7.3 2D Gradient NOESY Experiment

7.3.1 Introduction

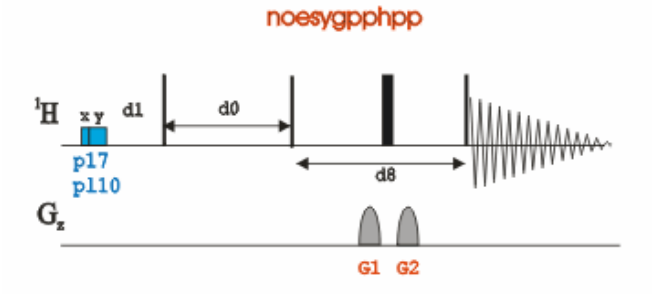
NOESY (Nuclear Overhauser Effect SpectroscopY) is a 2D spectroscopy method used to identify spins undergoing cross-relaxation and to measure the cross-relaxation rates. Most commonly, NOESY is used as a homonuclear 1H technique. In NOESY, direct dipolar couplings provide the primary means of cross-relaxation, and so spins undergoing cross-relaxation are those which are close to one another in space. Thus, the cross peaks of a NOESY spectrum indicate which protons are close to each other in space. This can be distinguished from COSY, for example, which relies on J-coupling to provide spin-spin correlation, and its cross peaks indicate which 1H atoms are close to other 1H atoms through the bonds of the molecule.

The basic NOESY sequence consists of three p/2 pulses. The first pulse creates transverse spin magnetization. This precesses during the evolution time t1, which is incremented during the course of the 2D experiment. The second pulse produces longitudinal magnetization equal to the transverse magnetization component orthogonal to the pulse direction. Thus, the basic idea is to produce an initial situation for the mixing period d8. Note that, for the basic NOESY experiment, d8 is kept constant throughout the 2D experiment. The third pulse creates transverse magnetization from the remaining longitudinal magnetization. Acquisition begins immediately following the third pulse, and the transverse magnetization is observed as a function of the time t2. The NOESY spectrum is generated by a 2D Fourier transform with respect to t1 and t2.

Axial peaks, which originate from magnetization that has relaxed during tm, can be removed by the appropriate phase cycling.

NOESY spectra can be obtained in 2D absorption mode. Occasionally, COSY-type artifacts appear in the NOESY spectrum; however, these are easy to identify by their anti-phase multiplet structure.

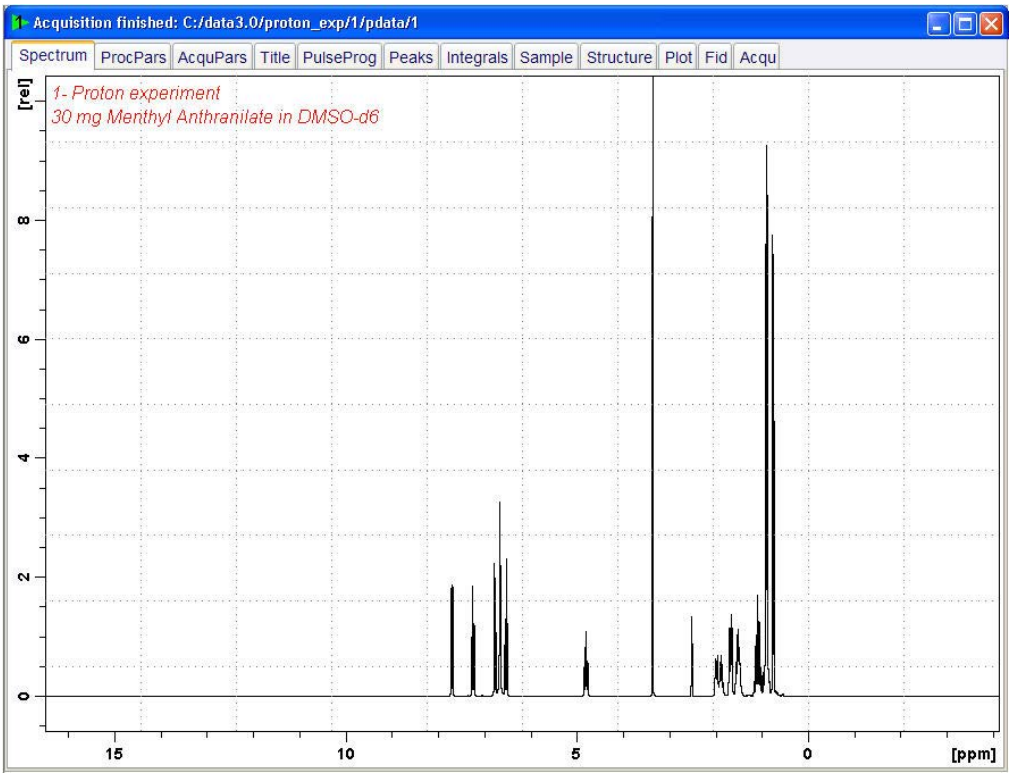
This section describes the acquisition and processing of a two-dimensional 1H phase sensitive NOESY. The standard Bruker parameter set is NOESYPHSW and includes the pulse sequence **noesygpphpp** shown in the next figure. It consists of the recycling delay, three radio-frequency (RF) pulses, separated by the increment delay D0 between the first and second pulse, a mixing time D8 between the second and third pulse and the acquisition time during which the signal is recorded. All three pulses are of 90^0.



The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

7.3.2 Preparation Experiment

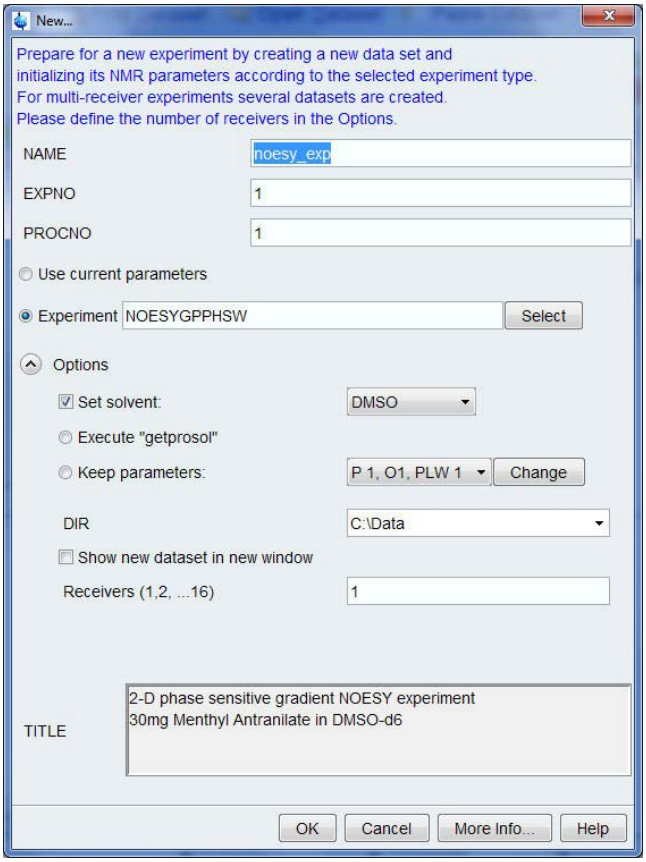
- Run a **1D Proton** spectrum, following the instructions in Chapter 1D Proton Experiment, *Experiment Setup [▶ 25]* through *Processing [▶ 30]*.



7.3.3 Setting up the NOESY Experiment

- On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.

- In the New window, enter or select:
  NAME = **noesy_exp**
  EXPNO = **1**
  PROCNO = **1**
  Experiment = **NOESYGPPHSW**
  Set Solvent = **DMSO**



DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

- In the New Dataset window, click **OK**.

Follow the instructions in the chapter *Setting up the COSY Experiment [▶ 58]* for performing **Prosol** and **SetLimits**. If you know what you're doing, this should give you all the necessary information. If you need more details, you're referred to those details from the COSY experiment.

- In the Dataset window, select the **AcquPars** tab.
- In the AcquPars tab toolbar click **Show pulse program parameters**. 
- In the Field D8[sec] enter **0.450**.

The mixing time depends on the size of the Molecule. The range for Bio-molecules is typically from **0.05 s** to **0.2 s**, medium size molecules from **0.1 s** to **0.5 s** and for small molecules **0.5 s** to **0.9 s**.

- In the Dataset window, select the **Spectrum** tab.

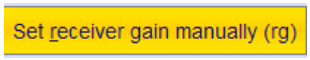
7.3.4 Acquisition

- On the Workflow button bar, click **Gain**.

EK: Important! Issue 'pulsecal' on a command line to calibrate proton pulse before adjusting the gain!

or

- On the **Gain** button, click the **drop-down** arrow to adjust the receiver gain manually.



- On the Workflow button bar, click **Go**.

or

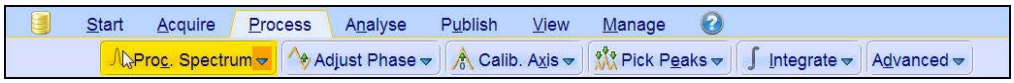
- On the **Go** button, click the **drop-down** arrow to see more options.

7.3.5 Processing

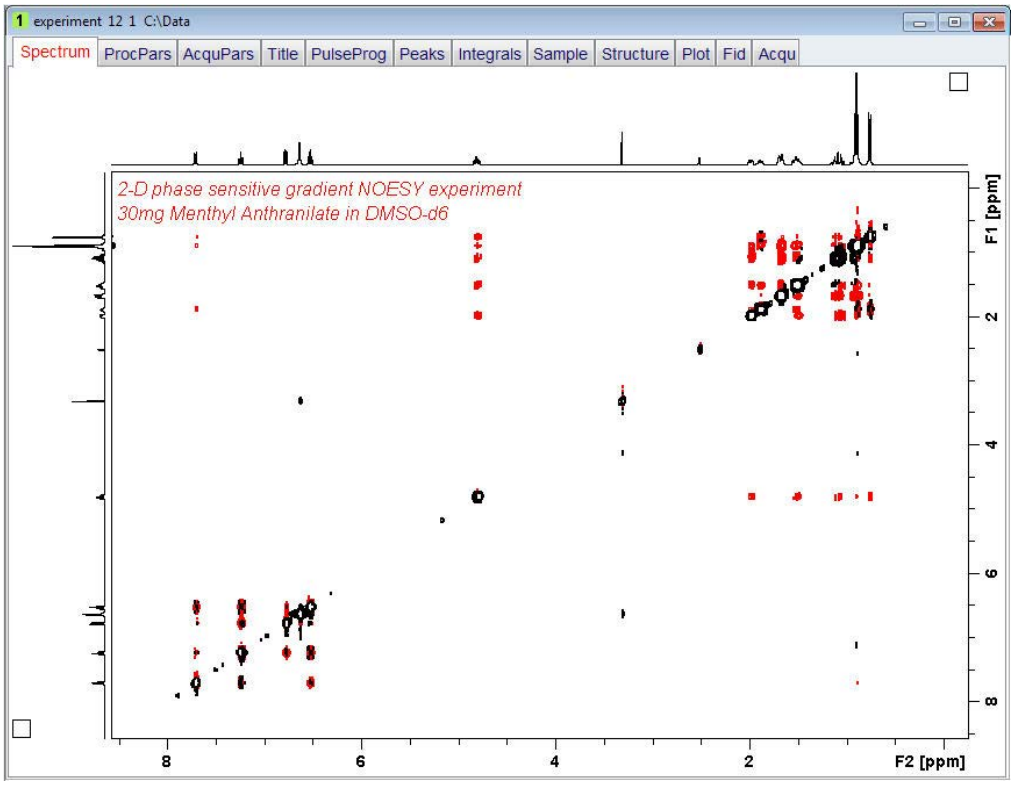
When the acquisition is finished:

- On the menu bar, click **Process**.

- On the Workflow button bar, click **Proc Spectrum**.



This executes a standard processing program **proc2d**. The **apk2d** option has to be enabled. To enable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow and configure the **Standard Processing (proc2d)** program.



7.3.6 Plotting the NOESY Spectrum

- Follow the plotting instructions in chapter *Plotting the COSY Spectrum [▶ 63]* in this chapter.