NOESY

Chapter 13

Goto

Introduction 13.1

Nuclear **O**verhauser **E**ffect **S**pectroscop**Y** is a 2D spectroscopy method whose aim is to identify spins undergoing cross-relaxation and to measure the cross-relaxation rates. Most commonly, NOESY is used as a homonuclear ^{1}H 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 ^{1}H's are close to which other ^{1}H's in space. This can be distinguished from COSY, for example, which relies on J-coupling to provide spin-spin correlation, and whose cross peaks indicate which ^{1}H's are close to which other ^{1}H's through the bonds of the molecule.

The basic NOESY sequence consists of three $\pi/2$ pulses. The first pulse creates transverse spin magnetization. This precesses during the evolution time t_1, 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 τ_m (the time during which cross relaxation occurs) where the longitudinal polarization of each spin is labelled by its resonance frequency. The longitudinal magnetization is allowed to relax during the mixing time τ_m. Note that, for the basic NOESY experiment, τ_m 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 t_2. The NOESY spectrum is generated by a 2D Fourier transform with respect to t_1 and t_2.

Axial peaks, which originate from magnetization that has relaxed during τ_m, 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.

References: J. Jeener, B. H. Meier, P. Bachmann, R. R. Ernst, *J. Chem. Phys.*, **69**, 4546 (1979); G. Wagner and K. Wüthrich, *J. Mol. Biol.*, **155**, 347 (1982).

Sample

The sample used to demonstrate NOESY in the chapter is 50 mM Gramicidin in DMSO-d6. This is the same sample that was used to demonstrate COSY.

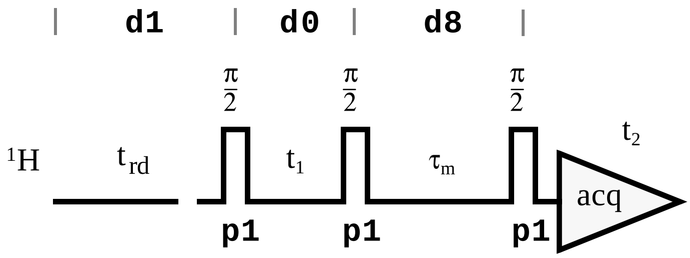
Pulse Sequence Diagram 13.2

The NOESY pulse sequence is shown in Figure 40. Notice that the pulse **p1** must be set to the appropriate 90° time found in Chapter 5 'Pulse Calibration'. The delay **d8** determines the length of the mixing period, during which NOE buildup occurs.

Goto

NOESY

Figure 40: NOESY Pulse Sequence



Acquisition and Processing

13.3

Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation.

It is generally recommended that NOESY, like all 2D experiments, be run without sample spinning.

^{1}H reference spectrum

Since NOESY is a homonuclear experiment only one reference spectrum is required. This ^{1}H spectrum will be used to determine **`o1`** and **`sw`** for the NOESY experiment, and can also be used as both the F1 and the F2 projections of the NOESY spectrum.

A ^{1}H reference spectrum of this sample was already created for the magnitude COSY experiment. This spectrum is found in the data set proton/5/1.

Create a new file directory for the 2D data set

Since the NOESY experiment is so similar to the COSY experiment, it makes sense to create the NOESY data set from the magnitude COSY data set. Enter **`re cosy 1 1`** to call up the data set cosy/1/1. Enter **`edc`** and change the following parameters:

NAME	noesy
EXPNO	1
PROCNO	1 .

Click **SAVE** to create the data set noesy/1/1.

If you do not wish to generate the NOESY data set from the COSY data set, it can be generated from the ^{1}H reference spectrum as follows: Enter **`re proton 5 1`** to call up the data set proton/5/1. Enter **`edc`** and change the following parameters:

NAME	noesy
EXPNO	1
PROCNO	1 .

Click **SAVE** to create the data set noesy/1/1.

Change to 2D parameter mode

If this data set was created from the magnitude COSY data set, it is already in 2D parameter mode. If not, enter **`eda`** and set PARMODE = 2D. Click on **SAVE** and ok the message “Delete ‘meta.ext’ files?”. The window now switches to a 2D display and the message “NEW 2D DATA SET” appears.

Set up the acquisition parameters

Enter **`eda`** and set the acquisition parameters as shown in Table 43. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters **`pl1`** and **`p1`** (^{1}H observe high power level and 90° pulse time).

The F2 parameters **`o1`** and **`sw`** (not shown in the table) should be identical to the values used in the optimized ^{1}H reference spectrum (proton/5/1). The F1 parameters **`sfo1`** and **`sw`** should be identical to the corresponding F2 values.

Finally, notice that **`in0`** and sw(F1) are not independent. A convenient way to set **`in0`** is to set the F1 parameters **`nuc1`** by clicking **`on`** **NUCLEI** for F1 parameters, **`nd0`**, and **`sw`** correctly. This automatically sets **`in0`** to the correct value.

Table 43. NOESY Acquisition Parameters

F2 Parameters		
Parameter	Value	Comments
PULPROG	noesytp	noesy using TPPI for quadrature detection in F1; see Figure 40 for pulse sequence diagram.
TD	1k	
NS	32	the number of scans must be 8*n for the phase cycling to work properly.
DS	16	number of dummy scans.
PL1		high power level on f1 channel (see “An Important Note on Power Levels” on page 7).
P1		90° ^{1}H high power pulse on f1 channel.
D0	3µsec	incremented delay (t_1); predefined.
D1	2sec	relaxation delay; should be about 1.25*T_1(^{1}H).
D8	350msec	mixing time for NOE buildup; should be on the order of T_1.
F1 Parameters		
Parameter	Value	Comments
TD	256	number of experiments.
ND0	2	there is one d0 period per cycle and MC2 = TPPI.
IN0	$1/(2*SW_H) = DW_H$	t_1 increment.

SW		sw of the optimized ^{1}H spectrum (proton/5/1); same as for F2.
NUC1		select ^{1}H frequency for F1; same as for F2.

Optimize d8

The parameter **d8** determines the length of the mixing period during which NOE buildup occurs. This should be on the order of T_1. The value listed in Table 43 is appropriate for this sample at 300 MHz and room temperature. In general, if the user is not sure of the appropriate value of **d8**, the following quick and easy procedure can be used.

First create a 1D data set from the NOESY 2D data set. Enter **edc**, set EXPNO to 2, and click **SAVE** to create the data set noesy/2/1. Enter **eda**, set PARMODE to 1D, click **SAVE** and ok the requests to delete acqu2, acqu2s and proc2, proc2s, and luta files.

In **eda** set PULPROG to zg (or equivalently, enter **pulprog** and then **zg** at the prompt). Set **ns** to 1 and **ds** to 0. Use **zg** and **ef** to acquire and process a 1D ^{1}H spectrum. Manually phase correct the spectrum and store the correction. Notice that this phase correction is necessary for a correct interpretation of the t1ir1d data below.

In **eda** change PULPROG to the pulse program to t1ir1d (or equivalently, enter **pulprog** and then **t1ir1d** at the prompt). This is an inversion recovery sequence. Set **d7** to approximately 500 msec (note that it is important to select a large enough starting value for **d7** since the **gs** routine will only allow the user to vary **d7** from 0 to twice the starting value).

Enter **acqu** to enter the acquisition window. Enter **gs** to start the go setup routine. Click the left mouse button to fix the “acquisition-gs” window somewhere on the screen, and then click on the box in the upper right hand corner of the window to send it away as an icon. Click on **Display->Phasing->PK phasing** to display the Fourier transformed data during **gs** and to apply the phase correction determined above to the Fourier transformed data. While monitoring the Fourier transformed data, adjust the value of **d7** (simply enter **d7** and then a new value at the prompt). Notice that for very short values of **d7**, all the signals are negative, while for very long values, all the signals are positive. Select the value of **d7** for which most of the signals have just passed through a null. This length of time is sufficient for NOE buildup in small molecules (in order to avoid spin diffusion in macromolecules, it may be necessary to use a shorter length of time).

Return to the NOESY data set by typing **re 1**. Enter **d8** and set this to the value of **d7** determined above.

Acquire the 2D data set

If this data set was created from the magnitude COSY spectrum cosy/1/1, the receiver gain is already set correctly.

Enter **zg** to acquire the time domain data. The approximate experiment time for NOESY with the acquisition parameters set as shown above is 5.8 hours.

Set up the processing parameters

Enter **`edp`** and set the processing parameters as shown in Table 44.

Table 44. NOESY Processing Parameters

F2 Parameters		
Parameter	Value	Comments
SI	1k	
SF		spectrum reference frequency (^{1}H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	2	choose pure cosine wave.
PH_mod	pk	apply 0- and 1st-order phase correction determined by phase correcting the first row.
PKNL	TRUE	necessary when using the digital filter.
BC_mod	quad	
F1 Parameters		
Parameter	Value	Comments
SI	512	
SF		spectrum reference frequency (^{1}H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	2	choose pure cosine wave.
PH_mod	no	first determine 0- and 1st-order phase correction with phasing subroutine.
BC_mod	no	
MC2	TPPI	determines type of FT in F1; TPPI results in a forward single real FT.

Process the 2D data set

Select window functions to optimize sensitivity or resolution. It is not possible with a window function, to suppress diagonal peaks relative to cross peaks.

Enter **`xfb`** to multiply the time domain data by the window functions and also perform the 2D Fourier transform. The 2D data set is displayed automatically.

Adjust the contour levels

The threshold level can be adjusted by placing the cursor on the ■ button, holding down the left mouse button, and moving the mouse up and down.

Since this is a phase-sensitive spectrum, click on **+/-** with the left mouse button until both positive and negative peaks are displayed.

The optimum display (both the threshold and which peaks are displayed) may be saved by clicking on **DefPlot**.

Phase correct the spectrum

To simplify the phasing of the 2D NOESY spectrum, it helps first to phase correct the first row. Enter `rser 1` to transfer the first row to the 1D data set ~TEMP/1/1. Enter `sinm` to apply the sine-bell windowing function, and enter `ft` to Fourier transform the data. Manually phase correct the spectrum as you would any 1D spectrum *except* that when you are finished, click **return** and select **save as 2D & return** to save the corrections `phc0` and `phc1` to the corresponding F2 parameters of the 2D data file noesy/1/1. Click **2D** with the left mouse button to return to the 2D data set noesy/1/1.

Now enter `xfb` to Fourier transform the NOESY spectrum again, this time applying the appropriate phase correction to F2. The spectrum should now require additional phase correction only in F1, and this can be accomplished in the 2D phasing subroutine.

Click on **phase** to enter the phase correction submenu. To phase correct a 2D spectrum in the F1 dimension (i.e., the columns), first select three columns as described below.

In the phase correction submenu, click on **col** with the left mouse button to tie the cursor to the 2D spectrum appearing in the upper left hand corner of the display. Move the mouse until the vertical cross hair is aligned with a column towards one end of the spectrum. This column should contain a diagonal peak. Select the column by clicking the middle mouse button. If the selected column does not intersect the most intense portion of the diagonal peak, click on **+** or **-** with the left mouse button until it does. Once the desired column is selected, click on **mov 1** with the left mouse button to move the column to window 1, appearing in the upper right hand corner of the display.

Click on **col** again and move the cross hair until it is aligned with a column containing a diagonal peak near the middle of the spectrum. Select the column by clicking the middle mouse button, adjust the selected column by clicking on **+** or **-** with the left and middle mouse buttons, and finally move the desired column to window 2 by clicking on **mov 2** with the left mouse button.

Repeat the above procedure to select a column with a diagonal peak at the other end of the spectrum. Move this column to window 3 by clicking **mov 3** with the left mouse button.

Now that three columns have been selected, the 0th- and 1st-order phase corrections in F1 are determined by hand. Click on **big: 1** with the left mouse button to set the pivot point to the biggest peak in window 1. Note that if the diagonal peak is not the biggest peak in the window, use **cur: 1** and the mouse to select the diagonal peak by hand.

Move the cursor to the **ph0** button. Hold down the left mouse button and drag the mouse to adjust the 0th-order phase correction. Recall that the 0th-order phase correction should be adjusted so that the peak at the pivot point is phased correctly (i.e., here the diagonal peak in window 1). Next, move the cursor to the `ph1` button and drag the mouse to adjust the 1st-order phase correction. Recall that the 1st-order phase correction should be adjusted so that the peak farthest from the pivot point is phased correctly (i.e., here the diagonal peak in window 3).

When you are satisfied with the phase correction, click on **return** and select **save & return** to save the results and confirm the xf1p option to apply this phase correction to the spectrum.

At this point, the spectrum should be phased correctly. If, however, the user wishes to make further adjustments, the above procedure can be repeated to adjust the F1 phasing. To further phase correct the spectrum in F2, repeat the above procedure using **row** rather than **col** to select three rows with diagonal peaks at one end of the spectrum, in the middle, and at the other end. Phase correct as described above and after selecting **save & return** after **return**, confirm the xf2p option.

Note that it is possible to exit the phase correction subroutine without saving the phase corrections by clicking on **return**. Select **save & return** without confirming the xf2p or xf1p option means that the new phase correction is saved to the **edp** menu, but not applied to the spectrum.

When the NOESY spectrum is properly phased, the diagonal peaks will be either all positive or all negative. The cross peaks may be positive or negative. Any anti-phase cross peaks are COSY artifacts.

If the data is to be retransformed with, e.g., different window functions, the phase correction determined above can be automatically applied by setting **PH_mod** to **pk** in both F1 and F2 of **edp**.

Plot the spectrum

Read in the plot parameter file standard2D, e.g., enter **rpar standard2D plot**. This sets most of the plotting parameters to values which are appropriate for this 2D spectrum, assuming that the paper size to be used here is the same as the default paper size defined when the spectrometer was configured.

More information about plotting parameters and the file standard2D can be found in Appendix C ‘1D and 2D Plotting Parameters’.

To set the region (full or expanded), threshold, and peak type (positive and/or negative), to be used in plotting the spectrum, first make sure the spectrum appears as desired on the screen, and then click **DefPlot** and answer the following questions.

Change levels?	y
Please enter number of positive levels?	6
Please enter number of negative levels?	3
Display contours?	n .

Enter **edg** to edit the plotting parameters.

Click the **ed** next to the parameter EDPROJ1 to enter the F1 projection parameters submenu. Edit the parameters from PF1DU to PF1PROC as follows:

PF1DU	u
PF1USER	(name of user for file proton/5/1)
PF1NAME	proton
PF1EXP	5
PF1PROC	1 .

Click **SAVE** to save these changes and return to the **edg** menu.

Click the **ed** next to the parameter EDPROJ2 to enter the F2 projection parameters submenu. Edit the parameters from PF2DU to PF2PROC as follows:

PF2DU	u
PF2USER	(name of user for file proton/5/1)
PF2NAME	proton
PF2EXP	5
PF2PROC	1 .

Click **`SAVE`** to save these changes and return to the **`edg`** menu.

Click **`SAVE`** to save all the above changes and exit the **`edg`** menu.

Next create a title for the spectrum. Enter **`setti`** to use the vi editor to open the title file. Write a title and save the file.

To plot the spectrum, simply enter **`plot`** (provided the correct plotter is selected in **`edo`**).

A NOESY spectrum of 50 mM Gramicidin in DMSO-d6 is shown in Figure 41.

Figure 41: NOESY Spectrum of 50 mM Gramicidin in DMSO-d6