8 COSY

8.1 Introduction

COSY (**CO**rrelation **S**pectroscop**Y**) is a homonuclear 2D technique that is used to correlate the chemical shifts of ^{1}H nuclei which are J-coupled to one another. In this chapter, two types of COSY sequences, magnitude COSY and double-quantum filtered DQF-COSY with and without pulsed field gradients, will be discussed. The different pulse sequences are quite simple and can be explained as follows: The first pulse creates transverse magnetization components which evolve chemical shift and homonuclear J-coupling during the evolution period t_1. The second pulse mixes the magnetization components among all the transitions that belong to the same coupled spin systems. The final distribution of labeled magnetization components is detected by measuring their precession frequencies during the detection period t_2. The COSY spectrum is processed by a 2D Fourier transform with respect to t_1 and t_2, and the cross peaks indicate which ^{1}H nuclei are J-coupled.

The sample used to demonstrate magnitude and DQF-COSY in this chapter is 50 mM Cyclosporin in benzene-d6.

8.2 Magnitude COSY

Several simple two-pulse programs can be used to record a magnitude mode COSY spectrum, e.g., cosy, cosy45, and cosy90. These vary with respect to the angle of the final pulse. Any value between 20^o and 90^o may be chosen for the final pulse angle. However, a pulse angle of 45^o is recommended because this yields the best signal-to-noise ratio together with a simple cross peak structure in the final spectrum.

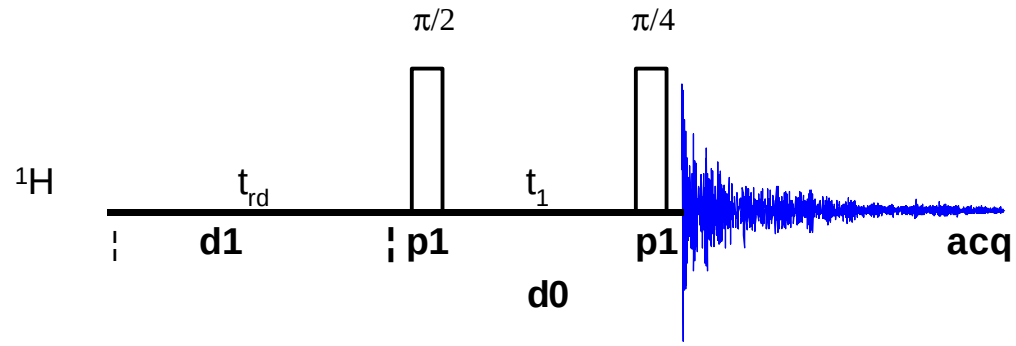
The signals acquired with one of these experiments have absorptive and dispersive lineshape contributions in both F1 and F2 dimensions. This means that it is impossible to phase the spectrum with all peaks purely absorptive, and, as a consequence, the spectrum must be displayed in magnitude mode. A typical spectral resolution of 3 Hz/pt is sufficient for resolving large scalar couplings. In order to resolve small J-couplings fine digital resolution is required, which significantly increases the experimental time. In general, the DQF-COSY experiment is recommended if a higher resolution is desired.

References: W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976); K. Nagayama, A. Kumar, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.*, **40**, 321 (1980).

8.2.1 Pulse Sequence

The COSY-45 pulse sequence is shown in Figure 21. The pulse **p1** must be set to the appropriate 90° pulse length found in Chapter 4.2.4

Figure 21: COSY-45 Pulse Sequence



8.2.2 Acquisition of the 2D COSY Spectrum

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 recommended to run all 2D experiments without sample spinning.

Record a ^{1}H reference spectrum to obtain the correct carrier frequency (**o1p**) and spectral width (**sw**) values: Enter **re proton 1 1** to call up the data set proton/1/1; enter **edc** and change the following parameters

NAME	cosy
EXPNO	1
PROCNO	1

Click SAVE to create the data set cosy/1/1.

Enter **rga** to perform an automatic receiver gain adjustment. Acquire and process a standard ^{1}H spectrum. Calibrate the spectrum, and optimize **sw** and **o1p** so that the ^{1}H signals cover almost the entire spectral width. Acquire an optimized spectrum.

Type **xau iexpno** (increment experiment number) to create the data set cosy/2/1.

Enter **eda** and set PARMODE to 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.

Enter **eda** and set the acquisition parameters as shown in Table 37. The F2 parameters **o1p** and **sw** should be identical to the values used in the optimized ^{1}H reference spectrum (cosy/1/1). Note that **in0** and sw(F1) are not independent from each other.

Table 37: COSY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	cosyqf	see Figure 21 for pulse sequence diagram
TD	1k	
NS	8	the number of scans should be 4 * n
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
P0	P1*0.5	^{1}H 45° pulse
D0	3	incremented delay (t_1); predefined
D1	3	relaxation delay; should be about 1.25 * T_1(^{1}H).
F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments
FnMODE	QF	absolute value mode
ND0	1	there is one d0 period per cycle
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2.
NUC1		select ^{1}H frequency for F1; same as for F2.

The receiver gain is already set correctly. Enter **zg** to acquire the data, which requires about 1.4 hours. (This can be estimated previously by entering **expt** into the command line).

8.2.3 Processing of the 2D COSY Spectrum

Enter **edp** and set the processing parameters as shown in Table 38.

Table 38: COSY Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	0	choose pure sine wave
PH_mod	no	this is a magnitude spectrum
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	0	choose pure sine wave
PH_mod	mc	this is a magnitude spectrum
BC_mod	no	
MC2	QF	determines type of FT in F1; QF results in a forward quadrature complex FT

Enter **xfb** to perform the 2D Fourier transformation.

For the magnitude COSY, sine-type window functions are selected to suppress the diagonal peaks relative to the cross peaks. Such a window function is also resolution enhancing, which is appropriate for a magnitude mode 2D spectrum. Adjust the threshold level by placing the cursor on the  button, holding down the left mouse button and moving the mouse up and down.

Since this is a magnitude spectrum, click on  with the left mouse button until only the positive peaks are displayed.

The region can be expanded with the  button followed by choosing the desired spectral region with the left mouse button depressed. The full spectrum is displayed again by clicking the  button.

The optimum may be saved by clicking on  and confirming the appearing questions as follows

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

8.2.4 Plotting the Spectrum

Read in the plot parameter file standard2D (**`rpar standard2D plot`**), which sets most of the plotting parameters to appropriate values.

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 as follows:

PF1DU	u
PF1USER	(name of user for file cosy/1/1)
PF1NAME	cosy
PF1EXP	1
PF1PROC	1

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

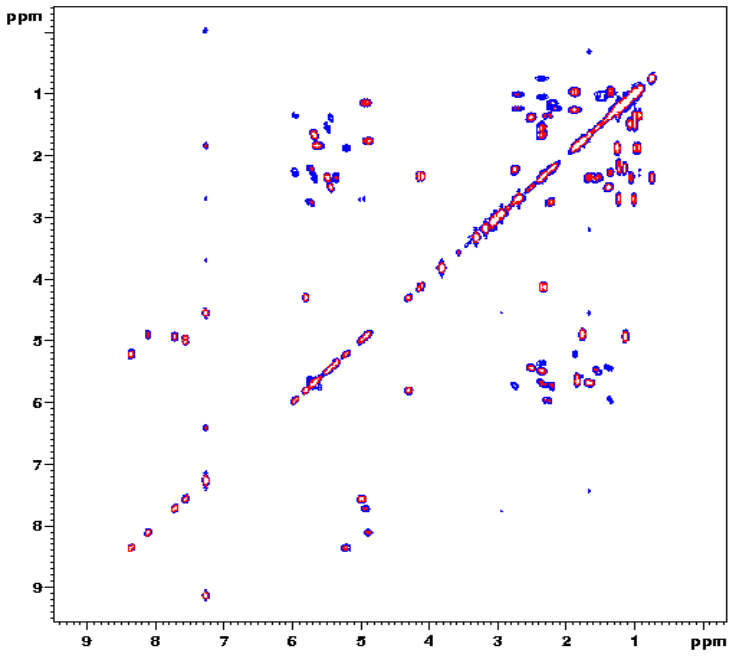
Click the **ed** next to the parameter EDPROJ2 to enter the F2 projection parameters submenu. Edit the parameters as follows:

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

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

Create a title for the spectrum (**`setti`**) and plot the spectrum (**`plot`**). A magnitude COSY spectrum of 50 mM Cyclosporin in C_6D_6 is shown in Figure 20.

Figure 22: COSY Spectrum of 50 mM Cyclosporin in C6D6



8.3 Double-Quantum Filtered (DQF) COSY

The DQF-COSY pulse sequence consists of three pulses, where the third pulse converts part of the multiple quantum coherence into observable single-quantum coherence, which is detected during the acquisition period.

One advantage of the DQF-COSY experiment is the phase-sensitivity, i.e., the cross peaks can be displayed with pure absorption lineshapes in both the F1 and the F2 dimension. In general, a phase-sensitive spectrum has a higher resolution than an otherwise equivalent magnitude spectrum because the magnitude lineshape is broader than the pure absorption lineshape.

Another advantage is the partial cancellation of the diagonal peaks in a DQF-COSY spectrum: Thus, the diagonal ridge is much less pronounced in a DQF-COSY spectrum than in a normal COSY spectrum.

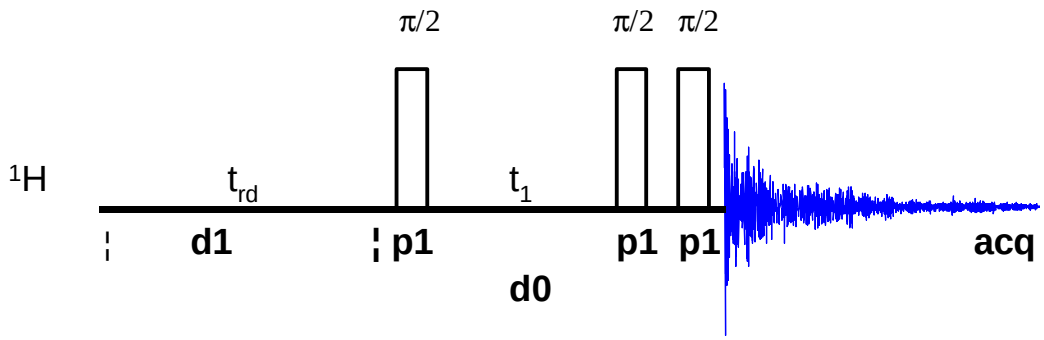
A third advantage of the double quantum filter is the elimination of strong signals, e.g., the solvent ^{1}H which do not experience homonuclear J-coupling.

References: M. Rance, O. W. Sørensen, G. Bodenhausen, G. Wagner, R. R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, **117**, 479 (1984); A. Derome and M. Williamson, *J. Magn. Reson.*, **88**, 117 (1990).

8.3.1 Pulse Sequence

The DQF-COSY pulse sequence is shown in Figure 23. The pulse **p1** must be set to the appropriate 90^o pulse length found in Chapter 4.2.4. Note that the DQF-COSY experiment is sensitive to high pulse-repetition rates, i.e., it is important to choose a long recycle delay time **d1** in order to avoid multiple-quantum artifacts in the spectrum. A suitable value for this sample is **d1** = 3 sec.

Figure 23: DQF-COSY Pulse Sequence



8.3.2 Acquisition and Processing

From the data set cosy/2/1, enter **edc** and change EXPNO to 3.

Click SAVE to create the data set cosy/3/1.

Enter **eda** and change the following acquisition parameters: It is recommended to use a larger value of **td** in both F1 (type **1 td 512**) and F2 (type **td 2k**) and a larger number of scans (**ns 16**) for a DQF-COSY experiment than for a magnitude COSY experiment. The pulse program must be set by typing **pulprog cosydfph** and the **FnMODE** in the F1 parameter list in the **eda** table must be set to "States-TPPI".

Enter **zg** to acquire the data. The approximate experiment time for the DQF-COSY using the acquisition parameters above can be estimated by the command **expt** and should be 5.5 hours.

Enter **edp** and set the processing parameters as shown in Table 39.

Table 39: DQF-COSY Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	2k	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	2	choose pure sine wave
PH_mod	pk	determine 0- and 1st-order phase correction with phasing subroutine
PKNL	TRUE	necessary when using the digital filter.
BC_mod	no	if aq_mod=DQD
F1 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 sine wave
PH_mod	pk	determine 0- and 1st-order phase correction with phasing subroutine
BC_mod	no	
MC2	States-TPPI	States-TPPI results in a forward complex FT

Enter **xfb** to perform the 2D Fourier transformation and adjust the displayed spectrum as described in Section 8.2.3.

8.3.3 Phase correct the spectrum

The phase correction of DQF-COSY spectra is best performed while examining the cross peaks rather than the diagonal peaks. When the

spectrum is phased properly, the cross peaks will be purely absorptive (i.e., they will not have the slowly decaying wings characteristic of dispersion peaks). However, since DQF-COSY peaks are antiphase (i.e., each multiplet has adjacent positive and negative peaks), it is not possible to phase the spectrum so that all peaks are positive.

Generally, a 2D spectrum is first phase corrected in the F2 dimension (rows), and then in the F1 dimension (columns). To phase correct the spectrum in F2, three rows each with a cross peak should be selected. The cross peak of one row should be to the far left of the spectrum, the cross peak of the second row should be close to the middle, and the one of the third row should be to the far right of the spectrum.

Enter the phase correction menu by clicking on the phase button. Select one row by clicking on row with the left mouse button to tie the cursor to the 2D spectrum appearing in the upper left corner of the display. Move the mouse until the horizontal cross hair is aligned with a row that has a cross peak. Select the row by clicking the middle mouse button. If the selected row does not intersect the most intense portion of the cross peak, click + -

with the left mouse button until it does. Once the desired row is selected, click on nov 1 with the left mouse button to move the row to window 1 appearing in the upper right hand corner of the display.

Repeat the selection of rows described above for a row with a cross peak in the middle and another row with a cross peak at the right edge of the spectrum and move them to window 2 and 3, respectively.

Now that three rows have been selected, the 0^{th}- and 1^{st}-order phase corrections in F2 are determined by hand exactly as described for the 1D spectrum in Section 3.8:

Click on the big 1 or the cur 1 button to tie the cursor to the biggest peak of the row in window 1. Phase Correct this row using the 0^{th}-order phase correction ph0 button ph1 . Correct the 1^{st}-order phase correction for the other two rows using the button and observe the rows in window 2 and 3, respectively.

Save the phase correction by returning to the main window (select **Save & return** at the prompt).

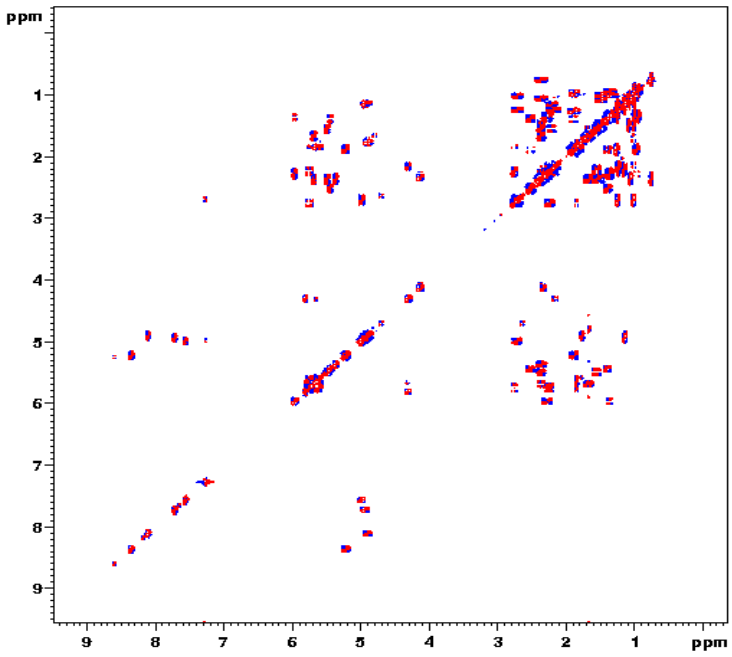
To phase correct the spectrum in F1, repeat the above procedure by selecting three columns rather than rows.

8.3.4 Plot the spectrum

See the plotting instructions given for the magnitude COSY spectrum in Section 8.2.4.

A DQF-COSY spectrum of 50 mM cyclosporin in C_6D_6 is shown in Figure 24.

Figure 24: DQF-COSY Spectrum of 50 mM Cyclosporin in C6D6



8.4 Double-Quantum Filtered COSY using Pulsed Field Gradients (GRASP-DQF-COSY)

The first high-resolution NMR experiments using pulsed field gradients (PFG) were the COSY experiments mainly to demonstrate that the application of PFGs can replace phase cycling. The quality in selecting a desired coherence pathway by PFGs turned out to be more efficient than phase cycling. In contrast to phase cycling, which requires several scans for each t_1 increment for coherence selection, field gradients allow coherence selection with only a single scan for each t_1 increment.

There are mainly two common PFG applications with COSY experiments:

1. Quadrature detection in the ω_1 dimension. The experiment time for such a COSY is in the order of a few minutes.
2. Double-quantum filter: the quality of the double-quantum filter using field gradients is very efficient. Therefore, solvent signals without homonuclear ^{1}H coupling (like water) can be suppressed very efficiently without additional solvent suppression techniques.

In this chapter we will describe the phase-sensitive double-quantum filtered COSY experiment and the pulse sequence is shown in Figure 25.

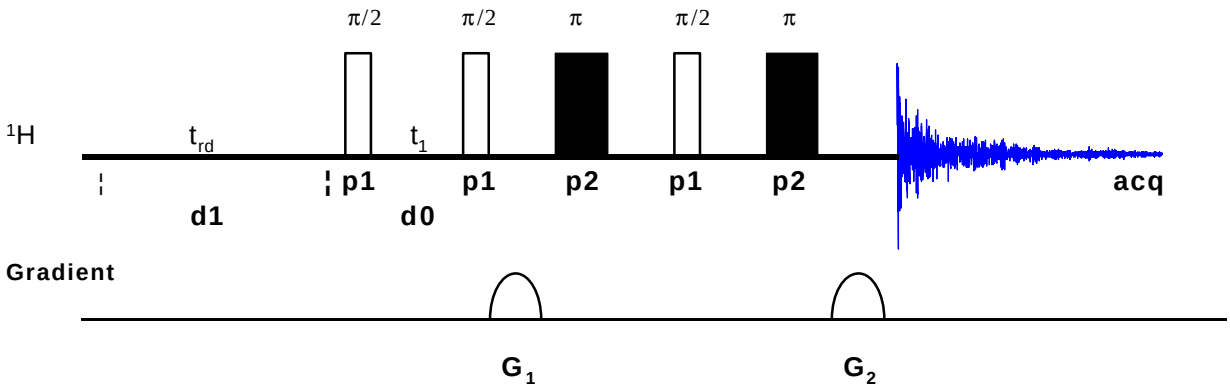
8.4.1 Pulse Sequence

The GRASP-DQF-COSY pulse sequence is very similar to the conventional DQF-COSY pulse program. After the second pulse, the spin system exhibits multiple-quantum coherence and the application of a PFG G_1 yields complete

dephasing of all coherences. In order to obtain a phase sensitive spectrum later on, the effect of chemical shift evolution during G_1 has to be eliminated by a spin echo. The third 90° pulse converts part of the multiple quantum coherence into observable single-quantum coherence, which is rephased by the PFG G_2 of proper intensity. All the unwanted magnetization stays dephased and can not be observed during the acquisition: Only spins J-coupled to at least one other spin are detected and solvent signals, especially water, are suppressed very efficiently.

The intensity ratio of the PFGs G_1:G_2 is 2: 1 for a double-quantum filter, and 3:1 for a triple quantum filter:

Figure 25: GRASP-DQF-COSY Pulse Sequence



8.4.2 Acquisition and Processing

Follow the instructions given in Sections 8.3.2 to 8.3.4 for the conventional DQF-COSY and create the data set cosy/4/1 starting out from the DQF-COSY data set (cosy/3/1).

Three parameters related to the PFGs G_1 and G_2 must be defined: The length of the PFG (**p16**), the recovery delay after the PFG (**d16**), and the shape and the intensity of the individual gradients.

Table 40: GRASP DQF-COSY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	cosygpmfph	
TD	2K	
NS	4	
DS	16	
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
D0	3u	incremented delay (t_1); predefined
D1	3	relaxation delay; should be about 1.25 * T_1(^{1}H).

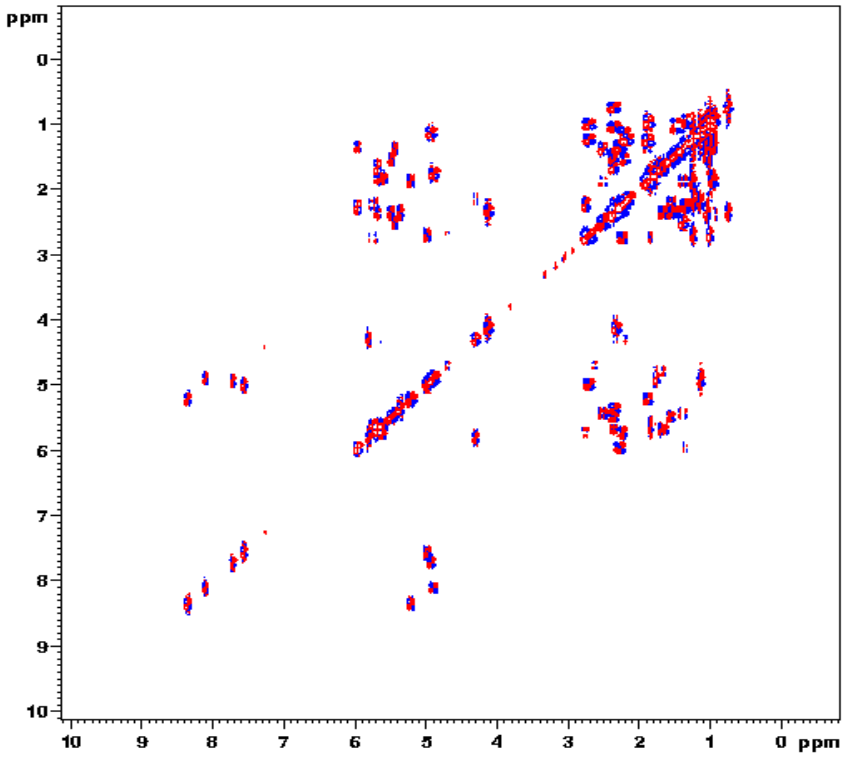
Gradient Parameters for the gp-syntax		
Parameter	**Value**	**Comments**
P16	1.5m	Length of gradient pulses
D16	150u	Gradient recovery delay
gpz1	10	% of the maximum gradient amplitude
gpz2	20	% of the maximum gradient amplitude 20 for double-quantum selection, 30 for triple-quantum selection
gpnam1	SINE.100	Gradient shape
gpnam2	SINE.100	Gradient shape
F1 Parameters		
Parameter	**Value**	**Comments**
TD	512	number of experiments
FnMODE	TPPI	
ND0	1	there is one d0 period per cycle
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2.
NUC1		select ^{1}H frequency for F1; same as for F2

Enter **zg** to start the DQF-COSY experiment. With the acquisition parameters shown above, the approximate experiment time is 1h.

Enter **edp** and set the processing parameters as shown in Table 39 for the conventional DQF-COSY except that the F1 parameter MC2 must be set to TPPI instead of States-TPPI.

Enter **xfb** to multiply the time domain data by the window functions and to perform the 2D Fourier transformation, adjust the threshold level, set the phase correction and plot the spectrum. A GRASP-DQF-COSY spectrum of 50 mM Cyclosporin in C_6D_6 is shown in Figure 26.

Figure 26: GRASP-DQF-COSY experiment of 50mM Cyclosporin in C6D6



9 TOCSY

9.1 Introduction

TOCSY (**TO**tal **C**orrelation **S**pectroscop**Y**) provides a different mechanism of coherence transfer than COSY for 2D correlation spectroscopy in liquids. In TOCSY, cross peaks are generated between all members of a coupled spin network. An advantage is that pure absorption mode spectra with positive intensity peaks are created. In traditional COSY, cross peaks have zero integrated intensity and the coherence transfer is restricted to directly spin-coupled nuclei. In TOCSY, oscillatory exchange is established which proceeds through the entire coupling network so that there can be net magnetization transfer from one spin to another even without direct coupling. The isotropic mixing which occurs during the spin-lock period of the TOCSY sequence exchanges all in-phase as well as antiphase coherences.

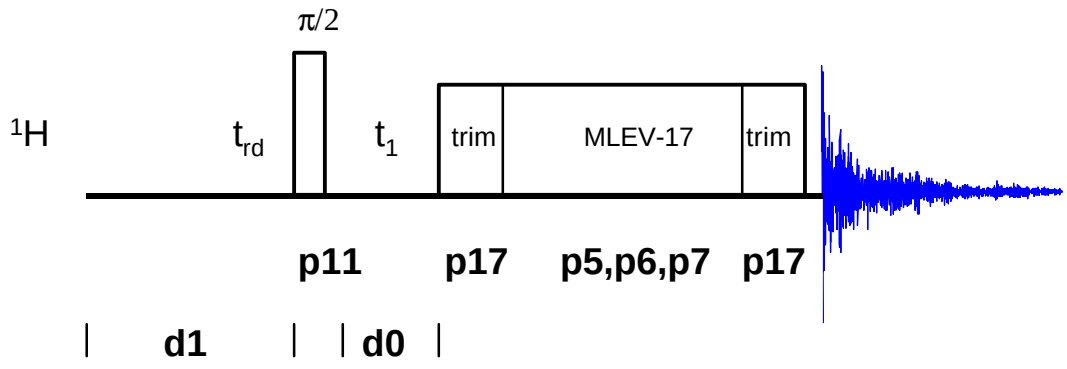
The coherence transfer period of the TOCSY sequence occurs during a multiple-pulse spin-lock period. The multiple-pulse spin-lock sequence most commonly used is MLEV-17. The length of the spin-lock period determines how far the spin coupling network will be probed. A general rule of thumb is that $1/(10\ J_{HH})$ should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

The sample used to demonstrate TOCSY in this chapter is 50 mM Cyclosporin in C_6D_6.

References: L. Braunschweiler and R. R. Ernst, *J. Magn. Reson.*, **53**, 521 (1983); A. Bax and D. G. Davis, *J. Magn. Reson.*, **65**, 355 (1985).

The TOCSY pulse sequence is shown in Figure 27. The pulse **p1** must be set to the appropriate 90° time found Section 4.2.4 and the MLEV-17 sequence used during the spinlock period requires the calibrated 90° time **p6** as determined in Section 4.2.5.

Figure 27: TOCSY Pulse Sequence



9.2 Acquisition

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 recommended to run 2D experiments without sample spinning.

Record a ^{1}H reference spectrum to determine the correct values for **o1p** and **sw**. A ^{1}H reference spectrum of this sample was already created for the magnitude COSY experiment (Section 8.2.2). This spectrum is found in the data set cosy/1/1.

The TOCSY data set can be created from the data set of any of the previous homonuclear 2D experiments run on this sample. For example, enter **re cosy 2 1** to call up the data set cosy/2/1. Enter **edc** and change the following parameters:

NAME	tocsy
EXPNO	1
PROCNO	1

Click SAVE to create the data set tocsy/1/1.

Enter **eda** and set the acquisition parameters as shown in Table 41.

The parameter **l1** determines the number of cycles of the MLEV spinlock sequence, and thus determines the length of the "mixing period". The mixing period typically lasts 20 to 100 msec, and so **l1** should be chosen so that the quantity [(**p6** * 64) + **p5**) ***11** + (**p17** * 2)] is 20 to 100 msec. The general rule of thumb is that a mixing time of $1/2J_{HH}$ or approximately 75 msec should be used.

The parameter **p17** determines the length of the trim pulses at the beginning and end of the mixing period. A good value for **p17** is 2.5 msec. The trim pulses are used to ensure that the final 2D spectrum can be phased. Note, however, that for aqueous samples only the first trim pulse should be used, in which case **l1** should be adjusted so that [(**p6** * 64) + **p5**) ***11** + **p17**] is 20 to 100 msec.

Table 41: TOCSY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	mlevph	
TD	1k	
NS	8	the number of scans should be 8 * n
DS	16	number of dummy scans
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
PL10		low power level on F1 channel (^{1}H) for MLEV-mixing as determined in Section 4.2.5

P1		^{1}H 90° pulse as determined in Section 4.2.4
P5		^{1}H 60° pulse, calculated from p6
P6		^{1}H 90° pulse as determined in Section 4.2.5
P7		^{1}H 180° pulse, calculated from p6
P17	2.5m	2.5 msec trim pulse
D1	2	relaxation delay; should about 1.25 * T_1 (^{1}H)
D9	80ms	TOCSY mixing time
L1	~ 30	loop for MLEV cycle ((p6 * 64) + p5) *11 + (p17 * 2) = mixing time); calculated internally
F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments
FnMODE	States-TPPI	
ND0	1	one d0 period
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2
NUC1		select ^{1}H frequency for F1; same as for F2.

Type **rga** to set the receiver gain and **zg** to acquire the time domain data. The approximate experiment time for the TOCSY with the acquisition parameters set as shown above is 1.3 hours.

9.3 Processing

Enter **edp** and set the processing parameters as shown in Table 42.

Table 42: TOCSY Processing Parameters

F2 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	pk	
PKNL	TRUE	
BC_mod	no	
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	pk	
BC_mod	no	
MC2	States-TPPI	

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

The threshold level can be adjusted by placing the cursor on the button, holding down the middle mouse button, and moving the mouse back and forth. The optimum may be saved by typing **defplot** and answering the questions which appear.

9.4 Phase Correction

To simplify the phasing of the 2D TOCSY spectrum, it helps to first phase 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 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 2D data file tocsy/1/1. Click 2D to return to the 2D data set tocsy/1/1.

Now enter **xfb** to Fourier transform the TOCSY 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.

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. Once the desired column is selected, move it to window 1, appearing in the upper right hand corner of the display (see Section 8.3.3).

Repeat the above procedure to select two further columns, one with a diagonal peak in the middle and one with a peak at the other end of the spectrum. Move these columns to window 2 and 3, respectively.

Now that three columns have been selected, the 0 - and 1st-order phase corrections in F1 are determined manually exactly as for the DQF-COSY spectrum (see Section 8.3.3). When the phase correction is satisfactory, click on 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 for rows rather than columns. Phase correct as described above and confirm the xf2p option.

It should be possible to phase correct the spectrum so that all TOCSY peaks are positive.

9.5 Plot the Spectrum

Set the region, the threshold and peak type (positive and/or negative) to be used for plotting the spectrum. Make sure the spectrum appears as desired on the screen, type **`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:

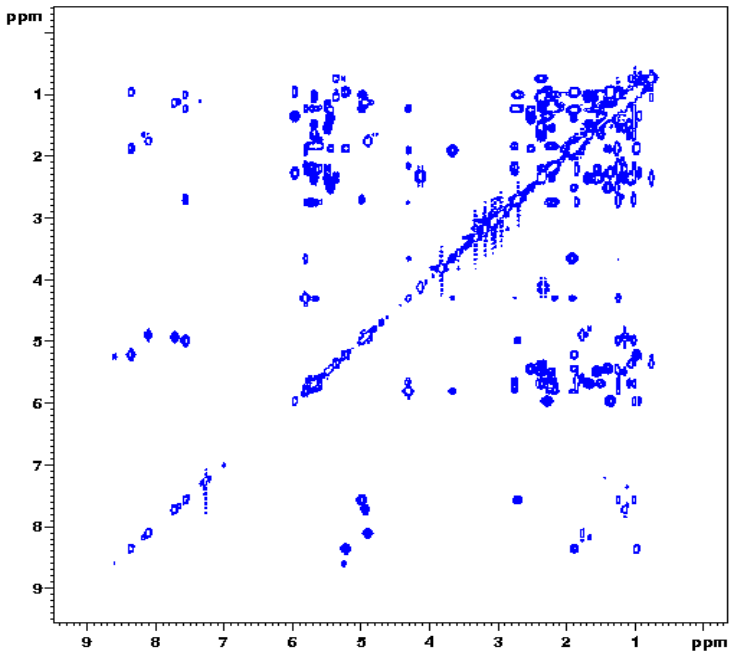
PF1DU	u
PF1USER	(name of user for file cosy/1/1)
PF1NAME	cosy
PF1EXP	1
PF1PROC	1

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 as described for EDPROJ1 above. Click SAVE to save all the above changes and exit the **edg** menu. Enter **`setti`** to open the title file and enter a title.

To plot the spectrum, simply enter **`plot`**. A TOCSY spectrum of 50 mM Cyclosporin in C_6D_6 is shown in Figure 28.

Figure 28: TOCSY Spectrum of 50 mM Cyclosporin in C6D6


ppm
1
2
3
4
5
6
7
8
9
9
8
7
6
5
4
3
2
1
ppm

10 ROESY

10.1 Introduction

ROESY (**R**otating-frame **O**verhauser **E**ffect **S**pectroscop**Y**) is an experiment in which homonuclear Nuclear Overhauser effects (NOEs) are measured under spin-locked conditions. ROESY is especially suited for molecules with motional correlation times (τ_c) such that $\omega\tau_c \sim 1$, were 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 which 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 to be positive for all values of τ_c.

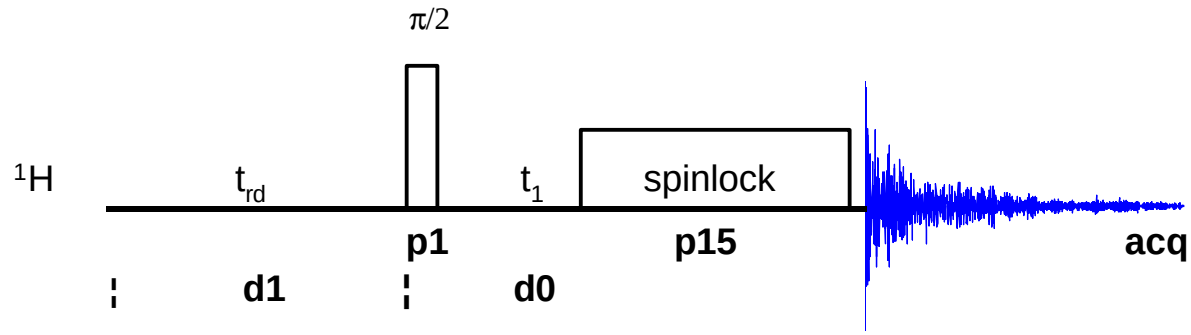
ROESY spectra can be obtained in 2D absorption mode. This is also useful for the identification of certain artifacts. Spurious cross peaks, both COSY-type and TOCSY-type, can be observed due to coherence transfer between scalar coupled spins. COSY-type artifacts (anti-phase) arise when the mixing pulse transfers anti-phase magnetization from one spin to another. TOCSY-type artifacts (which have the same phase as the diagonal peaks, while ROESY cross peaks have 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).

The sample used to demonstrate ROESY in this chapter is 50 mM Cyclosporin in C_6D_6.

The ROESY pulse sequence is shown in Figure 29.

Figure 29: ROESY Pulse Sequence



10.2 Acquisition

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 recommended to run 2D experiments without sample spinning.

Record a ^{1}H reference spectrum to determine the correct values for **o1p** and **sw**. A ^{1}H reference spectrum of this sample was already created for the magnitude COSY experiment (Section 8.2.2). This spectrum is found in the data set cosy/1/1.

The ROESY data set can be created from the data set of any of the previous homonuclear 2D experiments run on this sample. For example, enter **re cosy 2 1** to call up the data set cosy/2/1. Enter **edc** and change the following parameters:

NAME	roesy
EXPNO	1
PROCNO	1

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

Enter **eda** and set the acquisition parameters as shown in Table 43.

The pulse **p15** at **pl11** sets the length of the cw spinlock pulse. The value listed in Table 43 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.

Table 43: ROESY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	roesyph	
TD	1k	
NS	32	the number of scans must 8 * n
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
PL11		low power level on F1 channel (^{1}H) for spinlock as determined in Section 4.2.6
P1		^{1}H 90° pulse as determined in Section 4.2.4
P15	200m	spinlock pulse
D1	2	

F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments.
FnMODE	States-TPPI	
ND0	1	one d0 period per cycle
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2
NUC1		select ^{1}H frequency for F1; same as for F2

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.

10.3 Processing

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

Table 44: ROESY Processing Parameters

F2 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	pk	
PKNL	TRUE	
BC_mod	no	
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	pk	
BC_mod	no	
MC2	States-TPPI	

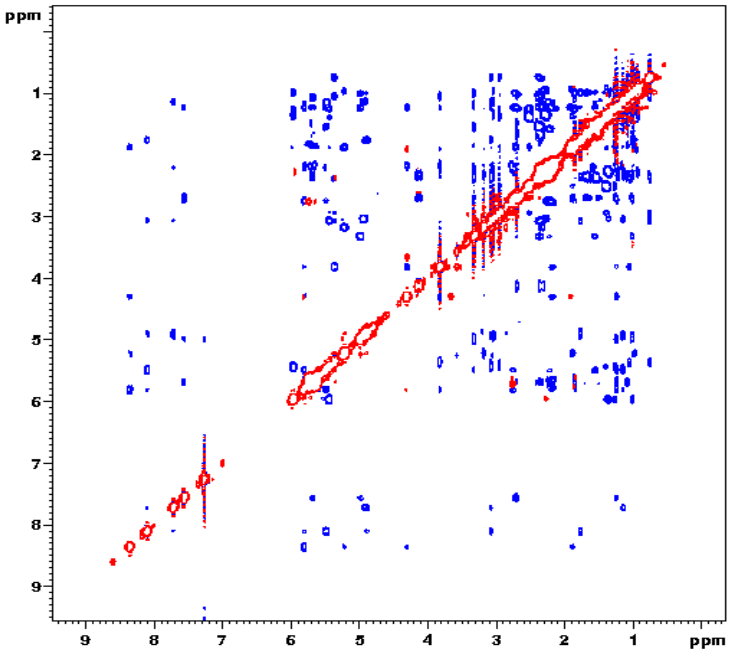
Enter **xfb** to multiply the time domain data by the window functions and to perform the 2D Fourier transformation.

The threshold level can be adjusted by placing the cursor on the button, holding down the middle mouse button, and moving the mouse back and forth. The optimum may be saved by typing **defplot** and answering the questions which appear.

10.4 Phase Correction and Plotting

For the phase correction procedure and the plotting procedure please follow the instructions given for the TOCSY spectrum in Sections 9.4 and 9.5, respectively.

Figure 30: ROESY Spectrum of 50 mM Cyclosporin in C6D6



11 NOESY

11.1 Introduction

NOESY (**N**uclear **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 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 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 Note that, for he 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.

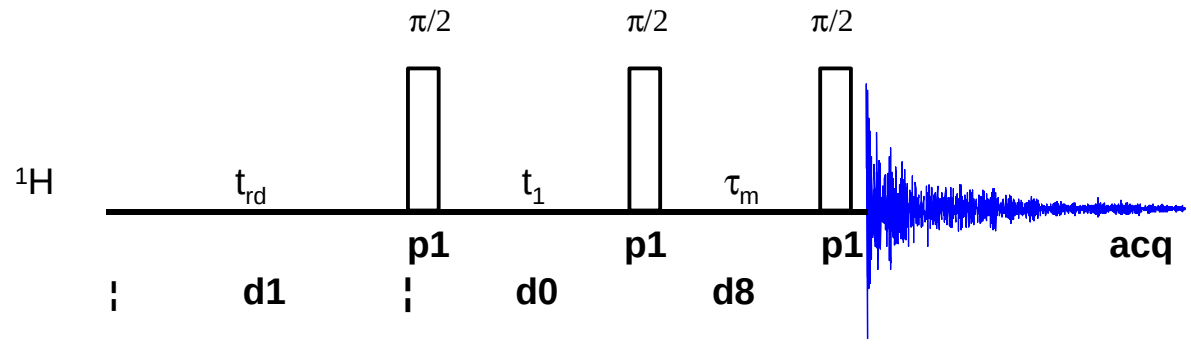
NOESY spectra can be obtained in 2D absorption mode. Occasionally, COSY-type artifacts appear in the NOESY spectrum; however, these are easy to identify by their anti-phase multiplet structure.

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).

The sample used to demonstrate NOESY in the chapter is 50 mM Cyclosporin in C_6D_6.

The NOESY pulse sequence is shown in Figure 31. The delay **d8** determines the length of the mixing period, during which NOE buildup occurs.

Figure 31: NOESY Pulse Sequence



11.2 Acquisition and Processing

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 recommended to run 2D experiments without sample spinning.

Record a ^{1}H reference spectrum to determine the correct values for **o1p** and **sw**. A ^{1}H reference spectrum of this sample was already created for the magnitude COSY experiment (Section 8.2.2). This spectrum is found in the data set cosy/1/1.

The NOESY data set can be created from the data set of any of the previous homonuclear 2D experiments run on this sample. For example, enter **re cosy 2 1** to call up the data set cosy/2/1. Enter **edc** and change the following parameters:

NAME	noesy
EXPNO	1
PROCNO	1

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

Enter **eda** and set the acquisition parameters as shown in Table 45.

Table 45: NOESY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	noesyph	
TD	1k	
NS	32	the number of scans must 8 * n
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
D8	350m	Mixing time
D1	2	
F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments.
FnMODE	States-TPPI	
ND0	1	one d0 period per cycle
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2
NUC1		select ^{1}H frequency for F1; same as for F2

11.2.1 Optimize Mixing Time

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 45 is appropriate for this sample at 300 MHz and room temperature. If no appropriate value of **d8** is available the following quick and easy procedure can be used.

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 and ok the requests to delete a number of files.

In **eda** set PULPROG to zg (or enter **pulprog zg**). 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.

In **eda** change PULPROG to the pulse program t1ir1d (or enter **pulprog t1ir1d**). This is a so-called inversion recovery sequence. Set **d7** to

approximately 1 msec (**d7 1m**), record and process a spectrum using **zg** and **efp**. The signals should all be negative.

To set **d7** to 1 sec, enter **d7 1** and record and process another spectrum using **zg** and **efp**. The signals should all be positive. Now find a value for **d7** in the range of 300-600ms, where all the signals are minimal. 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.

11.2.2 Acquire the 2D data set

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.

11.3 Processing

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

Table 46: NOESY Processing Parameters

F2 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	pk	
PKNL	TRUE	
BC_mod	no	
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	pk	
BC_mod	no	
MC2	States-TPPI	

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

The threshold level can be adjusted by placing the cursor on the button, holding down the middle mouse button, and moving the mouse back and forth. The optimum may be saved by typing **defplot** and answering the questions which appear.

11.4 Phase Correction and Plotting

For the phase correction procedure and the plotting procedure please follow the instructions given for the TOCSY spectrum in Sections 9.4 and 9.5, respectively. Note that for the NOESY spectrum recorded here, the first serial file should be chosen for the F2 phase correction: type **rser 1** instead of **rser 2**, as for the TOCSY and ROESY spectra.

Figure 32: NOESY Spectrum of 50 mM Cyclosporin in C_6D_6