KJM 5300

III

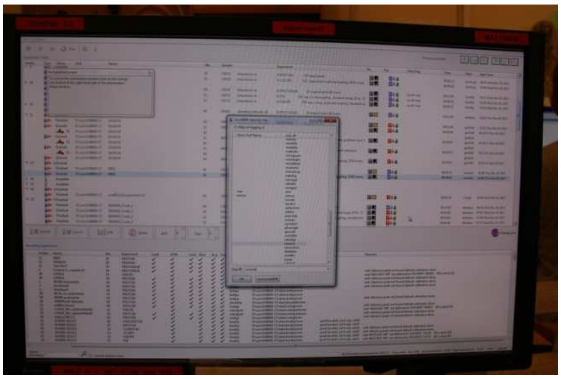
ICONNMR-BACS automation nmr

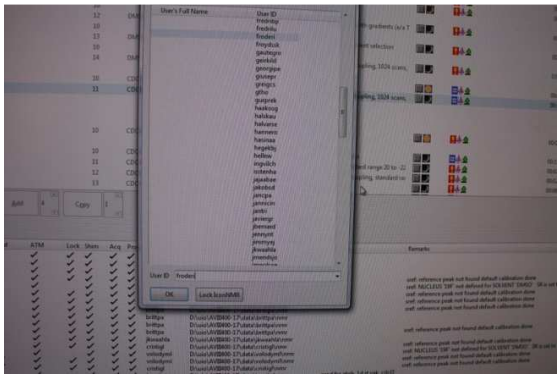
Version 1.0

Topspin 3.2 on AVIIIHD400 and AVII400

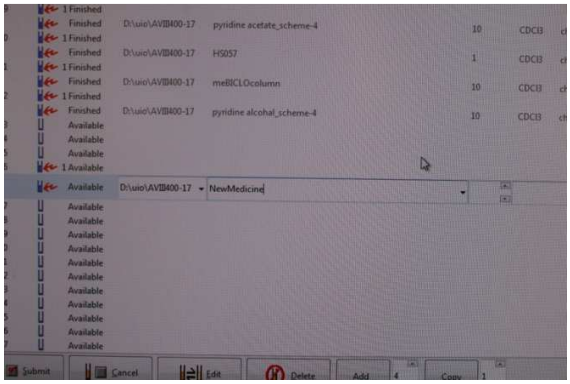
Windows 7/Windows Vista

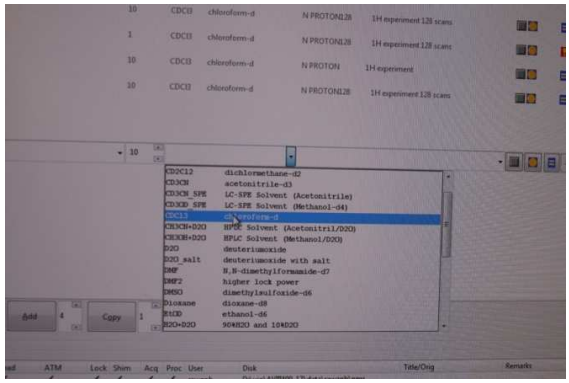
The combination of the BACS sample changer and the ICONNMR program that is running TopSpin for you greatly simplifies nmr acquisition, but reduces the freedom to fine tune the parameter selection. Before using the ICONNMR program you must define your password at a time when the PCs are not running experiments and your account must be activated inside the ICONNMR program by someone with administrator rights.



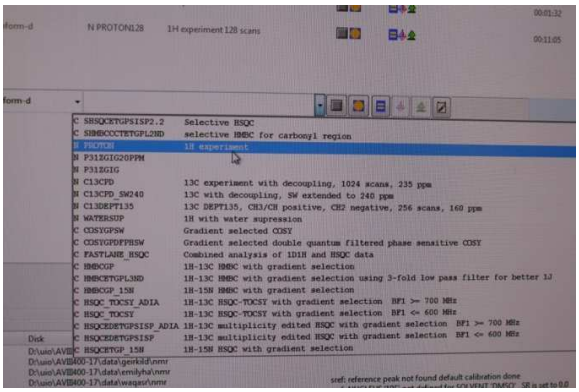


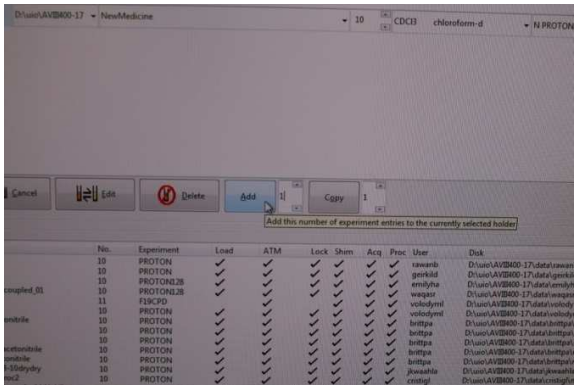
When this is done and the ICONNMR program is started by the user “tnmr” you will find your username in the list of users. Click on you name and enter your password in the pop up window.



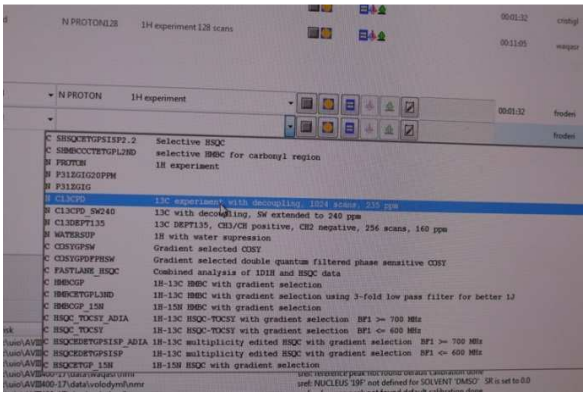


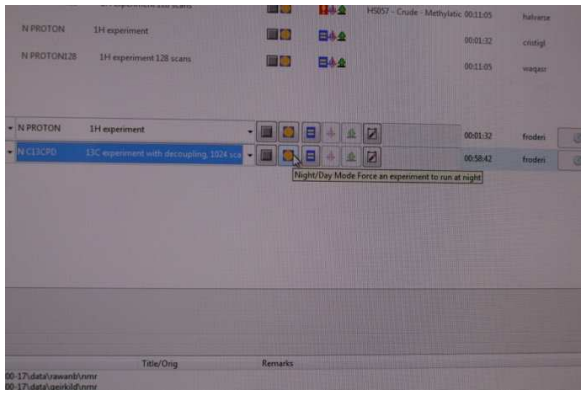
Then you look for an empty position in the BACS sample changer as close as possible after the last ran sample and add you sample with a correctly positioned holder (use the adjustment tool). The holder position must also be unused in the program of course. When your experiments are done you must come back to the lab ASAP to remove your sample and also delete your entry/entries in the ICONNMR program. When you have placed your sample in the BACS you can define your experiment(s) in ICONNMR. If you do it the other way e. g. you define your experiments first and the instrument/program is idle the program might decide to activate the changing arm to grab the sample that is not there – and the program crashes completely. Click on the empty line in ICONNMR. Start filling in information from the left to the right. First you fill in the name of the molecule/sample. Then you select the solvent.





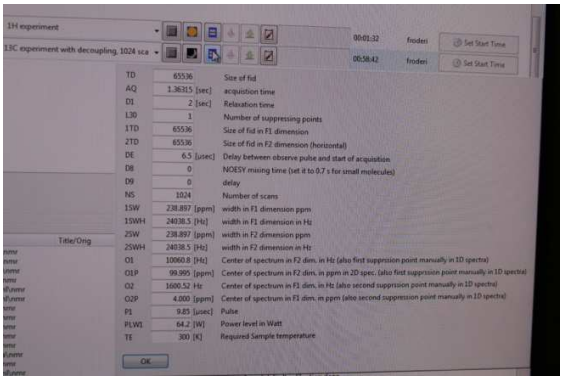
Followed by selecting the kind of experiment(s) you need. ALWAYS select PROTON first, even if you think you do not need it. For 99 % of the two dimensional nmr experiments obtained in ICONNMR the program needs a PROTON experiment to look at. If it is missing the 2D nmr experiment will fail completely and most likely crash the program. Then you click on the Add button.

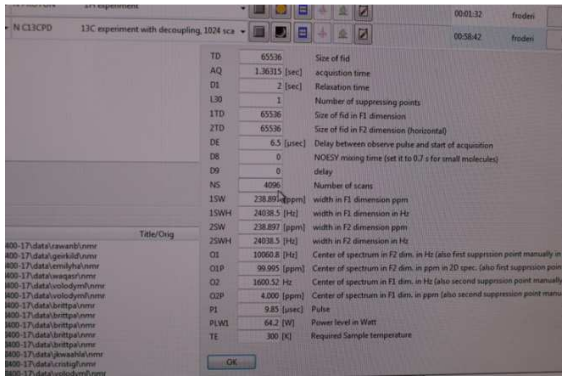




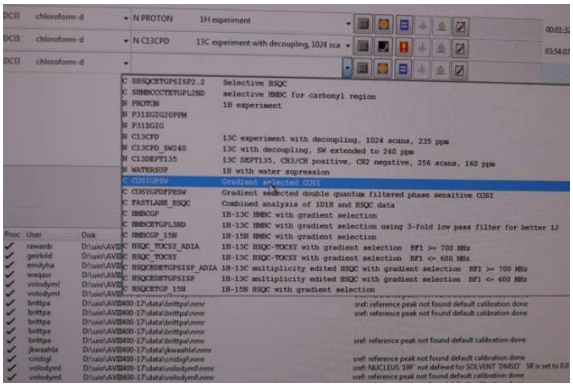
The next standard experiment to choose one either of the two 400 MHz instruments is the Carbon experiment C13CPD. Be aware that you must change the number of scans to 512 if you want to run this experiment on day time on AVIIIHD400 since this instrument is dedicated to fast experiments during day time. If selecting 1024 or higher for ns change to night time as shown above on this instrument. On the AVII400 instrument all experiments are running continuously so we do not distinguish between day and night experiments there. If you use ICONNMR on the AVI600 or AVII600 instruments please do not select C13CPD but use C13RESPECT instead. C13CPD gives an ugly rolling baseline in the spectrum on

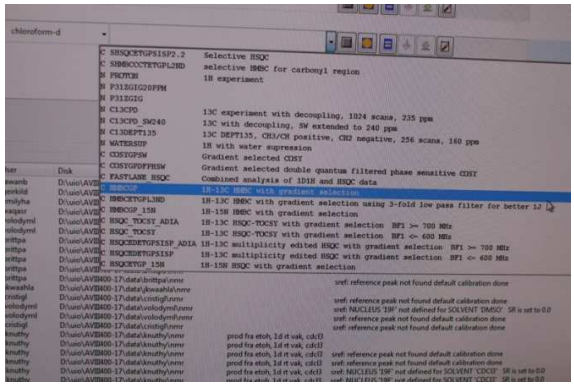
the 600s since the solid state ^{13}C atoms in a polymer in an insulating material between the sample held at ~300 Kelvin/ca 25 ^{o}C and the radio sender/receiver at 15 Kelvin give a very broad ^{13}C nmr signal. This signal is removed in C13REPECT.



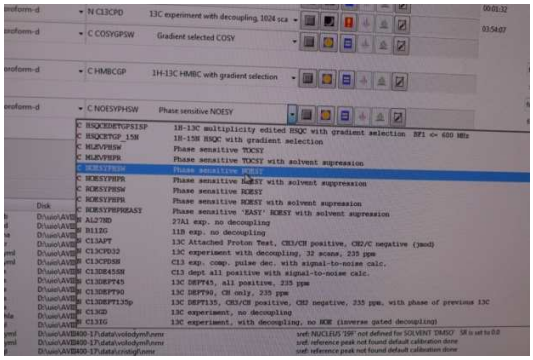


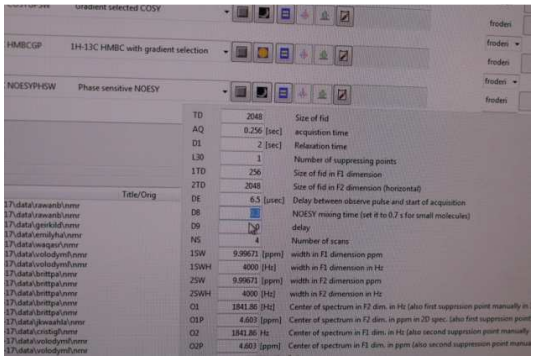
If your sample has low Molarity you need lots and lots of scans to get a decent 13C nmr signal. The button to the right of the day/night button brings up the list of parameters you are allowed to change. When you become experienced you might miss some parameters here. Ask and they will be added. Change for instance NS from 1024 to 4096 as shown above here. Click on OK at the bottom of the pop up window.



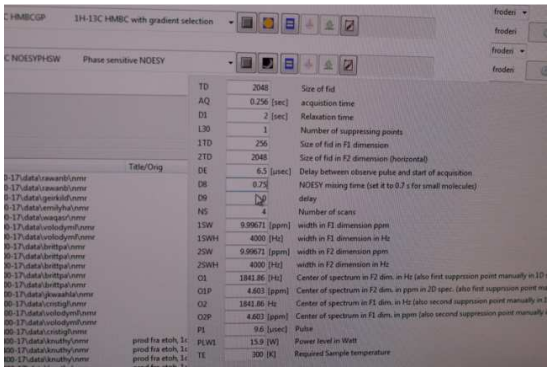


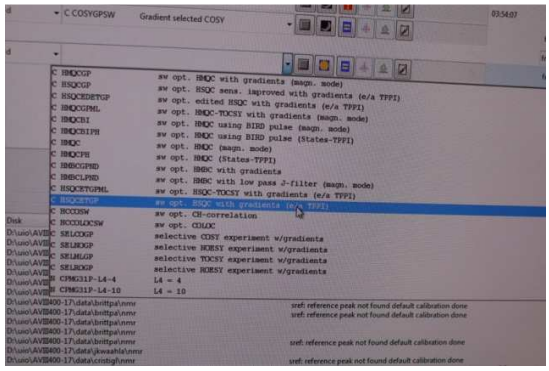
A series of two dimensional experiments and other experiments will now be selected. After clicking the ADD button (see earlier in this manual) you might select COSYGPSW. COSY shows you correlations between hydrogen atoms 2 to 4 bonds away. When this manual was made hastily the author chose to add HMBCGP, logically this is normally added last in an experiments setup, but since it is shown in the pictures it is commented here. HMBC experiments is connecting ^{1}Hs with ^{13}Cs atoms 2, 3 and 4 bonds and sometimes even 5 bonds away . The two letters GP seen here means (magnetic) gradient pulse – this is an invention that greatly speeds up your experiments when you have high Molarity samples. In old fashion 2D nmr so called phase cycling is used to suppress unwanted peaks. Often 4, 8, 16 or 32 numbers of scans had to be used for each increment in the indirect dimension even though one scan had been enough for fat samples. With the GP invention made and patented by the company Nicolet in Madison WI - 2D nmr became much faster for high Molarity samples since NS can be 1 in such cases.



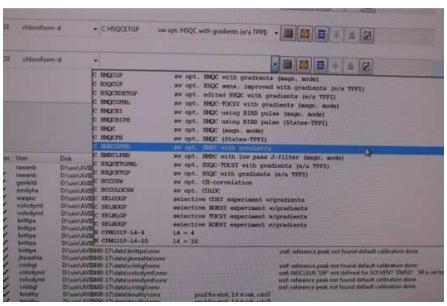


The next experiment selected here is the NOESY experiment. This experiment correlates ^{1}Hs that are close in space. Usually this experiment is used by Biochemists and one important parameter the so called mixing time is set to a default value that is suitable for large MW molecules. The D8 parameter must be changed to a higher number than what the program selects for you in order get meaningful results. Ideally you should have done a so called T1 inversion recovery experiment and found relaxation times for the hydrogens in your molecule and then used a formula to calculate D8. This is beyond the scope of KJM 5300. For small molecules MW 300-700 you simply change D8 to 0.75 seconds and hope for the best. One other problem with NOESY for small molecules is that you might have both COSY and NOESY correlations between the same hydrogens at the same time. The COSY correlations might infest or pollute your NOESY spectra. How to distinguish them is not explained here.

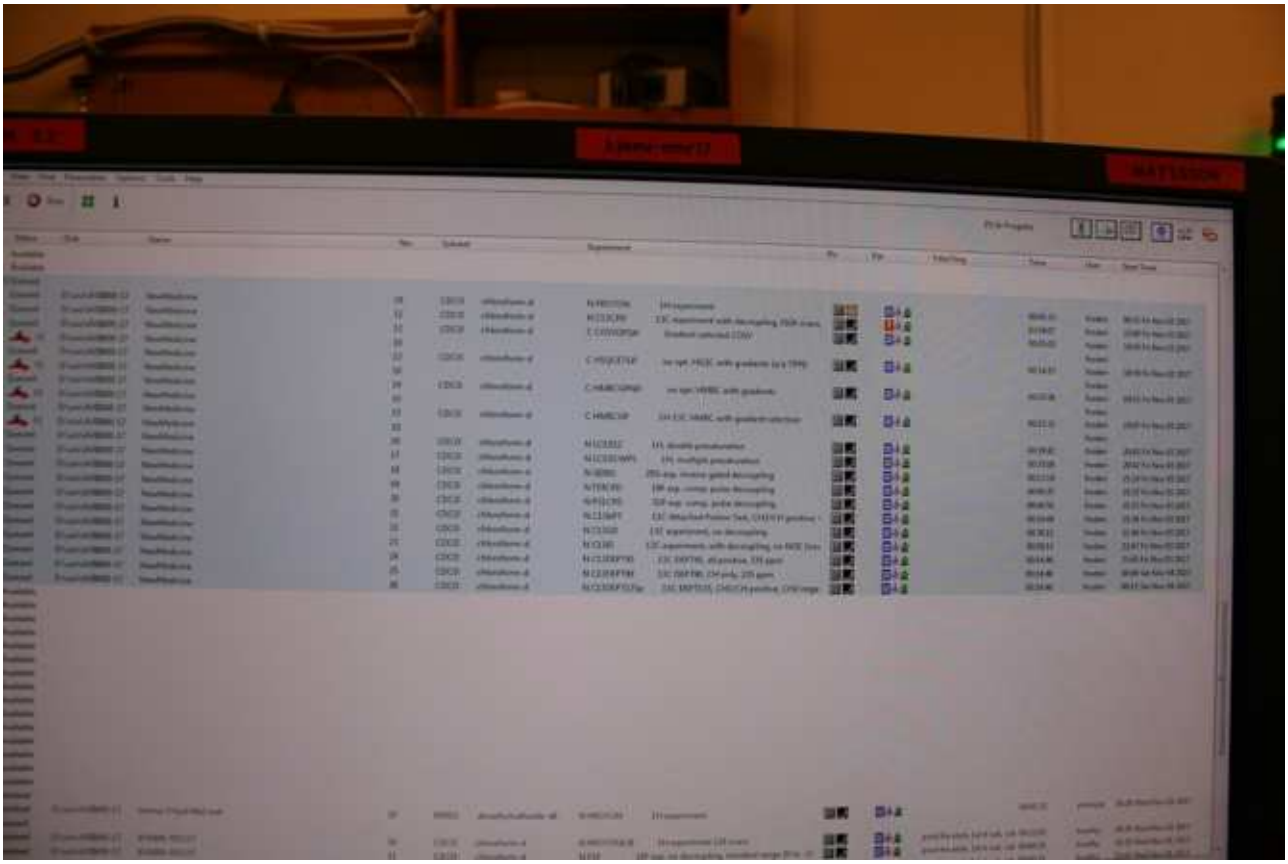




After adding a new experiment line HSQCETGP is selected here. This experiment gives correlation peaks between all pairs of ^{1}H and ^{13}C that are directly correlated (one bond).

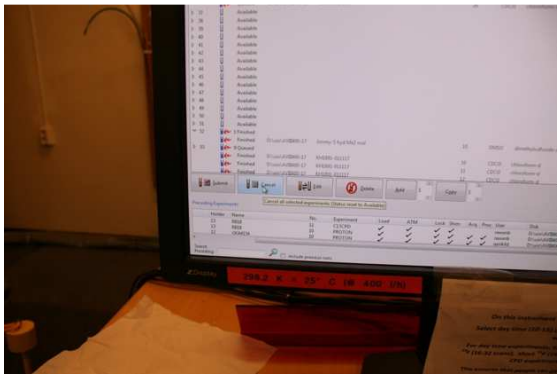


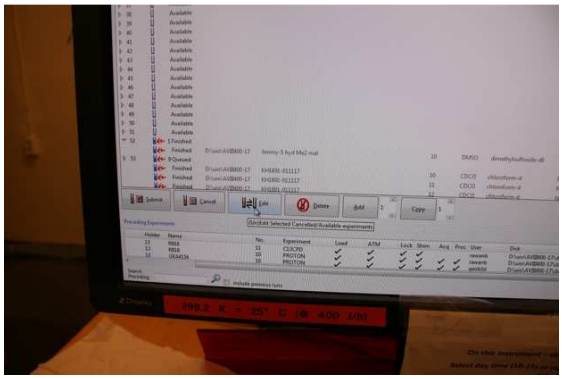
You might also choose to compare the results from HMBCGP with HMBCGPND (ND – no decoupling).



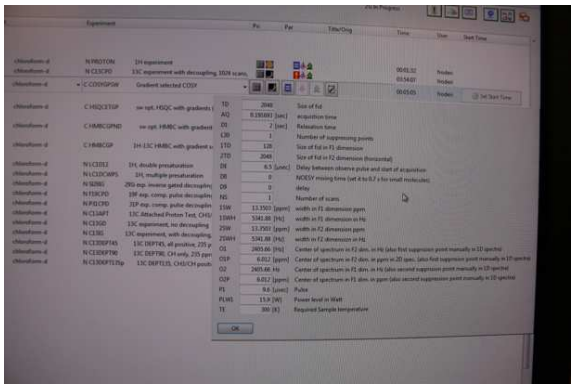
An extensive list of experiments is shown here. In a real scientific setting we select the experiments needed and not a lot of unnecessary experiments. To get the PC to actually add your experiments in the queue you must mark the experiments as shown above with the help of the mouse. Then you click on the Submit button.

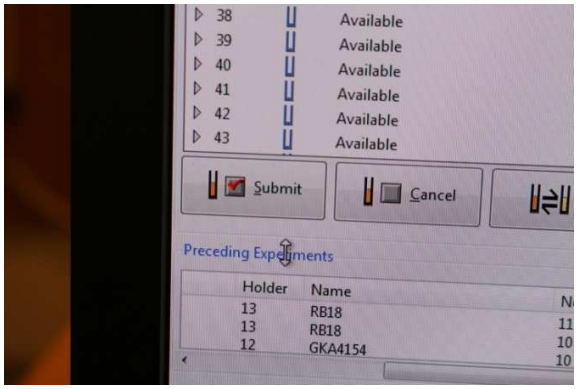
The 29Si, 19FCPD, 31PCPD experiments are hopefully self-explanatory. LC1D12 is a ^{1}H experiment with 2 solvent suppression points, the parameter L30 can be set to either 1 or 2 solvent points to be suppressed. The program is finding the largest peak(s) and kills it/them. LC1DCWPS is a program that suppresses multiple peaks. Change the parameter L30 to the number of peaks to be killed. C13APT sorts CH/ CH_3 (positive) from C /CH_2 (negative). C13GD is a 1D ^{13}C experiment with no 1H decoupling, it takes time to run since the intensity of the signals seen in C13CPD experiments are split in many peaks. C13IG is a ^{13}C experiment with no nuclear Overhauser enhancements, but with decoupling during acquisition to give singlets and not multiple signals. C13DEPT90 give CH carbons only. C13DEP45 give you all Cs except carbons with no protons on them. C13 DEPT135 is giving you CH_3 and CH as positive peaks and CH_2 negative.



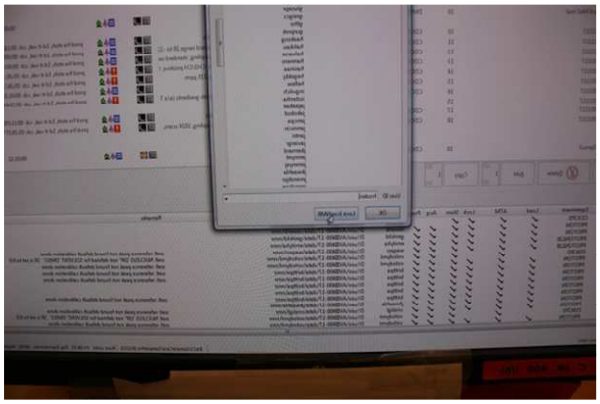


If you at some point you might realize that a parameter is in need of change, mark the corresponding line with the mouse that contains the experiment and click on Cancel and then on Edit.

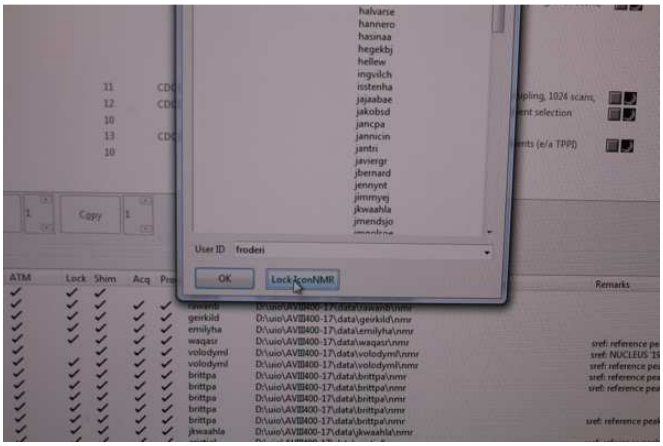




Then you do the necessary changes in the pop up window. Click OK. Be sure the line is blue marked. and click Submit.



Be aware that this experiment then will be the last experiment to be run. You cannot change anything in an experiment that is needed for another experiment later in the list. The experiment(s) that need the information from the one you changed will fail since the needed experiment will run later and after three failures the program will crash. If unsure cancel all experiments and submit the whole list again.



Bring up the Change user window (button located far to the right in the IconNmr window and click on Lock IconNMR. If you forget this and another distracted user arrives shortly after you left the instrument the screen is still open and that absent minded user might add her/his experiments with your identity.

Wait for e-mails telling you that your experiments are finished. See another manual for how to obtain your data for processing.