

## Setting Up for the Selective 1D TOCSY

Follow all of the instructions for the Selective 1D NOE experiment to obtain a 1D  $^1\text{H}$  spectrum and write down the frequency offsets (relative to the center of the spectral window) for the peaks of interest in the 1D spectrum. When you have written down the peak frequencies, set **SR** back to zero so you have a reasonable ppm scale for **Dual Display** comparisons.

In a new dataset, enter **rpar** and select **std-1D-TOCSY-n3**. Enter the **O1** and **SWH** values used for the 1D  $^1\text{H}$  spectrum. Enter **ased** and edit the acquisition parameters:

<b>SPOFFS1</b>	Set to the frequency of the selected peak
<b>SPOFFS2</b>	Set to the frequency of the selected peak
<b>L1</b>	Number of loops in TOCSY mixing cycle
	17 loops = 34.5 ms mixing time (similar to COSY)
	<b>30</b> loops = 60.9 ms mixing time (default - good mixing)
	35 loops = 71.0 ms mixing time (thorough mixing)
	40 loops = 81.2 ms mixing time (for long spin systems)

## Biselective 1D TOCSY

Set up exactly as you do for the Selective 1D TOCSY, with the same parameter set (you can clone the parameters from the "monoselective" TOCSY to a new experiment number using **edc**). Now click **Windows / ShapeTool** to enter the *ShapeTool* program. This will allow you to customize the shaped pulse so that it selects *two* different peaks. To select two peaks in the spectrum, the *ShapeTool* program calculates the rotation of the two desired  $B_1$  vectors in the x-y plane (in the rotating frame) at each time point (rectangular component) of the shaped pulse. The vector sum of the two  $B_1$  vectors at each time point is used to modify the amplitude and phase of that component of the Gaussian shaped pulse.

The Pulsed Field Gradient Spin Echo places the selected peak magnetization on the spin-lock axis in the x,y plane while destroying all magnetization from other peaks in the spectrum. To get especially good selectivity, this sequence is repeated so that there are actually two selective  $180^\circ$  pulses: a Double Pulsed Field Gradient Spin Echo (DPFGSE). Since there are two shaped pulses, we will have to construct two different shaped pulses in *ShapeTool* and save each one under a unique filename. We will be exciting two peaks in the spectrum with opposite phase (i.e., one will be upside-down), so we will specify two frequencies (<first frequency> and <second frequency>) and for the second time through the selective  $180^\circ$  pulse we will advance the phase by  $90^\circ$  for the second frequency selected relative to the first. In a spin echo, advancing the phase of the  $180^\circ$  pulse by  $90^\circ$  (e.g., from x to y) always inverts the phase of the final signal. This is what puts our second selected peak (and all of the members of its spin system) upside-down.

In the *ShapeTool* program create the two shaped pulses as follows:

**Shapes / Gauss** *Start with a Gaussian shape*  
Number of Points = **4096** *4096 rectangular pulse elements*  
Truncation Level = **5** *start and end the function at 5% of  
the maximum value*

**Manipulate / Phase Modulation acc. to Offset Freq.**

> Beginning at Phase 0 ( $I_y \rightarrow I_z$ )  
> No Reference Frequency Specified *Freq. relative to center*  
With Additional Phase Setting *Separate phase for each peak  
to be excited by the shaped pulse*  
Length of Pulse (in  $\mu\text{s}$ ) = **35000** *Total duration of shaped pulse*  
Number of Frequencies: **2** *Number of peaks to excite*

---OK---

Frequency List: Phase List:

1.) <first frequency> **0**  
2.) <second frequency> **0**

---OK---

**File / Save as**

Files:  
**gauss.nj1** *This saves the first pulse shape*

Now repeat the whole process for the second pulse exactly as you did above except that at the end use:

Frequency List: Phase List:

1.) <first frequency> **0** *Normal pulse phase*  
2.) <second frequency> **90** *Use a pulse phase advanced by 90°*

---OK---

**File / Save as**

Files:  
**gauss.nj2** *This saves the second pulse shape*

Now you can exit the *ShapeTool* program:

**File / Quit**

Enter **ased** at the XWinNMR prompt to edit the acquisition parameters:

**SPOFFS1** Set to zero  
**SPOFFS2** Set to zero  
**L1** Same as before, 30 cycles of mixing is 60.9 ms TOCSY mixing  
**SPNAM1** **gauss.nj1** *First shaped pulse created in ShapeTool*

<b>SPNAM2</b>	<b>gauss.nj2</b>	<i>Second shaped pulse created in ShapeTool</i>
<b>SP1</b>	<b>55.8 dB</b>	<i>Use 6 dB higher power than monoselective pulse</i>
<b>SP2</b>	<b>55.8 dB</b>	<i>Use 6 dB higher power than monoselective pulse</i>

The parameters **SPOFFS1** and **SPOFFS2** are set to zero since we have manually defined the offset frequencies of our custom-built shaped pulses. As before, **L1** defines the number of loops through the TOCSY mixing sequence. **SPNAM1** and **SPNAM2** are the filenames for the shaped pulse files; these contain the phase and amplitude of each of the 4096 elements of the shaped pulse. **SP1** and **SP2** are the power levels, on a scale of 120 dB (lowest) to 0 dB (highest) radio frequency power at the top amplitude of the shaped pulse. Note that these are vastly lower power levels than those we use for hard (non-selective) pulses, which are typically at 0 dB. But a 35 ms pulse is on for 3,500 times longer than a 10  $\mu$ s hard pulse, so its power must be reduced dramatically to get a 90° or 180° rotation.

The 35 ms gaussian pulse was calibrated to give a 180° selective rotation at a power level of 61.8 dB. Since we are now exciting two regions of the spectrum with a 180° rotation, we need twice the pulse amplitude (four times the power), which corresponds to 6 dB higher power. The decibel scale is logarithmic with 10 dB corresponding to a factor of 10 difference in power. A 6 dB increase in power is  $10^{0.6} = 4$  times more power, or twice the RF amplitude. Since lower dB values correspond to higher power (a Bruker tradition which is opposite to Varian's), we increase the shaped pulse power (**SP1** and **SP2**) to 55.8 dB for the biselective experiment.

Use **rga** to optimize the receiver gain (**RG**) and then **zg** to acquire. Phase correct carefully, since the first selected peak and the other members of its spin system should be absorptive positive (right side up) and the second selected peak and its spin system partners should be absorptive negative (upside down). Plot the spectrum with parameters and title.