KJM 5280

VII

^{1}H-NMR metabolomics

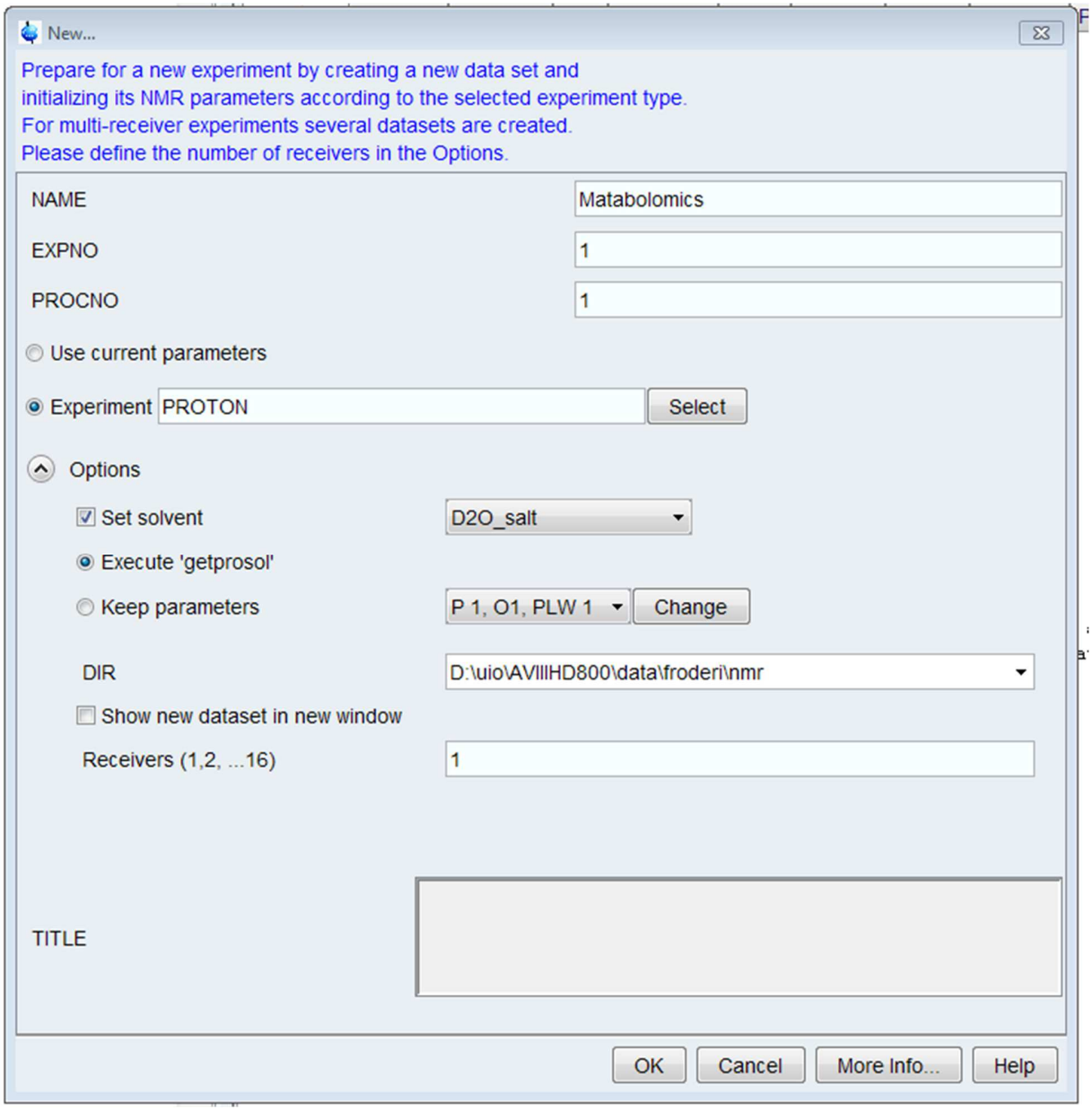
TopSpin 3.5

AVIIIHD800

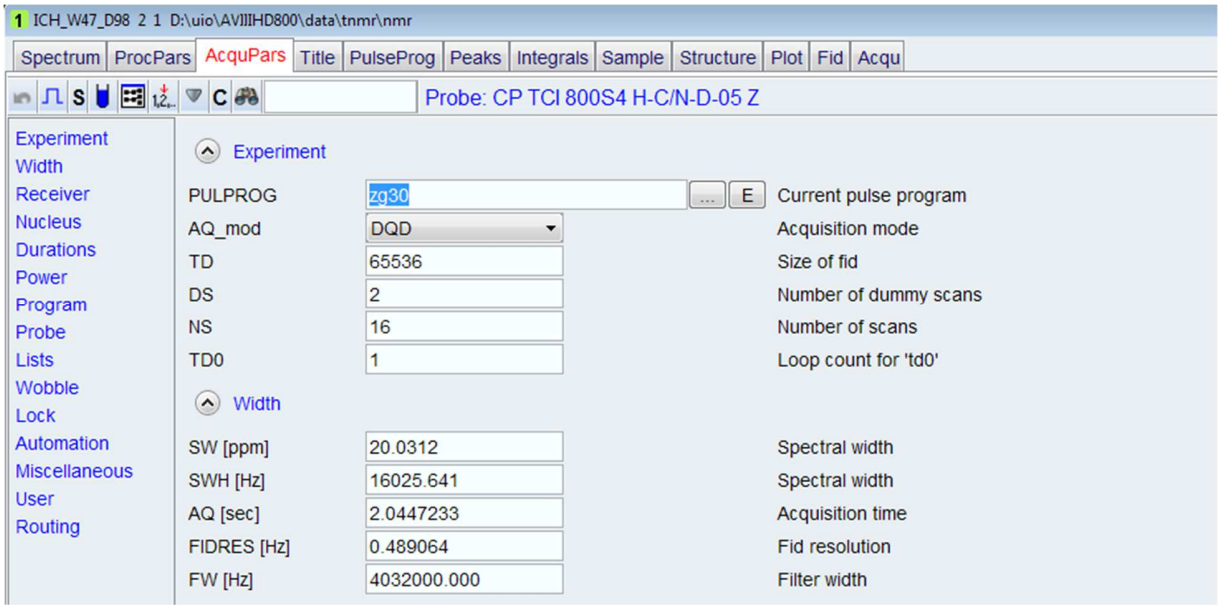
Version 1.0

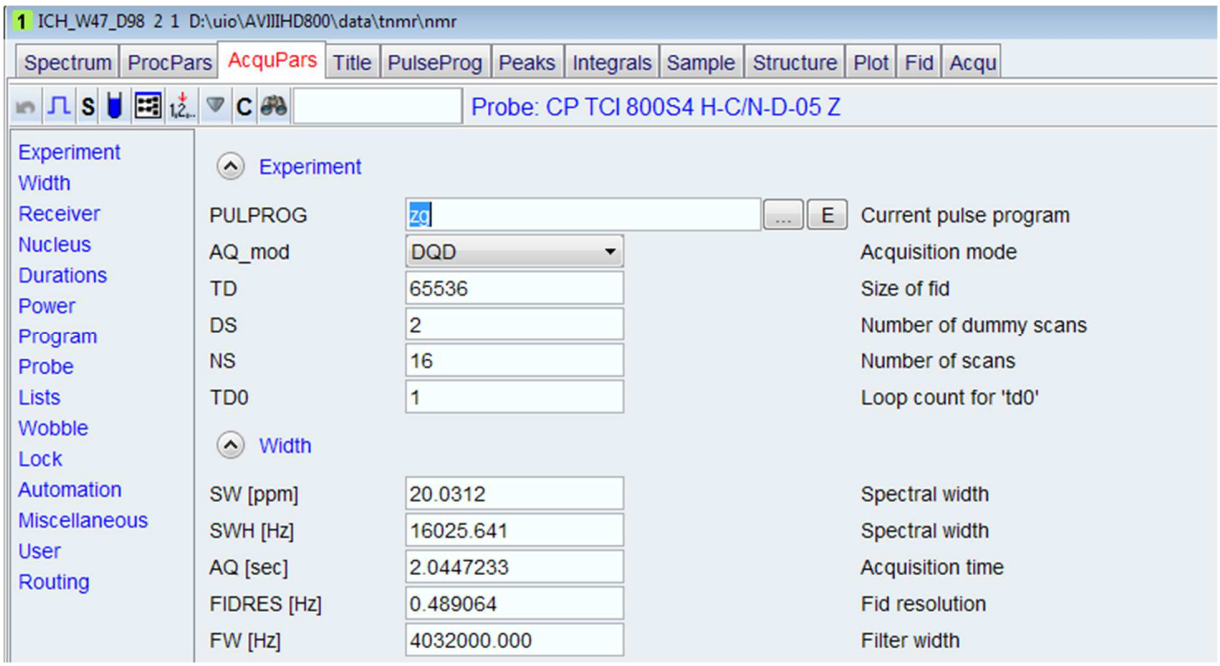
Crude and unfinished manual
May 2018

The goal of this document is to enable users to be able to obtain NMR–metabolomics data on the 800 MHz NMR instrument at the UiO NMR Center. Interpretation of spectra is not (yet) covered. Statistical treatment of the data is also not covered (yet). A database at Ohio State University, which can help you with identification of individual molecules in the metabolomics samples, is mentioned in this document, but the use is not (yet) covered. In order to understand the content of this document the reader need to know how to perform standard nmr-experiments on Bruker nmr-spectrometers.



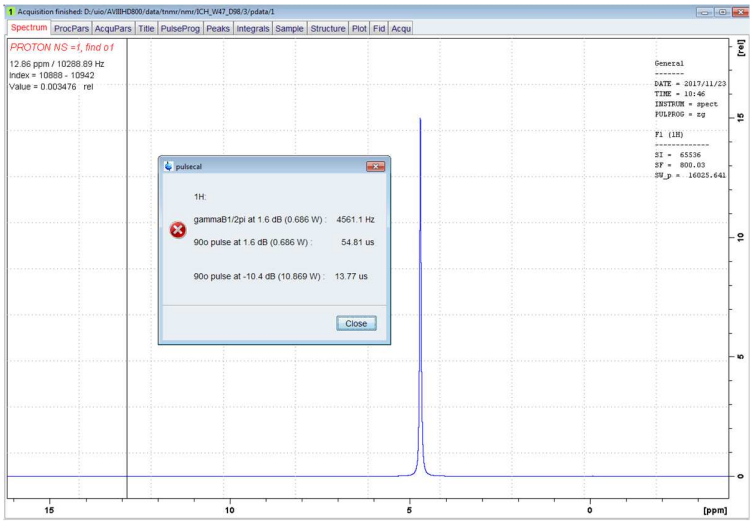
Run a regular proton experiment. Rpar PROTON all or do as above.





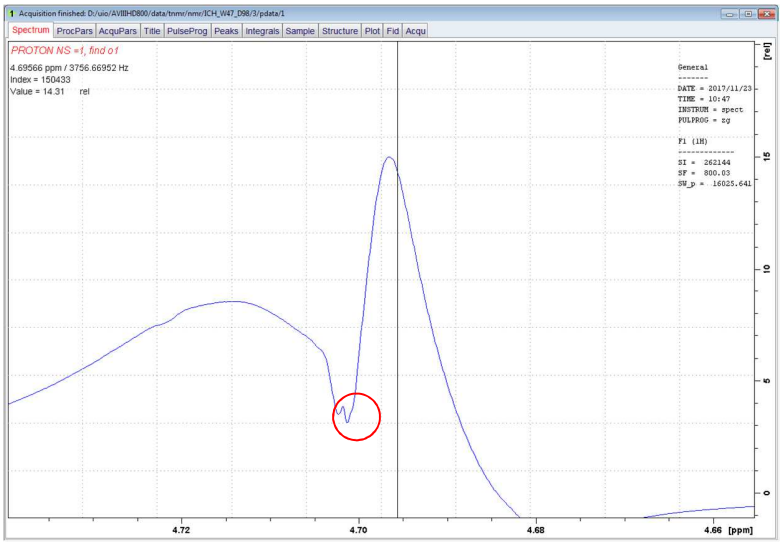
Change the pulse program from zg30 to zg and change ns to 1 and ds to 0.

Acquire the spectrum. Run pulsecal.

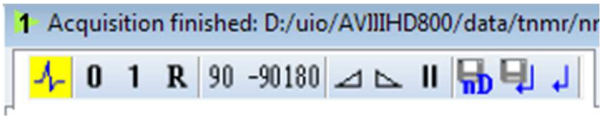


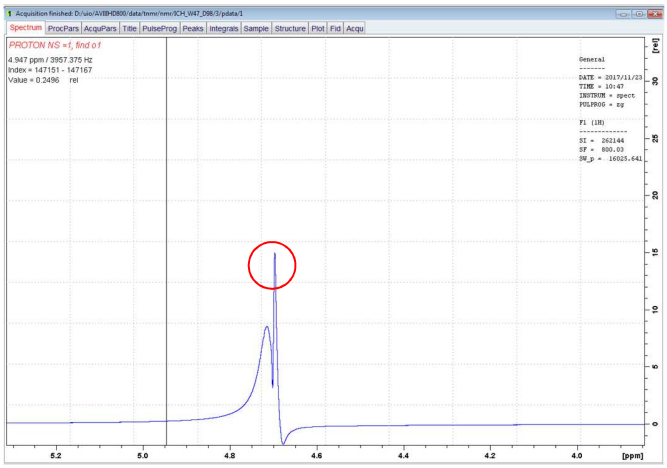
Multiply the new p1 with 4 and enter it by typing p1 and enter the 4 times larger number. (In the example here, 4 times 13.77).

Acquire the spectrum.



Go to Phasing and invert 180 degrees.



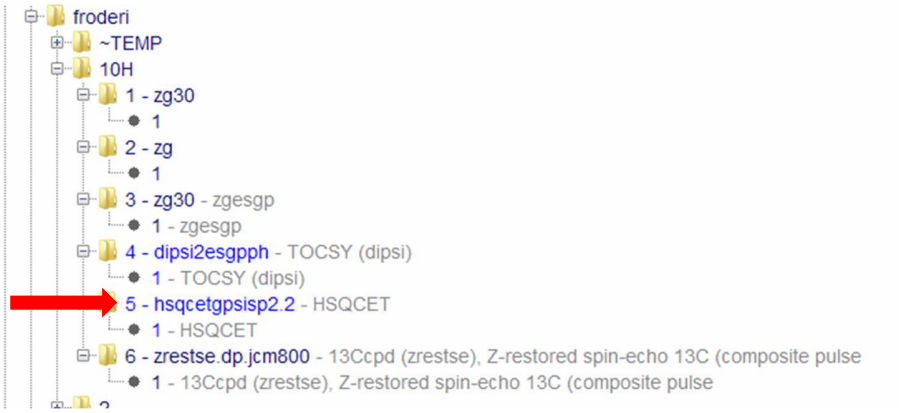


Zoom in to the sharp peak. That is the exact O1 value to be used in the metabolomics experiments. Write down this number (in Hz).

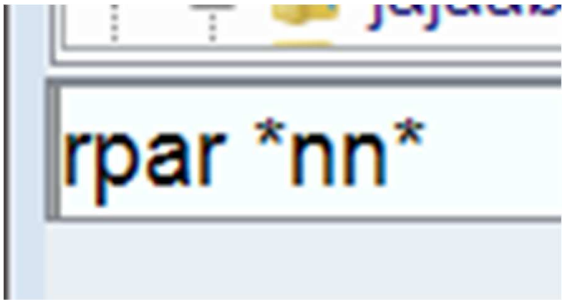
You will not get this result if lock is misadjusted, or the sample is badly shimmed, or if the temperature is not adjusted and has not reached stable temperature. **The sample must have been in the magnet for at least 15 minutes, preferably 20, before shimming and acquisition can start.** If a suitable solvent is available in the list of lock solvents (*e.g.* h2o_d2o_salt, then the standard o1 often works just as well as the one you find manually. A suitable lock solvent will need only small adjustments during tuning and matching. If it is far off, try a different lock solvent. In addition, you might be in more need of finding o1 yourself.

Finally, if the magnet is drifting a lot, you might have to refresh/find again the o1 value.

The three experiments which are routinely used at UiO are shown here, taken from the left pane of the TopSpin interface. The experiments are either manually run in TopSpin or in automation using ICONNMR.



For more extensive studies a $^{1}H^{13}C$ HSQC experiment is added. This 2D experiment is used in structural confirmation/elucidation of individual metabolites. As usual the main problem for newcomers is to figure out what rpar files to use. Nils Nyberg from Bruker has made some experiments that are well suited for NMR-metabolomics

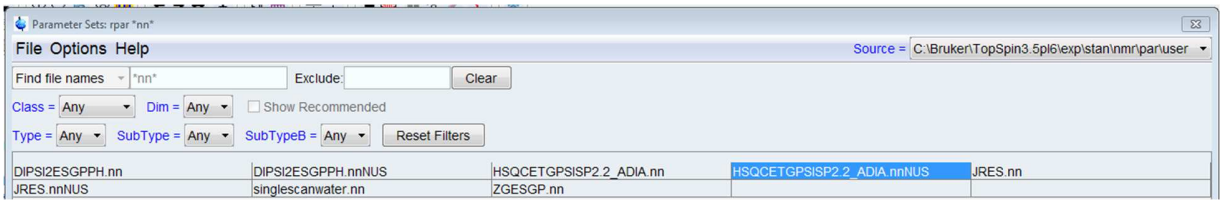


Rpar *nn* brings up all parameter files with nn or NN in the names, *i.e.* those made by Nils Nyberg.

Observe the line at the top right in the screenshot. \par\user is the location of the *nn* files.

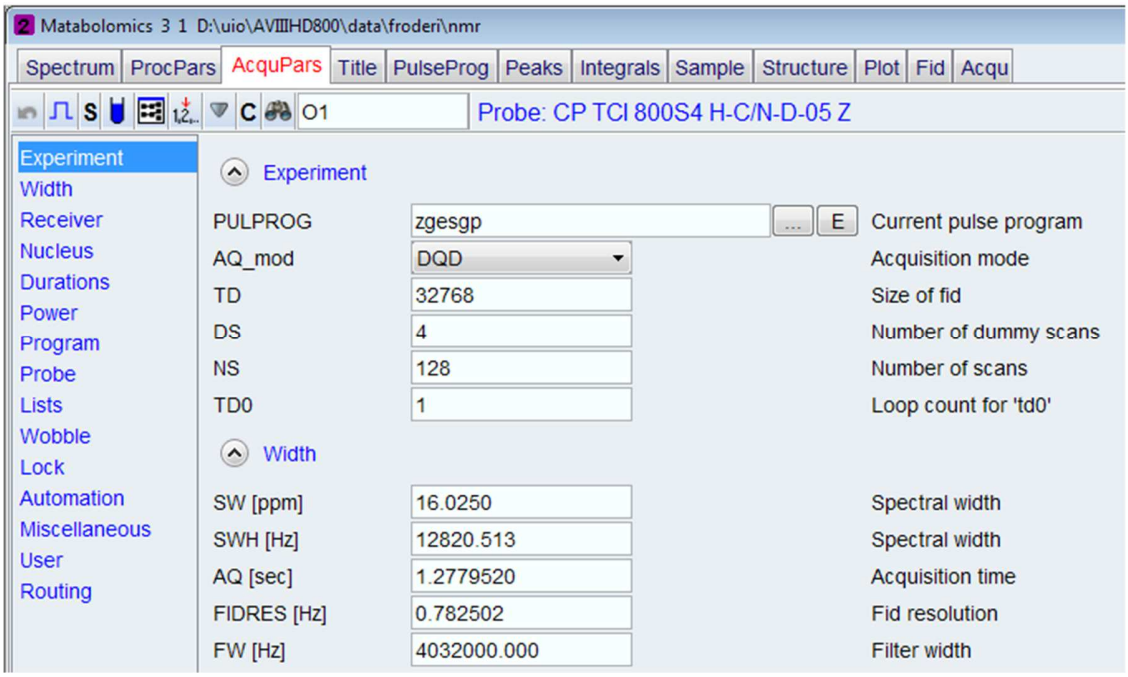
ZGESGP.nn is the proton experiment used for statistics.

DIPSI2ESGPPH.nnNUS is the TOCSY experiment we use. NUS stand for Non Uniform Sampling. The experiment time is cut to ¼ by using 25 % NUS. The computer will calculate the missing FIDs based on how the acquired FIDs "look" when the spectrum is Fourier transformed. The Fourier transformation can take up to 15 minutes. The DIPSI/TOCSY experiment is used online with a database at Ohio State University when the investigator needs to check or determine the molecular identity behind NMR resonances or peaks. A direct link to the 2D page is here: (http://spin.ccic.ohio-state.edu/index.php/toccata2/index). A useful manual is found here: (http://spin.ccic.ohio-state.edu/database/toccata_protocol.pdf). Please observe that you need a Linux computer or a dual boot computer to use this database, if you want to upload the files they ask for. The programs for making these files work best on Linux. Otherwise you have to manually read the peaks from TopSpin or MNova and write it into the search bar on the webpage.

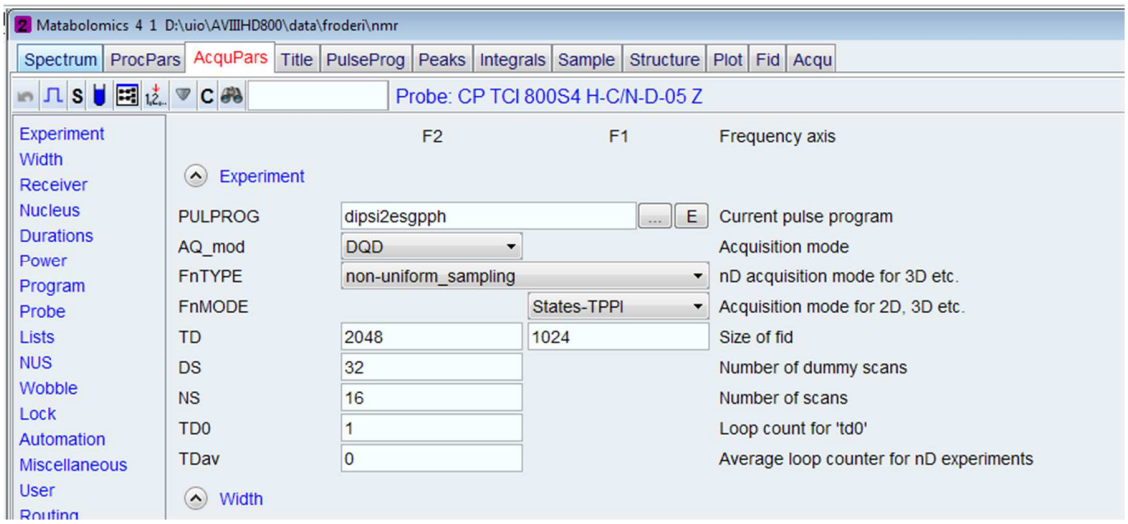


HSQCETGPSISP2.2_ADIA.nnNUS is the HSQC experiment used in NMR-metabolomics at the UiO NMR center.

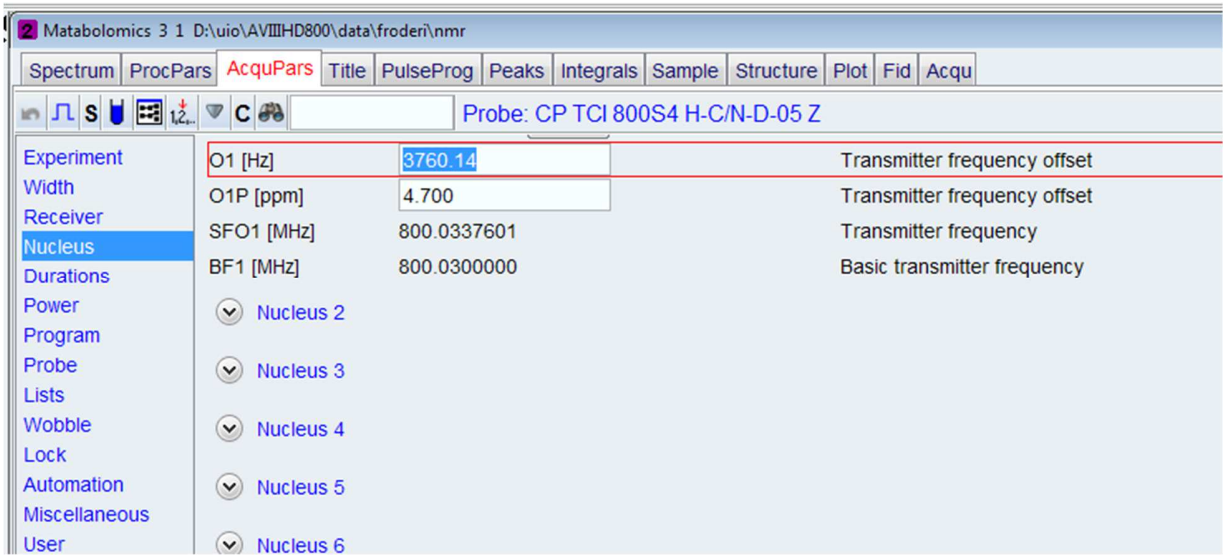
Please note that you might only see the pulse programs in the left pane of TopSpin and not the complete parameter names.

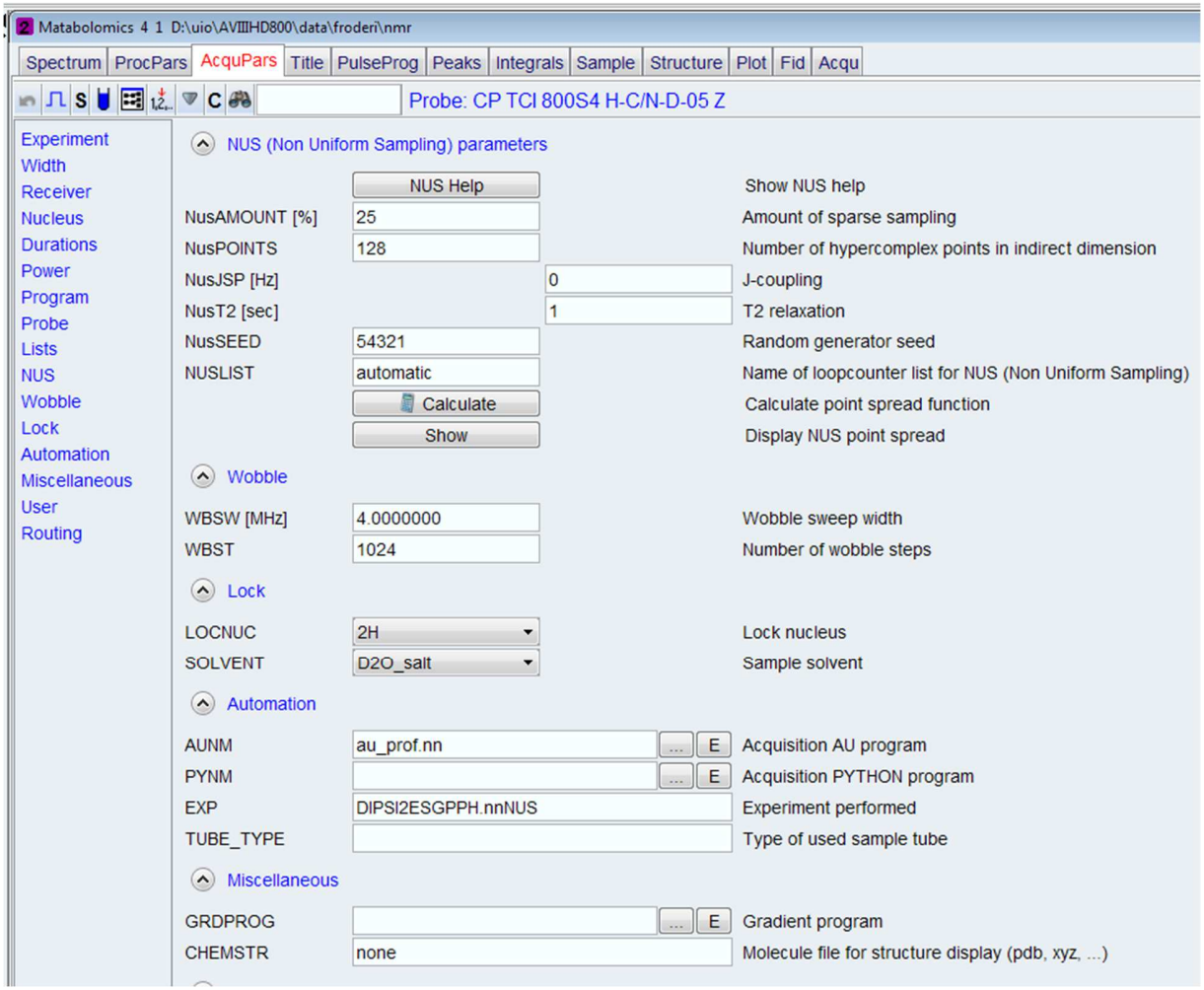


The top of the eda or AcuPars section of a zgesgp experiment.



The top of the eda or AcuPars section of a DIPSI2ESGPPH.nnNUS parameter set containing the dispsi2esgpph pulse program.





Examples of spectra will follow:

The OHIO database states that DSS must be used as an internal calibrant, but from experience of the authors of this manual, TSP works fine as well. However, it is ***strongly*** recommended to confirm the identity of the suggested molecule with 1D spectra and J resolved spectra. In other words, does the suggested molecule have the coupling shown in the J resolved spectra and do 1D databases agree with the 2D Ohio database?