Example of Setting Up 1D and 2D Experiments on the Bruker AV-III-400

In this example, the sample is dissolved in $CDCl_3$ in a 7" nmr tube with a Bruker (purple plastic) cap. The sample depth is 4 cm (liquid) and the sample is cleaned with ethanol on a KimWipe and placed in holder 65 of the carrousel. Here is how the 1D experiments are set up in the ICON-NMR automation window (upper half):

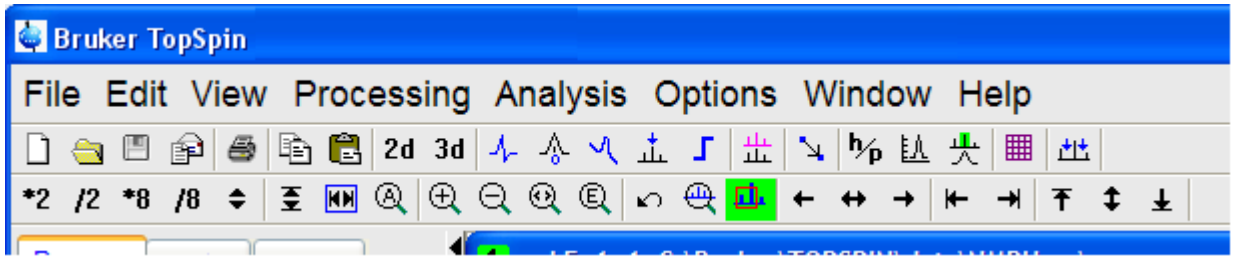
Holder		Status	Disk	DataSetName	Expno	Solvent	Experiment		Time	user
65	7	Queued								
		Queued	/opt/topspin	Unknown_13D	1	CDCl3	PROTON		00:00:50	catlin
		Queued	/opt/topspin	Unknown_13D	2	CDCl3	C13CPD_256		00:11:22	catlin
		Queued	/opt/topspin	Unknown_13D	3	CDCl3	C13DEPT90		00:04:38	catlin
		Queued	/opt/topspin	Unknown_13D	4	CDCl3	C13DEPT135		00:04:38	catlin

Note that the filename (DataSetName) is the same for all four experiments, and the experiment number (Expno) is incremented for each new experiment (1, 2, 3, 4). "Time" is the time for the data acquisition only; add about 5 minutes for sample insertion, spinning, automatic tune and match, automatic shimming and sample removal. Make sure the Solvent is correct for each of the experiments. The Status is "Available" while you are setting it up, "Queued" after you submit the experiment, "Running" when that particular experiment is actually being done, and "Finished" when the experiment has been run.

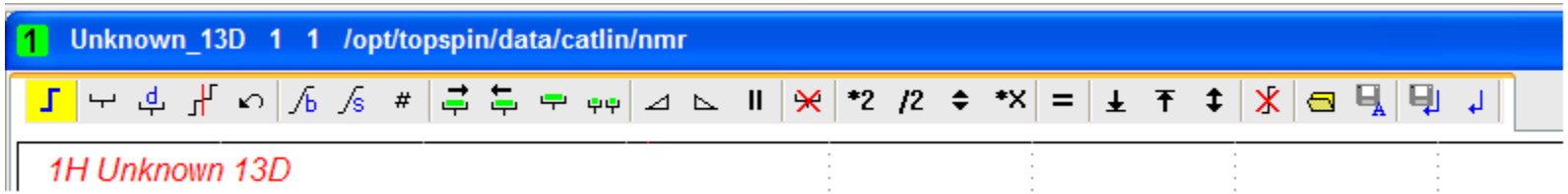
After the 1D experiments are finished, if you want to run 2D experiments you need to set the limits of the range of chemical shifts (ppm values) in the 1D experiments. This sets the "window" of chemical shifts that the 2D experiment will "cover". Because 2D experiments take longer and use much more data space, it's important to narrow the window to include JUST THE REGION WHERE YOUR SAMPLE HAS NMR PEAKS. This is done in the Bruker software ("TopSpin") by putting a single integral on the entire range of peaks in your 1D spectrum. The 2D spectrum uses this integral to define the window it will use. For a 2D-COSY, both dimensions are proton (1H), so you only need to "reference" the 2D COSY to an integrated 1D proton spectrum. In the lower part of the ICON-NMR automation window (shown below), scroll down until you find the history of your completed 1D proton spectrum. The top line is the most recent, and they continue down as you go backwards in time through the completed queue. Note that the four experiments submitted appear from bottom to top in the order they were run, starting with the proton spectrum. The check marks show completion of each step: Load (insert the sample in the magnet), ATM

Date	Time	Holder	Name	No.	Experiment	Load	ATM	Rotation	Lock	Shim	Acq	Proc	User
2010-03-23	16:16:36	65	Unknown_13D	4	C13DEPT135		✔	✔			✔	✔	catlin
2010-03-23	16:10:39	65	Unknown_13D	3	C13DEPT90		✔	✔			✔	✔	catlin
2010-03-23	15:57:53	65	Unknown_13D	2	C13CPD_256		✔	✔			✔	✔	catlin
2010-03-23	15:53:31	65	Unknown_13D	1	PROTON	✔	✔	✔	✔	✔	✔	✔	catlin

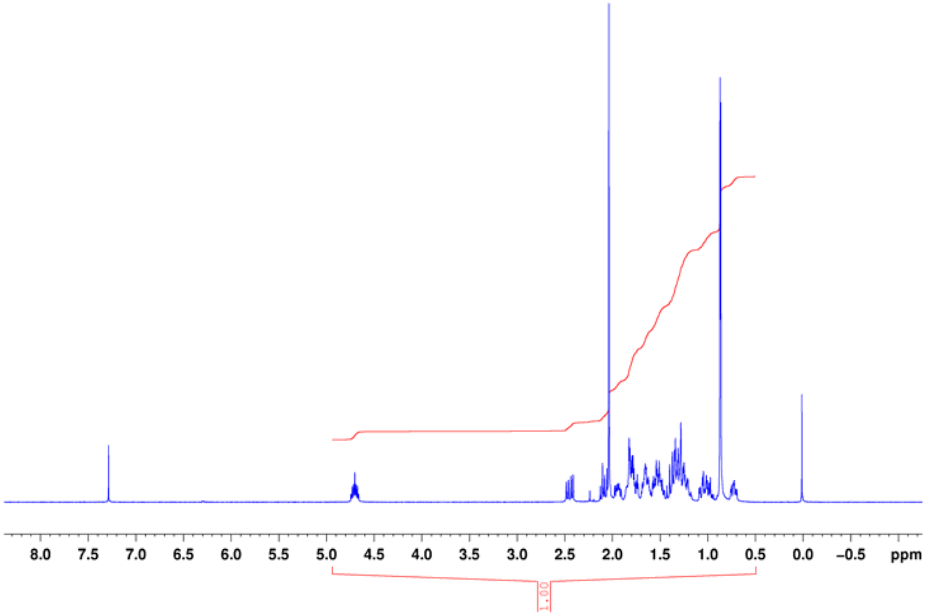
(auto tune and match), Rotation (spin the sample), Lock (lock on the deuterium signal of the solvent), Shim (automatic shimming), Acq (data acquisition) and Proc (data processing). Load, lock and shim only need to be done once on the first experiment. Double-click on the PROTON line and your spectrum will be displayed in the Bruker TopSpin software window. The upper left corner of the TopSpin window has a bunch of icons:



Click on the  icon (magnifying glass with an "A" in the center) to see the entire spectrum ("All"). Click on the blue integration icon () to enter the integration mode. This brings up a new row of icons just above the spectrum:

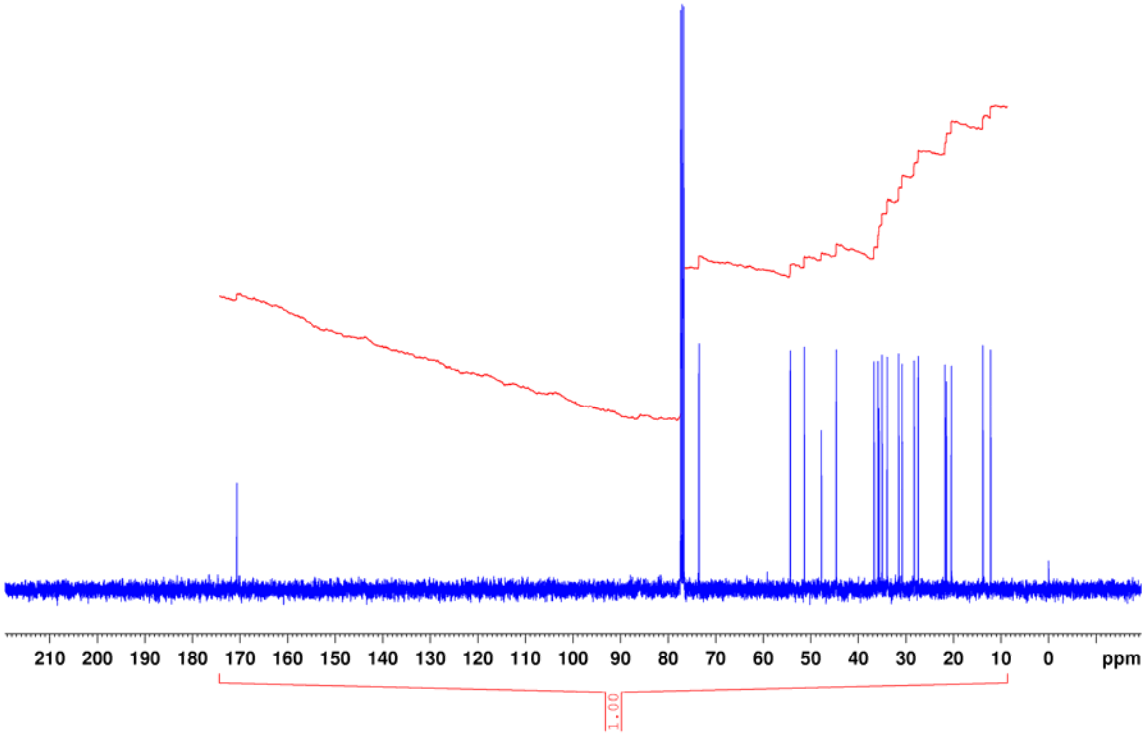


Click on the Select Region () icon to highlight it:  . Then use the vertical red line to sweep out the region of peaks for your compound. Position the line just to the left of the leftmost peak of your spectrum (ignoring the CHCl3 solvent peak or any impurity peaks) and click and drag to the right, releasing the mouse button just to the right of the rightmost peak of your spectrum (ignoring the TMS peak or any impurity peaks). This will put an integral of area 1.0 on the region of interest.

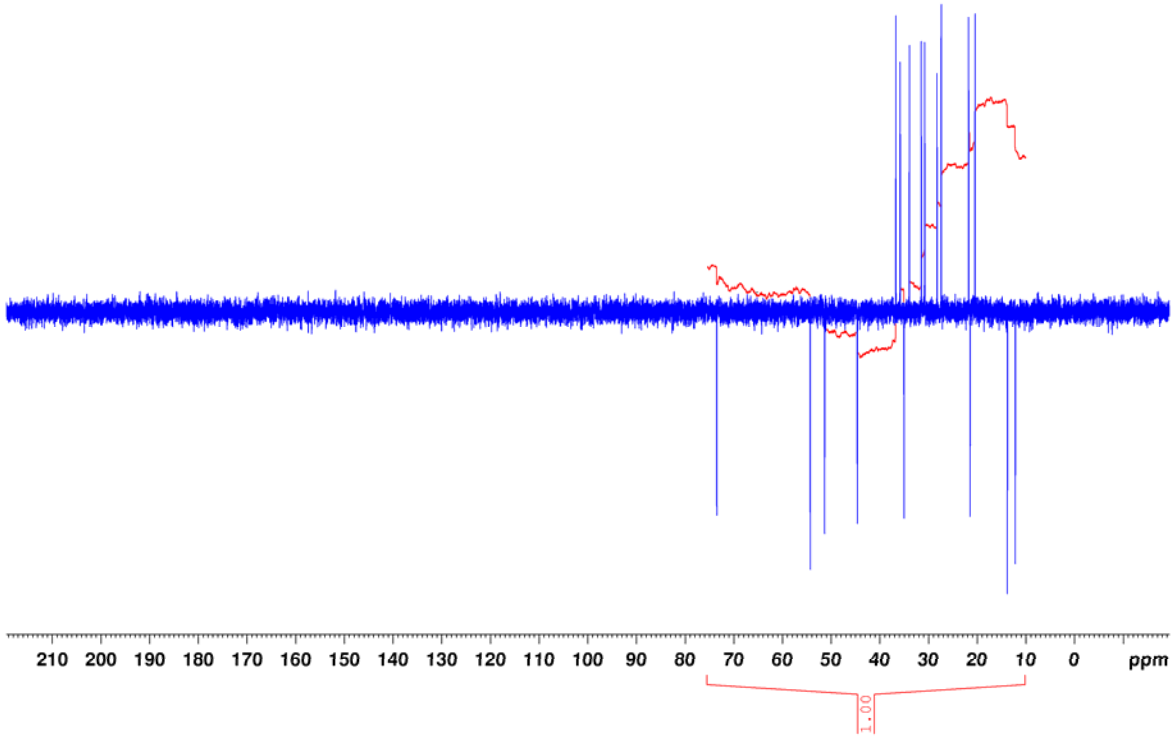


For example, your integrated proton spectrum might look like the ^{1}H spectrum on the left. Note that the $CHCl_3$ peak at 7.25 ppm and the TMS peak at 0.0 ppm are not included in the integral region. Only peaks from your compound, which will have integer values of peak area, should be included in the selected region. If you wish to delete the integral region and try again, click on  (select all integral regions) and  (delete selected regions) icons. Then repeat the click and drag to select the integral region. When you are satisfied with the integral, click on the  (save and return) icon. This exits the integration mode and saves the region you selected. YOU MUST SAVE the integral region or the 2D spectrum will fail.

Do the same for your ^{13}C spectrum, going back to the ICON-NMR automation window and double-clicking on the C13CPD_256 line of the completed experiments list. This will bring up your 13C spectrum. As before, use the integration tools to select an integral region a little bit larger than the region covered by your sample's ^{13}C peaks. The spectrum might look like this when you are finished:



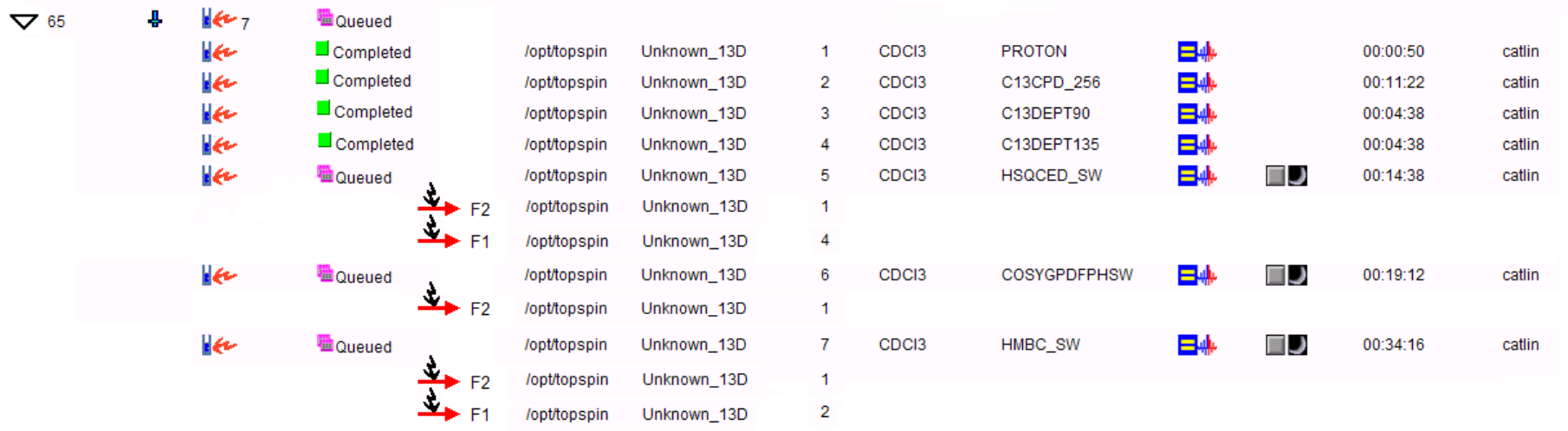
Finally, go back to the ICON-NMR automation window and double-click on your DEPT135 experiment. This will bring up your DEPT-135 spectrum in the TopSpin software. Select the region with ^{13}C peaks from your compound using the integration mode. Note that only the carbons with protons attached will appear in this spectrum, so the region may be smaller than in your ^{13}C spectrum. The integrated DEPT-135 spectrum might look like this:



It doesn't matter what the integral looks like, only that it starts and ends at the right place in the spectrum. Be sure to Save and Return from the Integration Mode in TopSpin.

Setting up the 2D Experiments. You are now ready to submit the 2D experiments. Make sure your sample is still in the same holder in the SampleJet, and that the solvent depth is still good ($CDCl_3$ evaporates significantly in a few days). Select your DEPT-135 experiment in the upper part of the ICON-NMR automation window and click on the ADD button to bring up a new line underneath the DEPT-135. Leave the sample name (e.g. "Unknown_13D") the same. The experiment number should automatically increment to experiment 5. Select the HSQCED_SW experiment. This will add two new lines below it for the 1D reference spectra. The first line, labeled "F2", is the reference for the horizontal (F_2) dimension of the HSQC spectrum. This dimension is the 1H dimension, so the filename should be the same and the experiment number should be 1. The second line, labeled "F1", is the reference for the vertical (F_1) dimension of the 2D HSQC spectrum. This dimension is the ^{13}C dimension, and the reference should be to the same filename with experiment number 4 (DEPT-135). The DEPT-135 spectrum is used because it has peaks in it for all carbons except the quaternary carbons. Quaternary carbons don't show up in the HSQC, so there is no need to include them in the F_1 window.

The COSY spectrum is set up in a similar way. It has ^{1}H in both dimensions, so it only needs one reference, to experiment 1. The intregral region from the ^{1}H spectrum is used in both the F_2 and the F_1 dimension to set the window. Finally, the HMBC spectrum is referenced to the ^{1}H spectrum (experiment 1, F_2 reference) and the ^{13}C spectrum (experiment 2, F_1 reference). Since even quaternary carbons can give crosspeaks in the HMBC spectrum, the entire region of the ^{13}C spectrum needs to be "covered" in the F_1 window. Here's how it looks once the experiments are submitted to the queue:



The little "moon" symbol ( ) indicates that the 2D experiment are submitted to the night queue, which begins at 7:00 p.m. on weekdays. These experiments will wait until that time and then will start.