

Operation of Bruker AV-III HD “Florence” with Prodigy cryoprobe and SampleXpress sample changer

Safety

Our lab’s common safety policies apply to this instrument.

The magnet’s fringe field is small (the 5 G line is within the circle marked by the magnet legs) but still present. Don’t let the swivel chairs roll close to the magnet.

Be careful around the stainless steel transfer line from the liquid nitrogen dewar to the cryoprobe, particularly when positioning the ladder near the magnet.



1 Front panel of the Prodigy cryocontroller

The Prodigy cryoprobe should only be used when it is cold and operating stably. The probe controller will show a steady blue light below the label “COLD” during normal operation. Do not submit any samples if any other lights (such as the error light) are on, or if the blue light is blinking.

The cryoprobe has an absolute lower limit for sample depth of 20 mm on the depth gauge, to avoid scratching the probe inside and lowering the resolution. The sample depth gauge is glued down at a safe height setting—do not attempt to move it. However, if your sample’s liquid column is short, move the tube up in the spinner

turbine to keep it centered around the probe center line marked on the depth gauge. It is safe to have the bottom of the tube higher than 20 mm as long as the top of the tube is not too high. The SampleXpress sample changer has an absolute upper limit for the top of the sample shown by a yellow line. Your tube must be short enough to go completely under this line—if it doesn’t, don’t use it, because the sample changer will break it. You cannot push the tube down to go under the yellow line because of the lower limit of the sample position in the probe. Do not use overly full, thick wall, or other unusually heavy types of sample tubes. Shigemi tubes give excellent results, but you must use a new Bruker style Shigemi tube that has no more than 8 mm of susceptibility matched glass in the bottom of the tube and a beveled lower edge. Varian style tubes have 14 mm of glass in the bottom, and cannot be used.

Sample preparation

You can successfully use a shorter liquid column in Bruker probes than in the Varian probes. A sample that is 3.5-4 cm long is satisfactory. Adding more solvent just dilutes your sample and makes the measurement time longer. Carefully remove fingerprints or other smudges from the outside of the lower part of the tube. The cryoprobe is so sensitive that it will show a “grease” signal from a fingerprint. Handle the spinners only with a Kimwipe. Dirty spinners gunk up the air bearing surface, which is quite difficult to clean on these instruments.

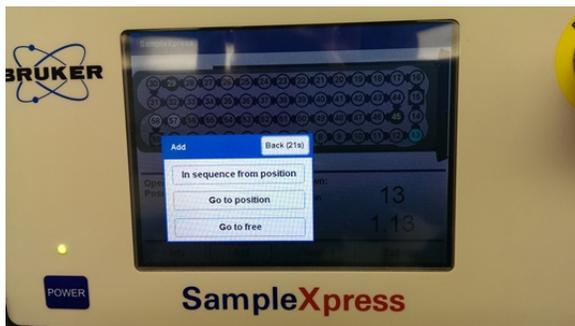
The ^{13}C speed is about 5 times that of indy or daytona for samples run the same way. The same will hold for other “X” nuclei like ^{31}P and ^{11}B . Florence does not do ^{19}F NMR. The instrument’s biggest impact will be with experiments that normally take a long time, or very small amounts of sample. A straightforward proton spectrum gets virtually no benefit from the cryoprobe, since most of the time is spent locking and shimming, rather than acquiring data.

Current schedule policy: day queue 9am-9pm. 40 minute limit per experiment, 2.5 hours per person. Night queue from 9pm-9am on weekdays. You must choose the night queue at the time of submission in order to bypass the day queue time limits. The instrument software operates on a first, first serve basis at all times, with the day queue basically being highest priority and night queue being lower priority. The software will accept an unlimited amount of submissions and in principle the night queue could be filled several days ahead. However we don’t want that to happen so we use a

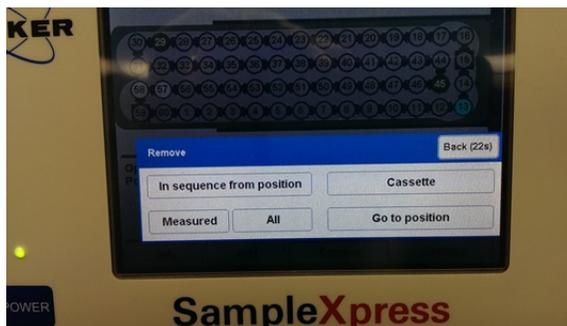
webcal reservation page to limit the night queue submissions to no more than 12 hours. Putting your name on the webcal page for the night queue does not guarantee that the time will actually be available if you submit your sample late in the day. Submit as early as possible even if it means you submit an empty tube and later replace it with the real one.

Sample entry

Put your samples into the sample changer first, then set up the experiments to be performed in the software. The SampleXpress robot has a touch screen control panel and software which is separate from the NMR workstation. The robot tracks each sample insertion and removal, so the display on the screen is kept up to date. To enter just one sample, you can just put it in the holder in the front right corner if that holder is empty. The SampleXpress will register its presence. You do not need to do anything else if you have only one sample. If that position is taken, or you have multiple samples, press Add and follow one of the options.



Add sample options

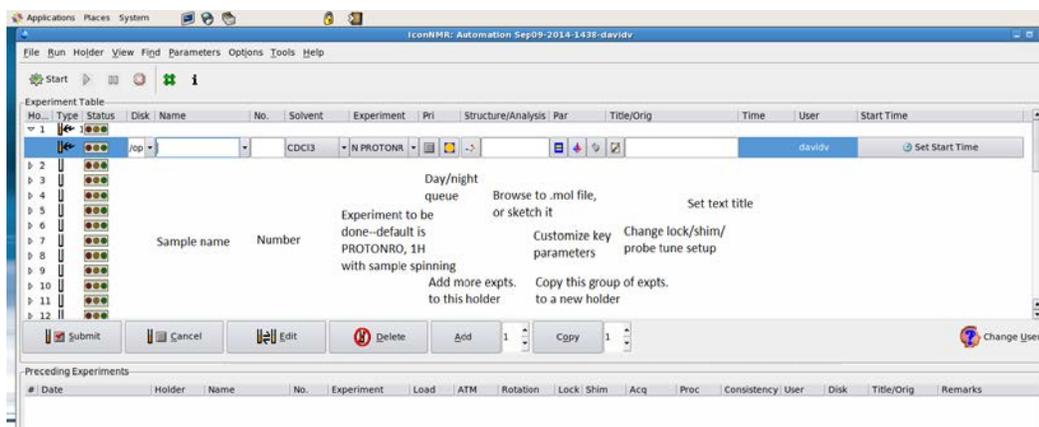


Remove sample options

To create space, or remove your sample after measurement, push the Remove button.

Adding your sample to the queue

The queue is controlled by the automation window of the ICON-NMR app within Topspin. After you have put your sample(s) in the SampleXpress, double click on a corresponding holder number to start the submission. You will need to supply a file name=sample name (no spaces or unusual characters), a number for the first experiment (a positive integer), the solvent, and choose a parameter set for the experiment you want. Optionally, you can supply on this line: a title; a structure file in .mol format for automated spectrum consistency check; customize a short list of key parameters such as the number of scans; turn off locking and shimming (for deuterium observe, for instance); direct an experiment to the night queue instead of the day queue; or choose a start time which is delayed. For experiment parameters not included on the short list of commonly adjusted ones, click on the Parameters tab to have access to all acquisition and processing parameters.



ICON-NMR has many predefined experiments and the ones which are similar to the ones on the VnmrJ “common” tab have been moved to the top of the list. If you need help choosing an appropriate parameter set for what you want, please ask. These 13 experiments work well:

- PROTONRO: 1H with sample spinning
- C13CPD: decoupled carbon. The probe has some carbon background signal, which can be taken out by baseline correction, or backwards linear prediction of the first few points of the fid.
- C13CPDSN: carbon with periodic signal to noise check; your acquisition is halted when you reach a number of scans you specify, or a S/N threshold, whichever comes first. This one is very useful to avoid overpaying for carbon spectra. S/N 75-100 is good for publication; less may be OK just to see the peaks.
- COSYGPSW: quick magnitude mode gradient COSY.
- HSQCEDETGPSISP: edited HSQC. Works OK with 25% non-uniform sampling. A ¹H spectrum is automatically added.
- HMBCEGTGPL3ND: HMBC. Works with 50% NUS sampling, works well with 50% sampling plus doubling the default number of points along the carbon axis. A ¹H spectrum is automatically added.
- NOESYPHSW: phase sensitive NOESY.
- C13DEPT135: CH₃ and CH up, CH₂ down, quaternary carbons suppressed.
- P31CPD: 1D 31P spectrum.
- B11ZG: 1D 11B spectrum. The probe does have a large boron background signal.
- WATERSUP: 1D preset solvent suppression.
- ZG2H: deuterium 1D spectrum through the lock channel. The sensitivity is excellent. Typically you would prepare the sample in a protio solvent. If so, switch off locking and shimming when you submit the sample.
- FASTLANE_HSQC: combination of HSQC and 1H spectra parameterized for the CMC-Assist program to do an automatic spectrum assignment and consistency check with your structure. Supply a .mol file for this option, or draw one in the JChemPaint program installed with Topspin. Your compound needs to be reasonably pure (85+%) for this to work well. You will also get a calculated sample concentration.

Some acquisition parameters can be quickly edited from within the submission entry form. To change any others, you need to go to the top menu Parameters and choose Edit Acquisition Parameters. This takes you back into Topspin where anything can be edited, but the number of parameters is very large—be careful about what you change.

The editable parameters are:

- ns: number of scans (or scans per block on a 2D)
- o1p: the center of the spectrum (or direct axis of a 2D)
- sw: sweep width (or direct axis of a 2D)
- d1: delay between scans. For most experiments, this defaults to 2 seconds; on most Agilent experiments, it is 1 second. For the cryoprobe, this delay should not be made shorter than 1 second.
- p1: observe pulse width, normally not changed
- SINO: S/N ratio desired; only checked in the C13CPDSN experiment
- TD: number of points (or in direct axis of a 2D)
- aq: acquisition time for the fid
- o2p: center of 1H decoupling on carbon spectrum, or center of indirect axis in 2D
- 1TD: number of points on indirect axis of 2D
- d8: mixing time (normally only applicable to noesy)
- ro: spin rate (set to 0 for top heavy tubes such as J. Young)

When you are done with sample entry, click on the “Change User” button on the ICON-NMR screen. This brings up the box for operator login. The software will automatically log you out after 10 minutes of inactivity. Your data is automatically saved as it is created. If you enter an email address in your user configuration, the instrument will email you PDFs of your spectrum plots, or the consistency check report if you have supplied a .mol file.

At the instrument, you can monitor the progress of experiments below the sample tray list as the software puts check marks as each operation (locking, shimming, etc.) is completed successfully. If a step fails, there will be an X instead, and the whole row will turn red. Some errors are not considered fatal, and acquisition may continue. To see the progress of the queue remotely, you can browse to <http://florence.caltech.edu:8015>.