Acquisition and Processing of 2D NMR Spectra using TOPSPIN 3.5

These notes follow on from the NMR handout *Introduction to the TOPSPIN program* (PDF file) and describe the procedures for the acquisition and processing of the most common 2D NMR experiments. The descriptions rely on the user having recorded a well shimmed proton spectrum on the same instrument before acquiring 2D experiments, and it is a **requirement that the user has been specifically trained** on the selected instrument(s) **by a member of the NMR staff**. These notes allow the operator to collect data using the default experiment parameter sets on the NMR basement instruments (listed using the command *rpar *xxx*, where *xxx* represents the probe identifier; see below).

Before experimenting with **any** parameters, please note that all 1-D and 2-D experiments have been carefully setup the NMR Staff in probe dependent parameter sets designed specifically for each instrument of relevance, which can be accessed with the command ***rpar** (read parameters),* see Table below;

Instrument	Probe parameter set	Example Parameter set
AVB400	***bbo**	*COSYbbo*
AVB500	***tfi**	*COSYtfi*
AVX500	***bbfo**	*COSYbbfo*
AV600	***bbo**	*COSYbbo*
AV700	***tci**	*COSYtci*

The following 2-D NMR experiments are described:

Experiment	Example Parameter file	Phase sensitive
^{1}H-^{1}H COSY	COSY90*xxx*	✘
^{1}H-^{13}C Edited HSQC	HSQCed*xxx*	✓
^{1}H-^{13}C HMBC	HMBC*xxx*	✘
^{1}H-^{1}H NOESY	NOESY*xxx*	✓

Note from the table above that the versions of 2D experiments which are regularly used that are phase sensitive are edited-HSQC and NOESY which require phase manipulation on processing. All 2-D experiments *do not require sample spinning*. It is advisable for all users to include titles incorporating solvent, temperature and date for each experiment.

Please ask NMR staff before altering parameters without due diligence, otherwise you do so at your peril and that of the instrument.

1) ^{1}H-^{1}H COSY

Used for establishing proton-proton correlations over 2 or 3 bonds.

Acquisition

The general layout for 2D NMR experiment parameters is shown below and can be accessed by using acquisition window (*AcquPars* tab on the toolbar) or the command *eda*;
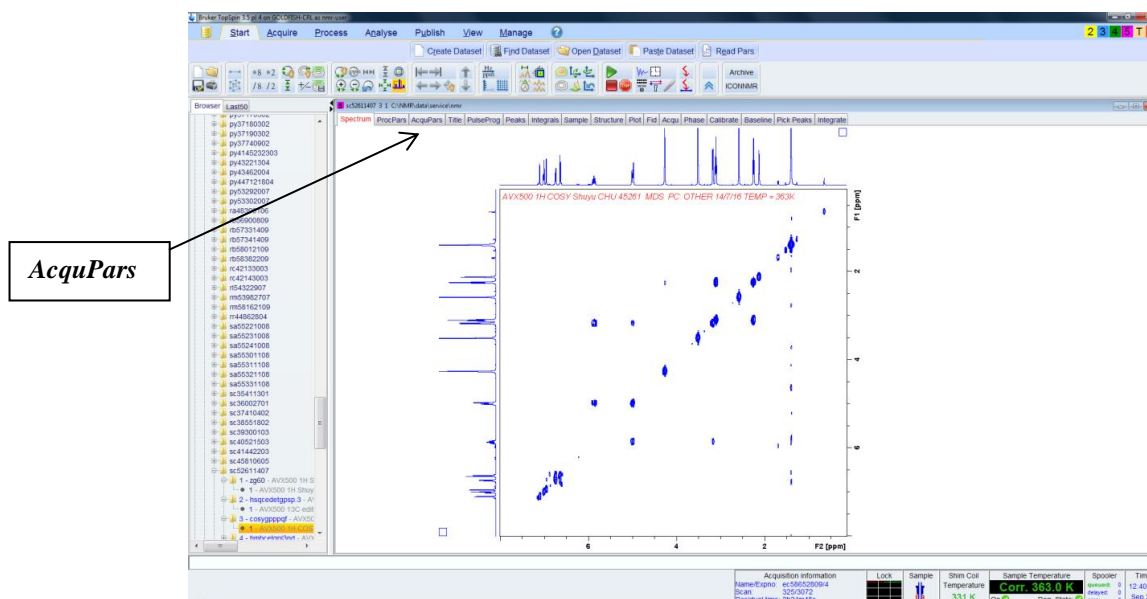

The first three tabs below spectrum are the main tabs of interest; '*show pulse program parameters*' in the form of a cartoon pulse (*ased*) – which lists only relevant parameters employed by the experiment; '*toggle status parameter view*'(*dpa* or **S** icon*)* which lists those actually recorded e.g. NS, actual number of scans or TE, actual temperature. Lastly '*getprosol*' (NMR tube icon) loads default pulse calibrations for the experiment. This should not be necessary if you are using the "home-made" parameter sets for the instrument, but is essential for any Bruker parameter sets you may use.

Pulse - *only parameters employed during the experiment are displayed (command **ased**)*

S [status] -*tells you what parameters were used during the experiment (command **dpa**)*

NMR-tube – *loads default pulse calibrations: there should be no need to use this for our default experiments (but is essential for the Bruker parameter sets) (command **getprosol**)*
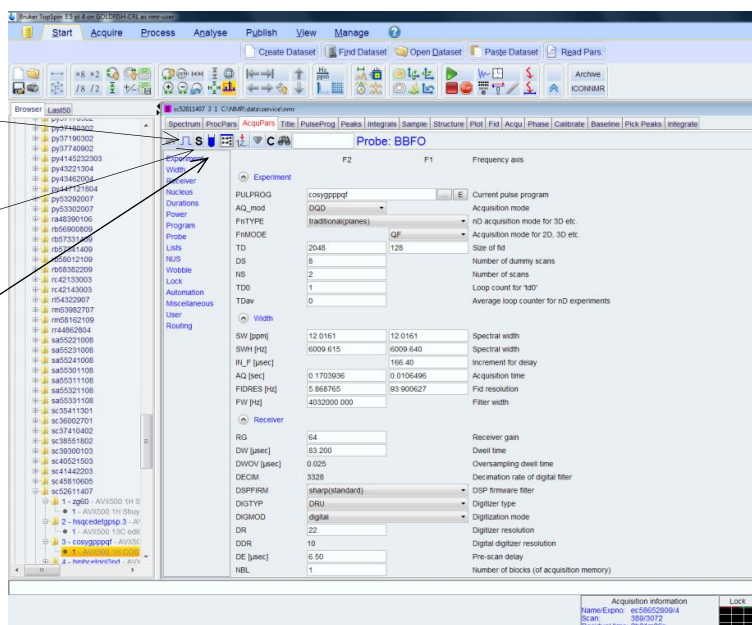
There are relatively few parameters you will need to adjust. Parameters to change for acquisition may include;

- Spectrum width (**SW**) for F2 must be equal to that for F1 – for all ^{1}H-^{1}H 2-D experiments (e.g. COSY, TOCSY and NOESY)

- **O1P**: in ppm – chemical shift corresponding to middle of the spectrum

- **NS:** number of scans- increase only for weaker samples

Do not change data sizes (TD) in F1 or F2. It is also advisable to specify solvent using the ***solvent*** command and ***te*** for the sample temperature you have used, for future reference. Please use the command ***expt*** for the experiment duration before starting the experiment, although the acquisition information in the lower right hand corner always records the time. The useful ***spooler*** feature allows you to queue several experiments with the command ***zg*** and will list the time taken for each one (in bottom right hand corner- double click window to open spooler table). Most default 2-D experiments do not need the command ***rga.*** To start the acquisition just use ***zg*** If "DRU" errors appear, stop the experiment (command ***stop***) then use ***rga*** then repeat ***zg***.

Processing

2D spectra may be transformed with the command ***xfb*** which replaces the 1D ***ft*** command (e**X**ecute **F**ourier transform in **B**oth dimensions). This can be executed at any time during and after the acquisition.

To set the 1D spectrum projections on both X (F2) and Y (F1) axes, right click anywhere over the projection and choose 'External projection…' to define the required 1D spectrum. Expansion of the 2D spectrum is a drag process (defining a boxed area), as for 1D expansion, and scaling is as for 1D spectrum scaling. 2D Spectra can also be overlaid using the multiple display mode (as for 1D) and similarly 1D spectra may be overlaid on top of 2D spectra.

To look at the processing parameters use ***edp*** or the **ProcPars** tab on the toolbar:
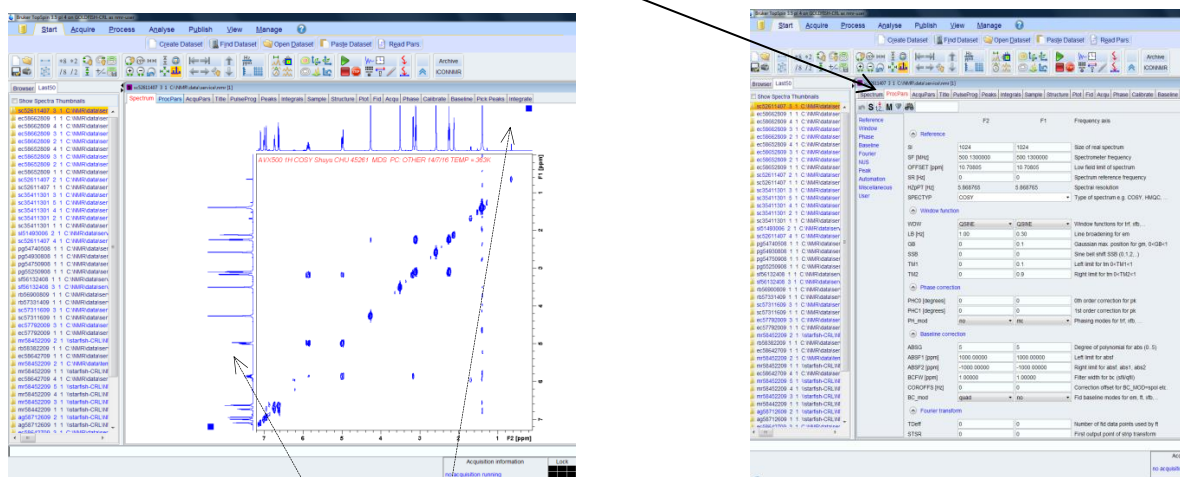
right click and insert name of external projection for 1-D spectrum

Standard COSY is not a phase sensitive experiment and so does not need phase correction. It is not advisable to change window functions as these are optimally set in the default parameters. If you want to enhance and add more contour, use the icons in the toolbar indicated by arrows
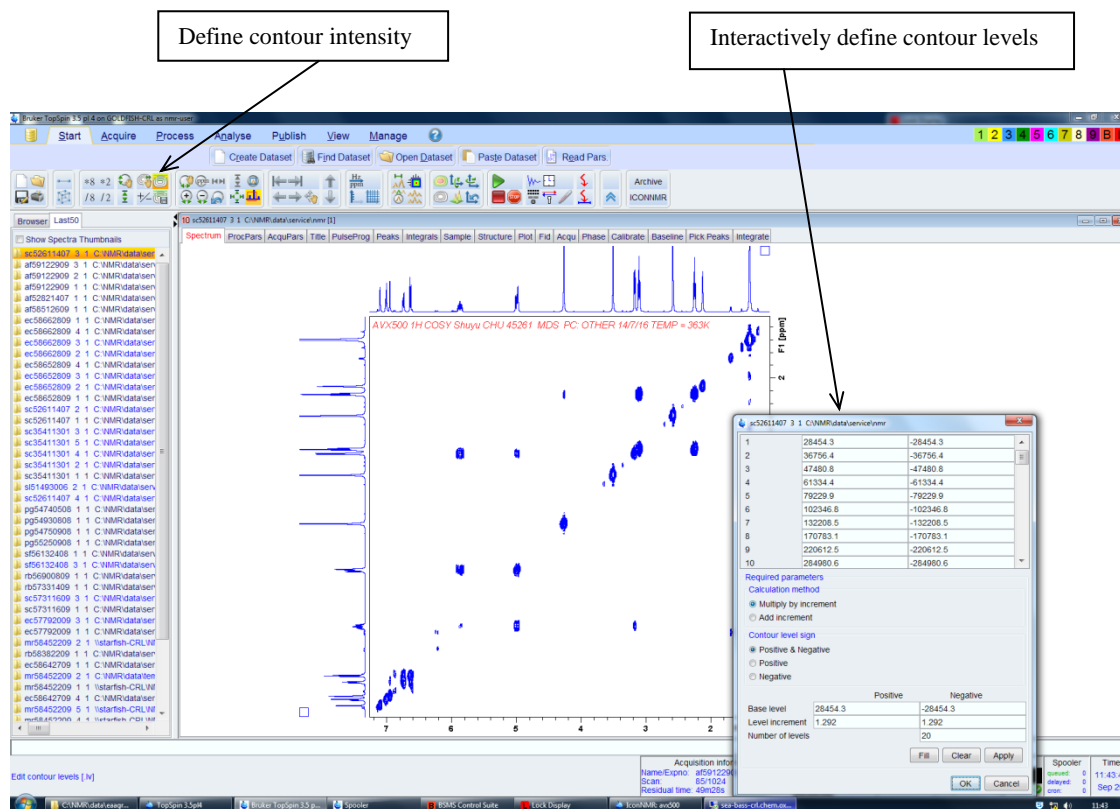
The number of contour levels may also be defined with the command **nlev** (number of levels; also in **edp**) and then use the command **levcalc** to set new contours.

2) ^1H-^{13}C (Edited) HSQC

HSQC provides one-bond heteronuclear correlations using proton observation. The Edited version (used by default) also includes multiplicity editing of cross peaks to distinguish CH_2 from CH/CH_3 groups and is therefore a more sensitive alternative to DEPT editing of ^{13}C 1D spectra.

Acquisition

It is preferable to use the edited-HSQC version that gives CH_2s phased oppositely to CHs and CH_3s, meaning the contours have opposite sign (colour) in the final spectrum. This experiment is therefore *phase sensitive* and must be phased corrected when processed to give the correct display.

Parameters to change for acquisition;

- **SW** (F2) spectrum width in ppm for ^1H

- **SW** (F1) spectrum width in ppm for ^{13}C (only for carbons with protons attached, typically 0-160 ppm without aldehydes present)

- **O1P** in ppm – middle of the ^1H spectrum

- **O2P** in ppm – middle of the ^{13}C spectrum

- **NS** number of scans (typically twice that needed for a good COSY)

- **CNST2** –this is the value for a standard $^1J_{CH}$ coupling constant and is set at 145 Hz by default - a compromise value for standard organic molecules. There is usually no need to alter it, unless you know that the compound has a critical $^1J_{CH}$ value that is very different from this default (see also the NMR experiment guide PDF)

Note that there is no need to perform *rga* on standard samples (<20mgs) as the default value normally meets most requirements. If your sample is particularly strong, *rga* ensures the receiver is not overloaded so the spectrum is then devoid of artefacts. Use *zg* to commence acquisition.

Processing

Again *xfb* can be used to monitor the 2D spectrum during and after acquisition. This is a phase-sensitive experiment and so will need phasing after initial data collection; use *edp* or the **ProcPars** tab to view parameters.

Phasing a 2-D spectrum

The easiest way is to perform automatic phasing with ***apk2d*** which is normally sufficient. If not, it is most likely that the phase correction will be required in the F2 (proton) dimension in edited-HSQC in which case the diamond shaped contour will have left and right halves of different colours (see below), whilst phase corrected crosspeaks should have one unified colour. Select the **Process** tab then the **Adjust Phase** icon from the tool bar.
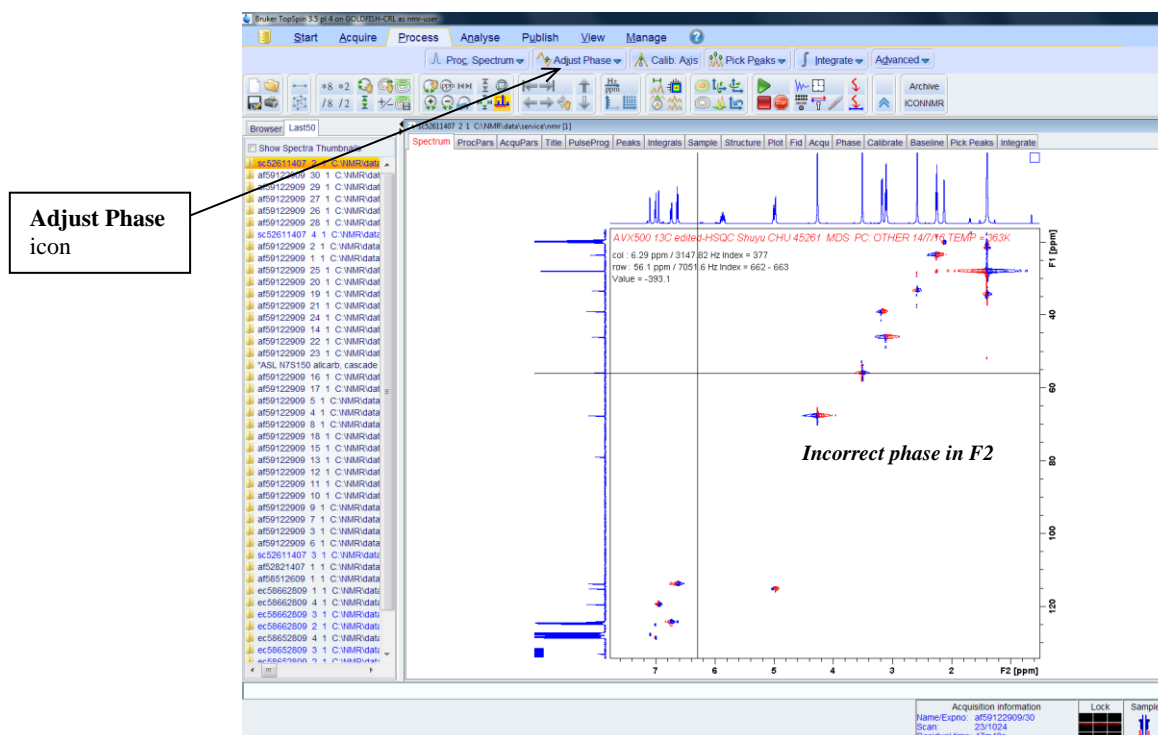

Right click on a crosspeak and select **Add** (see figure below), then repeat for another at a very different proton shift. Select the ***R*** icon to extract the rows and phase the peaks with the ***zero*** and ***first*** order phase corrections, as for 1D phasing. Save these corrections. If the contours require phase correction in F1, where the diamond shape contour have different colours for the top and bottom halves, repeat the process but select the ***C*** icon to extract

columns and repeat the phasing process. Note CH and CH$_3$ should have a different colours to the CH$_2$ peaks in the final HSQC spectrum.
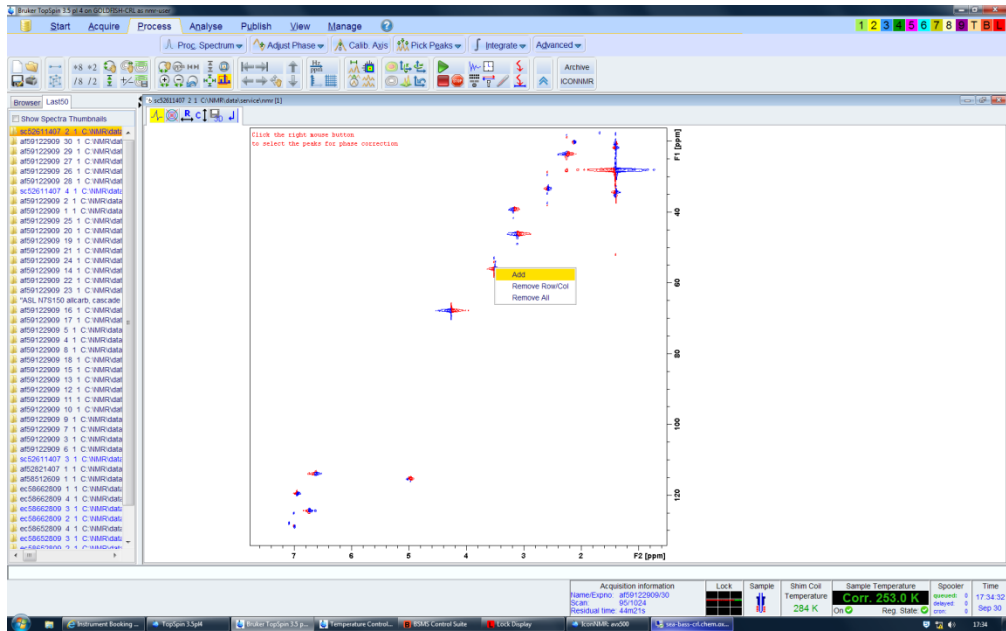
zero and *first* order phase correction: drag on each icon to correct phase; *Save and return* to transfer correction to 2D spectrum

Incorrectly phased rows

Correctly phased rows

Correctly phased 2D HSQC spectrum
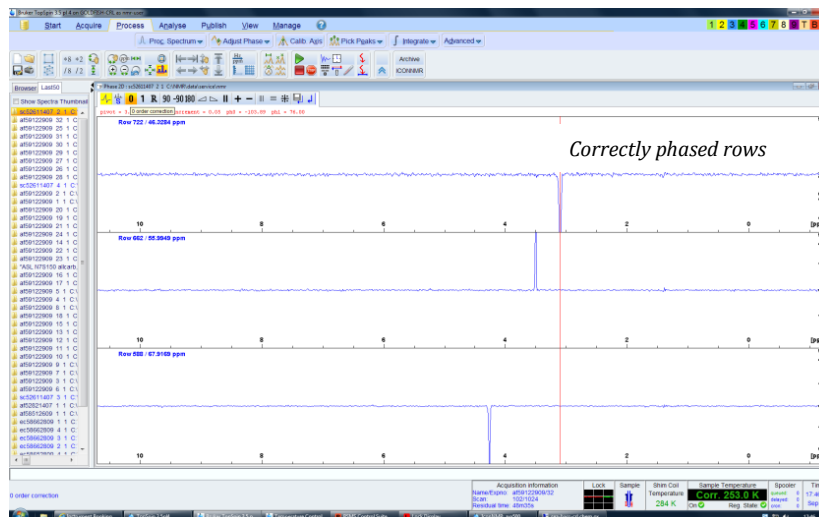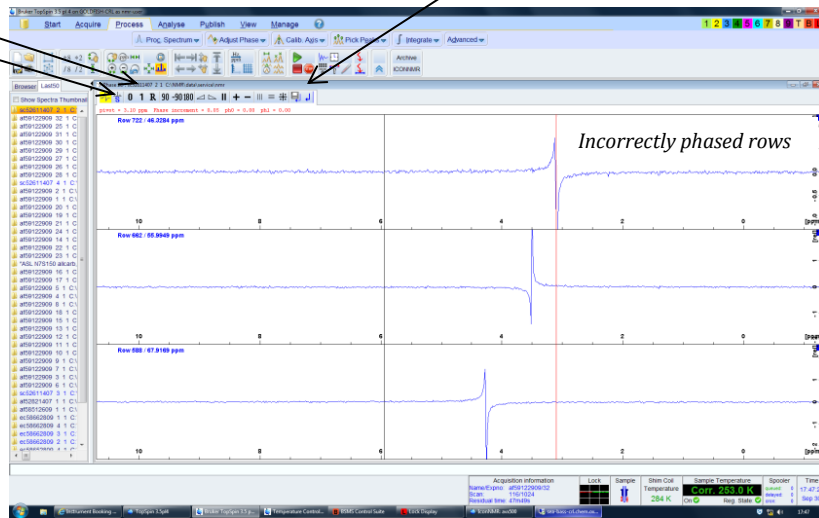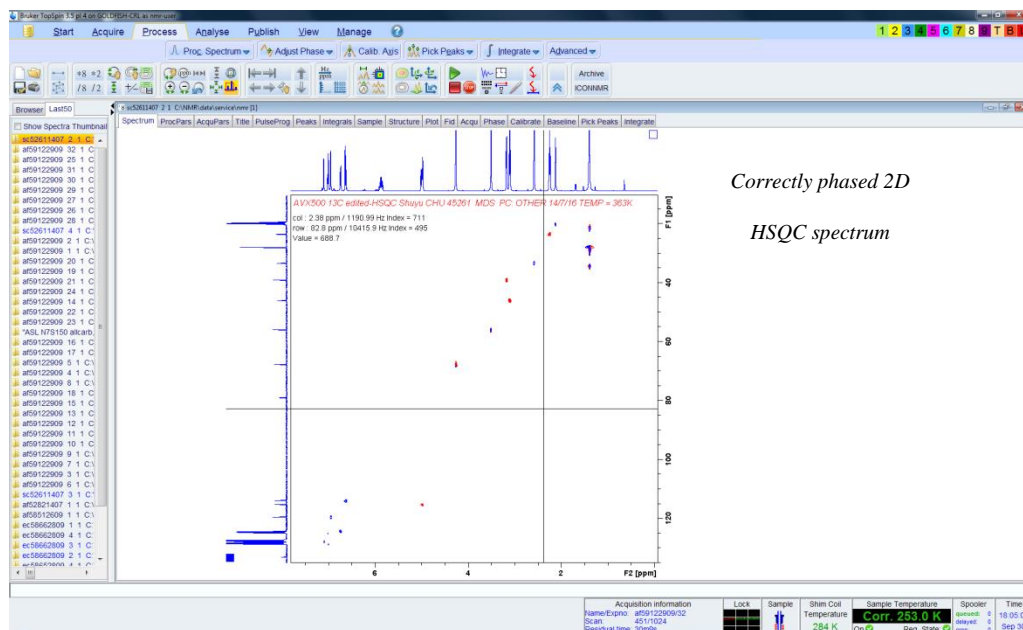
3) ^{1}H-^{13}C HMBC

HMBC provides long-range heteronuclear correlations with proton observation. Correlations are observed typically over 2 or 3 bonds (although weak correlations may sometimes be observed across 4 bonds in extended conjugated systems).

Acquisition

It is advisable to know where ^{13}C signals appear at either extremes of spectral width for HMBC since non-protonated carbons (such as acids, esters, amides and ketones) will also be included in this experiment.

Parameters to change for acquisition;

- **SW** (F2) spectrum width in ppm for ^{1}H

- **SW** (F1) spectrum width in ppm for ^{13}C (for **all** carbons in the molecule; typically 0-220 ppm)

- **O1P** in ppm – middle of the ^{1}H spectrum

- **O2P** in ppm – middle of the ^{13}C spectrum

- **NS** number of scans (typically four times that needed for adequate HSQC or eight times COSY)

- **CNST13** – this is the delay set for a $^2J_{CH}$ and $^3J_{CH}$ coupling constant is typically set at 8Hz, a compromise value for standard organic molecules. There is usually no need to alter this, unless you know that the compound has $^nJ_{CH}$ values are likely to be very low (< 4 Hz) or very high (> 12 Hz).

    Use *zg* to commence acquisition.

 Processing

Use *xfb* to process; this is not a phase sensitive experiment and does not require phasing, but may benefit from optimum setting of contour levels and threshold, as described above.
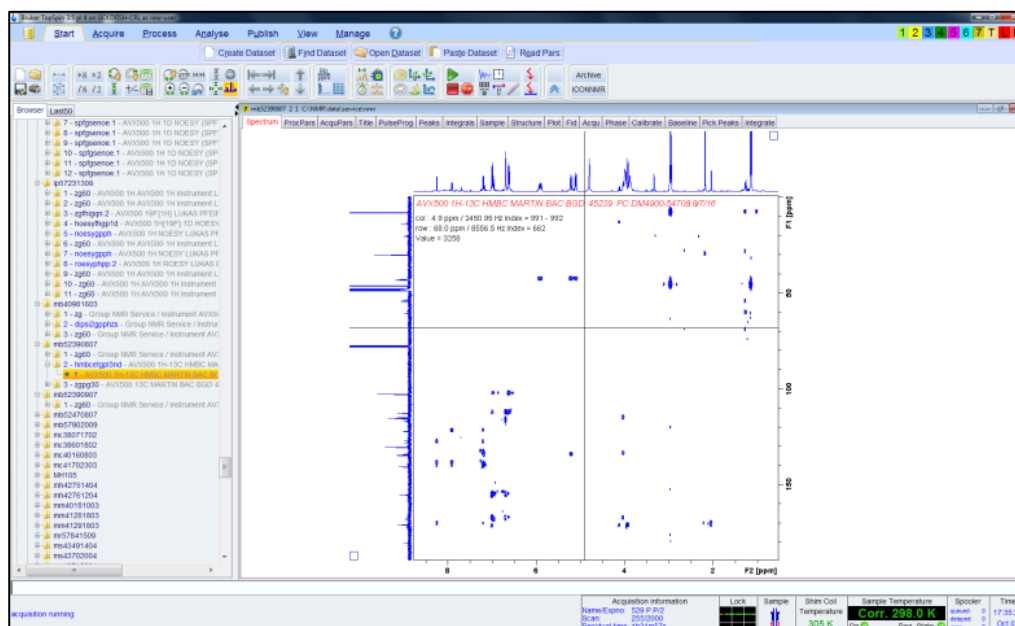
4) ^{1}H-^{1}H NOESY

NOESY establishes through space correlations between spatially close protons in a molecule and is the 2D NMR version of the (transient) NOE experiment. It is typically used for small (molecular weight < 600 Da; positive NOEs) and large (molecular weight >1500 Da; negative NOEs) molecules, but may not be applicable for intermediate sized molecules of molecular weight ~1000 Da, where the NOE has near zero intensity. Here ROESY is the method of choice (as the Tr-ROESY experiment); consult the NMR staff for advice.

Acquisition

This experiment is usually only performed at the end of an assignment process, after all other 2D experiments such as COSY, HSQC and possibly HMBC have been run and the proton spectrum properly assigned. The recorded ^{1}H spectrum should preferably have signals that are dispersed in chemical shift, if not, it may be preferable to dissolve sample in a different solvent before proceeding.

Compared to the other 2D techniques NOESY is a longer experiment, it often requires at least 8 or more transients and it is also very sensitive to shimming and instrument instabilities, especially temperature variation. Make sure you have shimmed the sample perfectly and the sample temperature is correctly regulated.

Parameters to change for acquisition;

- **SW** for F2 must be equal to SW for F1

- **O1P** in ppm – middle of the ^{1}H spectrum

- **NS** number of scans > 8 (usually multiples of 8 and similar to that needed for HMBC)

- **D8** the mixing time in which the NOE develops. Typically 0.8 s for small molecules (M_r < 400 Da), 0.6 s for mid-sized molecules ($400 < M_r < 800$ Da) or 0.3-0.4 s for large molecules (M_r 1500 Da or greater).

Use *zg* to commence acquisition.
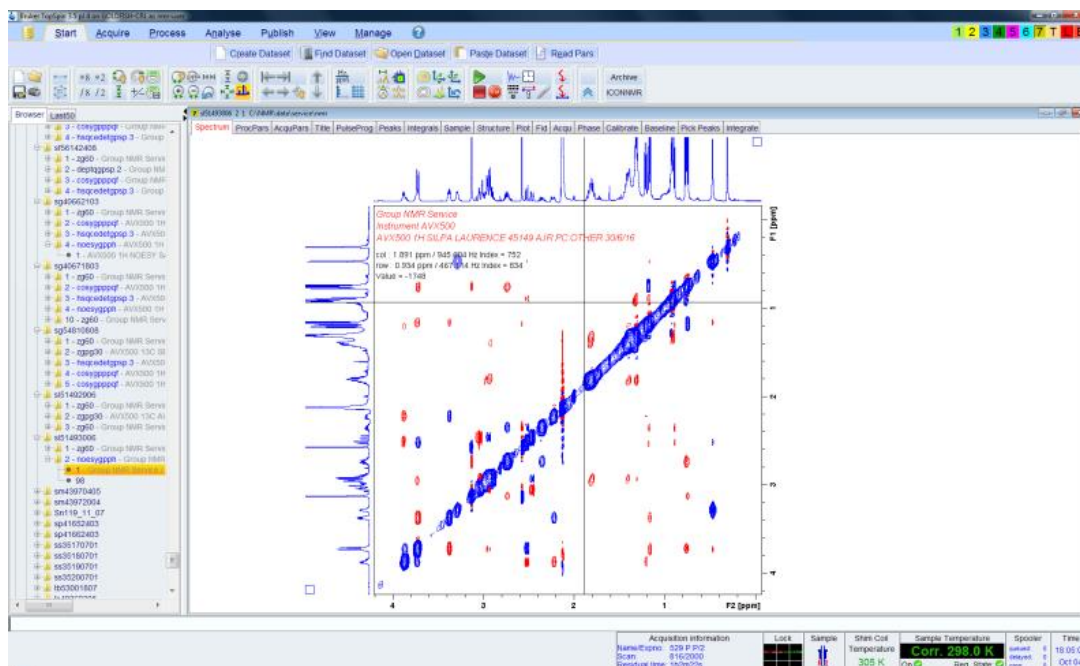
Processing

This is a phase sensitive experiment and will require phasing (you can try *apk2d* and/or manual phasing) in both F1 and F2 after processing with *xfb*, as described in the HSQC section. These spectra also benefit greatly from baseline correction in both dimensions: use *abs2* and *abs1* to apply automatic correction in F2 and F1 respectively.

NOE cross peaks (red cross peaks above) are of *opposite* sign to the diagonal peaks (in blue above) in small molecules whereas those arising from chemical exchange processes have the same sign as the diagonal (blue cross peaks above; here the exchange of rotamer cross peaks can be seen as well as exchange of the hydroxyl protons with water in toluene-d8). For large molecules NOE cross peaks have the *same* sign as the diagonal.

Please ask a member of the NMR staff if you need assistance in setting up or processing these experiments.