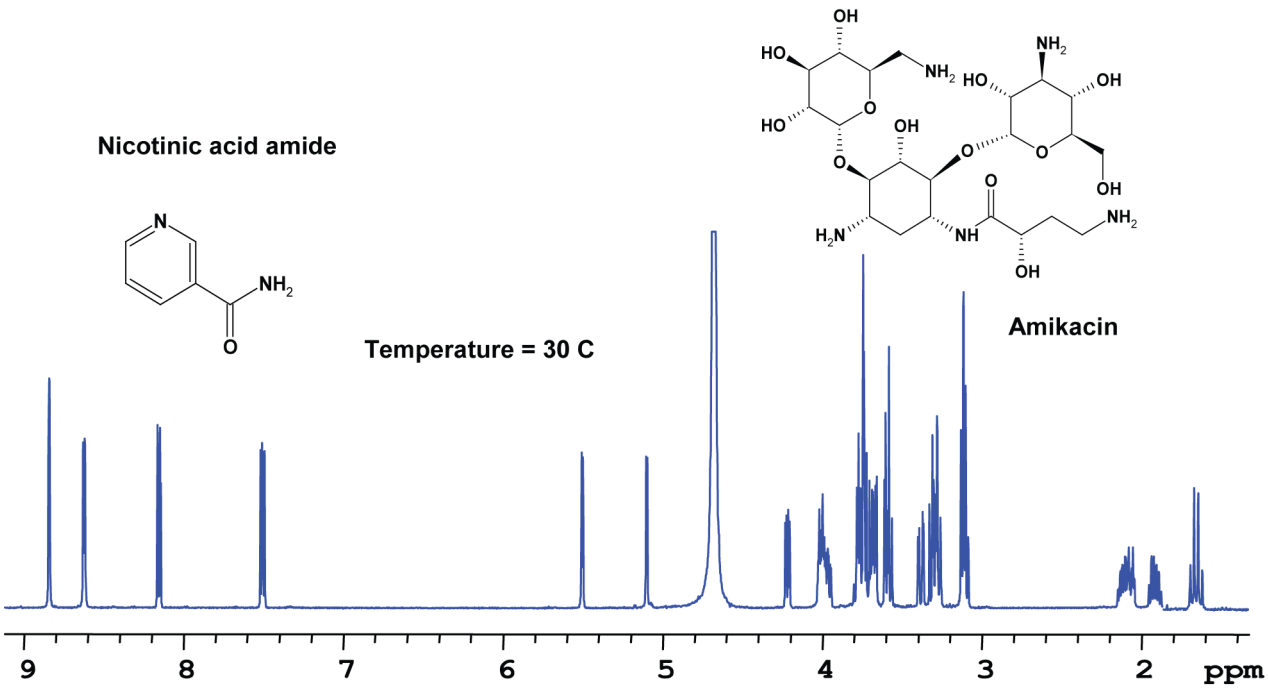


Figure 12 500 MHz proton spectrum of a nicotinic acid amide - amikacin mixture in D_2O at 30° C

The two pulse sequences at 30° C were run with identical diffusion delays (`del = 120 ms`), gradient duration (`gt1 = 2 ms`) and the same 20 values of gradient strengths (varied between 1 and 30 G/cm). A visual inspection of the signal

intensities (see Figure 13 on page 69) does not reveal obvious anomalies, for example, the individual lines show exponentially attenuated intensities with increasing gradient power (please note that the HDO signal is truncated in the first 9 spectra). The DOSY analysis (see Figure 13 on page 69), as expected, shows clear separation of the two components (and the solvent) along the diffusion axis. Practically all proton signals of the same molecule exhibit the same diffusion coefficient indicating high "relative" accuracy of the calculated D values. From this point of view, there is no difference between the results of the two different measurements. Consequently, if the only aim is the separation of the NMR spectra of the mixture components, then the `Dbppste` sequence may be preferred, as it has twice the sensitivity than that of its convection compensated counterpart (`Dbppste_cc`).

A comparison of the extracted D values, however, reveals that the coefficients with no convection compensation tend to be consequently bigger. The slower the diffusion of a certain component, the bigger is the deviation (21% on amikacin but only 9% on the water). We may conclude that convection is clearly having its "contribution" to the calculated D values leading to a false suggestion as if the molecules were having higher mobility than in reality.

Moving away from ambient temperatures will definitely increase the risk of convection. This is clearly demonstrated by repeating the previous pair of experiments at 60° C. In Figure 14 on page 70, the attenuation of the signal intensities are far from being exponential (in reality they show an oscillatory behavior) and apart from the amplitude distortions serious phase deviations may also occur (see the inset of 3rd spectrum in Figure 14 on page 70. The exponential fit of the signal amplitudes in the experiment with no convection compensation is extremely large. Therefore, the "resolution" of the 2D DOSY plot (see Figure 14 on page 70) is dramatically reduced and the experimental results are hardly usable. At the same time, the convection compensated pulse sequence provides excellent diffusion separation and reliable diffusion data.

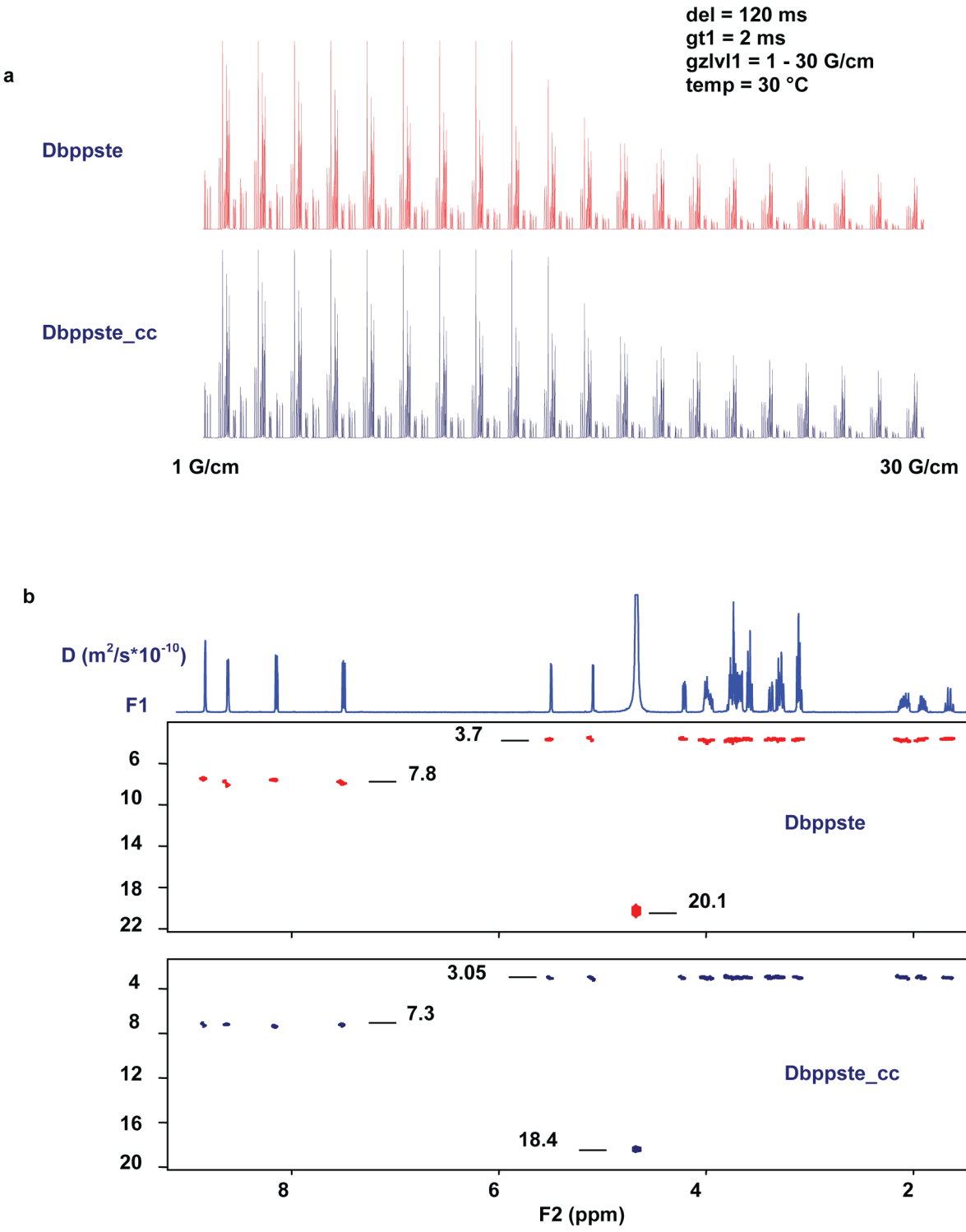


Figure 13 Signal intensities as a function of gradient power (a) and the 2D DOSY plots (b) of the Nicotinic acid amide - amikacin mixture at 30° C

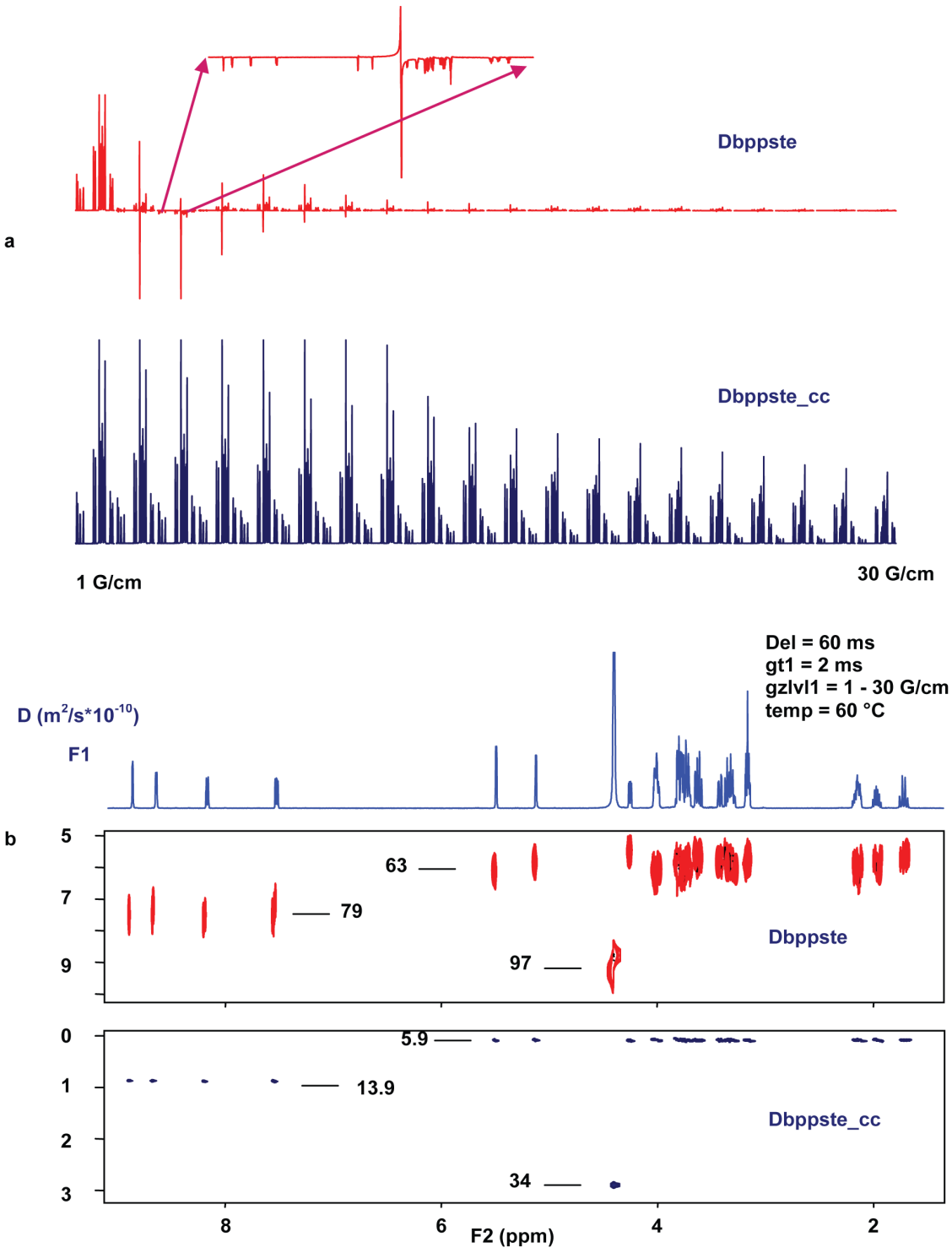


Figure 14 Signal intensities as a function of gradient power (a) and the 2D DOSY plots (b) of the nicotinic acid amide - amikacin mixture at 60° C

Processing 2D-DOSY Experiments

Once DOSY data have been acquired, they need to be processed to give a 2D DOSY spectrum. This involves the following steps:

1 Basic Fourier transformation of the raw data.

2 Reference deconvolution (`fiddle` command) - optional, but useful if the spectrum contains a suitable reference line which diffuses with comparable speed as the solutes in the diffusion sample.

3 Baseline correction (`fbc` macro) - also optional but strongly recommended.

4 Extraction of diffusion data from the spectra and synthesis of a 2D DOSY display (`dosy`).

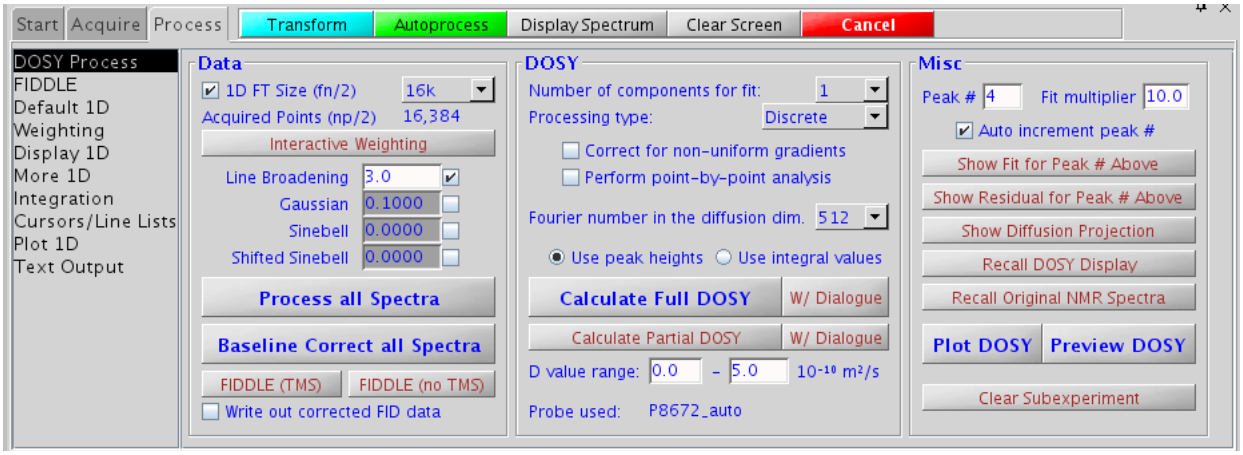


Figure 15 The VnmrJ DOSY Process panel for 2D-DOSY pulse sequences

5 After data acquisition with a DOSY experiment, a matching weighting function and zero filling (`fn`) should be chosen before Fourier transforming the FID array using the **Process all Spectra** button (executes `wft` command) on the **DOSY Process** panel (see Figure 15). Retrieving a DOSY FID from a disk usually leads to auto-processing - Fourier transformed array data arranged horizontally (`wft dssh`).

6 The `fiddle` program (**FIDDLE** panel, Figure 16 on page 72) allows reference deconvolution to be used to correct the line shapes, frequencies, phases and so forth of signals due to by instrumental imperfections, if a suitable reference signal is present in the spectrum (typically a singlet). Reference deconvolution of DOSY spectra removes systematic errors resulting from disturbance of the magnetic field and field/frequency lock caused by gradient pulses.

Typically, set the weighting function of the ideal lineshape to a value that is minimum as large as the widest signal in the spectrum. Select a reference line using two cursors, click **Select**, then **Do FIDDLE**. In case a TMS signal should be used for reference deconvolution, the **FIDDLE (TMS)** button on the **DOSY Process** panel or the **Include TMS satellite signals** check box on the **FIDDLE** panel should be used (required to fit the satellites of TMS correctly).

It is recommended to save the corrected data to disk by selecting the **Write out corrected data** option before clicking **Do FIDDLE** and use this data for further processing (using `fiddle` with the `"writefid"` option rewrites to the original FID file). After loading this corrected dataset, set all the weighting parameters to 'n' to before Fourier transforming and proceeding to the next step.

Further instructions for the use of FIDDLE are shown by the **Display FIDDLE manual** button and more information can be found in the *User Guide: Liquids NMR*, and in the *Command and Parameter Reference* manual.

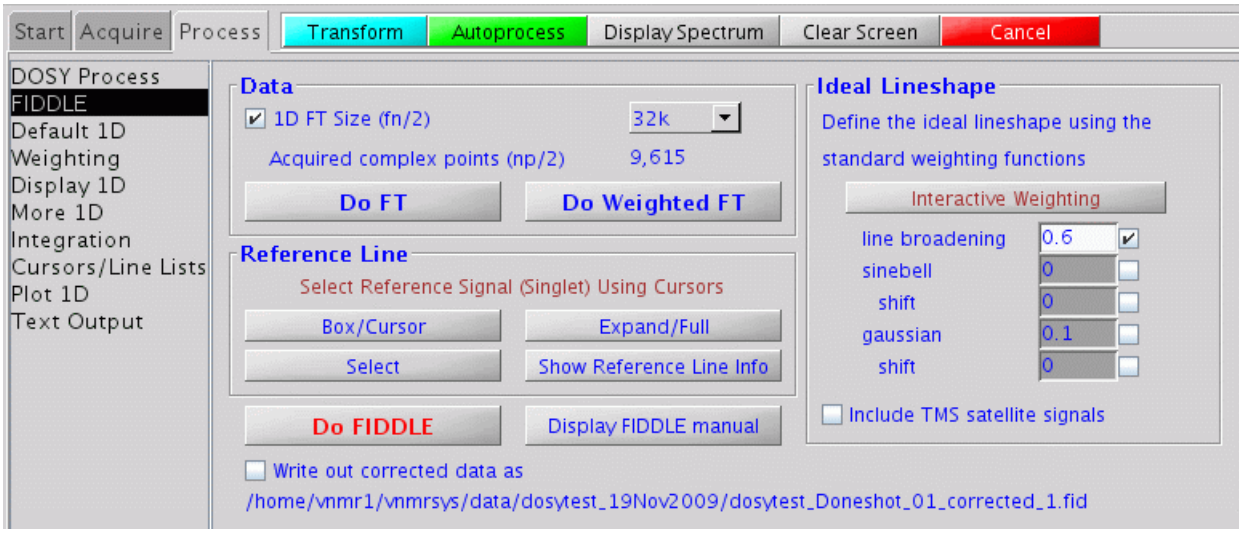


Figure 16 The VnmrJ FIDDLE panel for 2D-DOSY pulse sequences

7 Baseline correction can now be applied to all spectra in the array with the **Baseline Correct all Spectra** button (`fbc` macro). The partial integral mode should be used to set integral regions to include all signals, while leaving as large an area of baseline as possible blank. This minimizes systematic errors in diffusion coefficient fits caused by baseline errors.

8 **Calculate Full DOSY** now generates the final DOSY 2D display (`dosy` macro). This processing determines the heights of all signals above the threshold (`th`) using the commands `dll` and `fp`, and then fits the decay curve for each signal to a Gaussian (using the program `dosyfit`). After this, the 1D process panels are replaced with 2D process panels.

To return back to the original FIDs, use the **"Recall original NMR spectra"** button (`redosy` macro).

WARNING **Do not process the data with Calculate Full DOSY (`dosy` macro) until the acquisition has been completed. Data loss may occur.**

A summary of all diffusion coefficients and their estimated standard errors as well as various other results are stored in the directory userdir/expN/dosy:

- diffusion_display.inp
- diffusion_integral_spectrum
- diffusion_spectrum
- dosy_in
- fit_errors
- general_dosy_stats

The spectrum synthesized contains `fn1`/2 traces in the diffusion domain (`f1`), and `fn` complex points in the spectral domain (`f2`); `fn1` is limited to the range 128–1024. Normally, setting `fn` to 16–64k suffices; if `fn*fn1` is too large, spectral synthesis and display will be slow and/or VnmrJ may run out of disk space.

NOTE After displaying a 2D spectrum, the variable `ni` will be set to `fn1`/2 (this is required by `dconi`), so if more data are to be acquired or the sequence is to be displayed (`dps`), `ni` must be set back to zero.

By default, `dosy` uses all the experimental spectra and covers the whole diffusion range seen in the experimental peaks. If desired, the diffusion dimension can be calculated in part only, for example to have higher display resolution with a lower Fourier number (which "costs" calculation time). To do this, choose a *D* value range and click the **Calculate Partial DOSY** button.

Additionally, selected spectra (data points) can be disregarded during DOSY calculation by clicking the **W/ Dialogue** buttons

instead. A dialog in the command line will start which spectra shall be omitted.

These functions represent up to three arguments which can be supplied to the `dosy` macro:

- `dosy('prune')`- starts a dialog to allow one or more spectra to be omitted from the analysis.
- `dosy(d1,d2)` - where `d1` and `d2` are numbers causes the diffusion range of the synthesized spectrum to be limited to `d1`*10^{-10} m2/s and `d2`*10^{-10} m2/s;
- `dosy('prune',d1,d2)` - combines the above options.

The message “Systematic Gz deviations found” indicates that the decay curves are not purely exponential - very likely due to spatially non-uniform gradients. The non-linearity can be corrected during processing if NUG calibration data were available in the probe file when the experiment was set up.

The two-dimensional DOSY display (and plot) is constructed by taking the bandshape of a given signal from the first (lowest gradient area) spectrum, and convoluting it in a second dimension with a Gaussian line centered at the calculated diffusion coefficient and with a width determined by the estimated error of the diffusion coefficient obtained from the fitting process.

To extract spectra of the mixture components separated along the diffusion axis, select the region of interest using the two cursors in the interactive 2D display (`dconi`) mode, click on **Proj** (projection) and **Hproj(sum)** (horizontal projection). The spectrum can be ploted by the **Plot** menu.

When the DOSY processing is complete, two functions - **Show Fit for Peak # Above** (`showdosyfit` macro) and **Show Residual for Peak # Above** (`showdosyresidual` macro), provide a graphical display of the quality of the fit for each individual peak (see Figure 15 on page 71 that allows identifying systematic errors or may help to exclude erroneous data points from the analyses. When the **Auto increment peak #** option is active, each click of the **Show Fit...** or **Show Residual...** buttons advances the displayed peak number by one.

The **Show Diffusion Projection** function (`sdp` command) displays the integral projection of a DOSY dataset onto the diffusion axis. The macro uses the file `userdir`+'/expN/dosy/diffusion_spectrum' as input for the `sdp` command. Unlike in previous versions, in Dosy 3 the `sdp` command may be launched in the same experiment where the diffusion processing is taking place.

Plotting 2D-DOSY Experiments

DOSY data can be plotted in various ways:

- To plot the 1D array, use the **Auto Plot** or A**uto Plot Preview** buttons from the **Plot1D** panel or the **Plot DOSY/Preview DOSY** buttons on the **DOSY Process** panel. Alternatively, use the display and plotting tools of the vertical **ArrayedSpectra** panel.
- To auto-plot 2D DOSY data with a diffusion dimension, the same buttons from the **DOSY Process** panel can be used. In this case, a diffusion projection (`sdp`) is plotted on the diffusion axis while the spectrum axis (typically ^{1}H) depends on the availability of a PROTON which was stored via File - Auto Save (or during automation) prior to the auto-saving of the DOSY data. If a hi-res (PROTON) spectrum is found, it is loaded and printed on the spectrum axis automatically. The same principles as with plotting standard 2D spectra apply.

The **Plot2D** panel offers some more advanced plotting options including the choice of plotting high-resolution top/side spectra, from disk or from another workspace (see “Simple 2D DOSY Pulse Sequences” on page 41) or plotting projections. The diffusion dimension is always plotted as projection (`sdp`). If **"Projection"** is chosen for the frequency axis, a full projection along F2 is generated (via `proj` command) and plotted. A rotation of the diffusion/frequency axes is also respected.

5
Absolute Value 3D-DOSY Experiments

3D DOSY adds a diffusion domain to "conventional" 2D experiments such as COSY or HMQC. The package contains sequences for DOSY-COSY (**Dgcstecosy**) and DOSY-HMQC (**Dgcstehmqc**), but it is straightforward to add diffusion encoding for many other 2D experiments. The 3D DOSY sequences provide better resolving power than the 2D counterparts (the probability of overlapping cross-peaks in 2D spectra is much lower than the probability of overlapping lines in 1D proton detected experiments) at the expense of data size and experiment time.

An arrayed set of 2D experiments is performed using different values of gradient strength (`gzlvl1`), the data are doubly Fourier transformed, and the 1st 2D spectrum is used to define 2D volume integral regions automatically or manually. The dosy analysis then fits the integral volumes in successive increments to Gaussians, and synthesizes 2D integral projections of the 3D data set between defined diffusion limits. Full 3D display is not implemented, although with patience a similar effect can be achieved by performing a series of projections.

Setting up Absolute Value 3D-DOSY Experiments

Make sure that the "conventional" parameters of the COSY/HMQC experiment, such as pulse widths, transmitter offset, spectral windows etc. are set correctly. As with 2D DOSY, find suitable lower and upper bounds for the gradient strength `gzlvl1`. There is no need to run 2D experiments, for this purpose the 1st increment from a 2D run is normally adequate.

NOTE

In a COSY experiment with higher quantum filter (`qlvl` > 1), the first increment does not contain signals. Set the incremented delay (`d2`) to 0.05-0.1 during the gradient optimization process. Please do not forget to set `d2` back to zero when starting the 3D-DOSY experiment.

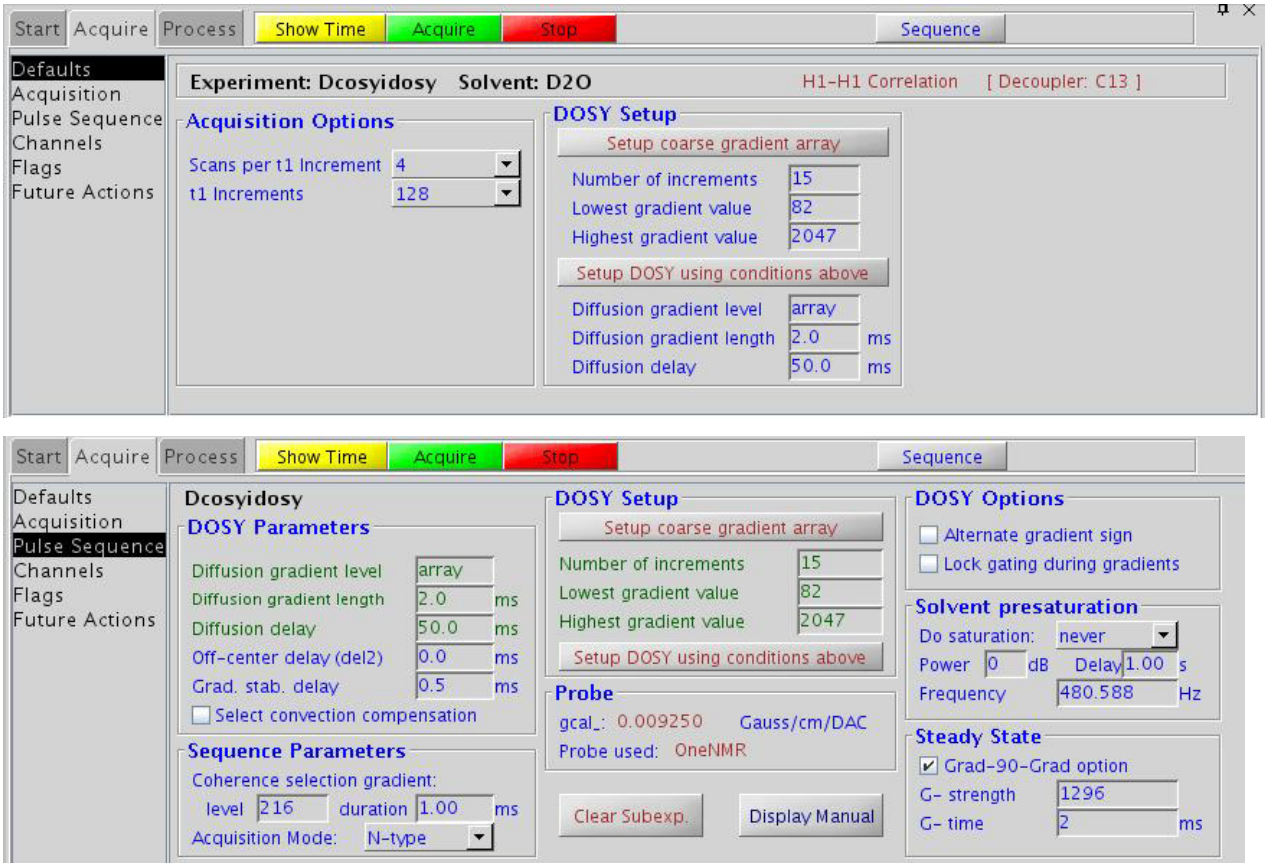


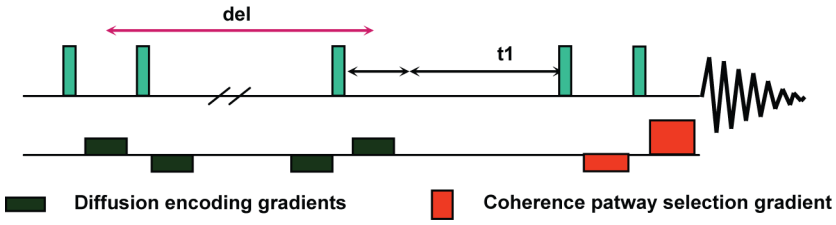
Figure 17 The VnmrJ Acquire/Defaults and Acquire/Pulse Sequence panel of an absolute value 3D-DOSY pulse sequence

Use the `setup_dosy` macro or the **Setup coarse gradient array** button to set up an array of trial `gzlvl1` values. Having

optimized the diffusion delay, `del`, and the corresponding `gzlvl1` range set the number of increments, the lowest and strongest diffusion strength and click on the **Setup DOSY using conditions above** button in the **Acquire/Pulse Sequence** panel (see Figure 17). Bear in mind the total experiment time when choosing the number of `gzlvl1` values, `ni`, and `nt`.

Absolute Value 3D-DOSY Sequences

Dgcstecosy (DOSY gradient compensated stimulated echo cosy) experiment (av mode)



Reference: D. Wu, A. Chen, C. S. Johnson, Jr., J. Magn. Reson. 1996, 121, (Series A), 88-91.

Table 23 Parameters

`del`	the actual diffusion delay
`gt1`	total diffusion-encoding pulse width
`gzlvl1`	diffusion-encoding pulse strength
`gstab`	gradient stabilization delay (~0.0002-0.0003 s)
`tweek`	tuning factor to limit eddy currents, (can be set from 0 to 0.2, usually set to 0.0)
`gzlvl2`	gradient power for pathway selection
`gt2`	gradient duration for pathway selection
`sspul`	flag for a GRD-90-GRD homospoil block
`gzlvlhs`	gradient level for sspul
`wet`	flag for optional wet solvent suppression
`satmode`	'yn' - turns on presaturation during satdly 'yy' - turns on presaturation during satdly and the diffusion delay the presaturation happens at the transmitter position (set `tof` right if presat option is used)
`satdly`	presaturation delay before the sequence (part of d1)
`satpwr`	saturation power level
`hsgt`	gradient duration for sspul
`alt_grd`	flag to invert gradient sign on alternate scans (default = 'n')

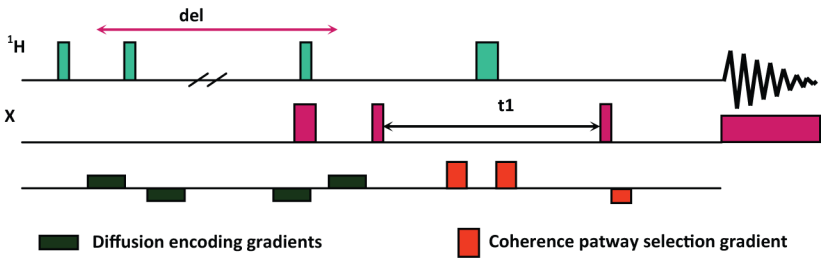
Table 23 Parameters

lkgate_flg	flag to gate the lock signal during diffusion gradient pulses
qlvl	quantum filter level (1=single quantum, 2=double quantum)
probe_	stores the probe name used to acquire the dosy experiment

Table 24 Processing parameters

nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
nugcal_[1-5]	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'; requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition
dosy3Dproc	'ntype' - calls dosy with 3D option with N-type selection

Dgcstehmqc (DOSY gradient compensated stimulated echo hmqc) experiment (AV mode)



Reference: H. Barjat, G. A. Morris and A. Swanson: JMR, 131, 131-138 (1998)

Table 25 Parameters

del	the actual diffusion delay
gt1	total diffusion-encoding pulse width

Table 25 Parameters (continued)

gzlvl1	diffusion-encoding pulse strength
gtE	coherence pathway selection gradient length in HMQC
gzlvlE	gradient power for gtE
EDratio	Encode/Decode ratio
gstab	gradient stabilization delay (~0. 2-0. 3 ms)
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
alt_grd	flag to invert gradient sign on alternate scans (default = 'n')
lkgate_flg	flag to gate the lock signal during diffusion gradient pulses
pwx	90 deg. X-pulse
pwxlvl	power level for pwx
jlxh	one-bond H-X coupling constant
jnxh	multiple-bond H-X coupling constant (for mbond='y')
mbond	flag to select multiple-bond correlations (HMBC)
c180	flag to make the 180 deg. X-pulse a composite pulse
satmode	presaturation flag 'yn' - does presat during satdly 'yy' - does presat during satdly and the diffusion delay
satfrq	saturation frequency
satdly	saturation delay
satpwr	saturation power
wet	flag for optional wet solvent suppression
probe_	stores the probe name used to acquire the dosy experiment

Table 26 Processing parameters

`nugflag`	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
`nugcal_[1-5]`	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'; requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition
`dosy3Dproc`	'ntype' - calls dosy with 3D option with N-type selection

The choice of decoupling method in the DOSY-HMQC experiment is crucial, as even relatively low values of `dpwr` can cause sufficient convection currents to invalidate DOSY results. Adiabatic decoupling schemes (WURST, STUD) are recommended.

Processing 3D-DOSY Experiments

In order to analyze 3D results, it is necessary to define the individual signal regions in the 2D spectrum automatically or manually:

2D Fourier transform the first increment of the 3D data set (for example, that with the lowest `gzlvl1` value), using proper weighting functions in both dimensions:

- `wft2d(1)` for N-type
- `wft2d(1,'ptype')` for P-type selected data

Set `vs2d` and `th` properly, then define the signal regions in the first spectrum using the standard `ll2d` command and its options (“**reset**”, “**volume**”, “**clear**”, “**combine**”, and so on.) The options have been made directly available for convenience on the **"DOSY Process"** panel (see Figure 18 on page 85) via the corresponding buttons.

As a general rule, all components of a given multiplet (cross-peak) should be included in a single signal region, provided there is no contamination by other signals. Grouping signals in this way maximizes the signal-to-noise ratio available for data fitting. This step offers the unique opportunity to exclude apparent spectral artifacts (t1-noise, decoupling sidebands, spurious peaks, etc. from the DOSY analyses). As the

manual peak selection is occasionally the most boring and time-consuming step of the whole procedure, once it is completed, it is worth storing the file (using the **Save peak assignment in FID file** button) in the original FID directory for later processing.

NOTE Please note that this action is only allowed if the DOSY fid has been previously saved and retrieved for processing for example, the parameter `file` is not an empty string but the full filename of the stored FID

Once the signal regions have been defined, call the dosy macro or press the **Process 3D DOSY** button. The macro then determines the volume of each region, for every value of `gzlvl1` (this involves, among other things, as many 2D Fourier transforms as the number of `gzlvl1` increments). The macro then fits the volumes as functions of `gzlvl1`, returning with a display in which each signal region is labeled with its diffusion coefficient (10^{-10} m^2/s), and with its standard error in brackets. The coefficients are displayed using the label facility of the `ll2d` command. Thus, 6.05(0.05) indicates a diffusion coefficient of $6.05*10^{-10}$ m^2/s (+/- $0.05*10^{-10}$ m^2/s). The 2D spectrum on which the display is based is that of the first 2D increment of the 3D experiment. A copy of the diffusion results is available from the file `userdir+/dosy/expN/diffusion_display_3D.inp`. This file contains 3 columns: the peak number (as obtained by `ll2dmode='nynn'`), the diffusion coefficient, and the standard error.

The display of diffusion coefficients as numbers on the screen can result in a very crowded display. The type of information displayed can be changed using the `ll2dmode` parameter (for details see the *Command and Parameter Reference Manua*l and see Figure 18 on page 85 and Figure 19 on page 85).

In order to make the analysis easier, you can use `sdp` to obtain the integral projection of the 3D data set onto the diffusion axis. This diffusion spectrum can be used to choose suitable diffusion regions for which to examine 2D projections of the 3D DOSY data.

In the experiment containing the 3D data, type `makeslice(d1,d2)`, where `d1` and `d2` are the diffusion limits (in units of 10^{-10} m^2/s) between which the 2D projection of the 3D DOSY spectrum is required. `makeslice` builds the slice and displays it after a few seconds. The `makeslice` command uses, among other things, the diffusion information in the file `userdir+'/expN/dosy/diffusion_display_3D.inp'`.

To return to the original spectrum, type `showoriginal`. This reverts to the original 2D spectrum for the first value of `gzlvl1`.

Both sequences are equipped with a VnmrJ **Process/DOSY Process** panel providing access to all necessary functions and parameters to process 3D DOSY data (see Figure 18).

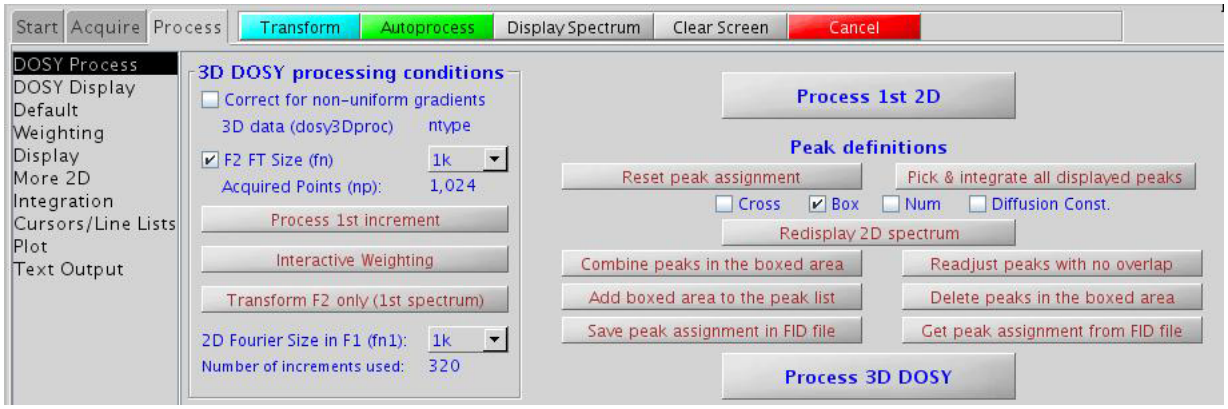


Figure 18 The VnmrJ DOSY Process panel of the absolute value 3D-DOSY pulse sequences

The 2D data display, the slice selection and the switching between the original NMR and the diffusion data can most conveniently be done by via the separate **Process/DOSY Display** panel (seeFigure 19).

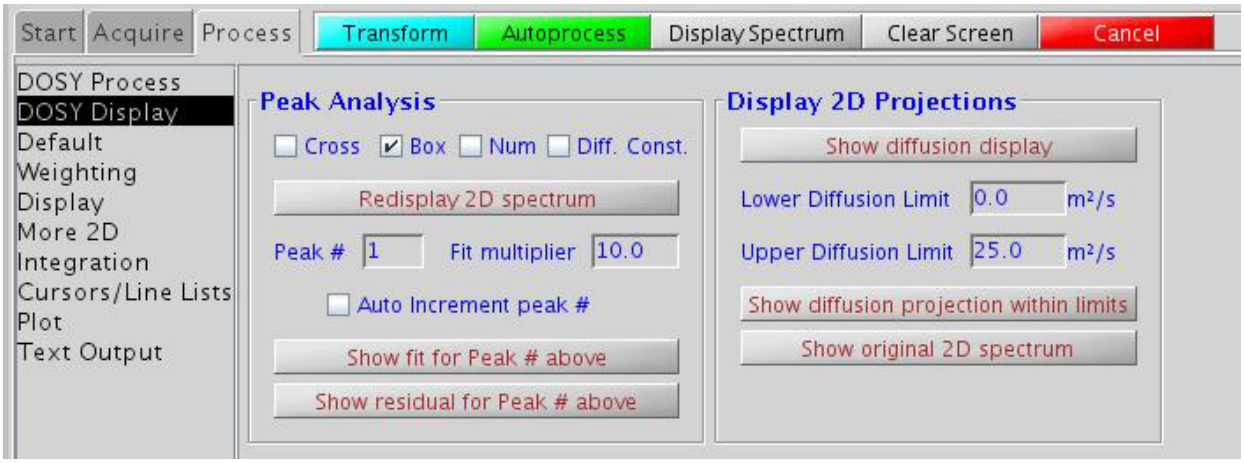


Figure 19 The VnmrJ DOSY Dispay panel of the 3D-DOSY pulse sequences

6
Phase-Sensitive 3D-DOSY Experiments

Setting up Phase-Sensitive 3D-DOSY Experiments

Make sure that the "conventional" parameters such as pulse width, transmitter offset, spectral window etc. are set correctly. Before the final setup the `alfa`, `rof2`, and the `ddrtc`, delays must be optimized to reach ideal phasing and baseline performance. As with 2D DOSY experiments, find suitable lower and upper bounds for the gradient strength `gzlvl1`. There is no need to run 2D NMR experiments. For this purpose, the 1st increment from a 2D run is normally adequate (with a sufficient number of transients to provide adequate signal/noise ratio). Use the `setup_dosy` macro or the **Setup coarse gradient array** button to set up an array of trial `gzlvl1` values. Having optimized the diffusion delay, `del`, and the corresponding `gzlvl1` range, set the number of increments, the lowest and strongest diffusion strength and click on the **Setup DOSY using conditions above** button in the **Acquire/Pulse Sequence** panel (see Figure 20). Bear in mind the total experiment time when choosing the number of `gzlvl1` values, `ni` and `nt`.

In every phase-sensitive 2D NMR experiment, the parameter `phase` needs to be arrayed (`phase=1,2`). Phase-sensitive 3D DOSY sequences, therefore, require a "double array", for example, simultaneous arraying of `gzlvl1` and `phase`. Data acquisition and processing in the VnmrJ 3 software is fully compatible with a double array.

For convenience, set up the double `phase, gzlvl1` array such that the array order is defined as: `array='gzlvl1,phase'`. If, by accident, the order was reversed at the time of data acquisition, using the `reorder3D` macro can still convert the data set to the format expected by the `dosy` macro.

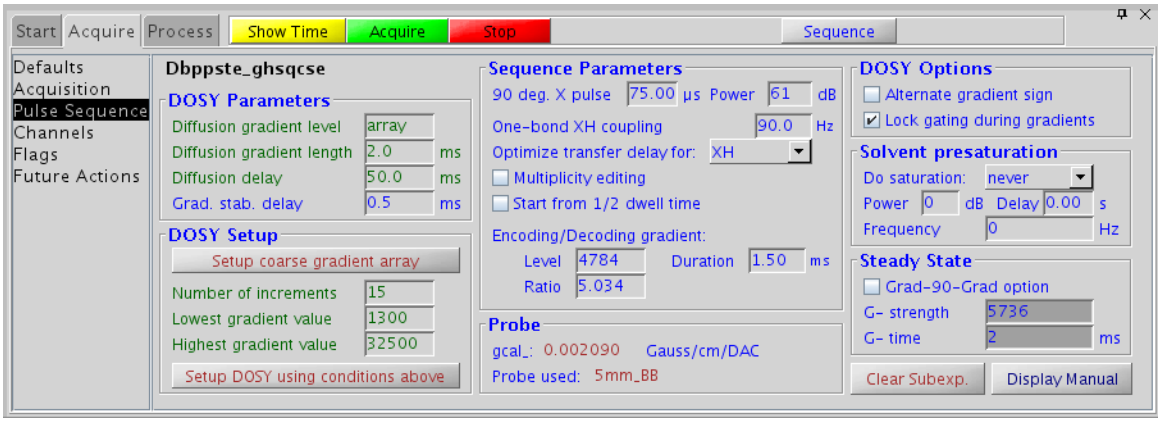


Figure 20 The VnmrJ Acquire/Pulse Sequence panel of the phase-sensitive 3D-Dbppste_ghsqcse pulse sequence

Phase-Sensitive 3D-DOSY Sequences

The `Dgcstehmqc_ps` and the `Dbppste_ghsqcse` pulse sequences have been designed and tested for ^{15}N-labeled peptide/protein samples. The `Dbppste_ghsqcse` has actually been taken over from `BioPack` and made compatible with the DOSY package. Therefore, the cancellation of ^{14}N or ^{12}C-bound protons in natural abundance may not provide perfect results.

Dgcstehmqc_ps (DOSY gradient compensated stimulated echo HMQC) experiment (phase-sensitive mode)

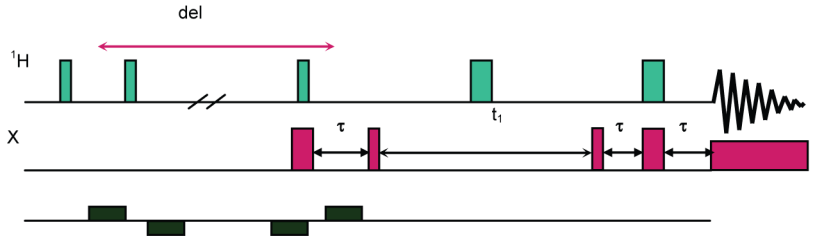


Table 27 Parameters

`del`	the actual diffusion delay
`gt1`	total diffusion-encoding pulse width
`gzlvl1`	diffusion-encoding pulse strength
`gstab`	gradient stabilization delay
`pwx`	90 deg. X-pulse
`pwxlvl`	power level for pwx
`jlxh`	one-bond H-X coupling constant
`c180`	flag to make the 180 deg. X-pulse a composite pulse
`satmode`	presaturation flag 'ynn' - does presat during satdly 'yyn' - does presat during satdly and the diffusion delay
`satdly`	saturation delay
`satpwr`	saturation power
`satfrq`	saturation frequency

Table 27 Parameters (continued)

`alt_grd`	flag to invert gradient sign on alternate scans (default = 'n')
`lkgate_flg`	flag to gate the lock signal during diffusion gradient pulses
`sspul`	flag for a GRD-90-GRD homospoil block
`gzlvlhs`	gradient level for sspul
`hsgt`	gradient duration for sspul
`wet`	flag for optional wet solvent suppression
`probe_`	stores the probe name used to acquire the dosy experiment
`phase`	1,2 for States-Haberkorn acquisition

Table 28 Processing parameters

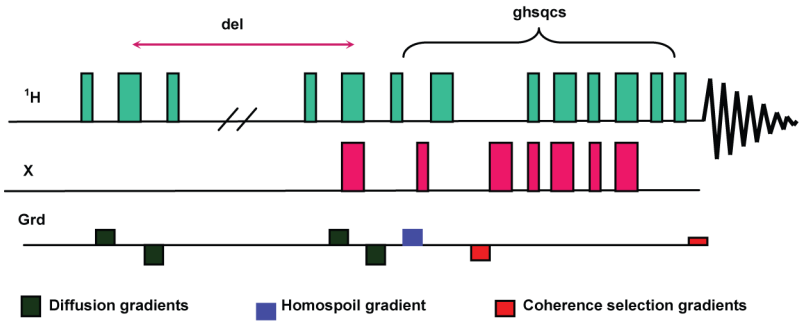
`nugflag`	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
`nugcal_[1-5]`	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'; requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.
`dosy3Dproc`	'y' - calls dosy with 3D option for phase-sensitive data

Run the phase-sensitive 2D HMQC spectra in the same experiment. To make the data set compatible with DOSY processing, it is recommended to set the array as follows: `array = 'gzlvl1,phase'`.

NOTE If the array order has been accidentally reversed, the `reorder3D` macro can be used to retain processing compatibility with the DOSY package.

The sequence was implemented for ^{15}N labeled protein samples for maximum sensitivity. Therefore, it is using States-Haberkorn acquisition, for example, no coherence selection is done by gradients.

Dbppste_ghsqcse (DOSY bipolar pulse pair stimulated echo gradient HSQC with sensitivity enhancement)



Reference: S. Rajagopalan, C. Chow, V. Vinodhkumar, C. G. Fry and S. Cavagnero; J. Biomol. NMR. 29. 505-516 2004

Table 29 Parameters

d1	relaxation delay
pw	90 degrees ^{1}H pulse
tpwr	^{1}H pulse power
pwx	90 degrees X pulse
pwxlvl	X pulse power level
jlxh	$^{1}J_{XH}$ in Hz (140 for ^{1}H-^{13}C)
xhn	'2','1' or '3' flag for signal selection in reverse INEPT sensitivity enhancement factors for different X-multiplicities against normal gHSQC: '1': CH(enh):2.0 CH2(enh):1.0 CH3(enh):1.0 '2': CH(enh):1.71 CH2(enh):1.41 CH3(enh):1.21 best for all '3': CH(enh):1.5 CH2(enh):1.37 CH3(enh):1.25
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
gzlvlE	gradient amplitude for coherence selection
gtE	gradient time for coherence selection (in seconds)
EDratio	Encode/Decode ratio
gstab	delay for stability (~ 0.0003 seconds)

Table 29 Parameters (continued)

edit	'y' makes multiplicity selection (CH & CH3 same sign CH2s opposite sign)
f1180	flag to set initial delay for t1 for phase (-90,180), marks aliased ("folded") signals as negative (180° phase shift)
satmode	'y' or 'n' turns presaturation on or off
satfrq	transmitter frequency for presaturation
satpwr	transmitter power for presaturation
satdly	duration of presaturation in seconds
del	diffusion delay
gzlvl1	gradient level for diffusion
gt1	gradient duration for gzlvl1
alt_grd	flag to invert diffusion gradient sign on alternate scans (default = 'n')
lkgate_flg	flag to gate the lock sampling off during the diffusion sequence
wet	flag for optional wet solvent suppression
probe_	stores the probe name used to acquire the dosy experiment
phase	1,2 for N & P-type selection

Table 30 Processing parameters

nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
nugcal_[1-5]	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'. Requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.
dosy3Dproc	'y' - calls dosy with 3D option for phase-sensitive data

Run the phase-sensitive 2D HSQC spectra in the same experiment. To make the data set compatible with DOSY processing it is recommended to set the array as follows: `array = 'gzlvl1, phase'`.

NOTE If the array order has been accidentally reversed, the `reorder3D` macro can be used to retain processing compatibility with the DOSY package.

Processing Phase-Sensitive 3D-DOSY Experiments

Load the 2D FID file into an experiment. Using the processing tools in Figure 21, select the window functions in both dimensions and correct phases in F2 and/or F1 in necessary. In VnmrJ 3, the `wft1da(x)` command may be used to process individual 2D spectra of a double array, where `x` is the serial number of the requested 2D data set. Transform the first data set, define the cross peaks, and do volume integration. Tools for integration, deleting, selecting, or combining cross peaks are easily accessible in the **Process/DOSY Process** panel and work in an identical way as for absolute value 3D DOSY spectra.

The final DOSY processing is done by the `dosy` macro or by clicking on the **Process 3D DOSY** button. At the end of the processing, the original 2D with the weakest gradient is displayed together with the individual diffusion coefficients and their standard deviation. "Sample FIDs to Practice DOSY Processing" on page 103. Processing contains one phase-sensitive 3D FID (`Si29-1H_Dghmqcidosy.fid`) to practice processing.

Slice selection is identical to that for absolute value 3D spectra and can conveniently be done via the **DOSY Display** panel (see Figure 19 on page 85).

Because of their three-dimensional nature, 3D-DOSY experiments cannot be plotted other than plotting individual 2D spectra of the array. This is no different than plotting "ordinary" 2D spectra - from the "Plot" panel.

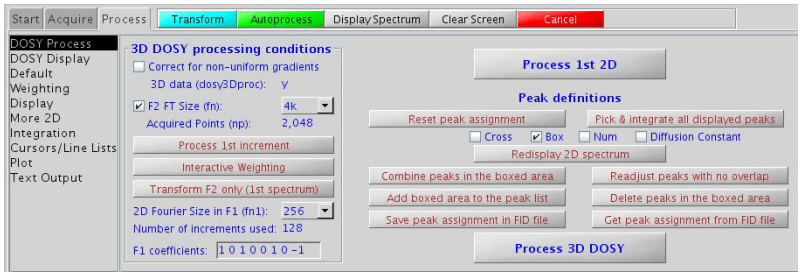


Figure 21 The VnmrJ **Process/DOSY Process** panel of the phase-sensitive 3D-DOSY sequences

7
IDOSY (Inclusive DOSY) Experiments

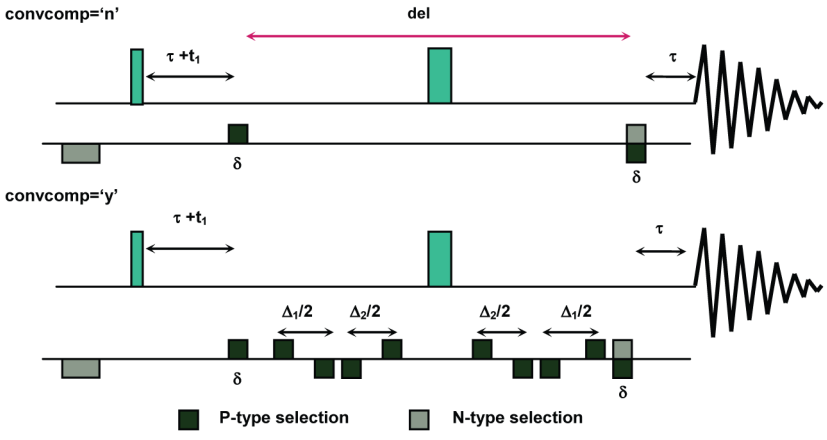
The Concept of I-DOSY

There are three strategies for creating a DOSY pulse sequence: prepending the diffusion encoding (DOSY-X), appending it (X-DOSY), and incorporating it internally (X-IDOSY). Almost all pulse sequences published before 2006 have been X-DOSY or DOSY-X type. However, where the parent pulse sequence either includes or can accommodate a diffusion delay Δ of a few tens of ms, the IDOSY approach can be simpler, quicker, and more sensitive. In the absolute value COSY-IDOSY and 2DJ-IDOSY sequences, the diffusion encoding is incorporated in the Hahn echo (or antiecho) and spin echo, respectively. In both cases, the coherence transfer pathways are identical to those in the parent experiment. Long-range HMQC allows the incorporation of two separate diffusion weighting segments, to form an HMQC-IDOSY sequence.

As the magnetization is transverse during the diffusion delays, there is an inherent possibility for convection compensation with no extra sensitivity loss. Every single I-DOSY sequence provides this option by simply selecting **Select convection compensation** on the **Acquire - Pulse Sequence** panel (or setting the `convcomp` flag to 'y').

I-DOSY Pulse Sequences

Dcosyidosy (COSY-IDOSY)



Reference: M. Nilsson, A. M. Gil, I. Delgadillo, G. A. Morris. Chem. Commun. 2005 1737-1739.

Table 31 Parameters

d1	relaxation delay
gt1	total diffusion-encoding pulse width
gzlvl1	diffusions gradient amplitude
gt1	gradient duration in seconds (0.001)
gstab	optional delay for stability
alt_grd	flag to invert diffusion gradient sign on alternate scans (default = 'n')
lkgate_flg	flag to gate the lock sampling off during the diffusion sequence
del2	delay parameter that can shift the convection compensation sequence elements off the center of the pulse sequence allowing to run a velocity profile can also be negative but in absolute value cannot exceed del/2 minus the gradient and gradient-stabilization delays (default value for diffusion measurements is zero)
satmode	'yn' turns on presaturation during satdly 'yy' turns on presaturation during satdly and del the presaturation happens at the transmitter position (set tof right if presat option is used)
satdly	presaturation delay (part of d1)
satpwr	presaturation power
sspul	gradient level for sspul
phase	1 (selects echo N-type coherence selection; default) 2 (selects antiecho P-type coherence selection)
convcomp	'y': selects convection compensated cosyidosy 'n': normal cosyidosy
wet	flag for optional wet solvent suppression
probe_	stores the probe name used to acquire the dosy experiment

Table 32 Processing parameters

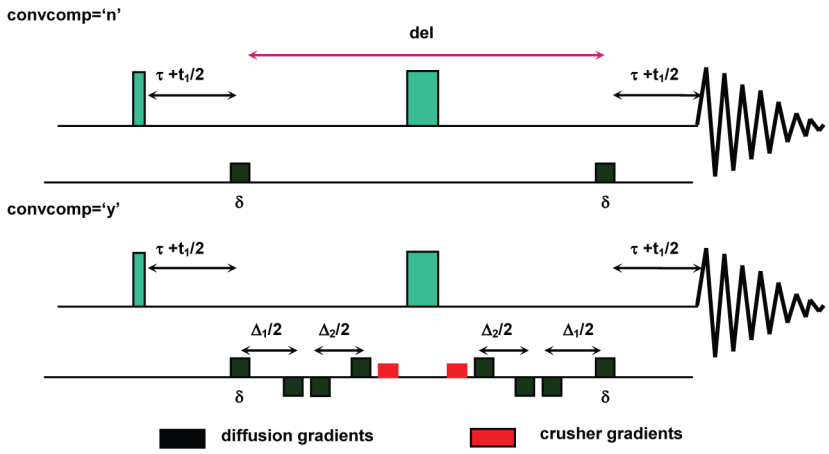
nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series

Table 32 Processing parameters (continued)

`nugcal_[1-5]`	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'; requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition
`dosy3Dproc`	'ntype' - calls dosy with 3D option with N-type selection 'ptype' - calls dosy with 3D option with P-type selection

NOTE In this experiment, the diffusion delay is part of the 2D evolution time. Therefore, if used with the presaturation option, the transmitter offset (`tof`) must be set on resonance to the solvent signal to be saturated.

Dhom2djidosy (Homonuclear 2D J-resolved IDOSY)



Reference: M. Nilsson, A. M. Gil, I. Delgadillo, G. A. Morris. Anal Chem 2004;76:5418-5422

Table 33 Parameters

`del`	the actual diffusion delay
`del2`	delay parameter that can shift the convection compensation sequence elements off the center of the pulse sequence allowing to run a velocity profile can also be negative but in absolute value cannot exceed del minus the gradient and gradient-stabilization delays (default value for diffusion measurements is zero)
`gt1`	total diffusion-encoding pulse width

Table 33 Parameters (continued)

gzlvl1	diffusion encoding gradient power
gzlvl2	gradient amplitude of the crusher gradients flanking the p1 pulse
gt2	gradient duration for gzlvl2
gstab	optional delay for stability
pw	90 degree xmtr pulse
p1	180 degree xmtr pulse
satmode	'yn' turns on presaturation during satdly 'yy' turns on presaturation during satdly and del the presaturation happens at the transmitter position (set tof right if presat option is used)
satdly	presaturation delay (part of d1)
satpwr	presaturation power
wet	flag for optional wet solvent suppression
alt_grd	flag to invert gradient sign on alternate scans (default = 'n')
lkgate_flg	flag to gate the lock sampling off during gradient pulses
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
nt	multiple of 1 (minimum) multiple of 16 (maximum and recommended)
convcomp	'y': selects convection compensated hom2djidosy 'n': normal hom2djidosy
probe_	stores the probe name used to acquire the dosy experiment

Table 34 Processing parameters

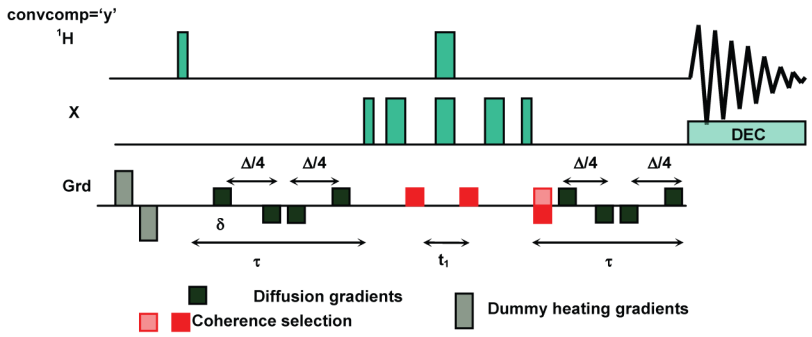
nugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series

Table 34 Processing parameters (continued)

`nugcal_[1-5]`	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'; requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.
`dosy3Dproc`	'ntype' - calls dosy with 3D option with N-type selection

NOTE In this experiment, the diffusion delay is part of the 2D evolution time. Therefore, if used with the presaturation option, the transmitter offset (`tof`) must be set on resonance to the solvent to be saturated.

Dghmqcidosy (DOSY gradient HMQC-IDOSY) DOSY for long-range couplings, phase-sensitive version



Reference: M. J. Stchedroff, A. M. Kenwright, G. A. Morris, M. Nilsson and R. K. Harris Phys. Chem. Chem Phys. 6, 3221-3227 (2004).

Table 35 Parameters

del	the actual diffusion delay
gt1	total diffusion-encoding pulse width
gzlvl1	gradient amplitude (-32768 to +32768)
gzlvlE	gradient amplitude for coherence selection
gtE	gradient duration for coherence selection
EDratio	Decode/Encode ratio
gzlvl_max	maximum gradient power (2048 for Performa I, 32768 for Performa II, IV, and Triax)

Table 35 Parameters (continued)

gstab	optional delay for stability
pwx	90 deg. X-pulse
pwxlvl	power level for pwx
alt_grd	flag to invert gradient sign on alternate scans (default = 'n')
lkgate_flg	flag to gate the lock sampling off during gradient pulses
jnxh	heteronuclear coupling for the transfer delay
satmode	'yn' turns on presaturation during satdly 'yy' turns on presaturation during satdly and del the presaturation happens at the transmitter position (set tof right if presat option is used)
satdly	presaturation delay (part of d1)
satpwr	presaturation power
wet	flag for optional wet solvent suppression
sspul	flag for a GRD-90-GRD homospoil block
gzlvlhs	gradient level for sspul
hsgt	gradient duration for sspul
phase	1,2 for phase-sensitive data
convcomp	'y': selects convection compensated hom2djidosy 'n': normal hom2djidosy
probe_	stores the probe name used to acquire the dosy experiment

Table 36 Processing parameters

ugflag	'n' uses simple mono- or multi-exponential fitting to estimate diffusion coefficients 'y' uses a modified S-T equation, in which the exponent is replaced by a power series
nugcal_[1-5]	a 5-membered parameter array summarizing the results of the calibration of non-uniform field gradients. Used if nugflag='y'. Requires a preliminary NUG-calibration by the Doneshot_nugmap sequence. The values are taken from the probe file at the time of the data acquisition.
dosy3Dproc	'y' - calls dosy with 3D option for phase-sensitive experiments

NOTE The jnxh coupling must be small enough to allow the diffusion delay embedded in the transfer delay. This pulse sequence was developed for ^{1}H-^{29}Si correlations with coupling constants of 7 Hz.

Processing I-DOSY Data

IDOSY sequences do not require special data processing. Depending on whether the data were acquired in the absolute value or phase-sensitive mode, the guidelines of "Phase-Sensitive 3D-DOSY Sequences" on page 89 or "Processing Phase-Sensitive 3D-DOSY Experiments" on page 93.

8
Sample FIDs to Practice DOSY Processing

The package includes a few 2D and 3D FIDs (in `/vnmr/fidlib/Dosy`) to practice DOSY processing. Each experiment type has at least one example to process.

Data Sets Collected Without NUG Correction

These FIDs have been collected well before NUG calibration was made available. Still, they may provide valuable data to practice data processing. When processing, please:

- Do NOT activate the NUG correction flag (leave `nugflag='n'`)
- Do NOT set `dosyproc` to "continuous" (leave it on "discrete")
- Do NOT activate the "point-by-point" analysis (leave `dosybypoints='n'`)

Dbppste.fid

The sample is a mixture of 3 dipeptides (Phe-Val, Phe-Glu, Phe-Gly) and 3(trimethylsilyl)-1-propane-sulfonic acid dissolved in D_2O.

Processing via the VnmrJ DOSY Process panel:

1 Load the data file into the experiment.

2 Select line broadening and the Gaussian window function and click on:

Process All Spectra

Baseline Correct All Spectra

3 Deselect line broadening (keep only the gaussian window function) flank the TMS line (including the satellites) by the two cursors and click on:

Fiddle(TMS)

Baseline Correct All Spectra

4 Select the threshold and click on:

Calculate Full DOSY

5 To zoom into the region of the dipeptides, set the lower and upper diffusion limits to 3.0 and 7.0, respectively and click on:

Recall Original NMR data

Calculate Partial DOSY spectrum

Manual processing via the command line:

1. Load the data file into the experiment.
2. Select `lb` and `gf` then type `wft` and adjust the phase of the 1st spectrum.
3. Set the cursor to the TSP singlet and type `nl rl(0)`.
4. Set the cursors 80 Hz either side of the TSP singlet, set `lb='n' and gf=0.75`, and type `fiddle('satellites','TMS')`. This performs reference deconvolution on all spectra, regularizing the lineshapes so that the peak heights in successive spectra accurately reflect the signal integrals.

   The integral regions have already been set in the parameters supplied. Display the integrals and see where the resets have been positioned.
5. Type `fbc` to perform baseline correction.
6. Set the threshold below the peaks of interest (`vs=500, th=3`).
7. Type `dosy`.
8. To zoom into the diffusion region of interest, type `undosy dosy(4,7)`.

The following examples describe how to process DOSY data in the command mode (left column) or by using the VnmrJ **Process/DOSY Process** panels (right column). Instructions or comments regarding both types of operation can be read in the middle column.

DgcsteSL.fid

The sample is a mixture of adenosine mono-, di-, tri-phosphate (AMP, ADP, ATP) and K_2HPO_4 in D_2O (pH=7). The data were acquired in a 3mm probe with direct ^{31}P observe

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('DgcsteSL.fid')`	

`lb=2 wft`	Fourier transform	*Process All Spectra*
`fbc`	Do baseline correction	*Baseline Correct All Spectra*
	Select threshold	

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`dosy`	Call dosy	*Calculate full DOSY spectrum*
	To have better diffusion resolution, you may calculate a partial dosy spectrum:	
`undosy`		*Recall original NMR spectra*
`dosy(6.1, 7.1)`	Select high and low diffusion limits and reprocess DOSY	*Calculate partial DOSY spectrum*
	To display (and plot) the diffusion spectrum, call `sdp`	

DgcsteSL_dpfgse.fid

This is a sample of 28-member polypeptide at 0.2 mmolar concentration in a H_2O/D_2O 9:1 mixture with some low molecular weight impurities. The aim of the experiment was not to separate mixture components, but to measure the diffusion coefficient to find out about possible aggregation.

The experiment does not require special processing. The steps above are completely applicable. It, however, may provide an example of what water suppression quality can (need to) be achieved when dealing with H_2O samples in extremely low concentrations.

Dbppsteinept.fid

The sample is a mixture of sucrose, methyl-alfa-D-glucopyranoside, 1,3,5,-O-methylidene-mio-inosytol and dioxane (as internal reference) in D_2O. The experiment was run using an AutoSwitchable gradient probe.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('Dbppsteinept.fid')`	

`lb='n' gf='n'`		(unset `lb` and `gf`)
`wft`	Fourier transform	*Process All Spectra*
`fbc`	Do baseline correction	*Baseline Correct All Spectra*
	Select threshold	
`lb=-0.4 gf=0.7`	Set weighting functions	(Activate `lb` and `gf`)

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	Expand the spectrum and put the two cursors around the most intense line (dioxane) ± 15 Hz.	
`fiddle`	Call fiddle	*FIDDLE (No TMS)*
`dosy`	Call dosy	*Calculate full DOSY spectrum*
`undosy`	To have better diffusion resolution, you may calculate a partial dosy spectrum	*Recall original NMR spectra*
`dosy(2.0, 5.0)`	Select high and low diffusion limits and reprocess DOSY	Select upper and lower diff. limits *Calculate partial DOSY spectrum*
	To display (and plot) the diffusion spectrum, call `sdp`	

Dgcstecosy.fid

The sample is a mixture of sucrose, methyl-alfa-D-glucopyranoside, and 1,3,5,-O-methylidene-mio-inosytol in D_2O. The experiment was run using an AutoSwitchable gradient probe.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('Dgcstecosy.fid')`	

`wft2d('t2dc',1)`	Fourier transform	*Process 1st 2D*
	The signal regions for this file have already been saved. Recall the ll2d file:	
`112d('readtext',file+'/112d_text')`		*Retrieve peak assignment from FID file*
`112dmode='nnyn'`	Check the preset signal regions, each crosspeak of interest is boxed	Set Box Unset Cross, Number and Diffusion coefficient
`dconi`	Display the 2D spectrum	*Redisplay 2D spectrum*
`dosy`	Call dosy	*Process DOSY spectrum*

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	When ready, the cosy spectrum is displayed again with each cross peak labeled by its diffusion coefficient and its error. Display the diffusion projection: `sdp` You will see a set of signals: 4.2-4.8 - 1,3,5,-O-methylidene-mio-inosytol 3.6-3.9 - methyl-alpha-D-glucopyranoside 2.8-3.1 - sucrose the other 3 lines between 3.2 and 3.6 D (10^{-10} m^2/s) are overlapping diagonal peaks.	
`112dmode='nnnn'`	Reset the peak labels	*Unset Cross, Box, Number and Diffusion coefficient*
`dconi`	Display the 2D spectrum	Redisplay 2D spectrum
`makeslice(4.2, 4.8)`	To display the inosytol spectrum	*Low. Lim.: 4.2, Up. Lim:4.8* *Show 2D projection within limits*
`makeslice(3.6, 3.9)`	To display the glucopyranoside projection	*Low. Lim.: 3.6, Up. Lim:3.9* *Show 2D projectionwithin limits*
`makeslice(2.8, 3.1)`	To display the sucrose projection	*Low. Lim.: 2.8, Up. Lim:3.1* *Show 2D projection within limits*

NOTE By accident, this cosy spectrum was run with an unusual parameter setting (`sw <> sw1`). It was absolutely unintended and should not affect the DOSY processing.

Dgcstehmqc.fid

The sample is a mixture of quinine, geraniol, camphene, (and TMS) in deutero-methanol. (see: J. Magn. Reson. 1998, 131, 131-138.)

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('Dgcstehmqc.fid')`	

`wft2d('ptype',1)`	Fourier transform	*Process 1st 2D*
	The signal regions for this file have already been saved. Recall the ll2d file:	

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`112d('readtext',file+'/112d_text')`		*Retrieve peak assignment from FID file*
`112dmode='nnyn'`	Check the preset signal regions, each crosspeak of interest is boxed	Set Box Unset *Cross*, Number and *Diffusion coefficient*
`dconi`	Display the 2D spectrum	*Redisplay 2D spectrum*
`dosy`	Call dosy	*Calculate DOSY spectrum*
When ready, the HMQC spectrum is displayed again with each crosspeak labeled by its diffusion coefficient and its error.		
`sdp`	Show diffusion projection	*Show diffusion display*
	You will see a set of signals: 7.0-8.5 - quinine 10.2-11.5 - geraniol 14.0-15.4 - camphene the other lines around 18 D (10^{-10} m^2/s) are methanol and TMS.	
`112dmode='nnnn'`	Reset the peak labels	Unset Cross, Box, Number, and Diffusion coefficient
`dconi`	Display the 2D spectrum	*Redisplay 2D spectrum*
`makeslice(7.0,8.5)`	To display the quinine spectrum	*Low. Lim.: 7.0,* *Up. Lim:8.5* *Show 2D projection within limits*
`makeslice(10.2,11.5)`	To display the geraniol projection	*Low. Lim.: 10.2,* *Up. Lim:11.5* *Show 2D projection within limits*
`makeslice(14.0, 15.4)`	To display the camphene projection	*Low. Lim.: 14.0,* *Up. Lim:15.4* *Show 2D projection within limits*

Si29-1H_Dghmqcidosy.fid

The sample is a mixture of cyclic dimethyl-siloxanes -$((CH_3)_2$-SiO$)_n$- (n = 3....~20) (see: Phys. Chem. Chem. Phys., 2004, 6, 3221-3227.) This is an example to process phase-sensitive 3D DOSY data.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('Dhmqcidosy.fid')`	

`wft2da(1)`	Process the first data set (This is a new feature in VnmrJ 3.)	*Process 1st 2D*
`112d('reset')`	Define volume integrals	Reset peak assignment
`112d`	Define volume integrals	
`112d('adjust')`	Define volume integrals	
`112d('volume')`	Define volume integrals	*Pick and integrate all displayed peaks*
`112dmode='nnyn'`	Check the selection	Set Box
`dconi`	Unset Cross, Number and Diff. Const.	*Redisplay spectrum*
`dosy`	Do 3D processing	*Process 3D DOSY*
`sdp`	Show diffusion projection	*Show diffusion display*
`makeslice(9.5,9.9)`	Show slice between 9.5-9.9 (n=4)	*Low. Lim.: 9.5,* *Up. Lim:9.9* *Show diffusion projection within limits*
`makeslice(8.8,9.0)`	Show slice between 8.8-9.0 (n=5)	*Low. Lim.: 8.8,* *Up. Lim:9.0* *Show diffusion projection within limits*

The rest of the processing is identical to that described at the absolute value 3D examples. The 2D DOSY projection shows all oligomers up to n = 16 completely resolved, except for the pairs 9,10 and 12,13.

NUG Mapping Data

Doneshot_nugmap_av.fid

The FID file contains NUG calibration data in the absolute value mode using a dilute H_2O sample in D_2O.

Measures apparent diffusion coefficient of the HDO signal as a function of frequency (z position), calculates the Stejskal-Tanner decay function, fits it with the exponential of a power series, and stores the value of `gcal_` used and the power series coefficients in the local parameter `nugcal_`, and optionally in the global parameter `nugcal` and in the probe file. These data are provided only as an example for NUG calibration. Please do not try to store these values in your current probe file, as the data are originated from a different probe/instrument.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('Doneshot_nugmap_av.fid')\`	

`wft`	Process the first data set	*Fourier Transform Profiles*
either `nugcalib` and follow dialogue: answer: 'd', '25', 'n', 'n' or `nugcalib('d',25,'n','n')`	Calculate NUG coefficients	Set Calibrant for grad mapping to 1% HOD Set Mapping data measured at: 25 *Calculate NUG coefficients*
`showgradfit`	To show the gradient fitting	*Show fitted gradient shape*
`shownugfit`	To show the fitted signal decay	*Show fitted signal decay*
`redosy`	To show the D distribution along z	*Show apparent D wrt position*

NOTE

The displays (and the optional plots) show the variation of the apparent diffusion coefficient with position, the power series fit of the gradient as a function of position, and the log of the power series fit to the attenuation as a function of nominal gradient squared. The latter fit should be good down to about –9 (more than a 1000-fold signal attenuation) for ordinary liquids gradient probes.

Doneshot_nugmap_ph.fid

The file contains NUG calibration data in the phase-sensitive mode using a dilute H_2O sample in D_2O.

Measures apparent diffusion coefficient of the HDO signal as a function of frequency (z position), calculates the Stejskal-Tanner decay function, fits it with the exponential of a power series, and stores the value of `gcal_` used and the power series coefficients in the local parameter `nugcal_`, and optionally in the global parameter `nugcal` and in the probe file. These data are provided only as an example for NUG calibration. Please do not try to store these values in your current probe file as the data are originated from a different probe/instrument.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('Doneshot_nugmap_av.fid')`	

`wft`	Process the first data set	*Fourier Transform Profiles*
`aph`	Adjust phase	Adjust phase manually
`fbc`	Do baseline processing	*Baseline Correct All Profiles*
either `nugcalib` and follow dialogue: answer: `'d', '25', 'n', 'n'` or `nugcalib('d',25, 'n','n')`	Calculate NUG coefficients	Set Calibrant for grad mapping to 1% HOD Set Mapping data measured at: 25 *Calculate NUG coefficients*
`showgradfit`	To show the gradient fitting	*Show fitted gradient shape*
`shownugfit`	To show the fitted signal decay	*Show fitted signal decay*
`redosy`	To show the D distribution along z	*Show apparent D wrt position*

NOTE The displays (and the optional plots) show the variation of the apparent diffusion coefficient with position, the power series fit of the gradient as a function of position, and the log of the power series fit to the attenuation as a function of nominal gradient squared. The latter fit should be good down to about –9 (more than a 1000-fold signal attenuation) for ordinary liquids gradient probes.

Data Sets with NUG Calibration

QGConeshot.fid

The sample is a mixture of quinine, geraniol, camphene, (and TMS) in deutero-methanol. The data have already been FIDDLEd to correct lineshape errors.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('QGConeshot.fid')`	

	Process the data	
`ft f full`	Fourier transform	*Process All Spectra*
`fbc`	Do baseline processing	*Baseline Correct All Spectra*
`th=2 vsadj`	Set threshold and vertical scale	Set threshold and vs manually
`dosyproc='discrete'`	Set processing type	Set *Processing type: Discrete*
`ncomp=1`	Define number of components	Set *Number of components for fit: 1*
`nugflag='n'`	Switch off NUG correction	Unset *Correct for non-uniform gradients*
`dosy`	Calculate DOSY	*Calculate Full DOSY*

NOTE This gives a normal 2D DOSY spectrum. The geraniol and camphene signals are somewhat overlapped, but the quinine shows nice clean signals (see Figure 22 on page 114).

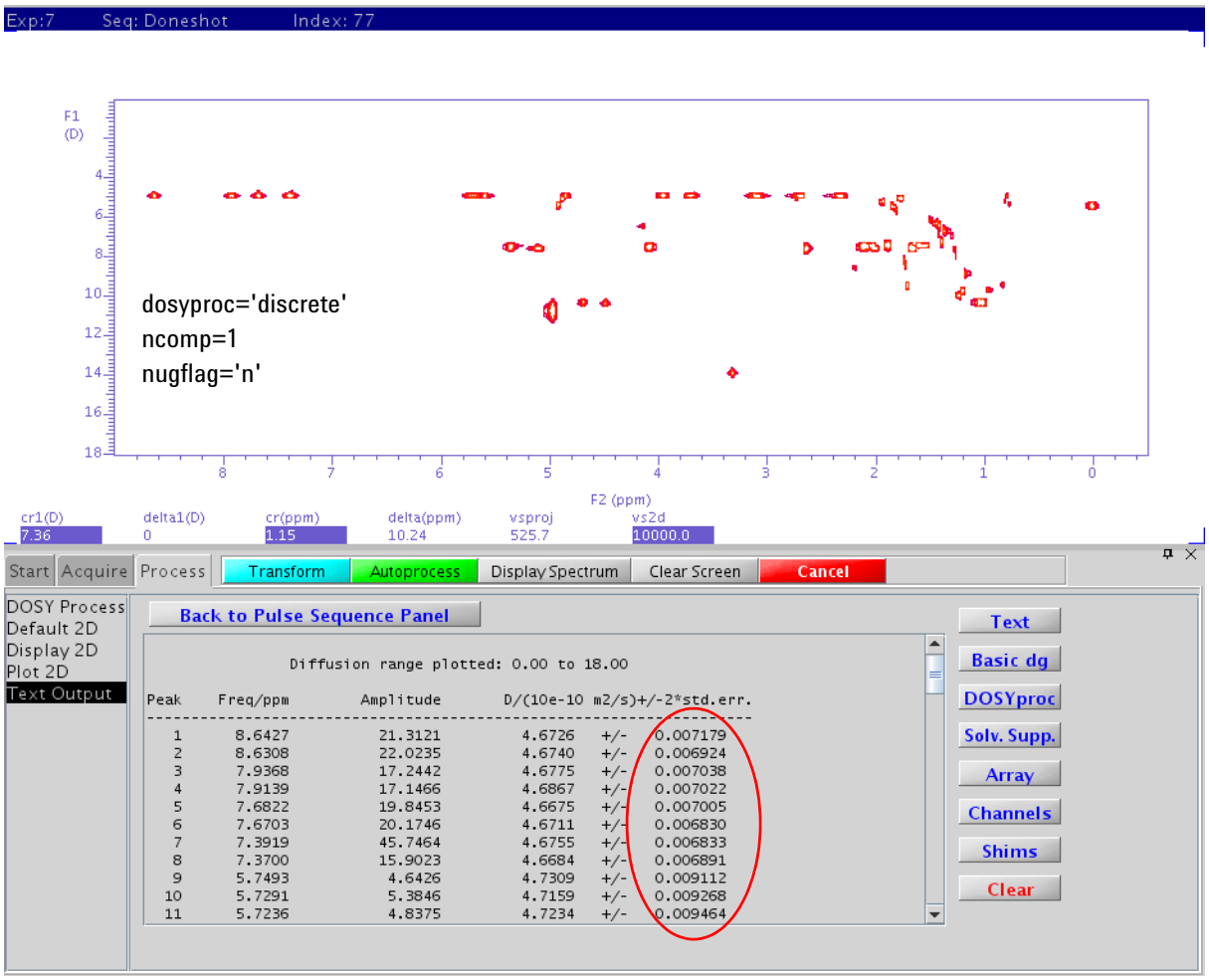


Figure 22 The 2D DOSY display of the QGC Doneshot data (dosyproc='discrete', ncomp=1, nugflag='n')

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`showdosyfit(1,100)`	Display the fitting results of peak #1	Set Peak: 1 and Fit multiplier: 100

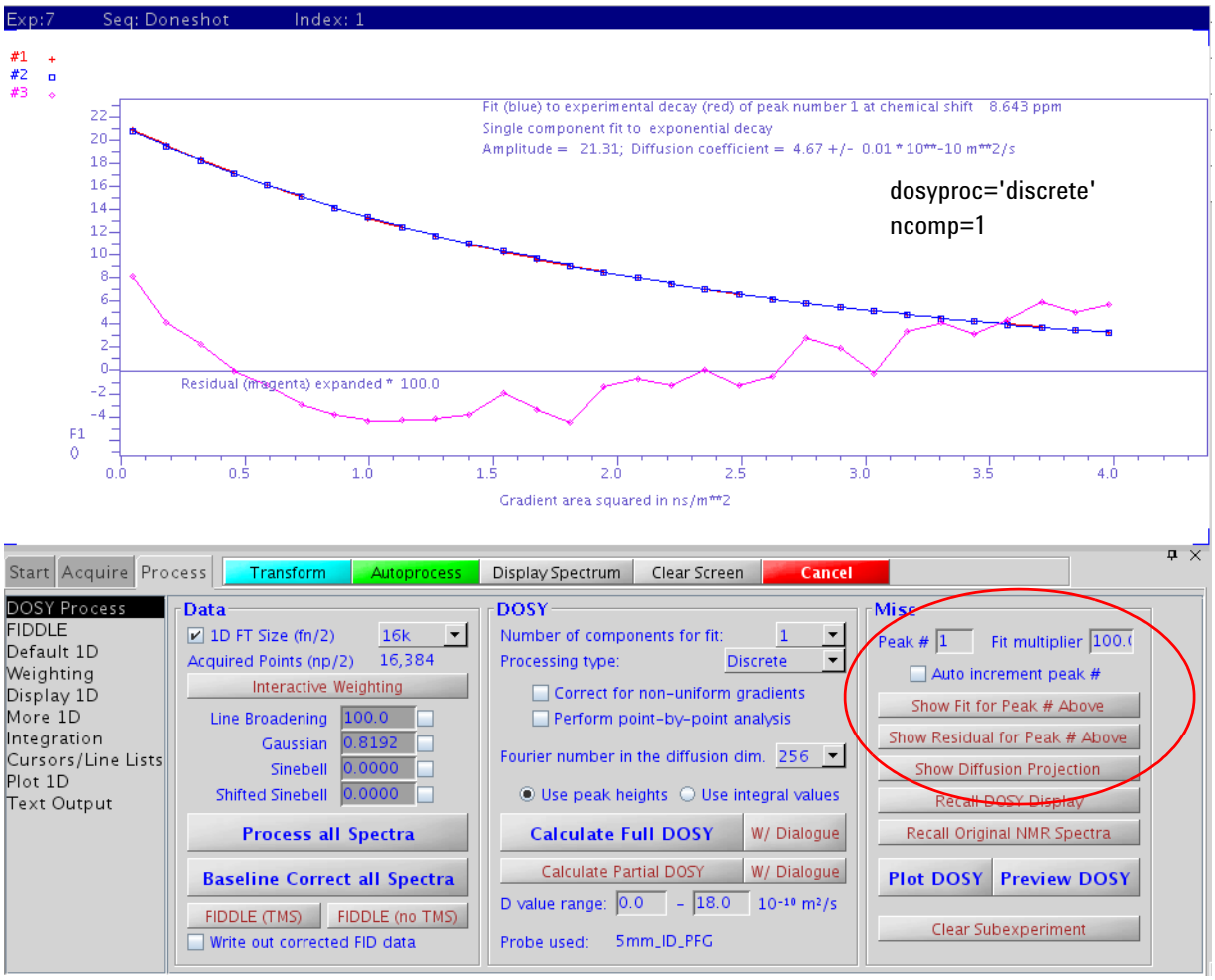


Figure 23 The results of the 2D DOSY fitting of the QGC Doneshot data (dosyproc='discrete', ncomp=1, nugflag='n'). Note the significant systematic errors caused by non-uniform gradients.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	Now reprocess the data with NUG correction	
`ft f full`	Fourier transform	*Process All Spectra*
`fbc`	Do baseline correction	*Baseline Correct All Spectra*

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`th=2 vsadj`	Set threshold and vertical scale	Set threshold and vs manually
`dosyproc='discrete'`	Set processing conditions	Set *Processing type: Discrete*
`ncomp=1`	Define number of components	Set *Number of components for fit: 1*
`nugflag='y'`	Switch on NUG correction	Set *Correct for non-uniform gradients*
`dosy`	Calculate DOSY	*Calculate Full DOSY*

NOTE The results are very similar to the one in Figure 23 on page 115 but the diffusion scale changes slightly, and the fitting statistics improve substantially (see Figure 24 on page 117).

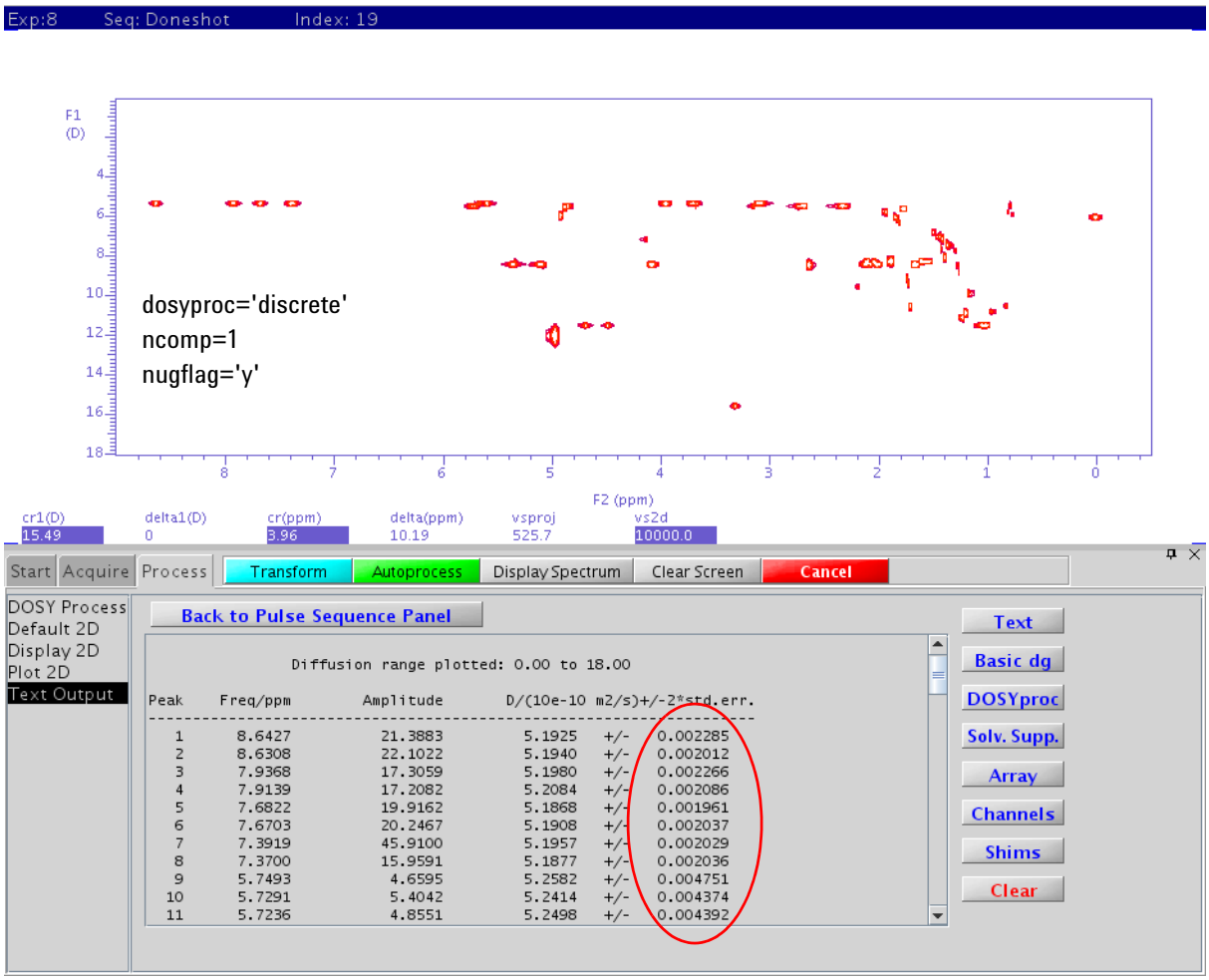


Figure 24 The 2D DOSY display of the QGC Doneshot data (dosyproc='discrete', ncomp=1, nugflag='y')

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`showdosyfit(1,100)`	Display the fitting results of peak #1	Set Peak #: 1 and Fit multiplier: 100

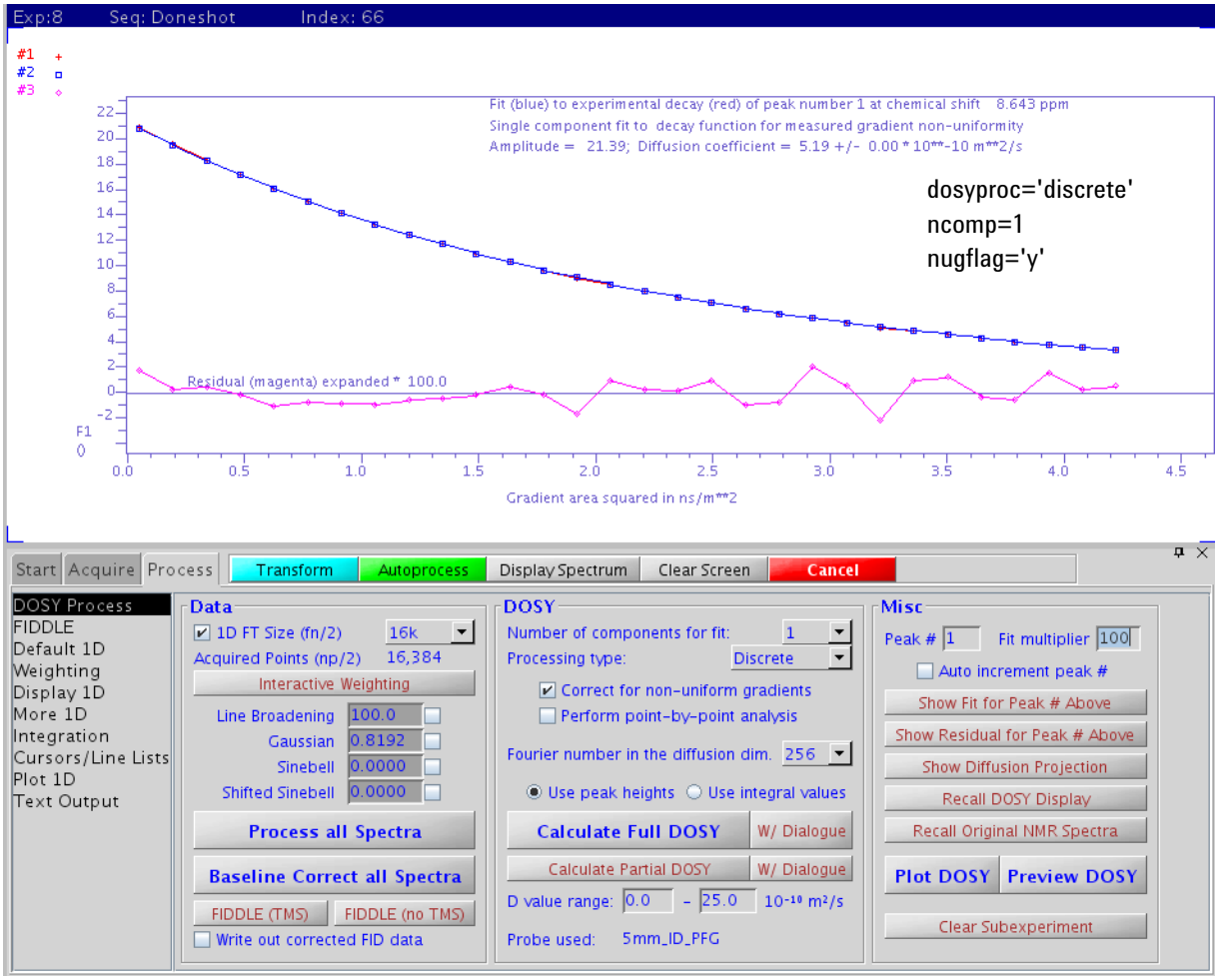


Figure 25 The results of the 2D DOSY fitting of the QGC Doneshot data (dosyproc='discrete', ncomp=1, nugflag='*y*')

Now the residual is almost pure noise, showing that the systematic errors have been corrected.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	Multiexponential processing without NUG correction	
`ft f full`	Fourier transform	*Process All Spectra*
`fbc`	Do baseline processing	*Baseline Correct All Spectra*
`th=2 vsadj`	Set threshold and vertical scale	Set threshold and vs manually
`dosyproc='discrete'`	Set processing conditions	Set *Processing type: Discrete*

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`ncomp=2`	Define number of components	Set *Number of components for fit: 2*
`nugflag='n'`	Switch off NUG correction	UnSet *Correct for non-uniform gradients*
`dosy`	Calculate DOSY	*Calculate Full DOSY*

Does a biexponential fit, because the data have high S/N, many lines appear to have two diffusion coefficients because of the gradient non-uniformity.

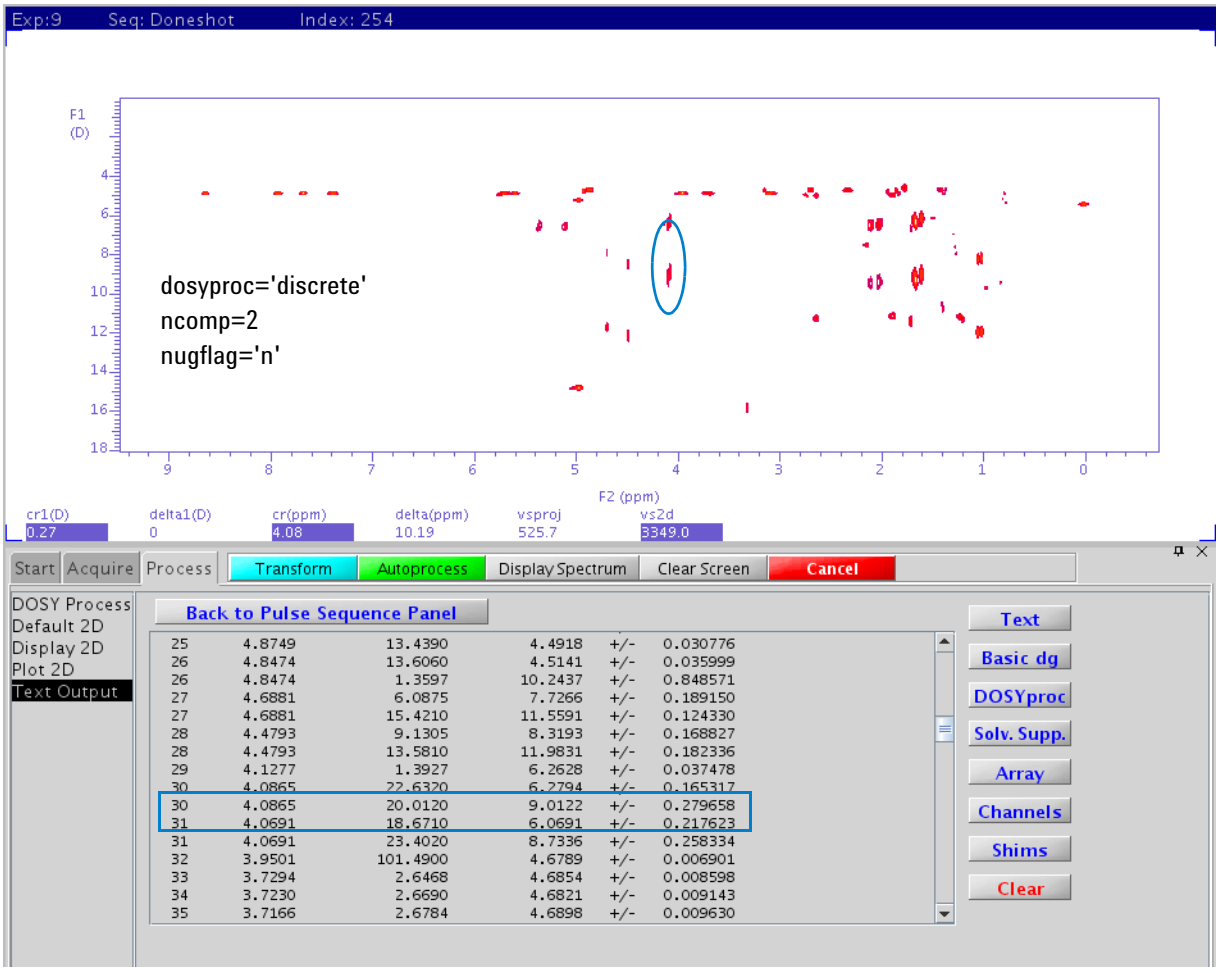


Figure 26 The 2D DOSY display of the QGC Doneshot data (dosyproc='discrete', ncomp=2, nugflag='n')

The residuals might look very good - a biexponential fit will accommodate the effects of non-uniform gradients very nicely, but both intensities and the diffusion coefficients will be incorrect!

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	Multiexponential processing with NUG correction	
`ft f full`	Fourier transform	*Process All Spectra*
`fbc`	Do baseline processing	*Baseline Correct All Spectra*
`th=2 vsadj`	Set threshold and vertical scale	Set threshold and vs manually
`dosyproc='discrete'`	Set processing conditions	Set *Processing type: Discrete*
`ncomp=2`	Define number of components	Set *Number of components for fit: 2*
`nugflag='y'`	Switch on NUG correction	Set *Correct for non-uniform gradients*
`dosy`	Calculate DOSY	*Calculate Full DOSY*

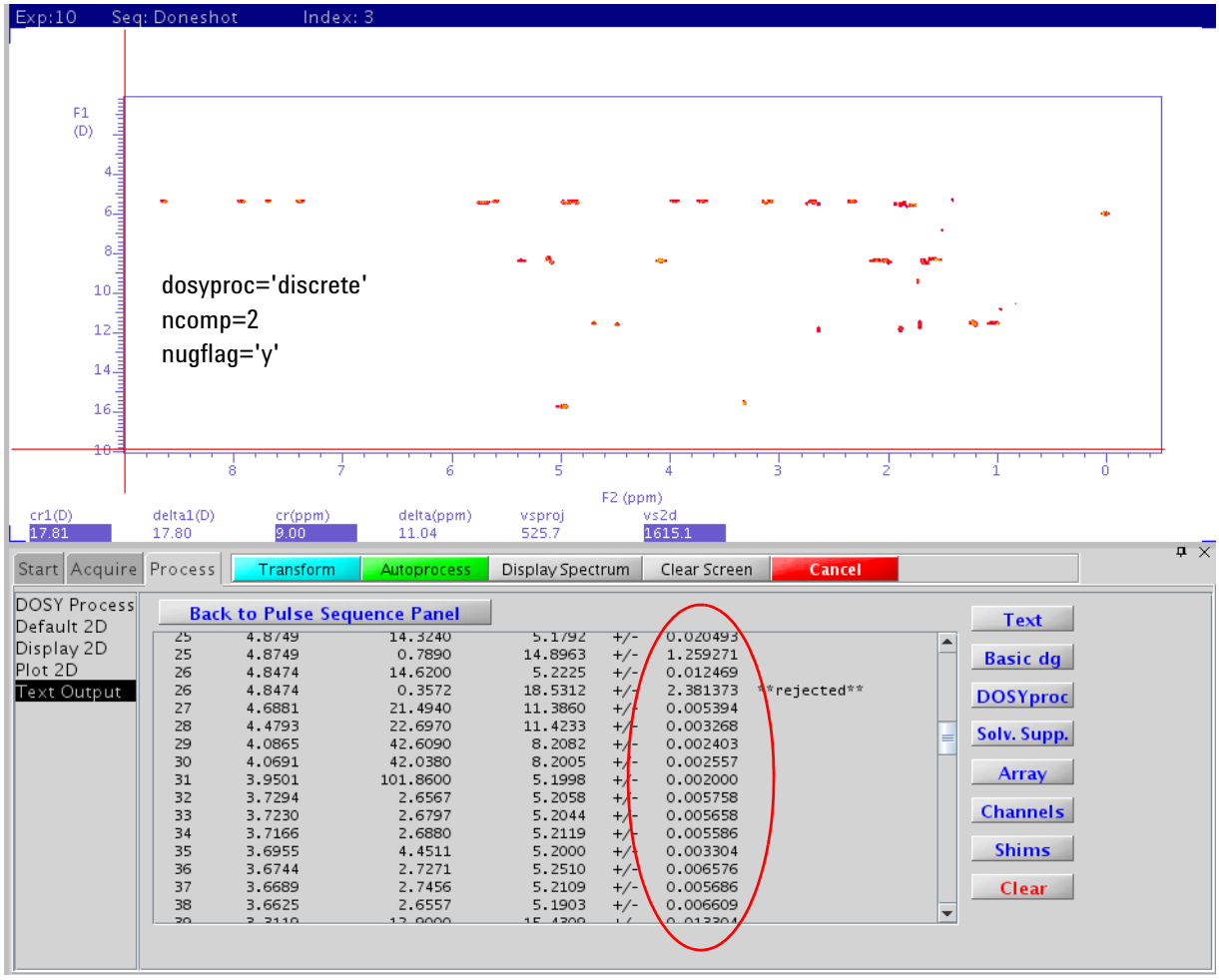


Figure 27 The 2D DOSY display of the QGC Doneshot data (dosyproc='discrete', ncomp=2, nugflag='y')

The biexponential fit with NUG correction shows a much cleaner spectrum and most lines show a single and correctly positioned diffusion peak with small residuals.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`showdosyfit(1,100)`	Display the fitting results of peak #30	Set Peak #: 30 and Fit multiplier: 100

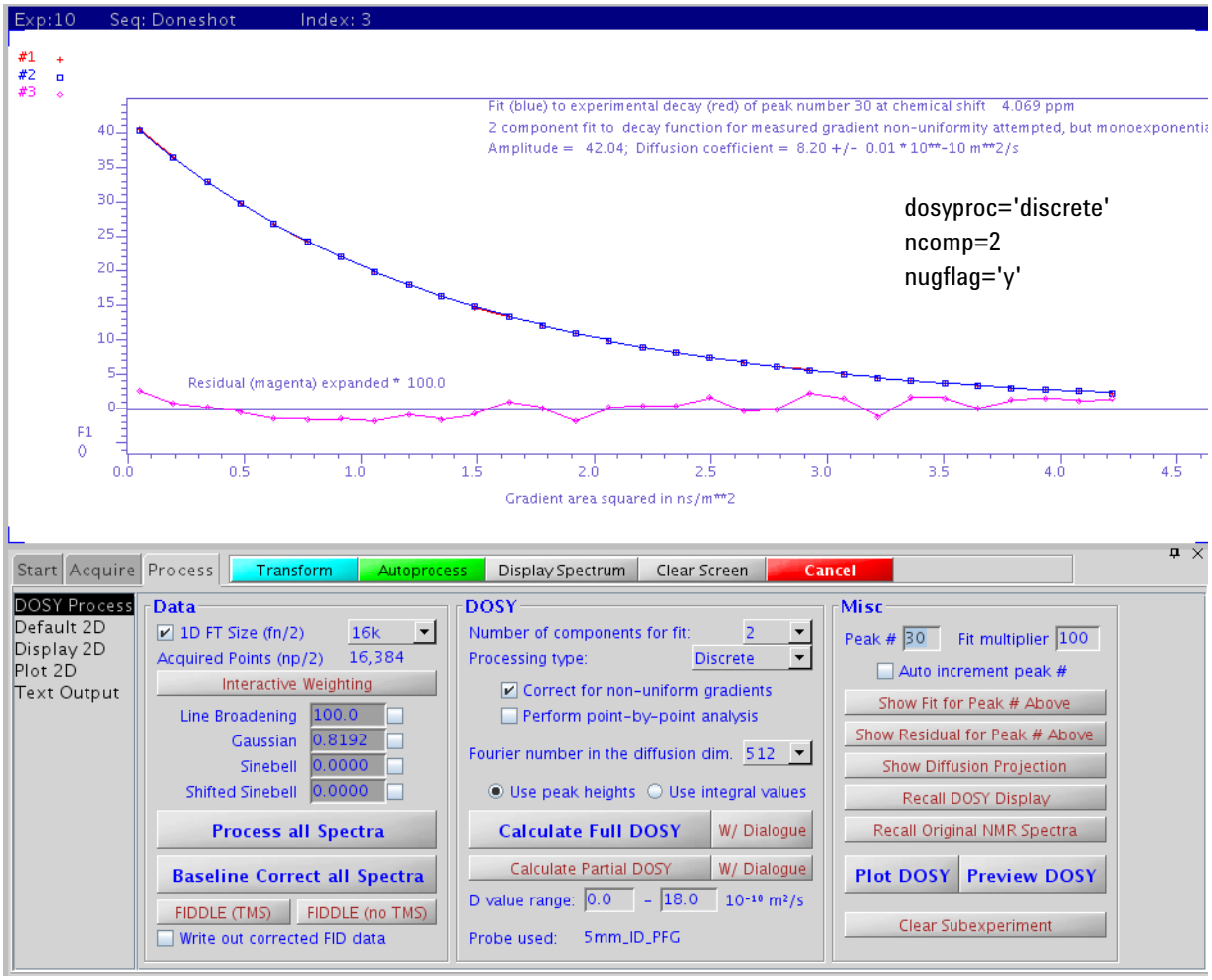


Figure 28 The results of the 2D DOSY fitting of the QGC Doneshot data (dosyproc='discrete', ncomp=2, *nugflag='y'*)

The same QGC Doneshot data set can be used to practice point-by-point, rather than peak-by-peak analysis. The effect of signal overlap then is to give signals that change apparent diffusion coefficient within a peak as the relative amounts of different overlapping signals change. Thus, a peak at 4.98 ppm, which is composed of overlapping contributions from quinine and methanol OH/water, is spread over a range of apparent diffusion coefficients.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	Monoexponential point-by-point processing without NUG correction	
`ft`	Fourier transform	*Process All Spectra*
`sp=4.86p wp=0.25p`	Expand the spectrum	Expand the spectrum manually between 4.75 and 5.22 ppm
`fbc`	Do baseline processing	*Baseline Correct All Spectra*
`th=2 vsadj`	Set threshold and vertical scale	Set threshold and vs manually
`dosyproc='discrete'`	Set processing conditions	Set Processing type: *Discrete*
`ncomp=1`	Define number of components	Set *Number of components for fit: 1*
`nugflag='y'`	Switch on NUG correction	Set *Correct for non-uniform gradients*
`dosybypoints='y'`	Select point-by-point analysis	Set *Perform point-by-point analysis*
`vs2d=400`	Adjust 2D vertical scale	Set vs2d=400
`dosy(0,20)`	Calculate DOSY	Set D value range: 0 and 20 *Calculate Partial DOSY*

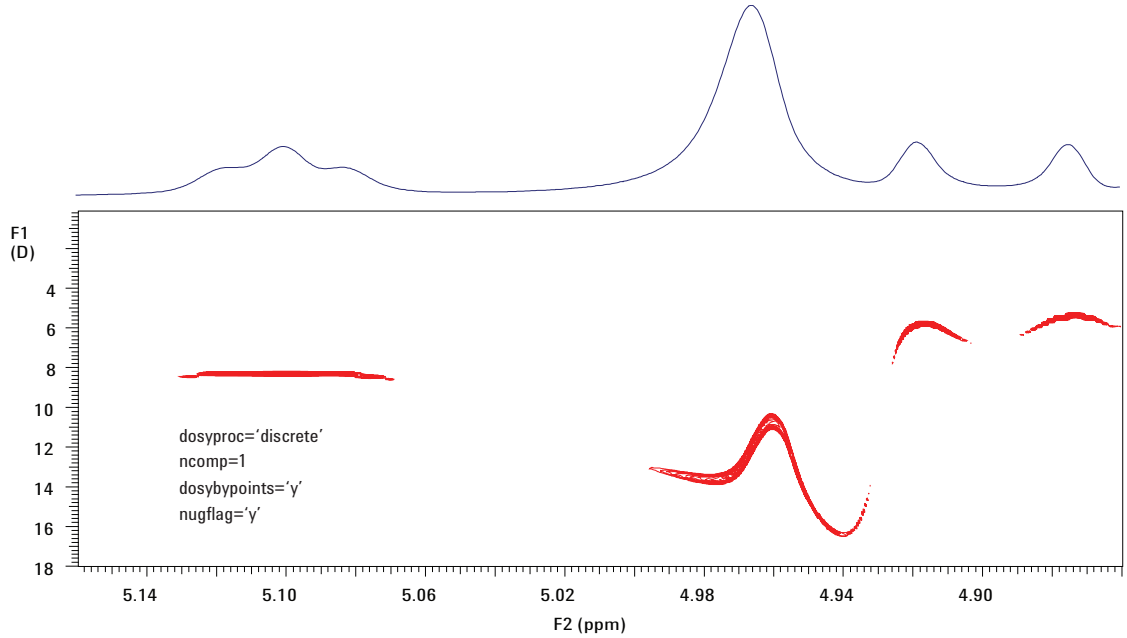


Figure 29 The 2D partial DOSY display of the QCDoneshot data (dosyproc='discrete', ncomp=2, nugflag='y', dosybypoints='y'

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	Biexponential point-by-point processing without NUG correction	
`ft`	Fourier transform	Process All Spectra
`sp=4.86p wp=0.25p`	Expand the spectrum	Expand the spectrum manually between 4.75 and 5.22 ppm
`fbc`	Do baseline correction	*Baseline Correct All Spectra*
`th=2 vsadj`	Set threshold and vertical scale	Set threshold and vs manually
`dosyproc='discrete'`	Set processing conditions	Set *Processing type: Discrete*
`ncomp=2`	Define number of components	Set *Number of components for fit: 2*
`nugflag='y'`	Switch on NUG correction	Set *Correct for non-uniform gradients*
`dosybypoints='y'`	Select point-by-point analysis	Set *Perform point-by-point analysis*
`vs2d=400`	Adjust 2D vertical scale	Set vs2d=400
`dosy(0,20)`	Calculate DOSY	Set D value range: 0 and 20 *Calculate Partial DOSY*

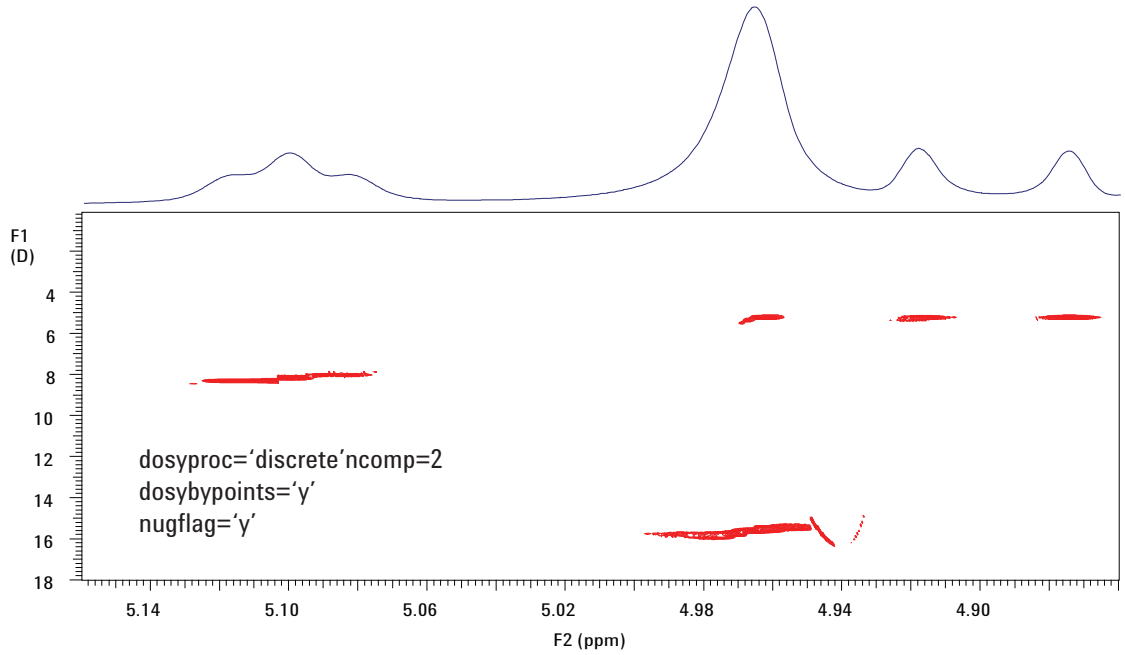


Figure 30 The 2D partial DOSY display of the QCDoneshot data (dosyproc='discrete', ncomp=2, nugflag='y', dosybypoints='y')

Using biexponential fitting now gives clean discrimination between the overlapping quinine and water signals. Note that the combination of point-by-point fitting and biexponential fitting is slow, so it is best to use this combination on narrow spectral regions rather than whole spectra.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	Processing with dosyproc='continuous'	
`ft f full`	Fourier transform	*Process All Spectra* Display the whole spectrum
`th=5 vsadj`	Set threshold and vertical scale	In the command line type: `th=5 vsadj`
`fn1=128`	Set Fourier number in F1	Set fn1 to 128
`dosyproc='discrete'`	Set processing conditions	Set *Processing type: Discrete*
`ncomp=2`	Define number of components	Set *Number of components for fit: 2*
`nugflag='y'`	Switch on NUG correction	Set *Correct for non-uniform gradients*
`dosyproc='continuous'`	Set processing conditions	Set *Processing type: Continuous*

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`dosybypoints='n'`	Select point-by-point analysis	Unset *Perform point-by-point analysis*
`vs2d=20`	Adjust 2D vertical scale	Set vs2d=20
`dosy`	Calculate DOSY	*Calculate Full DOSY*

This setting does a CONTIN fit of the most intense lines. CONTIN analysis is very computationally intensive, so it is best to keep both fn1 and the number of lines selected low. Obviously this analysis is more appropriate for a polydisperse sample.

DextranMix.fid

The data are from a sample containing glucose, dextran 6K and dextran 2M. The dextrans are polydisperse, but with a sufficient narrow distribution that we can successfully treat each as monodisperse. There is, naturally, severe spectral overlap of all three species. These data cannot easily be corrected by reference deconvolution due to the high molecular weights of the dextrans. A line broadening of 3 Hz is therefore used to reduce the effects of frequency drifts.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('DextranMix.fid')`	

	Process the data (Monoexponential processing)	
`lb=3 ft f full`	Fourier transform	*Process All Spectra*
`fbc`	Do baseline processing	*Baseline Correct All Spectra*
`th=2 vsadj`	Set threshold and vertical scale	Set threshold and vs manually
`dosyproc='discrete'`	Set processing conditions	Set *Processing type: Discrete*
`ncomp=1`	Define number of components	Set *Number of components for fit: 1*
`nugflag='y'`	Switch on NUG correction	Unset *Correct for non-uniform gradients*
`dosy`	Calculate DOSY	*Calculate Full DOSY*

This should give a normal 2D DOSY with a large difference between glucose and dextran signals where there is no overlap, but many peaks at intermediate apparent diffusion coefficients where signals do overlap.

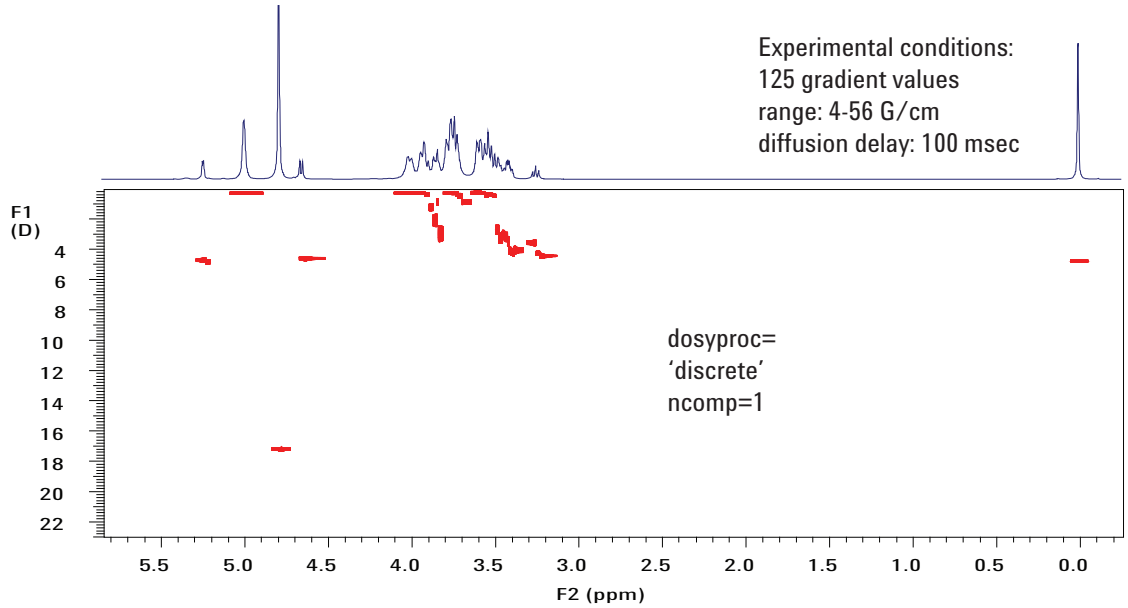


Figure 31 The 2D DOSY display of the DextranMix data (dosyproc='discrete', ncomp=1, nugflag='y')

The following parameter set makes a CONTIN fit of the most overlapped region:

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	Process the data (Triexponential processing)	
`lb=3 ft f full`	Fourier transform	*Process All Spectra*
`fbc`	Do baseline processing	*Baseline Correct All Spectra*
`th=2 vsadj`	Set threshold and vertical scale	Set threshold and vs manually
`dosyproc='discrete'`	Set processing conditions	Set *Processing type: Discrete*
`ncomp=3`	Define number of components	Set *Number of components for fit: 3*
`nugflag='y'`	Switch on NUG correction	Set *Correct for non-uniform gradients*
`dosy`	Calculate DOSY	*Calculate Full DOSY*

The triexponential analysis should show good separation of signals from all three components. Note that the water peak also shows biexponential behavior. This may stem from exchange, tight binding, or overlap with the slowly diffusing dextrans. Similarly, the TSP peak shows the presence of a small amount of TSP bound to dextran.

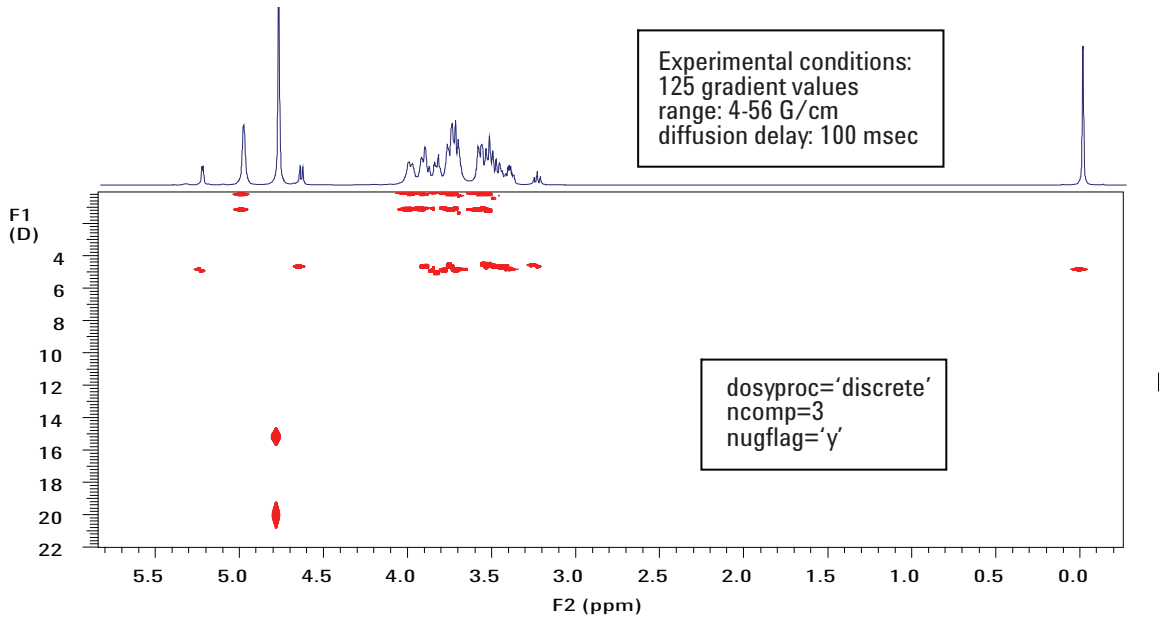


Figure 32 The 2D DOSY display of the DextranMix data (dosyproc='discrete', ncomp=3, nugflag='y')

The following parameter set makes a CONTIN fit of the most overlapped region:

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
	Process with dosyproc='continuous '	
`lb=3 wft f full`	Fourier transform	*Process All Spectra*
`th=3 vsadj`	Set threshold and vertical scale	In the command line type: `th=3 vsadj`
`fnl=256`	Set Fourier number in F1	Set fnl to 256
`sp=3.1p wp=1.0p`	Make expansion	Expand the spectrum between 3.1 and 4.1 ppm
`dosyproc='continuous'`	Set processing condition	Set *Processing type: Continuous*
`ncomp=3`	Define number of components	Set *Number of components for fit: 3*

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`nugflag='y'`	Switch on NUG correction	Set *Correct for non-uniform gradients*
`dosybypoints='n'`	Deselect point-by-point analysis	Unset *Perform point-by-point analysis*
`vs2d=10`	Adjust 2D vertical scale	Set vs2d=10
`dosy(0.001,10)`	Calculate DOSY	Set D value range: 0.001 and 10 *Calculate Partial DOSY*

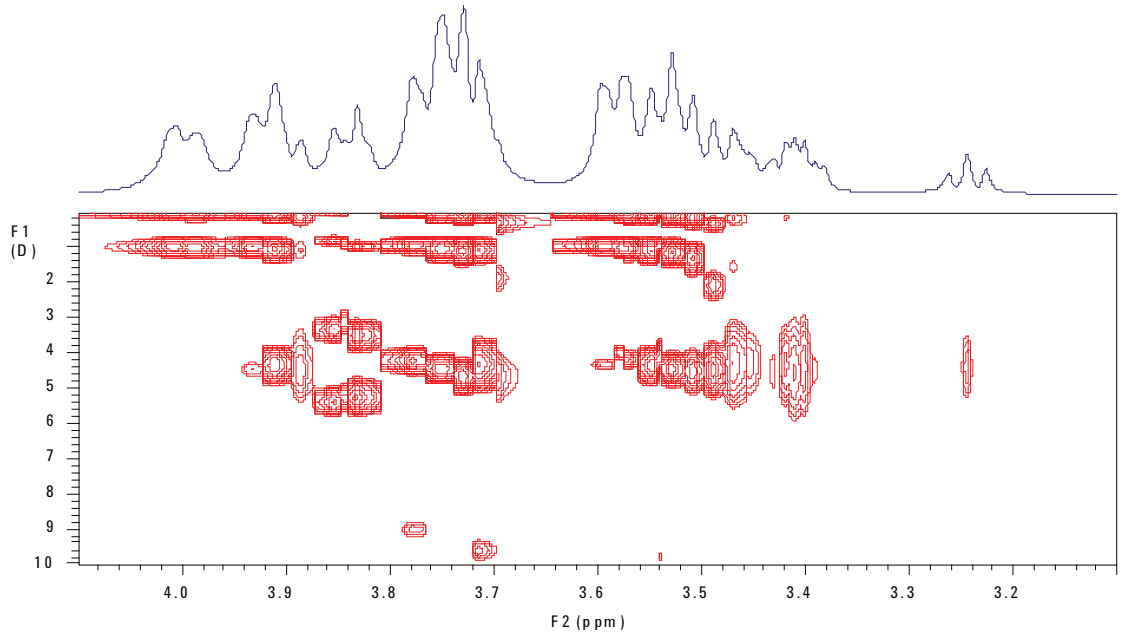


Figure 33 The 2D DOSY display of the DextranMix data (dosyproc='continuous', ncomp=3, nugflag='y')

GQC_quickCOSYiDOSY.fid

The sample is a mixture of quinine, geraniol, camphene (and TMS) in deutero-methanol.

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel

	Recall the FID :
	`cd('/vnmr/fidlib/Dosy')`
	`rt('GQC_quickCOSYiDOSY.fid')`	

Commands	Comments, instructions for both	Buttons in the DOSY Process Panel
`wft2d(1)`	Fourier transform	*Process the 1st 2D spectrum*
`112d('reset')`	Define volume integrals	*Reset peak assignment*
`112d`		
`112d('adjust')`		
`112d('volume')`		*Pick and integrate all displayed peaks*
`dconi`	Display the 2D spectrum	*Redisplay 2D spectrum*
`nugflag='y'`	Set processing conditions	Set *Correct for non-uniform gradients*
`dosy`	Call DOSY	*Process 3D DOSY*
`sdp`	Show diffusion projection	*Show diffusion display*
	You will see a set of signals: 6.5-8.0 - quinine 10.0-11.6 - geraniol 14.0-15.4 - camphene 17.5-18.5 - TMS 19.0-20.0 - Methanol	
`112dmode='nnnn'`	Reset the peak labels	Unset Cross, Box, Number and Diffusion coefficient
`makeslice(6.5,9.0)`	To display the quinine spectrum	Low.Lim.:6.5, Up. Lim:8.0 Show 2D projection within limits
`makeslice(10.0,11.5)`	To display the geraniol projection	Low.Lim.:10.0, Up. Lim:12.0 Show 2D projection within limits
`makeslice(6.5,9.0)`	To display the camphene spectrum	Low.Lim.:13.0, Up. Lim:15.4 Show 2D projection within limits

There is some overlap - the entire data set was recorded in 45 minutes - so the separation of the COSY planes is good but not perfect.

9
DOSY-Related Literature

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9 DOSY-Related Literature

10
DOSY Commands, Parameters, Macros

This section lists DOSY commands (C), parameters (P), and macros (M).

cleardosy (M) deletes any dosy data temporarily saved in the current experiment.

Description: This macro deletes any copies of dosy 1D or 2D data temporarily saved in the current experiment.

See Also: `dosy`

continflag (P) the command `ddif` creates a CONTIN display if `continflag='y'`

Description: Tells the command `ddif` to create a 2D display using data produced by the CONTIN program. Set by the `dosy` macros, does not normally need to be set manually.

Arguments:
```
continflag='y'
continflag='n'
```

See Also: `dosyproc`

continprepare (C) called by the macro `dosy` to prepare the input file for the CONTIN program

Syntax: `continprepare`

Description: `continprepare` takes the `dosy_in` file created in `dosy` and creates the file `dosy_contin.in` the format required by the CONTIN program.

(http://s-provencher.com/index.shtml).

Affected files:
```
curexp+'/dosy/dosy_in'
curexp+'/dosy_contin.in'
```

Arguments:
```
continflag='y'
continflag='n'
```

See Also: `continread, dosy, splmodprepare`

continread (C) called by the macro dosy to take the output of the CONTIN program and create an input file for ddif.

Syntax: continread

Description: continread takes a file dosy_contin.out as created by CONTIN program, run by the continrun shell script from the dosy macro, and creates the files. diffusion_display.contin and diffusion_spectrum used by ddif and sdp to display DOSY results.

Affected files:
curexp+'/dosy/dosy_contin.out'
curexp+'/diffusion_display.contin'

Arguments: continread takes no arguments

See Also ddif, dosy

decay_gen (C) calculates the form of diffusional attenuation expected for the measured gradient and signal maps in non-uniform gradient calibration

Syntax: decay_gen(D,ngrads)

Description: decay_gen takes the measured signal profile and gradient map as a function of position and calculates the predicted signal attenuation as a function of gradient strength.

Affected files:
curexp+'/dosy/NUG/Normalised_profile'
curexp+'/dosy/NUG/Gradient_coefficients'
curexp+'/dosy/NUG/Signal_atten_file'

Arguments: decay_gen takes two arguments: the diffusion coefficient (D) of the calibrant, and the number of gradient levels (ngrads) for which the attenuation is to be calculated. decay_gen is normally run only by the nugcalib macro.

See Also: nugcalib, gradfit, powerfit

Doneshot_nugmap (M)

Set up parameters for non-uniform gradient mapping.

See Also: `dosy, nugcalib, gradfit, powerfit`

dosy3Dflag (P)

used by the `dosy` macro to determine whether to use 2D or 3D DOSY processing

Description: `dosy3Dflag` is a parameter used by the `dosy` macro to determine whether to use 2D or 3D processing. It is normally set automatically, but can also be set manually, for example, to force 2D processing of one increment of a 3D dataset.

Arguments:
```
dosy3Dflag='y'
dosy3Dflag='n'
```

See Also: `dosy`

dosy

Process DOSY experiments (M)

Syntax:
```
dosy(<'prune'>,<lowerlimit,
upperlimit>)
```

Description: Performs a DOSY (diffusion ordered spectroscopy) analysis of the data in an array of spectra.
dosy uses the commands `dll` and `fp` to determine the heights of all signals above the threshold defined by the parameter th and then fits the decay curve for each signal to a Gaussian using the program dosyfit. It stores a summary of all diffusion coefficients and their estimated standard errors and various other results as follows:
- In the directory *$HOME/vnmrsys/Dosy: diffusion_display.inp, general_dosy_stats, calibrated_gradients, fit_errors, and diffusion_spectrum*
- In the current experiment: a second copy of `diffusion_display.inp`.
The command showdosy has been incorporated into `dosy`.

Arguments: `prune` starts a dialog to allow one or more spectra to be omitted from the analysis. `lowerlimit` is the lower diffusion limit (in units of 10^{-10} m^2/s) to be displayed. `upperlimit` is the upper diffusion limit (in units of 10^{-10} m^2/s) to be displayed. Without arguments, dosy uses all the experimental spectra and covers the whole diffusion range seen in the experimental peaks.

See also: `ddiff, redosy, setup_dosy, undosy`

`dosy3Dproc (P)` used by the `dosy` macro to determine whether to use 2D or 3D processing

Description: `dosy3Dproc` is a parameter used by the `dosy` macro to determine whether to use 2D or 3D processing, and what type of the latter. It is normally set automatically, but can also be set manually, for example, to force 2D processing of an increment extracted from a 3D dataset.

Arguments:
```
dosy3Dproc='n'
dosy3Dproc='ntype'
dosy3Dproc='ptype'
dosy3Dproc='y'
```

See Also: `dosy`

dosybypoints (P) determines whether peak picking is used by the dosy macro

Description: Determines whether dosy produces a 2D display based on whole peaks (the default) or point by point (much slower) in the spectral dimension.

Arguments: 'n' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum.

'y' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the threshold th.

See Also: ddif, dosy

dosyfit (C) fits 2D or 3D DOSY data to obtain diffusion coefficients, amplitudes and statistics.

Syntax:
```
dosyfit
dosyfit('version')
dosyfit('3D')
dosyfit('3D', avgnoise)
```

Description: dosyfit performs monoexponential least squares fitting on signal intensities from 2D and 3D datasets, summarizing the results in various files.

Affected files:
```
curexp+'/dosy/dosy_in'
curexp+'/diffusion_display.inp'
curexp+'/dosy/diffusion_display_3D
.inp'
curexp+'/dosy/diffusion_spectrum
curexp+'/dosy/fit_errors'
curexp+'/dosy/general_dosy_stats'
curexp+'/peaks.bin'
curexp+'/peaks.bin.<n>'
```

Arguments: dosyfit takes 0, 1, or 2 arguments: 'version' returns the version umber of the software, '3D' invokes processing of cross-peak volumes stored in the files peaks.bin.<n> rather than peak heights stored in the file dosy_in. In the case of 3D processing, the parameter avgnoise allows correction for the average baseplane noise in absolute value data.

See Also: ddif, dosy

dosyfrq (P) the spectrometer frequency of the encoded (diffusing) nucleus in a DOSY experiment

Description: `dosyfrq` is set to the relevant spectrometer frequency by the macro `makedosyparams` invoked when a DOSY pulse sequence is run, and does not normally need to be set manually.

See Also: `dosy, dosygamma`

dosygamma (P) the magnetogyric ratio of the encoded (diffusing) nucleus in a DOSY experiment

Description: `dosygamma` is calculated from `dosyfrq` by the macro `makedosyparams` invoked when a DOSY pulse sequence is run, and does not normally need to be set manually.

dosyproc (P) determines the type of processing performed by the `dosy` macro

Description: Determines whether `dosy` produces a discrete or a continuous diffusion spectrum.

Arguments: 'discrete' invokes monoexponential fitting with `dosyfit` if `ncomp`=1, and multiexponential fitting with the external program SPLMOD if `ncomp`>1.

'continuous' invokes processing with the external program CONTIN and gives a continuous distribution in the diffusion domain.

See Also: `dosy`

For information about the programs SPLMOD and CONTIN, please visit

http://s-provencher.com/index.shtml.

dosypeaks (P) determines whether peak picking is used by the dosy macro

Description: Determines whether dosy produces a 2D display based on whole peaks (the default) or point by point (much slower) in the spectral dimension.

Arguments: 'y' divides the spectrum into individual peaks, creating one cross-peak for each individual peak found in the 1D spectrum.

'n' performs a diffusion fit for every point in the displayed region of the spectrum that lies above the threshold th.

See Also ddif, dosy

dosytimecubed (P) Stejskal-Tanner time parameter used in DOSY

Description: dosytimecubed collects the three time parameters in the Stejskal-Tanner equation for signal attenuation as a function of field gradient. It is typically equal to the square of the diffusion-encoding gradient pulse width multiplied by the effective diffusion time.

dosytimecubed is set by the macro makedosyparams invoked when a DOSY pulse sequence is run, and does not normally need to be set manually.

See Also: dosy, dosyfrq, dosygamma

gcal_ (P) local value of the conversion factor between gradient in DAC points and gradient in G/cm

Syntax: `gcal_`

Description: `gcal_` is a local copy of the conversion factor from DAC points to G/cm for the probe used. `gcal_` is set equal either to the value in the current probe file, if available, or to the global value `gcal`, by the macro `makedosyparams` invoked when a DOSY pulse sequence is run, and does not normally need to be set manually.

See Also: `gcal`

gradfit (C) calculates fit coefficients describing the variation of gradient strength with position in calibration of non-uniform pulsed field gradients

Syntax:
```
gradfit(lowfrq,highfrq,D)
gradfit(lowfrq,highfrq,D,ncoef)
```

Description: `gradfit` calculates the coefficients of a power series to fit the measured variation of gradient strength with position during the calibration of non-uniform pulsed field gradients.

Affected files:
```
curexp+'/dosy/diffusion_display.inp'
curexp+'/dosy/NUG/write_file'
curexp+'/dosy/NUG/Gradient_coefficien
ts'
curexp+'/dosy/NUG/Gradient_fit_stats'
curexp+'/dosy/NUG/Gradient_fit_stats'
```

Arguments: `gradfit` takes 3 or 4 arguments: `lowfrq` is the lower frequency limit of the signal profile, `highfrq` the high frequency limit, D the diffusion coefficient of the calibrant, and `ncoef` is the number of coefficients in the power series (default is 8).

See Also: `nugcalib, nugcal, powerfit`

int_flg (P) determines whether dosy uses integrals or peak heights for DOSY fitting

Description: int_flg determines whether dosy uses integrals or peak heights for DOSY fitting. int_flg='y' requires that valid integral resets be defined.

Arguments: in_flg='y' invokes fitting of peak integrals
in_flg='n' invokes fitting of peak heights

See Also: dosy

makedosyparams (M) stores the dosyfrq, dosytimecubed and probe_ parameters of a DOSY experiment

Syntax: makedosyparams(dosytimecubed,dosyfrq)

Description: This macro is run automatically when a new-style DOSY pulse sequence is run. It stores the correct values of dosyfrq (the Larmor frequency of the diffusion-encoded nucleus) and dosytimecubed (the time cubed factor in the expression for diffusional attenuation), creating the parameters if necessary.

makedosyparams should not normally need to be run manually. If it is run, it takes the values of dosytimecubed and dosyfrq as arguments.

See Also: dosy, probe_, dosytimecubed, dosyfrq

makeslice (C) Synthesizes an integral projection between specified diffusion limits of a 3D DOSY spectrum onto the frequency frequency plane.

Syntax:
```
makeslice(d1,d2)
makeslice(mode,d1,d2)
```

Description: The `makeslice` command synthesizes an integral projection between specified diffusion limits of a 3D DOSY spectrum onto the frequency plane. It requires the first 2D increment of the 3D DOSY data to have been transformed.

Arguments: The `makeslice` command requires two arguments d1 and d2, the limits in units of 10-10 m2/s between which integration is required. An optional extra argument mode ('i' or 's') may precede d1 and d2. 's' only includes in the integration peaks whose diffusion coefficient lies between the specified limits, whereas 'i' includes the 'tails' of diffusion peaks which lie outside the range between d1 and d2. The default mode is 'i'.

See Also: `dosy, showoriginal`

ncomp (P) the number of components to be used in discrete DOSY fitting

Description: `ncomp` determines the number of components to be used in fitting the signal decay in DOSY when the parameter `dosyproc`='discrete'.

Arguments: `ncomp` should be an integer >0

See Also: `dosy`

nugcal (P) — a parameter array containing calibration information from calibration of non-uniform field gradients

Syntax: `nugcal` is an array with elements `gcal,c1,c2,c3,c4`

Description: `nugcal` is a parameter array summarizing the results of a calibration of non-uniform field gradients. The first value is the gradient calibration value `gcal` used. `c1-c4` are the coefficients of a fourth order power series in the exponent of the Stejskal-Tanner equation.

`nugcal` is a global parameter specific for a a given probe and pulse sequence. The parameter `nugcal_` is a local copy that is set when a `dosy` experiment is run, to ensure that the correct parameters are available for subsequent processing if `nugflag`='y'.

See Also: `dosy, nugcalib, nugflag`

nugcalib (M) — The nugcalib macro calculates the probe/pulse sequence specific coefficients from an experiment designed to map the non-uniformity (NUG) of the pulsed field gradients.

Syntax:
```
nugcalib
nugcalib(calibrant,(T|D),saveglobal,saveprobe)
```

Description: `nugcalib` calculates a set of four coefficients that relate the nominal gradient strength per DAC point, `gcal`, to the calculated diffusional signal attenuation as a function of gradient for a given probe and pulse sequence.

As input, `nugcalib` requires:

- the calibrant used ('w' for pure water, 'd' for dilute HDO, 'o' for other;
- the temperature (T) in Celsius if 'w' or 'd', or the diffusion coefficient (D) in units of 10^{-10} m^2/s if 'o';

- decisions on whether or not to save the results in the global parameter file and/or in the current probe file.

This information is supplied either as four arguments (see below) or by dialog. The macro:

- takes a set of signal profiles measured under a read gradient, performs monoexponential DOSY fitting on each point across the profile, and uses the resultant data and the known diffusion coefficient for the calibrant to obtain a map of relative gradient strength as a function of position.
- fits this map with `gradfit` to obtain a set of coefficients.
- uses these coefficients to extrapolate into regions of small signal.
- normalizes the signal profile with `profile_int`.
- takes the gradient coefficients and signal profile and uses `deacy_gen` to calculate the diffusional attenuation as a function of nominal gradient strength.
- and uses `powerfit` to fit this decay to the exponential of a power series in the Stejskal-Tanner exponent, storing the results in the array `nugcal_` (and optionally in the global parameter nugcal and/or the current probe file)

Affected files:

```
curexp+'/dosy/dosy_in'
curexp+'/dosy/NUG/fit_coefficients'
curexp+'/dosy/NUG/fit_coeff_stats'
curexp+'/dosy/NUG/fit_coeff_stats_exp
l'
curexp+'/dosy/NUG/Gradient_coefficien
ts'
curexp+'/dosy/NUG/Gradient_fit_stats'
curexp+'/dosy/NUG/Gradient_fit_stats_
expl'
curexp+'/dosy/NUG/Normalised_profile'
curexp+'/dosy/NUG/Signal_atten_file'
curexp+'/dosy/NUG/Signal_profile'
```

Arguments

```
nugcalib('w',temperature,('n'|'y')
```

```
,('n'|'y'))
nugcalib('d',temperature,('n'|'y')
,('n'|'y'))
nugcalib('o',diffusion
coefficient,('n'|'y'),('n'|'y'))
```

See Also: `decay_gen, dosy, gcal, gcal_, gradfit, nugcal, nugcal_, nugflag, powerfit, profile_int`

`nugflag (P)` tells the macro `dosy` to use processing with correction for non-uniform field gradients

Syntax:

```
nugflag='y'
```

```
nugflag='n'
```

Description: When `nugflag='n'`, DOSY processing invoked by the dosy macro uses simple mono- or multi-exponential fitting to estimate diffusion coefficients by fitting to the Stejskal-Tanner equation. When `nugflag='y'`, a modified Stejskal-Tanner equation is used in which the exponent is replace by a power series, the coefficients for which are stored in the array nugcal.

Correction for non-uniform gradients is available in both 2D and 3D DOSY, but only for discrete fitting (`dosyproc='discrete'`) and not for CONTIN.

See Also: `nugcal, nugcalib, dosy, dosyproc`

`powerfit (C)` fits the diffusional attenuation calculated by `decay_gen` to the exponential of a power series in the calibration of the non-uniformity of pulsed field gradients.

Syntax:

```
powerfit()
```

```
powerfit(ncoef)
```

Description: Used in the calibration of non-uniform field gradients to fit the diffusional decay calculated by `decay_gen` to the exponential of a power series.

Affected files: `curexp+'/dosy/NUG/Signal_atten_file'`
`curexp+'/dosy/NUG/write_file'`
`curexp+'/dosy/NUG/fit_coefficients'`
`curexp+'/dosy/NUG/fit_coeff_stats'`
`curexp+'/dosy/NUG/fit_coeff_stats_expl'`

Arguments: `powerfit` has one optional argument, the number of coefficients in the power series. The default is 8.

See Also: `decay_gen, gradfit, nugcalib, profile_int`

`probe_ (P)` probe name used for DOSY experiments

Syntax: `probe_`

Description: `probe_` is a local copy of the parameter probe, set when a dosy experiment is run to ensure that the correct parameters are available for subsequent processing.

See Also: `dosy, makedosyparams, probe`

`profile_int (C)` normalize the experimental signal profile during calibration of non-uniform pulsed gradients.

Syntax: `profile_int(lowfrq,highfrq)`

Description: Integrates the signal in the file `Signal_profile`, normalizes it, and writes it to the file `Normalised_profile`.

Affected files: `curexp+'/dosy/NUG/Normalised_profile'`
`curexp+'/dosy/NUG/Signal_profile'`

Arguments: `profile_int` takes two arguments: `lowfrq` is the lower frequency limit of the profile, `highfrq` is the high frequency limit of the profile

See Also: `decay_gen, gradfit, nugcalib, powerfit`

redosy (M) Restore 2D DOSY display from sub experiment

Description: Restores the previous 2D DOSY display (if one exists) by recalling the data stored by the `dosy` macro in the file `subexp/dosy2Ddisplay` in the current experiment. `undosy` and `redosy` enable easy switching between the 1D DOSY data (spectra as a function of `gzlvl`) and the 2D DOSY display (signal as a function of frequency and diffusion coefficient).

See also: `dosy`, `undosy`

reorder3D (M) reorders array elements in arrayed phase sensitive 2D experiment

Syntax: `reorder3D`

Description: Exchanges the order of the two arrayed parameters in an arrayed phase sensitive 2D experiment. Useful if 3D DOSY data are acquired with `array='phase,gzlvl1'` instead of `array='gzlvl1,phase'`.

See Also: `dosy`

sdp (M) shows the diffusion projection of a 2D or 3D dosy spectrum with high digital resolution

Syntax:
```
sdp
sdp(n)
```

Description: Constructs a diffusion spectrum equivalent to the projection of the current 2D or 3D DOSY spectrum onto the diffusion axis, but with high digital resolution, either in the current experiment (no argument) or in experiment n. If no argument is given the original data may be recovered with the macro `undosy`, or the DOSY spectrum with `redosy`.

Affected files: `curexp+'/dosy/diffusion_spectrum'`

Arguments:	This macro takes one optional argument which is the number of the experiment in which the dosy projection should be displayed.
See Also:	`dosy, redosy, undosy`

WARNING `sdp` **overwrites the current parameters in the destination experiment.**

setup_dosy (M) sets up the array of gradient levels for a DOSY experiment

Syntax:
```
setup_dosy
setup_dosy(n)
setup_dosy(n,gmin,gmax)
setup_dosy('panelread')
```

Description: `setup_dosy` initialises DOSY parameters with `makedosyparams`, and sets up an array of n values of `gzlvl1` ranging from `gmin` to `gmax` in approximately equal steps of gradient squared, rounded to the nearest 5 DAC points.

Called with no arguments it initiates a dialogue to establish `n`, `gmin` and `gmax`. With one numercial argument it uses `n` equal steps up to 20% below maximum gradient, and with three numercial arguments it uses `n` equal steps from `gmin` to `gmax`, all rounded to the nearest 5 DAC points. If called with the 'panelread' argument, `setup_dosy` reads the values of `n, gmin` and `gmax` from the **'Acquire-Defaults'** and/or **'Acquire-Pulse Sequence'** panels.

Arguments: 0, 1 or 3 arguments as above.

See Also: `dosy`

showdosyfit (M) plots the experimental signal attenuation, fitted attenuation and residual for one peak from a 2D or 3D DOSY experiment

Syntax:
```
showdosyfit(peaknr)
showdosyfit(peaknr,expFac)
```

Description: Displays using `expl` the result of fitting peak `peaknr` using `dosy`. Experimental data points are in red, fitted points in blue, and residuals in magenta.

Affected files:
```
curexp+'/dosy/general_dosy_stats'
curexp+'/dosy/dosyplot'
```

Arguments: The macro takes one or two arguments (`peaknr, expFac`), which are the peak number and the expansion factor of the

residual respectively. When `expFac` is not given it defaults to 1.

See Also: `dosy`

`showdosyresidual (M)` plots the residual for one peak from a 2D or 3D DOSY experiment

Syntax:
```
showdosyresidual(peaknr)
showdosyresidual(peaknr,expFac)
```

Description: Displays using `expl` the residuals of fitting peak `peaknr` using dosy.

Affected files:
```
curexp+'/dosy/general_dosy_stats'
curexp+'/dosy/dosyplot'
```

Arguments: The macro takes one or two arguments (`peaknr, expFac`), which are the peak number and the expansion factor of the residual respectively. When `expFac` is not given it defaults to 1.

See Also: `dosy`

`showgradfit (M)` plots the experimental gradient variation with position and the power series fit in non-uniform gradient calibration

Syntax: `showgradfit`

Description: Displays (using `expl`) the result of fitting the experimental variation of gradient strength with position, measured during non-uniform gradient calibration, and the result of fitting with a power series. Experimental data points are in red and fitted points in blue.

Affected files:
```
curexp+'/dosy/Gradient_fit_stats'
curexp+'/dosy/Gradient_fit_stats_expl'
```

See Also: `gradfit, nugcalib, powerfit, shownugfit`

shownugfit (M)	plots the logarithm of the calculated diffusional attenuation and of the power series fit in non-uniform gradient calibration
Syntax:	`shownugfit`
Description:	Displays (using `expl`) the result of fitting the calculated signal attenuation as a function of gradient squared to the exponential of a power series. Calculated data points are in red and fitted points in blue.

Affected files:	`curexp+'/dosy/Signal_atten_file'`
	`curexp+'/dosy/fit_coeff_stats'`
	`curexp+'/dosy/fit_coeff_stats_expl'`

See Also:	`gradfit, nugcalib, powerfit, shownugfit`

showoriginal (M)	restores the first 2D spectrum in a 3D DOSY experiment
Syntax:	`showoriginal`
Description:	Restores the first 2D spectrum in a 3D DOSY experiment after it has been saved by the `dosy` macro.
Affected files:	`curexp+'/subexp/original2d'`
See Also:	`dosy, makeslice`

splmodprepare (C) used by the dosy macro to prepare data for the program SPLMOD

Syntax: splmodprepare

Description: splmodprepare takes a dosy_in file as created by dosy and creates the file dosy_splmod.in in a format suitable for the SPLMOD program (http://s-provencher.com/index.shtml).

Affected files: curexp+'/dosy/dosy_in
curexp+'/dosy/dosy_splmod.in'

See Also: splmodread, continread, continprepare, dosy

splmodread (C) used by the dosy macro to convert the output of the SPLMOD program into a form suitable for ddif

Syntax: splmodread

Description: splmodread takes the file dosy_splmod.out, created by SPLMOD (run by the splmodrun shell script from the dosy macro) and creates the files diffusion_display.inp and diffusion_spectrum in a suitable format for the ddif and sdp commands respectively.

Affected files: curexp+'/dosy/dosy_in
curexp+'/dosy/dosy_splmod.in'

See Also: splmodprepare, continprepare, continread, dosy

undosy (M) restores 1D DOSY data stored by the dosy macro

Syntax: undosy

Description: undosy restores 1D DOSY data stored by the dosy macro (if they exist), recalling the data stored in the file subexp/dosy1Ddata in the current experiment. The macros undosy and redosy allow easy switching between the 1D DOSY data (spectra as a function of gzlvl1) and the 2D DOSY display (signal as a function of frequency and diffusion coefficient).

See Also: redosy, dosy

undosy3D (M) restores 2D DOSY data stored by the dosy macro in 3D DOSY

Syntax: undosy3D

Description: undosy3D restores 2D DOSY data stored by the dosy macro (if they exist), recalling the data stored in the file subexp/original2d in the current experiment.

See Also: dosy

Agilent Technologies

Printed in USA, February 2011