ROESY

Introduction 14.1

Rotating-frame **O**verhauser **E**ffect **S**pectroscop**Y** is an experiment in which homonuclear NOE effects are measured under spin-locked conditions. ROESY is especially suited for molecules with motional correlation times (τ_c) such that $\omega\tau_c \sim 1$, where ω is the angular frequency $\omega = \gamma B$. In such cases the laboratory-frame NOE is nearly zero, but the rotating-frame NOE (or ROE) is always positive and increases monotonically for increasing values of τ_c. In ROESY the mixing time is the spin-lock period. During this time spin exchange occurs among spin-locked magnetization components of different nuclei (recall that spin exchange in NOESY occurs while magnetization is aligned along the z axis). Different spectral density functions are relevant for ROESY than for NOESY and these cause the ROE's to be positive for all values of τ_c.

ROESY spectra can be obtained in 2D absorption mode. This is also useful for the identification of certain artifacts. Spurious cross peaks, both COSY-type and TOCSY-type, can be observed due to coherence transfer between scalar coupled spins. COSY-type artifacts (anti-phase) arise when the mixing pulse transfers anti-phase magnetization from one spin to another (the long spin-lock pulse acts like the mixing pulse in COSY). TOCSY-type artifacts (which have the same phase as the diagonal peaks, while ROESY cross peaks have the opposite phase) arise when the Hartmann-Hahn condition is met (e.g., when spins A and B have opposite but equal offsets from the transmitter frequency or when they have nearly identical chemical shifts). In general, to minimize these artifacts, it is suggested to limit the strength of the spin-locking field.

Reference: A. Bax and D. G. Davis, *J. Magn. Reson.*, **63**, 207 (1985).

Sample

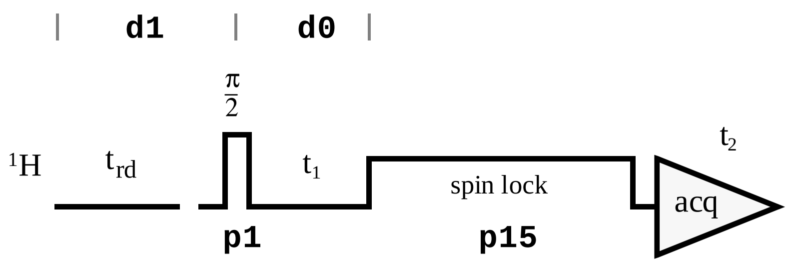
The sample used to demonstrate ROESY in this chapter is 50 mM Gramicidin in DMSO-d6. This is the same sample that was used to demonstrate COSY and NOESY.

Pulse Sequence Diagram 14.2

The ROESY pulse sequence is shown in Figure 42. Notice that the pulse **p1** must be set to the appropriate 90° time found in Chapter 5 'Pulse Calibration'. The pulse **p15** is the cw spinlock pulse, during which ROE buildup occurs.

ROESY

Figure 42: ROESY Pulse Sequence



Acquisition and Processing 14.3

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 ROESY, like all 2D experiments, be run without sample spinning.

^{1}H reference spectrum

Since ROESY is a homonuclear experiment only one reference spectrum is required. This ^{1}H spectrum will be used to determine **o1** and **sw** for the ROESY experiment, and can also be used as both the F1 and the F2 projections of the ROESY 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 ROESY experiment is so similar to the NOESY experiment, it makes sense to create the ROESY data set from the NOESY data set. From the data set noesy/1/1, enter **edc** and change the following parameters:

NAME	roesy
EXPNO	1
PROCNO	1 .

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

Change to 2D parameter mode

If this data set was created from the NOESY data set, it is already in 2D parameter mode.

Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 45. 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), and **pl11** (^{1}H low power level for ROESY spinlock).

The parameter **p15** sets the length of the cw spinlock pulse. The value listed in Table 45 is appropriate for this sample. For other samples with different relaxation properties, optimal results may be achieved with slightly different values. The typical range for **p15** is from 50 to 300 msec. A good rule of thumb is that **p15** for the ROESY experiment of a molecule should be about the same as **d8** for the NOESY experiment of that molecule.

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 45. ROESY Acquisition Parameters

F2 Parameters		
Parameter	Value	Comments
PULPROG	roesytp	see Figure 42 for pulse sequence diagram.
TD	1k	
NS	32	the number of scans must 8*n in order 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).
PL11		ROESY spin-lock power level on f1 channel.
P1		90° ^{1}H high power pulse on f1 channel.
P15	200 msec	cw pulse for ROESY spin lock; should be approximately Ý$T_{1\rho}$.
D0	3 µsec	incremented delay (t_1); predefined.
D1	2 sec	relaxation delay; should be about 1.25*T_1(^{1}H).
D12	20 µsec	delay for power switching; predefined.
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 ^1H spectrum (proton/5/1); same as for F2.
NUC1		select ^1H frequency for F1; same as for F2.

Acquire the 2D data set

If this data set was created from the NOESY spectrum noesy/1/1, the receiver gain is already set correctly.

Enter **zg** to acquire the time domain data. The approximate experiment time for ROESY with the acquisition parameters set as shown above is 5.5 hours.

Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 46.

Table 46. ROESY Processing Parameters

F2 Parameters		
Parameter	Value	Comments
SI	512	
SF		spectrum reference frequency (^1H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	2 (3, 4)	choose pure cosine wave (or optimize the phase shift of the sine function).
PH_mod	pk	apply 0- and 1st-order phase correction determined by phase correcting the second row.
PKNL	TRUE	necessary when using the digital filter.
BC_mod	quad	
F1 Parameters		
Parameter	Value	Comments
SI	512	
SF		spectrum reference frequency (^1H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	2 (3, 4)	choose pure cosine wave (or optimize the phase shift of the sine function).
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

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

Adjust the contour levels

The threshold level can be adjusted by placing the cursor on the button, holding down the middle or right mouse button, and moving the mouse back and forth.

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 ROESY spectrum, it helps first to phase correct the second row. Enter **`rser 2`** to transfer the second 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 & retrun** to save the corrections **`phc0`** and **`phc1`** to the 2D data file roesy/1/1. Click **2D** to return to the 2D data set roesy/1/1.

Now enter **`xfb`** to Fourier transform the ROESY 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 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 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 0^{th}- and 1^{st}-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 0^{th}-order phase correction. Recall that the 0^{th}-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, hold down the left mouse button and drag the mouse to adjust the 1^{st}-order phase correction. Recall that the 1^{st}-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 **return** followed by **save & return**, confirm the xf2p option.

Note that it is possible to exit the phase correction subroutine without saving the phase corrections by selecting **return** after clicking on **return**. Selecting **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 ROESY spectrum is properly phased, the diagonal peaks will be all positive and the true ROESY cross peaks all negative (or vice versa).

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 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 ROESY spectrum of 50 mM Gramicidin in DMSO-d6 is shown in Figure 43.

ROESY

Figure 43: ROESY Spectrum of 50 mM Gramicidin in DMSO-d6