PLEASE NOTE: This document assumes the user has a basic understanding of how to run 1H and 13C experiments on the Bruker 400 and 401.

‘Essential’ 1H-detected 2D Pulse Sequences:

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

**TOCSY** – **TO**tal **Correlation** **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). (see separate training guide for TOCSY).

**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.

PLEASE NOTE: If you want to run your VT experiment at a different temperature, please contact a DCIF staff member for VT training. Do not change the probe temperature unless you have been trained.
Summary of Methodology

In other words, what you need to do.

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

1. Lock, tune and shim.
   a. Check that the spinning is shut off.
   b. Shim the magnet: X, Y, Z1-Z5
   c. Make sure you tune (atma) each channel in use during the 2D experiment
      i. $^1$H ONLY: COSY, NOESY, TOCSY
      ii. $^1$H and $^{13}$C: HSQC and HMBC

2. 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 $^1$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._

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.**
Experiment 100 (or any other new experiment)

Type `rpar` on the command line or use Create Dataset.

In the upper right corner of the Parameter Sets window, click the arrow next to Source, and make sure `/opt/topspin3.1/exp/stan/nmr/par/` is selected. This will open the master list of all the Bruker standard parameter sets. Select the one you want, then click Set selected item in editor.

If you check the Show Recommended box, this will display a list of Bruker’s most commonly used small molecule experiments.
Suggested parameter sets and pulse programs

PLEASE NOTE that these are suggestions only. These may or may not work for your particular sample.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Parameter Set</th>
<th>Pulse Program</th>
</tr>
</thead>
<tbody>
<tr>
<td>gCOSY</td>
<td>COSYGPSW</td>
<td>cosygpppqf</td>
</tr>
<tr>
<td>gNOESY</td>
<td>NOESYPHSW</td>
<td>noesygpphpp</td>
</tr>
<tr>
<td>ROESY</td>
<td>ROESYPHSW</td>
<td>roesyppp2.2</td>
</tr>
<tr>
<td>gHSQC</td>
<td>HSQCGPPH</td>
<td>hsqcgpph</td>
</tr>
<tr>
<td>gHMBC</td>
<td>HMBCGP</td>
<td>hmbcgplpndqf</td>
</tr>
<tr>
<td>TOCSY</td>
<td>MLEPHSW</td>
<td>mlephpp</td>
</tr>
</tbody>
</table>

**COSY experiments**

COSYGPSW (cosygpppqf) – magnitude mode COSY (qf) with gradients (gp) and purge pulses (pp).

COSYGPDPFPHSW (cosygpmfphpp) – COSY with gradient pulses (gp), multiple quantum filter (mf), phase sensitive (ph), and purge pulses (pp). Difficult for a beginner to phase.

**NOESY experiment**

NOESYPHSW (noesygpphpp) – NOESY with gradient pulses during mixing time, phase sensitive (ph), and purge pulses (pp).

**ROESY experiment**

ROESYPHSW (roesyppp2.2) – ROESY sequence, phase sensitive (ph), and purge pulses (pp), using 180x-180x pulses for spin lock to suppress TOCSY artifacts (.2)

**HSQC experiments**

HSQCGPPH (hscqcgpph) – simple HSQC, phase sensitive (ph).

HSQCETGP (hscqetgp) – simple gradient HSQC, non-Edited.

HSQCEDDETGP (hscqedetgp) - simple Multiplicity Edited gradient HSQC.

HSQCEDDETGPSISP_ADIA (hscqedetgpsisp2.3) – Multiplicity Edited (ed), adiabatic pulses (sp), sensitivity improved (si). Gives you DEPT type information in addition to the $^1$H-$^{13}$C connectivity.

HSQCETGPSISP_ADIA (hscqetgpsisp2.2) – NOT Multiplicity Edited, adiabatic pulses (sp), sensitivity improved (si). Simple and all peaks are positive.
HMBC experiments

HMBCGP (hmblcplndqf) – Gradients for coherence selection (gp), low pass filter (lp), no decoupling during acquisition (nd), and magnitude mode (qf). Simple and no 180° pulses.

HMBCETGPL3ND (hmbcetgpl3nd) – Echo Anti Echo (et), gradients for coherence selection (gp), 3rd order low pass filter (l3). More difficult to process (xfb + xf2m).

TOCSY experiments

MLEVPHSW (mlevphpp) – Homonuclear Hartman-Hahn using MLEV17 sequence, phase sensitive (ph), and purge pulses (pp).

MLEVPHPR (mlevphpr.2) - Homonuclear Hartman-Hahn using MLEV17 sequence, phase sensitive (ph), and presat (pr).

3. Check pulse program (in AcqPar tab) and make sure the correct pulse program (PULPROG) has be loaded (see above table). If you click the button with the three periods (…) next to the PULPROG window, it will open the list of available pulse programs.

4. Load the prosol parameters by typing getprosol or by clicking the Acquire → Prosol button.

5. Edit the basic parameters based on the information from the 1D experiments (the values you recorded in step #2).

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 measured the 90° pulse).

- **pl1** Power Level p1
- **pl2** Power Level for p2
6. Check **Experiment Specific Parameters** (listed below) and adjust as needed.

7. Optional parameter changes
   - 2 td  F2 Number of points
   - 1 td  F1 Number of points

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

9. Data Processing

   *TopSpin’s 2D display buttons. If you want to know what a button does, hover your mouse over the button, without pressing, and the software will tell you what the button does.*

   a. Fourier Transform the data by typing **xfb**
   b. Phase the data (if needed).
      i. Type **ph** or hit the **Adjust Phase** button.
      ii. Choose manual phasing (if given the option).
      iii. Right click on two or 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.
   c. Homonuclear experiments ONLY may be symmetrized to reduce the noise. Type **sym** to symmetrize.

10. Load the 1D projections. Right click in the 2D spectrum window, at the top OR on the left side, and select External Projection. Fill in the name of the 1D experiment you want as that projection.

11. To edit the contour lines, you can right click in the middle of the 2D spectrum and select Edit Contour Levels. Edit as desired.

12. 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

**NOESY**
- **d8**: Mixing time. Default = 0.3 seconds. Recommend 400-500 ms for small molecules, 100-200 ms for larger

**ROESY**
- **p15**: mixing time for dipolar or through-space interactions. Default = 200 milliseconds.
- **ds**: dummy scans to establish thermal equilibrium

**HSQC**
- **d1**: Delay time

**CNST2**
- (average value for $^1$J(XH) will affect d24 $1/(8 * ^1$J(XH))

**TOCSY**
- **d9**: Mixing time. Default = 0.08 seconds.

*See the NMR Guide in TopSpin for more details.*