

2D NMR SPECTROSCOPY OVERVIEW

BRUKER

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'Essential' ¹H-detected 2D Pulse Sequences:

COSY – **C**orrelation **S**pectroscop**Y**, good for determining basic connectivity via J-couplings (through-bond).

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).

NOESY – **N**uclear **O**verhauser **E**ffect **S**pectroscop**Y**, allows one to see through-space effects, useful for investigating conformation and for determining proximity of adjacent spin systems. Not so useful for MWs in the 1 kDa range due to problems arising from the NMR correlation time.

ROESY – **R**otational **O**verhauser **E**ffect **S**pectroscop**Y**, same as **NOESY**, but works for all molecular weights. Has the disadvantage of producing more rf heating, hence it requires more steady state scans.

HMQC – **H**eteronuclear **M**ultiple **Q**uantum **C**orrelation, allows one to pair NH or CH resonances. Often uses X-nucleus decoupling and hence gives rise to rf heating, requires reasonably well calibrated pulses and many steady state scans.

HSQC – **H**eteronuclear **S**ingle **Q**uantum **C**orrelation, provides the same information as **HMQC**, but gives narrower resonances for ¹H-¹³C correlations. Also requires X-decoupling and hence a large number of steady state scans and is also more sensitive to pulse imperfections.

HMBC – **H**eteronuclear **M**ultiple **B**ond **C**orrelation, a variant of the **HMQC** pulse sequence that allows one to correlate X-nucleus shifts that are typically 2-4 bonds away from a proton.

Information of individual 2D pulse sequences can be found in the TopSpin Menu [Help]>[NMR GUIDE]

Experiment	Parameter Set	Pulse Program	Minimum NS
gCOSY	2D-gCOSY-dcif	cosygpqf	1
gNOESY	2D-gNOESY-dcif	noesygpqh	2
ROESY	2D-ROESY-dcif	roesyetgp	8
TOCSY	2D-TOCSY-dcif	mlevegtp	8
gHMQC	2D-gHMQC-dcif	hmqcgpqp	1
gHSQC	2D-gHSQCdcif	hsqcetgpsi2	1
gHMBC	2D-gHMBC-dcif	hmbcgplpndqf	1

Bruker Pulse Program Abbreviations	
qf	absolute value
gp	gradient pulse
ph	phase sensitive
et	phase sensitive (Echo/Anti-Echo TPPI)
si	sensitivity improved
nd	no decoupling
pr	presat

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 ^1H spectra, set: reference, sweep width, and transmitter frequency.
 4. Reacquire 1D ^1H spectra with reduced sweep width, and then determine the number of scans required. Record parameter values.
 5. Repeat for 1D ^{13}C spectra to run HSQC or HMBC (if needed).
 6. Calibrate the 90 degree pulse for ^1H (if needed).
 7. Load 2D parameter set.
 8. Check 2D pulse program.
 9. Load **prosol** parameters and setup the reference, sweep width, transmitter frequency, number of scans, and the number of points.
 10. Set receiver gain, acquire.
 11. Transform 2D data, phase and load projections.
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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

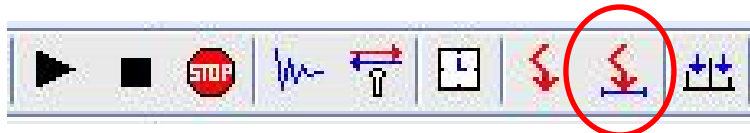
2. Lock, tune and shim.

- a. Check that the spinning is shut off.
- b. Shim the magnet: X, Y, Z1-Z5
- c. Tune each channel in use during the 2D experiment
 - i. ¹H ONLY: COSY, NOESY, TOCSY
 - ii. ¹H and ¹³C: HSQC and HMBC

3. Collect a good 1D spectra

Experiment 1 (EXPNO)

- a. **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).



- b. 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 ^1H 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.*

Write these parameters to experiment 10 - **wrpa 10** (for pulse calibration).

Experiment 2

- c. **X Nucleus** (only if acquiring HMQC, HSQC, or HMBC), Acquire a 1D and reference. Zoom in and display all X signals leaving 0.5 ppm of baseline side. Type **.setsw** to set the transmitter offset (o1p) and sweep width (sw).
- d. Reacquire “reduced-sweep width spectra”. This dataset will become the X projection.

Write down the following values:

o1p:_____

sw:_____

sr:_____

****These values will be used in F1 (indirect) dimension.****

How to calibrate the ^1H 90° Pulse on the Bruker NMRs

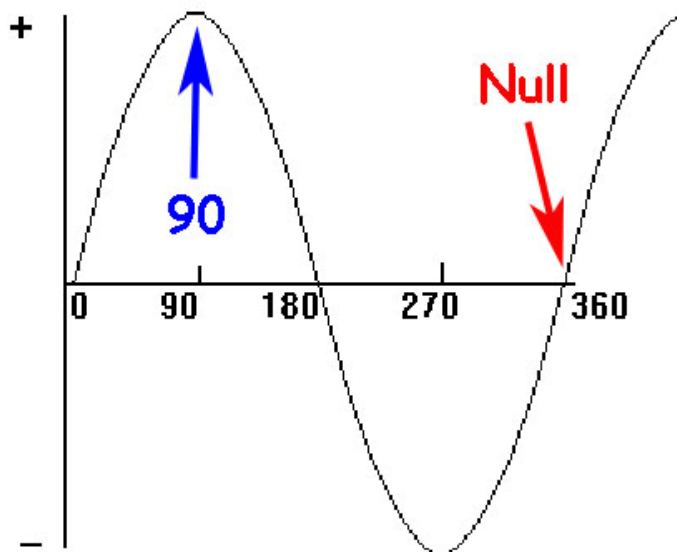
Background Information:

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° , 45° , 180° , 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° pulse width (p1). The 90° 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° 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° pulse width for proton NMR experiments is about 10-20 microseconds on most modern spectrometers. The exact value of the 90° 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° pulse for every sample you need to perform 2D experiments on. Lucky for us, the proton 90° pulse is typically quite similar for all the protons in your sample.

Measuring the 90° pulse width is simple enough. Remember that the 90° 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° pulse. Because it is difficult to discern maximum signal intensities by comparing similarly intense peaks (i.e. comparing an 89° , a 90° , and a 91° pulse.), we look at the 180° or the 360° 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.

4. Calibrate 90° pulse (if needed!).

Experiment 10 (or any other new experiment)

The Bruker nitty gritty:

- **re 10** and obtain a well-shimmed ^1H spectrum.
- Type **p1**, hit enter and notice the current value for the 90° pulse. Record p1 and p11
- Type **pulprog zg**. Typically, Bruker uses a 30° pulse (zg30) for a proton 1D. This resets this to a 90° pulse.
- Change parameters (**ns 1; ds 0; d1 60**), reacquire, and phase. The value for d1 should be $5 \times T_1$, hence using a value of 60 here is an estimate. If you have a slow relaxer or know your value for T_1 , you might want to set d1 to a larger value.
- 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
- Start the acquisition by executing the AU **popt** program.
 1. Check Optimize button
 2. Enter *parameter* to modify: p1
 3. Choose *optimum* value: zero
 4. Enter *startval* value: 8
 5. Enter *endval* value: 64
 6. Enter the number of experiments (*nexp*) 8
 7. Enter the increment variation mode (*varmod*) lin(ear)
 8. Enter parameter increment (inc) 8
 9. Click [start optimize]

store as 2D data (ser file)
 The AU program specified in AUNM will be executed
 Perform automatic baseline correction (ABSF)
 Overwrite existing files (disable confirmation Message)
 Run optimisation in background

OPTIMIZE	PARAMETER	OPTIMUM	STARTVAL	ENDVAL	NEXP	VARMOD	INC
<input checked="" type="checkbox"/>	p1	ZERO	8	64	8	LIN	8

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

p1: _____ power level for p1

5. Load 2D parameter set.

Experiment 100 (or any other new experiment)

rpar <PARAMETER SET>

Experiment	Parameter Set	Pulse Program	Minimum NS
gCOSY	2D-gCOSY-dcif	cosygpqf	1
gNOESY	2D-gNOESY-dcif	noesygpqh	2
ROESY	2D-ROESY-dcif	roesyetgp	8
TOCSY	2D-TOCSY-dcif	mleveltgp	8
gHMQC	2D-gHMQC-dcif	hmqcgppf	1
gHSQC	2D-gHSQCdcif	hsqcetgpsi2	1
gHMBC	2D-gHMBC-dcif	hmbcgplpndqf	1

6. Check pulse program and make sure the correct one has been loaded (see above table).
7. Load the prosol parameters by typing **getprosol**.
8. Edit the basic parameters based on the information from the 1D experiments (the values you recorded in [step #3](#)).

Homonuclear Experiments

- **ns** Number of Scans
- **p1** Pulse width (us) (90 degree Pulse)
- **o1p** F2 Transmitter frequency (ppm)
- **sw** Sweepwidth (ppm). Enter the value for F2 and F1 dimensions.
- **sr** Reference (Hz). Enter the value for F2 and F1 dimensions.

Inverse experiments (1H vs 13C) require additional parameters

- **p2** Pulse width (us)
- **o2p** F1 Transmitter frequency (ppm)
- **1 sw** F1 Sweepwidth (ppm)
- **1 sr** F1 Reference(Hz)

Check these parameters, loaded during **getprosol**. Adjust as needed (eg. if you did measure the 90° pulse).

- **pl1** Power Level p1
- **pl2** Power Level for p2

9. Check **Experiment Specific Parameters** (listed below) and adjust as needed.
10. Optional parameter changes
 - **2 td** F2 Number of points
 - **1 td** F1 Number of points

11. Set receiver gain **rga** and acquire **zg**.
Use **multizg** to start multiple incremental experiments (if desired).

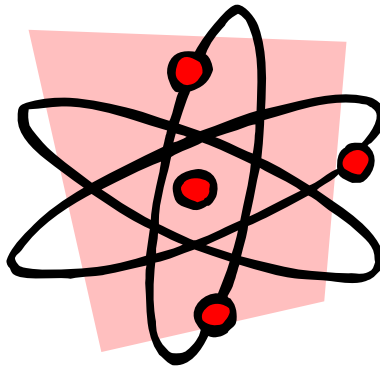
12. Data Processing

- a. Fourier Transform the data: **xfb**
- b. Phase the data.
 - i. Type **ph**
 - ii. Choose manual phasing
 - iii. RMB on three peaks that span your spectrum
 - iv. LBM on the “R” button to start phasing the rows
 1. Click and drag on the “0” and “1” to adjust the zero and first order phasing
 2. When finished, click save and return
 - v. LBM on the “C” button to start phasing the columns
 1. Click and drag on the “0” and “1” to adjust the zero and first order phasing
 2. When finished, click save and return
 - vi. Click return to go back to the spectra
- c. Homonuclear experiments may be symmetrized to reduce the noise. Type **sym** to symmetrize.

13. Load the 1D projections

- a. Type **edc**
 - i. Fill out the name, EXPNO and PROCNO information for both F2 and F1.

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



Experiment Specific Parameters

(step #9)

COSY

- **p1** 90 degree pulse
- **pl1** power level for p1 pulse

TOCSY:

- **d9** mixing time 60ms by default, typically 0.03 to 0.3
- **ds** dummy scans to establish thermal equilibrium

NOESY:

- **d8** mixing time, 400-500 ms for small molecules, 100-200 ms for larger

ROESY:

- **d?** mixing time for dipolar or through-space interactions
- **ds** dummy scans to establish thermal equilibrium

HSQC

- **d1** Delay time
- **CNST2** (average value for $^1J(XH)$ will affect $d24 \frac{1}{(8 * ^1J(XH))}$)

