

# TOCSY SPECTROSCOPY OVERVIEW

## BRUKER

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**TOCSY** – **T**otal **C**orrelation **S**pectroscop**Y**, same as **COSY**, but also able to generate cross peaks via intermediate spins (mix). Uses a spin lock that produces rf heating of the sample and hence requires many steady state scans (ds).

## Summary of Methodology

### **In other words, what you need to do.**

1. Set up variable temperature control (if needed).
2. Lock, tune, and shim.
3. Acquire 1D  $^1\text{H}$  spectra, set: reference, sweep width, and transmitter frequency.
4. Reacquire 1D  $^1\text{H}$  spectra with reduced sweep width, and then determine the number of scans required. Record parameter values.
5. Calibrate the 90 degree pulse for  $^1\text{H}$  (not necessary, but a good idea).
6. Load 2D TOCSY parameter set (**MLEVPHSW**).
7. Check 2D pulse program (should be **mlevph**).
8. Load **prosol** parameters and setup the reference, sweep width, transmitter frequency, number of scans, and the number of points.
9. Set receiver gain, acquire.
10. Transform 2D data, phase and load projections.

## 1. Regulate the temperature (if desired).

*Make sure you have had Bruker VT training BEFORE adjusting the temperature.*

Open temperature controller: **edte**

- a. Select the Carrier Gas: Compressed Air (10-40 °C) or Nitrogen.
  - i. Turn off the compressed Air (may keep 401 magnet legs on compressed air). The valve is closed when the handle is perpendicular to the pipe.
  - ii. Turn on the nitrogen.
- b. Select: [**Corrections**] and verify that no correction is applied.
- c. Select: [**Ramp**] enter a ramp rate of 2 degrees/min, enable ramp.
- d. Normal Conditions: [**Main Display**]
  - i. Sample Temp= 20 °C Thermocouple located below tube.
  - ii. Target Temp= 20 °C
  - iii. Heater= OFF (Set Max = 10% )
  - iv. Gas Flow= 270 L/h
  - v. Cooling= Empty
- e. Increase Gas Flow
  - i. 270 L/h normal, 800 for high/low temp [**+/-**]
    - a. Extreme temperatures will need a higher flow rate
  - ii. Turn the heater [**on**]
  - iii. Check the maximum heater power [**Set Max**] 10%. Increase the heater power if unable to obtain the desired temperature.
- f. Set temperature at 25 °C. The liquid nitrogen dewar is not required for 25 °C
- g. Within the edte window open [**Monitoring**]
  - i. Use auto scale for both y-axis':
    - a. Left: Temperature
    - b. Right: Heater Power
  - ii. Let sample equilibrate for 5 to 15 minutes
- h. Open [**Self tune**], run Self-tune program

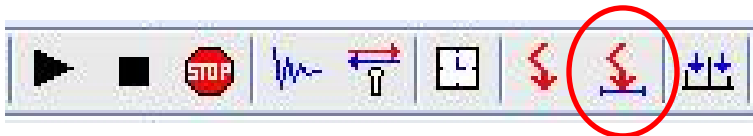
## 2. Lock, tune and shim.

- a. Check that the spinning is shut off.
- b. Shim the magnet: X, Y, Z1-Z5
- c. Tune for <sup>1</sup>H.

**3&4.** Collect a good 1D spectra

### Experiment 1 (EXPNO)

**Proton:** Acquire a 1D and reference. Zoom in and display all proton signals leaving 0.5 ppm of baseline on each side. Type **setsw** (or click on the icon) to set the transmitter offset (**o1p**) and sweep width (**sw**).



Reacquire “reduced-sweep width spectra” with the number of scans (ns) needed to get good signal to noise and phase. This dataset will become the  $^1\text{H}$  projection.

Write down the following values:

o1p:\_\_\_\_\_

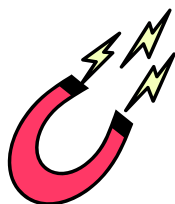
sw:\_\_\_\_\_

sr:\_\_\_\_\_

ns:\_\_\_\_\_

**\*\*These values will be used in F2 (direct) dimension.\*\***

*Type the parameter in the command line, hit enter, and TopSpin will display the value for you.*



# How to calibrate the $^1\text{H}$ $90^\circ$ Pulse on the Bruker NMRs

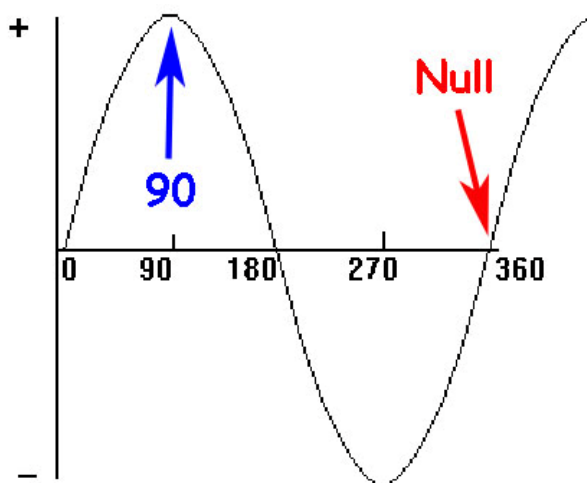
## Background Information:

For many NMR experiments such as DEPT, TOSCY, NOESY, and HMBC, the pulse sequence requires that many specific pulses or a series of pulses ( $90^\circ$ ,  $45^\circ$ ,  $180^\circ$ , etc.) be applied. Without properly calibrated pulses, many of these experiments will yield meaningless results, or most likely, fail outright.

Since each compound (and each nucleus) has a different chemical environment, each had a distinct  $90^\circ$  pulse width (p1). The  $90^\circ$  pulse is defined as the duration, in microseconds, that the rf signal must irradiate your sample in order to tilt the magnetizations into the XY-plane,  $90^\circ$  away from the Z-axis of the NMR's magnetic field. Another way to think of it is how long you must pulse in order to tip all the spins into the XY plane. This pulse is often referred to as the  $\pi/2$  pulse.

The  $90^\circ$  pulse width for proton NMR experiments is about 10-20 microseconds on most modern spectrometers. The exact value of the  $90^\circ$  pulse width depends on the sample (nucleus, solvent, etc.) as well as the instrument (probe, transmitter power, etc.). It may be 5 microseconds long, 17 microseconds, or 35 microseconds, or some other number determined experimentally. For this reason, it is necessary to measure the  $90^\circ$  pulse for every sample you need to perform 2D experiments on. Lucky for us, the proton  $90^\circ$  pulse is typically quite similar for all the protons in your sample.

Measuring the  $90^\circ$  pulse width is simple enough. Remember that the  $90^\circ$  pulse tilts the sample magnetization into the XY plane, which contains the detector. A simple pulse sequence of irradiate-observe should show a maximum for the pulse duration corresponding to a  $90^\circ$  pulse. Because it is difficult to discern maximum signal intensities by comparing similarly intense peaks (i.e. comparing an  $89^\circ$ , a  $90^\circ$ , and a  $91^\circ$  pulse.), we look at the  $180^\circ$  or the  $360^\circ$  pulse.



The 360° pulse corresponds to a ‘null’ – no signal is observed at this irradiation. Searching for this null is easier to determine and has the added advantage of minimizing the time required between pulses due to relaxation issues.

5. Calibrate 90° pulse (if needed!).

## Experiment 10 (or any other new experiment)

### The Bruker nitty gritty:

1. **re 10** and obtain a well-shimmed <sup>1</sup>H spectrum.
2. Type **p1**, hit enter and notice the current value for the 90° pulse. Record p1 and p11
3. Type **pulprog zg**. Typically, Bruker uses a 30° pulse (zg30) for a proton 1D. This resets this to a 90° pulse.
4. Change parameters (**ns 1; ds 0; d1 60**), reacquire, and phase. The value for d1 should be 5xs T1, hence using a value of 60 here is an estimate. If you have a slow relaxer or know your value for T1, you might want to set d1 to a larger value.
5. Fourier transform (**ft**) and phase (**apk**). Type **dpl1** to set the display regions. Type **phmod pk** to use the same phase values for all spectra
6. Start the acquisition by executing the AU popt program.

Check Optimize button

```

Enter parameter to modify:      p1
Choose optimum value:         zero
Enter startval value:         8
Enter endval value:           64
Enter the number of experiments (nexp)      8
Enter the increment variation mode (varmod) lin(ear)
Enter parameter increment (inc)           8
Click [start optimize]
  
```

<input type="checkbox"/>	store as 2D data (ser file)							
<input type="checkbox"/>	The AU program specified in AUNM will be executed							
<input checked="" type="checkbox"/>	Perform automatic baseline correction (ABSF)							
<input type="checkbox"/>	Overwrite existing files (disable confirmation Message)							
<input type="checkbox"/>	Run optimisation in background							
OPTIMIZE	PARAMETER	OPTIMUM	STARTVAL	ENDVAL	NEXP	VARMOD	INC	
<input checked="" type="checkbox"/>	p1	ZERO	8	64	8	LIN	8	
Start optimize		Halt optimize		Read protocol		Add parameter		Restore
Save		Read array file		Save array file as ...		Help		

7. In PROCNO 999, the finished array will be displayed, similar to Figure 1.

8. On the screen, you should see a series of spectra that start positive, pass through a null at 180°, become negative, and pass through a second null at 360°. Estimate the point where the signal goes from negative values through zero then become positive.

This is the location of your 360° pulse. (If you do not see a clear null at 360°, you may need to run **popt** again, adjusting the entered values.)

9. Run your array again, to determine the 360° pulse width  $\pm 0.5 \mu\text{s}$  (i.e. array 60 to 63 with an increment of 0.5)

10. Calculate the 90° pulse by dividing the p1 value of the null by 4. Use this number for your p1 in your subsequent experiments on this sample.

p1:\_\_\_\_\_ 90° pulse

pl1:\_\_\_\_\_ power level for p1

6. Load 2D parameter set.

### Experiment 100 (or any other new experiment)

rpar MLEVPHSW

7. Check pulse program and make sure the correct one has been loaded (**mlevph**).

8. Load the prosol parameters by typing **getprosol**.

a. Edit the basic parameters based on the information from the 1D experiments (the values you recorded in **step #3**).

b. Set **pl10** from **step #5** (or leave as default if you did not run **step #5**).

c. **d9** by default is 60ms, but it is typically 0.03 to 0.3ms.

i. **d9** is the duration of the TOCSY mixing time, which consists of:

**pl10**: the power level for the TOCSY spin-lock (determined during **step #5**).

**p5**: 60° <sup>1</sup>H pulse at pl10 power level.

**p6**: 90° <sup>1</sup>H pulse at pl10 power level (about 3-35 microseconds)

**p7**: 180° <sup>1</sup>H pulse at pl10 power level

**p17**: trim pulse (2 milliseconds)

d. **ns** is set to 8 and **ds** to 4, by default. Modify as needed.

9. Set receiver gain **rga** and acquire **zg**.

10. Process the recorded data with **xfb**.

a. By default, **SI2=SI1=1K** and pure cosine squared sine window functions (**WDW2=WDW1=QSINE**) are applied to both dimensions.

b. **SSB2=SSB1=2** using MC2 (TPPI, States-TPPI) as defined in **FnMODE**.

11. Load projections by typing **edc**. Fill out the name, **EXPNO** and **PROCNO** information for both **F2** and **F1**.

12. When finished, remember to **ro off**, **lock off**, and eject (**ej**) your sample.

