

TOCSY

Introduction

15.1

Total Correlation Spectroscop**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 the “net” coherence transfer produced can be arranged to create pure absorption mode spectra with positive intensity peaks (rather than “differential” coherence transfer which causes spectra with equal positive and negative intensities). In traditional COSY, cross peaks have zero integrated intensity and 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 $1/(10J_{HH})$ should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

The TOCSY spectrum can be phased so that all cross peaks and diagonal peaks have positive intensity.

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).

Sample

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

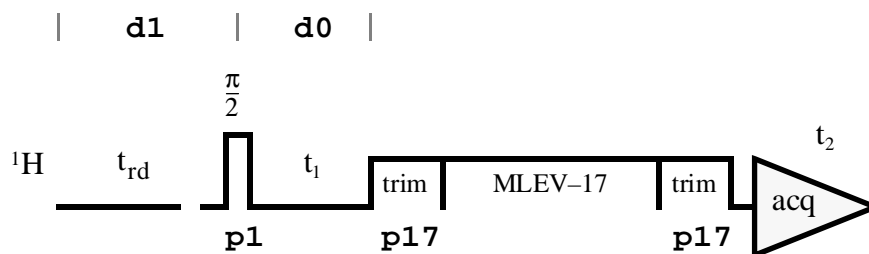
Pulse Sequence Diagram

15.2

The TOCSY pulse sequence is shown in Figure 44. Notice that the pulse **p1** must be set to the appropriate 90° time found in Chapter 5 ‘Pulse Calibration’. The MLEV-17 sequence used during the spinlock period requires the calibrated 90° time **p6**.

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Figure 44: TOCSY Pulse Sequence



Acquisition and Processing

15.3

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 ^1H observation.

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

 ^1H reference spectrum

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

A ^1H 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

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 roesy 1 1** to call up the data set roesy/1/1. Enter **edc** and change the following parameters:

NAME	tocsy
EXPNO	1
PROCNO	1 .

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

Change to 2D parameter mode

If this data set was created from a previous 2D 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 47. Use the values determined in Chapter 5 'Pulse Calibration' for the parameters **p11** and **p1** (^1H observe high power level and 90° pulse time), and **p110** and **p6** (^1H low power level and 90° pulse time for MLEV spinlock).

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 100msec, and so **l1** should be chosen so that the quantity $[(p6 * 64) + p5) * l1 + (p17 * 2)]$ is 20 to 100msec. The general rule of thumb is that $1/10J_{HH}$ should be allowed for each transfer step, and typically five transfer steps are desired, which means a mixing time of $1/2J_{HH}$ or approximately 75msec.

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 2.5msec. The trim pulses are used to ensure that the final 2D spectrum can be phased easily. Note, however, that for aqueous samples only the first trim pulse should be used, in which case **l1** should be adjusted so that $[(p6 * 64) + p5) * l1 + p17]$ is 20 to 100msec.

The F2 parameters **o1** and **sw** (not shown in the table) should be identical to the values used in the optimized 1H reference spectrum (proton/5/1). The F1 parameters **sf01** 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 47. TOCSY Acquisition Parameters

F2 Parameters		
Parameter	Value	Comments
PULPROG	mlevtp	see Figure 44 for pulse sequence diagram.
TD	1k	
NS	8	the number of scans should be 8*n in order for the phase cycling to properly.
DS	16	number of dummy scans.
PL1		high power level on f1 channel (see “An Important Note on Power Levels” on page 7).
PL10		MLEV spin-lock power level on f1 channel.
P1		90° 1H high power pulse on f1 channel.
P5		60° 1H low power pulse on f1 channel; calculated internally.
P6		90° 1H low power pulse on f1 channel.
P7		180° 1H low power pulse on f1 channel; calculated internally.
P17	2.5 msec	trim pulse to defocus non-spinlocked transverse magnetization.
D0	3 μsec	incremented delay (t_1); predefined.
D1	2 sec	relaxation delay; should be about $1.25 * T_1 (^1H)$.
D12	20 μsec	delay for power switching; predefined.

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D13	3 μsec	short delay; predefined.
L1	~30	loop for MLEV cycle $((p6 * 64) + p5) * 11 + (p17 * 2) = \text{mixing time}$.
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 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 roesy/1/1, or the data set of any of the other homonuclear 2D experiments run on this sample, the receiver gain is already set correctly.

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

Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 48.

Table 48. TOCSY 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	choose pure cosine wave.
PH_mod	pk	apply 0- and 1 st -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 (¹ H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	2	choose pure cosine wave.
PH_mod	no	first determine 0- and 1 st -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 **thres** 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 **p/n/a/u** 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 TOCSY spectrum, it helps first to phase correct the second row. (Note that because of the phase-cycling routine used here, no spin-locking occurs during the acquisition of the first row, so the second row is the earliest row containing TOCSY signal which can be phase corrected.) 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 & return** to save the corrections **phc0** and **phc1** to the 2D data file tocsy/1/1. Click **to2D** 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. 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 and middle mouse buttons until it does. Once the desired column is selected, click on **mov1** 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, hold down the left mouse 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 **return** and selecting **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.

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

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 TOCSY spectrum of 50 mM Gramicidin in DMSO-d₆ is shown in Figure 45.

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Figure 45: TOCSY Spectrum of 50 mM Gramicidin in DMSO-d6

