

# Selective Excitation

## Introduction

## 8.1

The hard pulses used in the other chapters of this manual are meant to excite the entire spectral width uniformly. In this chapter we discuss using a soft pulse to excite only one multiplet of a  $^1\text{H}$  spectrum selectively. Important characteristics of a soft pulse include its shape, its amplitude, and its length, where (although roughly) the pulse shape may be correlated with the shape of the excitation profile, the pulse amplitude with the flip angle, and the pulse length with the pulse selectivity (i.e., the width of the excited spectral region).

For example, the pulse shape used in this chapter is a Gaussian. The excitation profile of a Gaussian pulse falls off quickly and has no side lobes in the frequency domain (unlike the excitation profile of a square pulse). This means that a Gaussian pulse fulfills the condition of selectivity to a high degree of accuracy, i.e., it has a minimal effect on the rest of the spectrum.

The Gaussian pulse envelope must be truncated at some suitably low level so that the rf field is extinguished before the signal acquisition or application of other pulses begins. Truncation when the intensity falls below 1% of the maximum is acceptable, and is used here. The pulse width is then defined as the width between the 1% points.

In cases where the resonances of a given spin are well separated from all other resonances of the spin system, the effect of a selective  $90^\circ$  Gaussian pulse is (nearly) equivalent to the effect of a hypothetical nonselective  $90^\circ$  pulse applied selectively to that given spin, followed by a delay of suitable length.

The pulse length used in this chapter is 80 msec. The selectivity of a pulse is measured by its ability to excite a chosen resonance (or group of resonances) without appreciably affecting near neighbors. For a given net flip angle, the selectivity is determined by the duration of the soft pulse. Generally, the peaks to be excited should lie within  $\pm 1/2t_p$  Hz of the transmitter frequency, where  $t_p$  is the length of the selective pulse. The peaks to be left unperturbed should lie more than  $\pm \pi/t_p$  Hz from the transmitter frequency.

Since the length of the selective pulse affects its selectivity, the length is selected based on the selectivity desired and then the pulse amplitude (i.e., power level) is adjusted to give a  $90^\circ$  (or  $270^\circ$ ) flip angle.

Notice that it is necessary to set the transmitter offset frequency of the selective pulse to the frequency of the desired resonance. This transmitter frequency does not have to be the same as  $\omega_1$  (the offset frequency of the hard pulses), but for reasons of simplicity, they are often chosen to be identical.

Most selective excitation experiments rely on phase cycling, and thus subtraction of spectra, to eliminate large unwanted signals. It is important to minimize possible sources of subtraction artifacts, and for this reason it is generally suggested to run selective experiments non-spinning.

**References:** C. J. Bauer, R. Freeman, T. Frenkiel, J. Keeler, and A. J. Shaka, *J. Magn. Reson.*, **58**, 442 (1984); H. Kessler, H. Oschkinat, C. Griesinger, and W.

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Bermel, *J. Magn. Reson.*, **70**, 106 (1986); L. Emsley and G. Bodenhausen, *J. Magn. Reson.*, **82**, 211 (1989).

### Sample

The sample used to demonstrate selective pulse experiments in this chapter is 50 mM Gramicidin in DMSO-d<sub>6</sub>.

**NOTE:** Selective excitation experiments using shaped pulses are possible on DSX, DMX, and DRX spectrometers but *not* on DPX spectrometers since they have no rf-shape modulator.

## Selective Pulse Calibration

## 8.2

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

It is recommended that selective experiments be run without sample spinning.

Before performing selective excitation experiments, it is first necessary to calibrate the selective pulse. The steps involved in this procedure are first, to obtain a <sup>1</sup>H reference spectrum and determine the resonance frequency of the desired resonance; second, to define the shaped pulse; and third, to perform the pulse calibration experiment.

### <sup>1</sup>H reference spectrum

The first step is to acquire and process a standard <sup>1</sup>H spectrum. This <sup>1</sup>H spectrum will be used to determine **o1**, **sw**, and the resonance for selective excitation.

Enter **re proton 2 1** to call up the data set proton/2/1. Enter **edc** and change the following parameters:

NAME	selex
EXPNO	1
PROCNO	1 .

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

Enter **rga** to perform an automatic receiver gain adjustment. Acquire and process a standard <sup>1</sup>H spectrum. Throughout this chapter, the ornithine peptide N-H resonance at 8.65 ppm will be used for selective excitation. Change **o1** to the frequency of this resonance as follows: Expand the spectrum about the peak at 8.65 ppm. Click on **utilities** to enter the calibration submenu. Click on **O1** with the left mouse button to select **o1** calibration. Move the cursor to the center of the doublet and click the middle mouse button to assign **o1** to this frequency. Click on **return** to exit the utilities submenu and return to the main window.

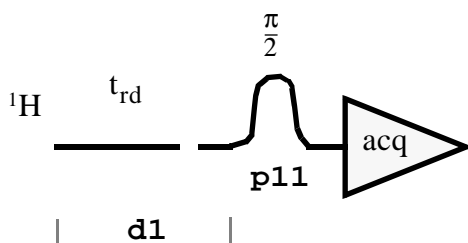
Make sure **sw** is large enough to include the entire <sup>1</sup>H spectrum, even with this new value of **o1**. Acquire and process a second <sup>1</sup>H spectrum with this new **o1**.

(Notice that it is possible to perform selective excitation experiments off-resonance, but for the sake of simplicity, only on-resonance experiments will be described here).

### Selective one-pulse sequence

The pulse sequence used to calibrate the selective pulse is shown in Figure 22. Notice that this sequence is identical to the standard one-pulse sequence shown in Figure 1 on page 19, except that now the pulse is a low-power, shaped pulse. The pulse length **p11** and the pulse strength **sp1** must be adjusted so that the pulse is 90° or 270° (see below). In addition, the actual shape of the pulse must be defined.

Figure 22: Selective One-Pulse Sequence



### Define the pulse shape

The shape of the selective pulse is defined by the program **xShape**, which is run outside of XWIN-NMR. Open a UNIX shell and enter `/u/prog/<version>/xShape`, where **<version>** is the name of the current XWIN-NMR, e.g. **xwin-nmr1.1**, to start the program. This opens a window entitled “RF Pulse and Gradient Shapes.” The window includes a region in which the pulse shape will be plotted. Below this is a menu of options that the user can select. These options are selected by entering the appropriate number or letter at the command line at the bottom of the window.

The following describes how to set up a standard set of parameters for generating a Gaussian pulse shape:

At the prompt “Which function do you want>”, enter **I** to define the size of the shaped pulse. At the prompt “# of points to calculate (multiple of 2, real size)>” enter **1024**.

Again the prompt “Which function do you want>” appears. This time enter **2** to select a Gaussian shape. At the prompt “truncation level: (0.001% < level % < 100%)>” enter **1** to select a truncation level of 1%.

The selected shape is now plotted in the window. This shape should now be saved as follows: At the prompt “Which function do you want>”, enter **W** to write the file. The prompt “Storage as RF (=0), GRADIENT (=1) or GRADPROG (=2) shape (0/1/2)>” then appears. Enter **0** to indicate an RF shape. Next the prompt “Storage of whole file (=0) or fractional part of it (=1) (0/1)>” appears. Enter **0** to store the whole file, then the prompt “Apply attenuation Y/N” and enter **n**. Finally, at the prompt “Enter filename>” enter the filename **gaus1.1k**.

The message “going to write: /u/exp/stan/nmr/lists/wave/gaus1.1k” appears on the comment line.

Exit the shape program by typing **q** at the prompt “Which function do you want>”.

Return to the window running UXNMR.

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### Acquire and process the selective one-pulse spectrum

First, create a new data set for the 1D selective experiment. From the data set `selex/1/1` enter **edc** and change EXPNO to 2. Click **SAVE** to create the data set `selex/2/1`. Then set up the acquisition parameters as shown in Table 28.

**Table 28. Selective One-Pulse Acquisition Parameters**

Parameter	Value	Comments
PULPROG	selzg	see Figure 22 for pulse sequence diagram.
TD	8k	
NS	1	no need for signal averaging yet.
DS	0	no need for dummy scans yet.
SP1	80dB	shaped pulse power level on f1 channel.
P11	80msec	90° shaped pulse on f1 channel.
D1	10sec	relaxation delay; select a long delay time to ensure correct pulse calibration results.
D12	20μsec	delay for power switching; predefined.
SP	edit	enter this array to edit power level, offset, and filename for the shaped pulse.

To enter the power level, offset, and filename for the shaped pulse, click on the **edit** button next to the parameter **SP07** towards the bottom of the **eda** table. This calls up the table “Power for shaped pulses”, which has four columns: one for the shaped pulse index number (Index), one for the power level (Power[dB]), one for the offset frequency (Offset-Frequency), and one for the filename of the shaped pulse (Filename). The pulse program **selzg** makes use of shaped pulse **1** only, so here the user need only be concerned with entries in the row corresponding to index number **1**.

In row 1, set the power level for the shaped pulse to 80dB. (This parameter is also known as **sp1**.) Then, for on-resonance selective excitation, make sure that the offset frequency is set to 0Hz. Finally, click on the filename box with the right mouse button to call up the menu of possible shape files. From this list, select `gauss1.1k` with the left mouse button.

All other acquisition parameters should be the same as for the reference spectrum, in particular **td**, **o1**, **sw**, and **rg**.

Acquire and process a selective one-pulse spectrum. The spectrum should be processed with the command **efp** so that the *same phase correction as was used for the reference spectrum* is applied. The ornithine N-H resonance should appear in the middle of the window and no other peaks should be visible. Phase correct the ornithine N-H resonance using the 0<sup>th</sup>-order correction only. Note this value, but click **return** to return to the main menu *without* storing the phase correction. This additional phase correction might to be applied to the shaped pulse only, not to the hard pulses (used in the pulse programs **selco** and **selmlzf** below).

## Selective Pulse Calibration

To apply the additional phase correction to the shaped pulse it must be entered into the PHCOR(1) menu within **eda**. Enter **eda** and select the array PHCOR near the bottom of the table. This opens the table “correction angle for phase program,” which lists the correction in degrees for phases [0] to [31]. Since **ph1** is the phase used for the shaped pulse, enter the phase correction beside “[1]”. Click on **SAVE**. (Rather than using the PHCOR table, it is also possible to type **2 phcor 1** and enter the phase correction directly.)

Now if the spectrum is reacquired and processed with **efp**, the ornithine peptide N-H should be phased properly.

Expand the spectrum so that the ornithine N-H doublet occupies approximately the center quarter of the window (e.g., so that the region from approximately 9.2 ppm to 8.1 ppm is displayed). Save this as a plotting region by clicking on **DP1** with the left-hand mouse button and hitting return in response to the questions. This plotting region will be used by the au program **paropt**, below.

### Perform the pulse calibration

As described in Chapter 5 ‘Pulse Calibration’, the au program **paropt** may be used to perform an automatic pulse calibration. Simply enter **xau paropt** and answer the questions as follows:

Enter parameter to modify:	sp1
Enter initial parameter value:	90
Enter parameter increment:	-2
Enter # of experiments:	20 .

At the end of the experiment, the message “paropt finished” and a value for **sp1** are displayed. This value is the approximate  $^1\text{H}$  transmitter power level for a  $90^\circ$  pulse time of 80 msec.

Note that the maximum intensity, at the  $90^\circ$  pulse, should occur at approximately 6dB less attenuation than the null at the  $180^\circ$  pulse.

To obtain a more accurate  $90^\circ$  pulse, repeat **paropt** using a smaller increment for **sp1**. (At this point it may be useful to repeat the above procedure for a range of **p11** pulse lengths.)

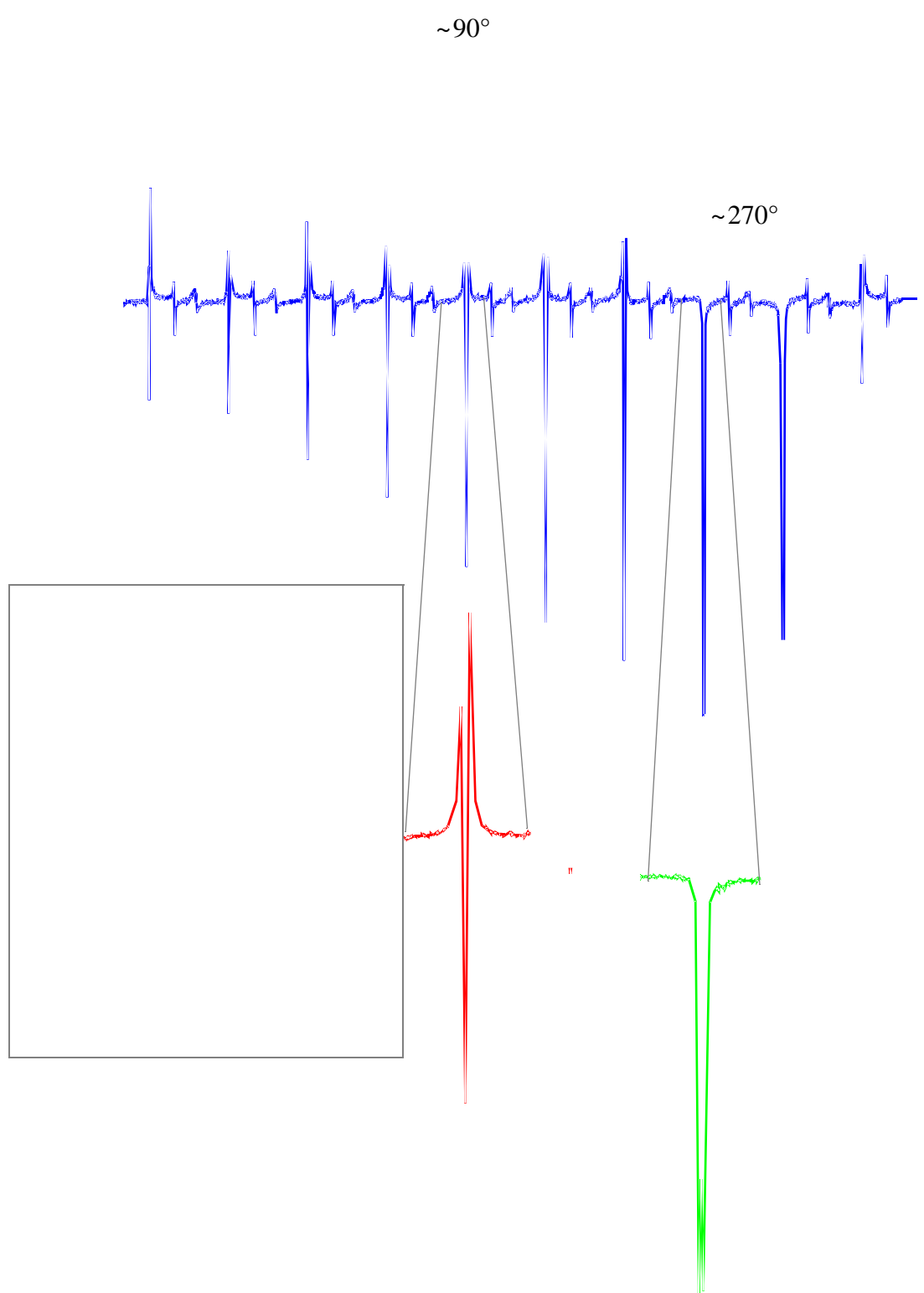
Paropt results of selective excitation of the ornithine N-H resonance are shown in Figure 23. Notice that the peak corresponding approximately to a  $270^\circ$  pulse has a better lineshape than that corresponding approximately to a  $90^\circ$  pulse. This is because a  $270^\circ$  Gaussian pulse causes refocusing of evolution due to J-coupling that occurs during the pulse. The  $270^\circ$  pulse can be thought of as a  $90^\circ$  pulse followed by a  $180^\circ$  refocusing pulse. Since the  $180^\circ$  pulse is also selective, the evolution of the magnetization due to J-coupling is refocused in exactly the same way as if it were due to chemical shifts.

In fact, in many cases, a  $270^\circ$  Gaussian pulse is preferable to a  $90^\circ$  Gaussian pulse. For this reason, it is also suggested to use the above procedure to determine the correct **sp1** value for a  $270^\circ$  pulse of 80 msec.

A selective one-pulse  $^1\text{H}$  spectrum of the ornithine N-H resonance of Gramicidin, together with the reference spectrum, is shown in Figure 24. The selective spectrum was obtained using a  $270^\circ$  Gaussian pulse.

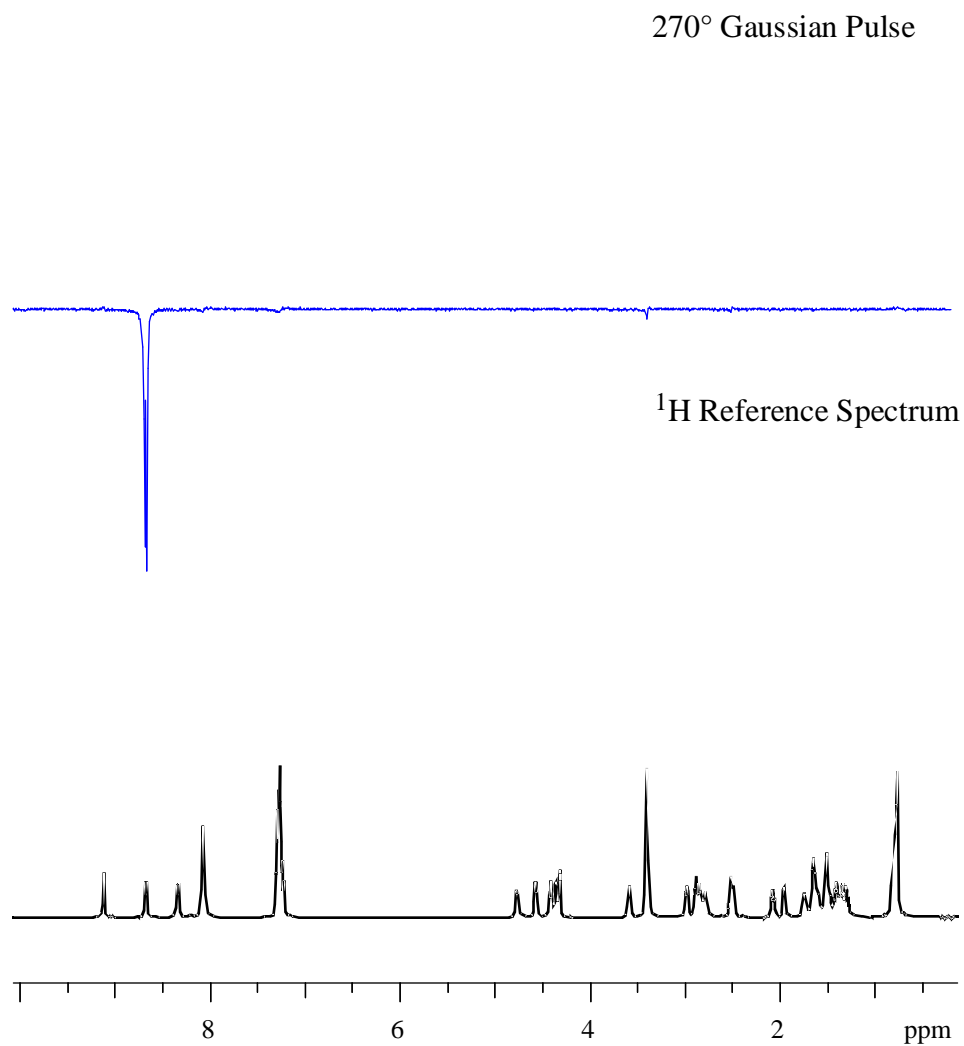
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Figure 23: Selective One-pulse Paropt Results



## Selective Pulse Calibration

Figure 24: Selective One-pulse Spectrum of 50 mM Gramicidin in DMSO-d<sub>6</sub>



Many 2D NMR experiments can be converted to analogous 1D experiments by using Gaussian pulses. A 1D sequence is advantageous when a limited amount of information is desired, which is often the case for medium-sized molecules. When this is the case, the total experiment and data manipulation times are shorter for the 1D experiment than for the 2D experiment, and the data storage capacity requirements are less.

The 2D COSY experiment is very effective at indicating coupling except in cases where the  $^1\text{H}$  chemical shifts are closely crowded together so that many cross-peaks overlap. Selective COSY gives the same  $^1\text{H}$  coupling information one site at a time, without involving a 2D Fourier transform. This is useful for probing regions of the spectrum where the  $^1\text{H}$  shifts are densely packed, provided that some  $^1\text{H}$  resonances are sufficiently well separated that they can be picked out for selective irradiation.

The selective COSY sequence begins with a  $90^\circ$  frequency selective excitation pulse. This is followed by a fixed delay (rather than the variable evolution period of the 2D COSY sequence) during which antiphase coherence is created by evolution due to J-coupling. The duration of this delay is measured from the middle of the Gaussian envelope. As with 2D COSY, the second (or coherence transfer) pulse is a hard  $90^\circ$  pulse. This pulse creates observable magnetization from the antiphase coherence present at the end of the fixed delay. The acquisition period follows immediately after the second pulse.

The frequency of the selective pulse is set to the chemical shift of a multiplet and the selectivity is chosen so that adjacent multiplets are unperturbed. The spectral width is set large enough to cover the entire chemical shift range whatever the transmitter offset. The intensity of the transferred signal depends on the magnitude of the appropriate coupling constant and on the length of the fixed delay, and varies in a sinusoidal fashion. There is a chance that a particular transfer falls accidentally at a null, in which case a coupling path would be overlooked. This risk can be minimized by selecting the precession interval short compared with the reciprocal of the largest expected coupling constant. The lower level of the delay is one half the Gaussian duration needed to get the required selectivity.

Since the final pulse gives coherence transfer to spins whose couplings are in antiphase to the selectively excited spin, 1D selective COSY gives rise to antiphase multiplets (which will unavoidably have adjacent positive and negative intensities). Thus, direct extraction of the coupling constants may be complicated due to annihilation of individual lines within the multiplet.

Notice that the final pulse also converts any longitudinal magnetization into transverse magnetization. The resulting signals are intense for all  $^1\text{H}$  sites other than the one excited by the selective pulse. These signals are eliminated by the same phase cycling as is used in 2D COSY; however, the corresponding signals in the 2D experiment are much weaker, and so are more easily eliminated by the phase cycling.

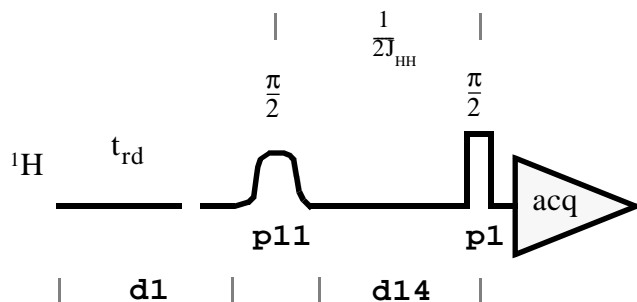
## Pulse Sequence Diagram

8.3.1

The selective COSY pulse sequence is shown in Figure 22. Notice that it is very similar to the standard COSY sequence shown in Figure 35 on page 134, except that here the first pulse is a low-power shaped pulse and the delay between the two pulses (**d14**) is not incremented.

The high-power pulse **p1** must be set to the appropriate  $90^\circ$  time found in Chapter 5 'Pulse Calibration', and the shaped pulse **p11** to the appropriate  $90^\circ$  time found in Section 8.2 'Selective Pulse Calibration'. The delay **d14** should be set so that  $(\mathbf{p11})/2 + \mathbf{d14} = 1/(2J_{\text{HH}})$  for the selected resonance. Notice that **p11** may also be chosen to be a  $270^\circ$  Gaussian pulse, in which case the delay **d14** should be set to  $1/(2J_{\text{HH}})$ .

Figure 25: Selective COSY Pulse Sequence



## Acquisition and Processing

8.3.2

If this experiment is carried out directly after the pulse calibration described in Section 8.2, the correct sample is already in the magnet, the probehead tuned and matched, and the magnetic field shimmed and locked.

For best results, run selective COSY experiments non-spinning.

**Create a new file directory**

From the data set `selex/2/1`, enter **edc** and change the following parameters:

NAME	selco
EXPNO	1
PROCNO	1 .

Click **SAVE** to create the data set `selco/1/1`.

 **$^1\text{H}$  reference spectrum**

The reference spectrum for the selective COSY experiment is the standard  $^1\text{H}$  spectrum of Gramicidin with **o1** set to the ornithine peptide N-H resonance. This may be found in `selex/1/1`.

## Selective Excitation

### Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 29. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters **p11** and **p1** (<sup>1</sup>H observe high power level and 90° pulse time), and the values determined in Section 8.2 ‘Selective Pulse Calibration’ for the parameters **sp1** and **p11** (<sup>1</sup>H selective low-power level and 90° pulse time).

**Table 29. Selective COSY Acquisition Parameters**

Parameter	Value	Comments
PULPROG	selco	see Figure 22 for pulse sequence diagram.
TD	32k	
NS	64	number of scans must be 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).
SP1		shaped pulse power level on f1 channel.
P1		90° <sup>1</sup> H high power pulse on f1 channel.
P11	80msec	90° <sup>1</sup> H shaped pulse on f1 channel.
D1	2sec	relaxation delay; should be 1-5 *T <sub>1</sub> ( <sup>1</sup> H).
D13	3µsec	short delay; predefined.
D14	35 msec	delay for evolution after shaped pulse ((p11)/2 + d14 = 1/(2J <sub>HH</sub> )).
PHCOR(1)		additional phase correction applied to shaped pulse; use value determined on page 86.

Notice that in this pulse sequence, the delay **d14** is to ensure that the magnetization is antiphase when the second pulse is applied. There are two ways to accomplish this. The first, which is appropriate when the selective pulse is 90°, is to choose **d14** such that  $(p11)/2 + d14 = 1/2J_{HH}$  (i.e., **d14** = 31 msec, assuming **p11** is 80 msec and J<sub>HH</sub> is 7 Hz). The second, which is appropriate when the selective pulse is 270°, is to choose **d14** to be  $1/2J_{HH}$  (i.e., 71 msec). Here it is recommended to use a 90° selective pulse so that the shorter **d14** value may be used.

### Acquire the data

Perform a routine acquisition with **zg**. The approximate experiment time for Selective COSY with the parameters set as shown above is 4 minutes.

**Set up the processing parameters**

Enter **edp** and set the processing parameters as shown in Table 30.

**Table 30. Selective COSY Processing Parameters**

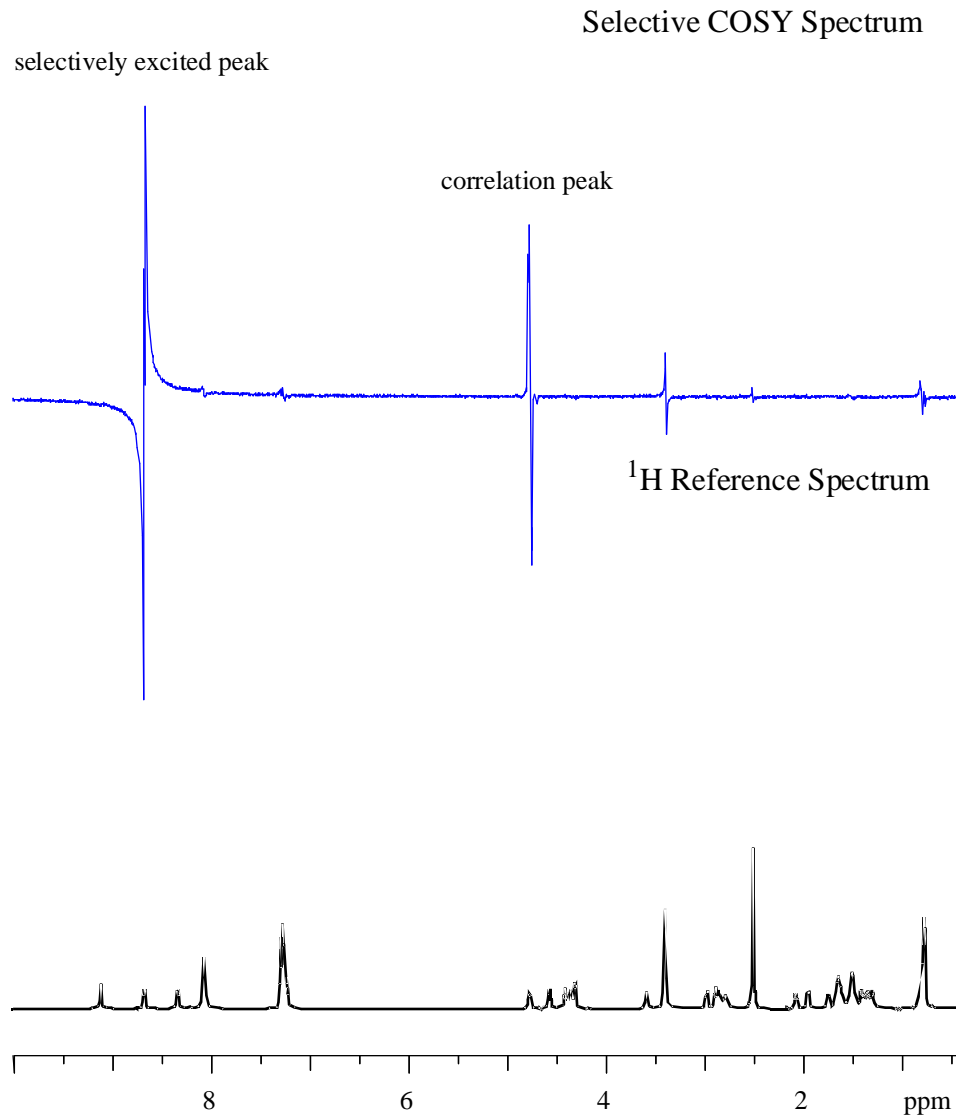
Parameter	Value	Comments
SI	16k	
WDW	EM	
LB	0.30	
PKNL	TRUE	necessary when using the digital filter.

**Process the data**

Add line broadening and Fourier transform the time domain signal with the command **ef**. Manually phase correct the spectrum. The resulting spectrum should look like that in Figure 26.

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Figure 26: Selective COSY Spectrum of 50 mM Gramicidin in DMSO-d<sub>6</sub>



## Selective TOCSY

## 8.4

In practice it is often desirable to elucidate the entire spin system which is coupled to a given nucleus. In TOCSY a spin-locking pulse train distributes the magnetization of one nucleus over the whole spin system. There can be net magnetization transfer from one spin to another even without direct J-coupling. (In addition to connectivities via J-coupling, magnetization transfer via dipolar coupling and chemical exchange is observed during the spin-locking pulse train, but these will have a different sign in the phase-sensitive TOCSY spectrum.)

Selective TOCSY gives the same  $^1\text{H}$  coupling information as 2D TOCSY one site at a time and without involving a 2D Fourier transform. The selective TOCSY sequence begins with a  $90^\circ$  frequency selective excitation pulse. This is followed by a fixed delay (rather than the variable evolution period of the 2D TOCSY sequence) during which in-phase coherence is created by evolution due to J-coupling. The duration of this delay is measured from the middle of the Gaussian envelope. Next, the coherence transfer occurs during the 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  $1/(10J_{\text{HH}})$  should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

Immediately following the spin-lock period is a z-filter, which is used to make the final 1D TOCSY spectrum easier to phase correct. Following this is the acquisition period. Since the TOCSY correlation peaks arise from magnetization that was in-phase during the fixed delay, they can be phase corrected to be positive and absorptive.

## Pulse Sequence Diagram

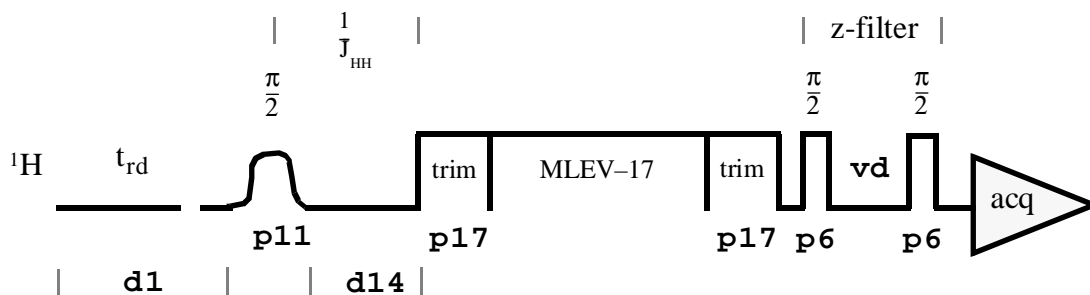
## 8.4.1

The selective TOCSY pulse sequence is shown in Figure 27. Notice that it is very similar to the standard TOCSY sequence shown in Figure 44 on page 168, except that here the first pulse is a low-power shaped pulse, the following delay (**d14**) is not incremented, and the spin-lock period is followed by a z-filter.

The high-power pulse **p1** and the low-power pulses **p6** and **p17** must be set to the appropriate  $90^\circ$  times found in Chapter 5 ‘Pulse Calibration’. The shaped pulse **p11** must be set to the appropriate  $90^\circ$  time found in Section 8.2 ‘Selective Pulse Calibration’. If **p11** is chosen to be a  $90^\circ$  pulse, the delay **d14** should be set so that  $(\text{p11})/2 + \text{d14} = 1/(J_{\text{HH}})$  for the selected resonance. On the other hand if **p11** is chosen to be a  $270^\circ$  pulse, **d14** should be set to  $20\mu\text{sec}$  (the time required for power switching).

## Selective Excitation

Figure 27: Selective TOCSY Pulse Sequence



### Acquisition and Processing

### 8.4.2

If this experiment is carried out directly after the pulse calibration described in Section 8.2, the correct sample is already in the magnet, the probehead tuned and matched, and the magnetic field shimmed and locked.

For best results, run selective TOCSY experiments non-spinning.

#### Create a new file directory

From the data set `selco/1/1`, enter **edc** and change the following parameters:

NAME	seltoc
EXPNO	1
PROCNO	1 .

Click **SAVE** to create the data set `seltoc/1/1`.

#### $^1\text{H}$ reference spectrum

The reference spectrum for the selective TOCSY experiment is the standard  $^1\text{H}$  spectrum of Gramicidin with **o1** set to the ornithine peptide N-H resonance. This may be found in `selex/1/1`.

### Write the variable delay list

The z-filter in the selective TOCSY experiment requires a variable delay list. To create the variable delay list, first enter **edlist**. A menu of list types appears. Select **vd** from this menu. This calls up a menu of existing vdlst filenames and gives the user the option of creating a new file ('Type new name'). Simply type the name **zf**. This calls up the vi editor. Enter the delays desired, some appropriate values are listed below:

```
0.004 s
0.016 s
0.010 s
0.006 s
0.004 s
0.010 s
0.017 s
0.011 s
0.018 s
0.012 s .
```

When the list is complete, save the file and exit the vi editor.

### Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 31. Use the values determined in Chapter 5 'Pulse Calibration' for the parameters **p11** and **p1** (<sup>1</sup>H observe high power level and 90° pulse time), and **p110** and **p6** (<sup>1</sup>H low power level and 90° pulse time for MLEV spinlock). Use the values determined in Section 8.2 'Selective Pulse Calibration' for the parameters **sp1** and **p11** (<sup>1</sup>H selective low-power level and 90° pulse time).

The parameter **11** 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 **11** should be chosen so that the quantity  $[(p6 * 64) + p5] * 11 + (p17 * 2)$  is 20 to 100msec. The general rule of thumb is that  $1/10J_{HH}$  should be allowed for each coherence 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 **11** should be adjusted so that  $[(p6 * 64) + p5] * 11 + p17$  is 20 to 100msec.

Be sure to set **vdlst** to the name of the appropriate variable delay list (here **zf**). This may be done in the **eda** menu either by typing **zf** in the box next to the parameter VDLIST, or by clicking with the right mouse button on this box to call up the menu of possible vdlsts and then selecting **zf** with the left mouse button. Also be sure to set **14** equal to the number of entries in the vdlst (here 10).

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**Table 31. Selective TOCSY Acquisition Parameters**

Parameter	Value	Comments
PULPROG	selmlzf	see Figure 27 for pulse sequence diagram.
TD	32k	
NS	32	the number of scans must be 16*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).
PL10		MLEV spin-lock power level on f1 channel.
SP1		shaped pulse power level on f1 channel.
P5		60° <sup>1</sup> H low power pulse on f1 channel; calculated internally.
P6		90° <sup>1</sup> H low power pulse on f1 channel.
P7		180° <sup>1</sup> H low power pulse on f1 channel; calculated internally.
P11	80msec	90° <sup>1</sup> H shaped pulse on f1 channel.
P17	2.5msec	trim pulse.
D1	2sec	relaxation delay; should be 1–5*T <sub>1</sub> ( <sup>1</sup> H).
D11	30msec	delay for disk I/O; predefined.
D12	20μsec	delay for power switching; predefined.
D13	3μsec	short delay; predefined.
D14		delay for evolution after shaped pulse ((p11)/2 + d14 = 1/J <sub>HH</sub> ).
L1	30	loop for MLEV cycle ((p6*64) + p5)*11 + (p17*2) = mixing time; this is generally between 15 and 55.
L4	10	number of delays in vclist.
VCLIST	zf	name of vclist used for z-filter.
PHCOR(1)		additional phase correction applied to shaped pulse; use value determined on page 86.

Notice that in this pulse sequence, the delay **d14** is to ensure that the signals are in-phase at the beginning of the mixing period (not antiphase, as for the selective COSY sequence). There are two ways to accomplish this. The first, which is appropriate when the selective pulse is 90°, is to choose **d14** such that  $(p11)/2 + d14 = 1/J_{HH}$  (i.e., **d14** = 103msec, assuming **p11** is 80msec and  $J_{HH}$  is 7 Hz). The second, which is appropriate when the selective pulse is 270°, is to choose **d14** to be 20μsec (in theory **d14** should be 0, but in practice 20μsec are required for power

switching). Here it is recommended to use a  $270^\circ$  selective pulse so that the shorter **d14** delay may be used.

#### Acquire the data

Perform a routine acquisition with **zg**. The approximate experiment time for selective TOCSY with the parameters set as shown above is 18 minutes.

#### Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 32.

**Table 32. Selective TOCSY Processing Parameters**

Parameter	Value	Comments
SI	16k	
WDW	EM	
LB	0.30	
PKNL	TRUE	necessary when using the digital filter.

#### Process the data

Add line broadening and Fourier transform the time domain signal with the command **ef**. Manually phase correct the spectrum using the 0<sup>th</sup>-order phase correction. The resulting spectrum should look like that in Figure 28.

**Selective Excitation***Figure 28: Selective TOCSY Spectrum of 50 mM Gramicidin in DMSO-d<sub>6</sub>*

## Selective TOCSY Spectrum

