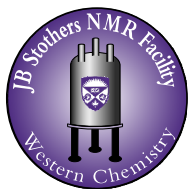


Operation of the Bruker 400

JB Stothers NMR Facility
Department of Chemistry
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Basic Operation of the Bruker 400

1. INTRODUCTION

1.1. Overview of the Bruker 400 NMR Spectrometer

The Bruker 400 NMR spectrometer runs in automation, which means that your sample is first placed into one of the 24 positions in the autosampler, then into the sample queue. When your sample is up in the queue, the autosampler will insert the NMR sample into the NMR magnet, and all of your chosen NMR experiments will run automatically. When all the NMR experiments have completed for your sample, your sample will eject from the spectrometer and be returned to the autosampler.

The Bruker 400 is equipped with an automatic tuning module and thus is capable of acquiring 1D ^1H , ^{11}B , ^{13}C , ^{19}F , ^{31}P , ^{77}Se , ^{119}Sn and ^{125}Te NMR spectra, plus 2D ^1H - ^1H , ^1H - ^{13}C , ^1H - ^{29}Si and ^1H - ^{31}P NMR spectra, all in automation.



Figure 1: (A) A side view of the Bruker 400 magnet and autosampler. (B) View of the top of the autosampler's carousel showing the 24 sample positions.

1.2. Overview of Software

The Bruker 400 utilizes the IconNMR program for automation. IconNMR software screen is separated into two main sections, an upper section where the 24 sample positions are displayed and samples are submitted to the queue, and a lower section that displays all the completed NMR spectra as well as the status of the active sample. In addition, there is a “Change User” button that is used to log into, and out of, your group's account, plus six action buttons that are used for a variety of tasks.

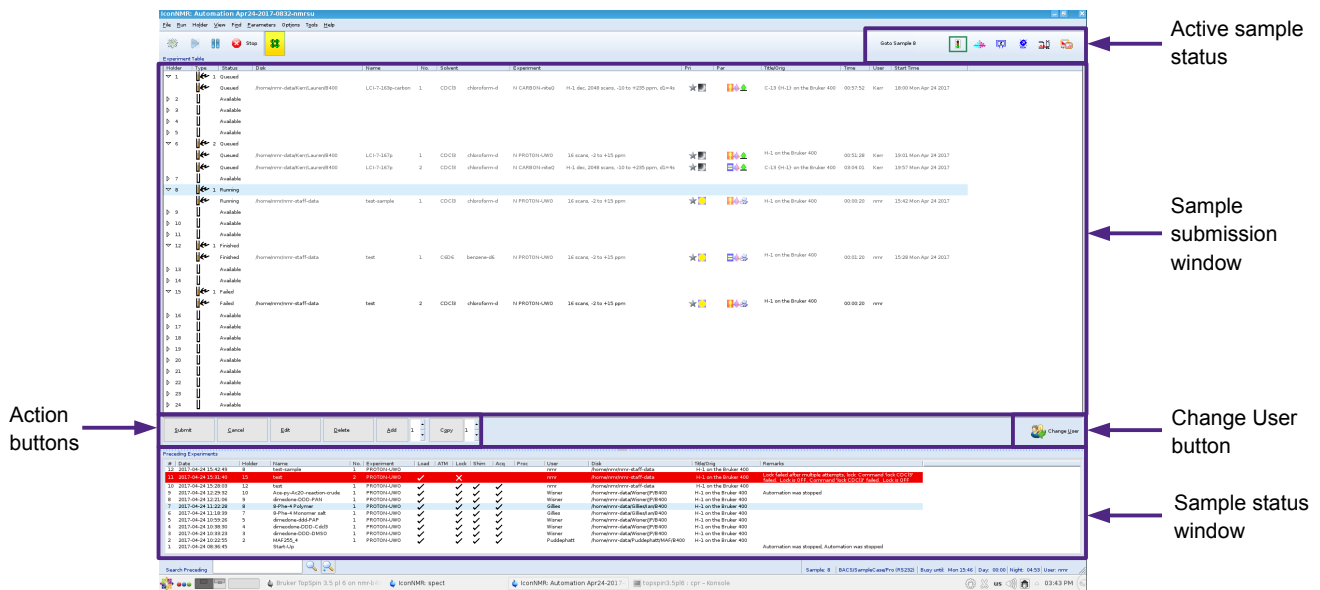


Figure 2: Overview of the Icon5 NMR software after logging in. There are two main sections: the upper “Sample submission window” where the 24 sample positions are displayed and your sample submission is performed, and the lower “Sample status window” that displays the current sample and all completed samples.







1.2.1. Active sample status

In the top right-hand corner of IconNMR is a message window and 6 icons that show the status of the current NMR experiment of the active sample (see Figure 4). When the action is active, the icon will flash and be outlined in green. When the action is completed, the icon will no longer flash, but will still be outlined in green.



Figure 3: The sample status icon bar that appears on the top right-hand side of the IconNMR screen. Each icon represents a specific spectrometer action being performed on the current sample. When the icon is flashing and is outlined in green, that action is currently in process. When the action is completed, the icon will be outlined in green, but will no longer flash.

From left to right the icons represent:

-  : the sample is being inserted into the NMR magnet
-  : deuterium lock
-  : sample shimming
-  : optimization of receiver gain
-  : acquisition of NMR spectrum
-  : processing of NMR spectrum

Basic Operation of the Bruker 400

1.2.2. Sample submission window

The sample submission window is on the top half of the IconNMR screen. The sample submission window shows all 24 sample positions, the status of each sample position, plus loads of other information about any sample in the sample position (see Figure 5).

Holder	Type	Status	Disk	Name	No.	Solvent	Experiment	Pri	Par	Title/Orig	Time	User	StartTime	
1	1	Queued	/home/nmr-data/Kerr/LaurenB400	LCI-7-167p-carbon	1	CDCl3	chloroform-d N CARBON-nitro	H-1 dec, 2048 scans, -10 to +235 ppm, d1=4s	★	🚦	C-13 {H-1} on the Bruker 400	00:57:52	Kerr	18:00 Mon Apr 24 201
2		Available												
3		Available												
4		Available												
5		Available												
6	2	Queued	/home/nmr-data/Kerr/LaurenB400	LCI-7-167p	1	CDCl3	chloroform-d N PROTON-UW0	16 scans, -2 to +15 ppm	★	🚦	H-1 on the Bruker 400	00:51:28	Kerr	19:01 Mon Apr 24 201
		Queued	/home/nmr-data/Kerr/LaurenB400	LCI-7-167p	2	CDCl3	chloroform-d N CARBON-nitro	H-1 dec, 2048 scans, -10 to +235 ppm, d1=4s	★	🚦	C-13 {H-1} on the Bruker 400	03:04:01	Kerr	19:57 Mon Apr 24 201
7		Available												
8	1	Running	/home/nmr/nmr-staff-data	test-sample	1	CDCl3	chloroform-d N PROTON-UW0	16 scans, -2 to +15 ppm	★	🚦	H-1 on the Bruker 400	00:00:20	nrrr	15:42 Mon Apr 24 201
9		Available												
10		Available												
11		Available												
12	1	Finished	/home/nmr/nmr-staff-data	test	1	CDCl3	benzene-d6 N PROTON-UW0	16 scans, -2 to +15 ppm	★	🚦	H-1 on the Bruker 400	00:01:20	nrrr	15:28 Mon Apr 24 201
13		Available												
14		Available												
15	1	Failed	/home/nmr/nmr-staff-data	test	2	CDCl3	chloroform-d N PROTON-UW0	16 scans, -2 to +15 ppm	★	🚦	H-1 on the Bruker 400	00:00:20	nrrr	

Figure 4: A closeup of the Sample Submission Window.

In the sample submission window, there are 14 columns of information, which from left to right are:

- **Holder:** *the sample location number in the autosampler*
- **Type:** *displays whether the location is full or empty*
- **Status:** *several status values can be displayed*
 - Available (no sample submitted to that position, position can be used for your sample)
 - Queued (sample is in the position, but has yet to be run, position not available for your sample)
 - Running (active sample, position not available for your sample)
 - Finished (sample has completed, position can be used for your sample)
 - Failed (sample has failed, position not available for your sample)
- **Disk:** *directory where your NMR data will be saved, chosen from drop-down list, should be /home/nmr-data/group-name/your-name/B400/*
- **Name:** *name of your sample*
- **No.:** *experiment number, usually 1 for the first experiment*
- **Solvent:** *name of your solvent, chosen from a drop-down list*
- **Experiment:** *the desired NMR experiment, chosen from a drop-down list*
- **Pri:** *sample priority: 🟡 represents day queue, while 🟤 represents night queue*
- **Par:** *NMR parameters: 🟩 means default parameters have been used, 🟥 means that at least one NMR parameter has been modified*
- **Title/Orig:** *text stored with your NMR experiment*
- **Time:** *duration of your NMR experiment*
- **User:** *group name*
- **Start Time:** *estimated start time for your NMR experiment*

1.2.3. Action buttons

The actions button toolbar is displayed in Figure 6. These buttons are used to perform a variety of important tasks.



Figure 5: Action buttons used to Submit samples to the queue, Edit samples that have been submitted to the queue, Delete samples from the queue, Add additional NMR experiments to your sample, and to Copy your sample to the next available location.

1.2.4. Sample status window

The sample status window is on the bottom half of the IconNMR screen. The sample status window displays information about each completed NMR experiment, plus the status of the active NMR experiment (see Figure 6).

#	Date	Holder	Name	No.	Experiment	Load	ATM	Lock	Shim	Acq	Proc	User	Disk	Title/Orig	Remarks
12	2017-04-24 15:42:49	8	test-sample	1	PROTON-LJWO							nmr	/home/nmr/nmr-staff-data	H-1 on the Bruker 400	
11	2017-04-24 15:35:40	15	test	2	PROTON-LJWO	✓	✗					nmr	/home/nmr/nmr-staff-data	H-1 on the Bruker 400	Lock failed after multiple attempts. Lock Command 'lock CDCIS' failed. Lock is OFF. Command 'lock CDCIS' failed. Lock is OFF
10	2017-04-24 15:28:03	12	test	1	PROTON-LJWO	✓	✓	✓	✓	✓		nmr	/home/nmr/nmr-staff-data	H-1 on the Bruker 400	
9	2017-04-24 12:29:32	10	Accepy-Ac20-reaction-crude	1	PROTON-LJWO	✓	✓	✓	✓	✓		Wisner	/home/nmr-data/Wisner/JP/B400	H-1 on the Bruker 400	
8	2017-04-24 12:21:06	9	dimedone-DDD-PAH	1	PROTON-LJWO	✓	✓	✓	✓	✓		Wisner	/home/nmr-data/Wisner/JP/B400	H-1 on the Bruker 400	Automation was stopped
7	2017-04-24 11:22:28	8	8-Phe-4 Polymer	1	PROTON-LJWO	✓	✓	✓	✓	✓		Gillas	/home/nmr-data/Gillas/lan/B400	H-1 on the Bruker 400	
6	2017-04-24 11:18:39	7	8-Phe-4 Monomer salt	1	PROTON-LJWO	✓	✓	✓	✓	✓		Gillas	/home/nmr-data/Gillas/lan/B400	H-1 on the Bruker 400	
5	2017-04-24 10:59:26	5	dimedone-dddPAP	1	PROTON-LJWO	✓	✓	✓	✓	✓		Wisner	/home/nmr-data/Wisner/JP/B400	H-1 on the Bruker 400	
4	2017-04-24 10:38:30	4	dimedone-DDD-Cdc3	1	PROTON-LJWO	✓	✓	✓	✓	✓		Wisner	/home/nmr-data/Wisner/JP/B400	H-1 on the Bruker 400	
3	2017-04-24 10:39:23	3	dimedone-DDD-DMSO	1	PROTON-LJWO	✓	✓	✓	✓	✓		Wisner	/home/nmr-data/Wisner/JP/B400	H-1 on the Bruker 400	
2	2017-04-24 10:22:55	2	MAF255_4	1	PROTON-LJWO	✓	✓	✓	✓	✓		Puddephatt	/home/nmr-data/Puddephatt/MAF/B400	H-1 on the Bruker 400	
1	2017-04-24 08:36:45		StartUp	1	PROTON-LJWO	✓	✓	✓	✓	✓					Automation was stopped. Automation was stopped

Figure 6: Sample status window. The sample status window shows details about all completed NMR experiments and the active NMR experiment.

In the sample status window, there are 16 columns of information and each column can be sorted by clicking on the column heading. From left to right, the columns are:

- #: *the chronological number of the NMR experiment in the run*
- Date: *date and time the NMR experiment started*
- Holder: *sample holder position in the autosampler's carousel*
- Name: *sample name*
- No.: *experiment number; usually 1 for the first experiment*
- Experiment: *name of completed/active NMR experiment*
- Load: *sample loaded into NMR magnet: ✓ when completed correctly, ✗ when failed*
- ATM: *automatic tune and match (ATM): ✓ when completed correctly, ✗ when failed*
- Lock: *deuterium lock: ✓ when completed correctly, ✗ when failed*
- Shim: *sample shimming: ✓ when completed correctly, ✗ when failed*
- Acq: *acquisition of NMR spectrum: ✓ when completed correctly, ✗ when failed*
- User: *group name*
- Disk: *directory where the NMR data was/will be saved, should be /home/nmr-data/group-name/your-name/B400/*
- Title/Orig: *text stored with your NMR experiment*
- Remarks: *errors and other IconNMR messages related to the NMR experiment*

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1.3. Sample Changer Overview

An overview of the 24 sample positions will be displayed in a pop-up window on the right monitor.

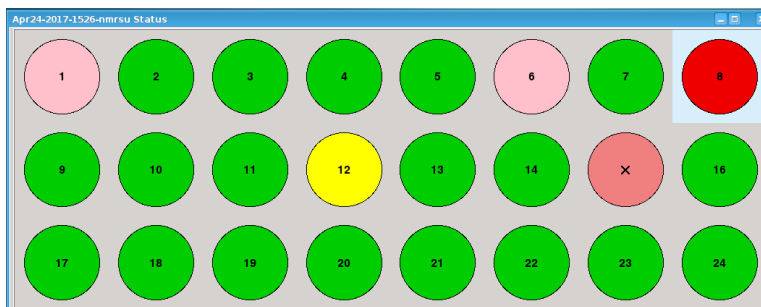


Figure 7: Sample position display on the Bruker 400. Each colour represents the status of the sample position (see text for description). Any green or yellow position can be used for your sample.

In the sample changer overview, each of the 24 positions within the autosampler will be a certain colour, where the colour indicates the status of that particular sample position. The possible colours are:

- unused, position is available for your sample
- sample has completed in this position, position is now available for your sample
- active sample, position is not available for your sample
- queued, position is not available for your sample
- sample failure, position is not available for your sample

1.4. Day Queue and Night Queue

The Bruker 400 utilizes a day queue and night queue system for samples submitted on weekdays between 8:00 am and 6:00 pm. The day queue is limited to NMR experiments less than 30 minutes in duration. NMR experiments longer than 30 minutes can be submitted to the Bruker 400 at any time, but will be forced to run in night queue. The night queue begins after 6:00 pm and after all day queue NMR experiments have completed. There is no day/night queue on weekends, thus the 30 minute sample limit does not apply.

1.5. Glossary of Available Bruker 400 NMR Parameters

Every NMR experiment has numerous NMR parameters that must be set correctly. On the Bruker 400, you may modify some of the most common NMR parameters. A list of these parameters and a brief description of each parameter can be found below.

number of scans (ns)

Number of repetitions of the NMR pulse sequence. More scans results in better signal-to-noise, but longer experiment times. Depends primarily on the nuclear isotope, NMR experiment and sample.

relaxation delay (d1)

Time between scans, which allows for nuclear spins to relax back to equilibrium. Typically set between 1 – 3s. Depends on the nuclear isotope and sample.

spectral width (sw)

Total chemical shift range of the NMR spectrum. Depends primarily on the nucleus of interest. For 2D NMR experiments sw is the spectral width of the direct dimension (^1H for ^1H - ^1H 2D and for ^1H - ^{13}C 2D).

centre of spectrum (o1p)

Centre of the spectral width. Combined with sw, determines the upper-most and lower-most chemical shift regions that will be probed. For 2D NMR experiments o1p is the centre of the spectral width of the direct dimension (^1H for ^1H - ^1H 2D and for ^1H - ^{13}C 2D).

number of increments (1td)

2D only. Total number of increments. More increments results in better resolution in the indirect dimension, but longer experiment times. Typically set to either 128 or 256.

spectral width of indirect dimension (1sw)

2D only. Total chemical shift range of the indirect dimension (eg. ^1H for ^1H - ^1H 2D, ^{13}C for ^1H - ^{13}C 2D). Depends primarily on the nucleus of the indirect dimension.

centre of spectral width of indirect dimension (o2p)

2D only. Centre of the spectral width of the indirect dimension (eg. ^1H for ^1H - ^1H 2D, ^{13}C for ^1H - ^{13}C 2D). Combined with 1sw, determines the upper most and lower most chemical shift regions that will be probed in the indirect dimension.

1.6. Standard NMR Experiments and Default NMR Parameters

Table 1.1. Standard 1D NMR Experiments and Default Parameters

Experiment	ns	d1 (s)	o1p (ppm)	sw (ppm)	F1 (ppm)	F2 (ppm)	Time (hr:min:s)
PROTON-UWO	16	1	+6.5	17	+15	-2	0:01:20
PROTON-F19dec	16	1	+6.5	17	+15	-2	0:01:20
CARBON-dayQ	512	1	+112.5	245	+235	-10	0:20:24
CARBON-niteQ	2048	3	+112.5	245	+235	-10	3:04:01
FLUORINE	32	1	-85	250	+40	-210	0:01:17
FLUORINE-H1dec	16	1	-85	250	+40	-210	0:00:38
PHOSPHORUS	64	1	+75	300	+225	-75	0:02:54

Table 1.2. Standard 2D NMR Experiments and Default Parameters

Experiment	Nuclei	ns	d1 (s)	1td	o1p (ppm)	sw (ppm)	o2p (ppm)	1sw (ppm)	Time (hr:min:s)
gCOSY	^1H - ^1H	4	1.5	128	+6	14	+6	14	0:15:17
gDQF-COSY	^1H - ^1H	4	1.5	128	+6	14	+6	14	0:15:01
TOCSY	^1H - ^1H	4	1.5	256	+6	14	+6	14	0:31:26
NOESY	^1H - ^1H	16	1.5	256	+6	14	+6	14	2:19:46
ROESY	^1H - ^1H	16	1.5	256	+6	14	+6	14	2:13:36
gHSQCAD	^1H - ^{13}C	4	1.5	128	+6	14	+80	180	0:15:08
gHMBC	^1H - ^{13}C	4	1.5	256	+6	14	+105	230	0:30:21

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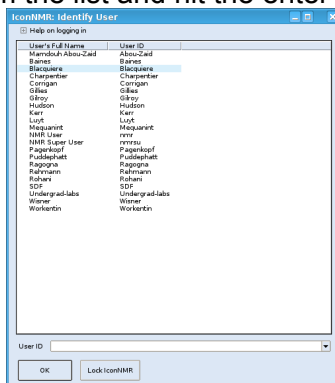
2. SUBMITTING SAMPLES TO THE SAMPLE QUEUE

2.1. Insert your NMR Sample in the Autosampler

- 1) On the spectrometer, look at the sample tray display; any of the green or amber carousel positions can be used for your sample. Or any samples labelled finished or available in the IconNMR screen.
- 2) At the autosampler's carousel, if there is a sample in the carousel's position that you want to use, remove the sample and place it in the "Current Week" tray.
- 3) Place the spinner in the depth gauge, insert your sample until it reaches the bottom of the depth gauge.
- 4) Remove the spinner/NMR tube from the depth gauge, clean the bottom of the sample with a Kim-Wipe, and place the spinner+sample into the carousel position that you have chosen.

2.2. Submit your Sample to the Queue

- 1) Select your supervisor's name from the list and hit the enter key.



- 2) Enter your group's password and hit the enter key.



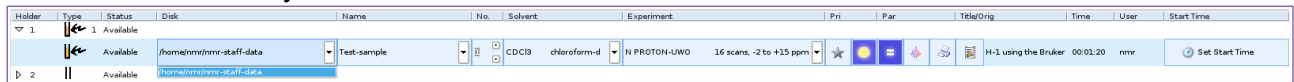
- 3) In the top section of the software, find the sample number you have chosen for your sample; you may only use positions labelled "Available" or "Finished".
 - a) if the sample position says "Available", double click on the sample position

- b) if the sample position says "Finished", click on the sample position, then click on

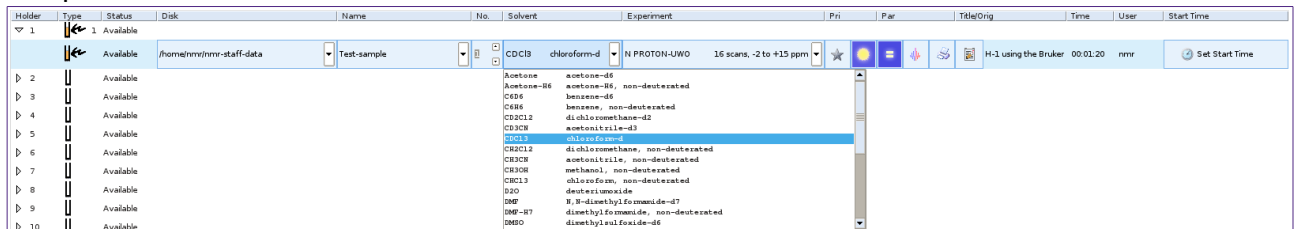
											Delete		
6	2	Queued	/home/nmr-data/Kerr/Lauren@B400	LCI-7-167p	1	CDCB	chloroform-d	N PROTON-JWQ	16 scans, -2 to +15 ppm	H-1 on the Bruker 400	00:51:28	Kerr	19:01 Mon Apr 24 2017
7	2	Queued	/home/nmr-data/Kerr/Lauren@B400	LCI-7-167p	2	CDCB	chloroform-d	N CARBON-rheQ	H-1, dec, 2048 scans, -10 to +235 ppm, d1=4s	C-13 (H-1) on the Bruker 400	03:04:01	Kerr	19:57 Mon Apr 24 2017
8	1	Running	/home/nmr/nmr-staff-data	test-sample	1	CDCB	chloroform-d	N PROTON-JWQ	16 scans, -2 to +15 ppm	H-1 on the Bruker 400	00:00:20	nmr	15:42 Mon Apr 24 2017
9		Available											
10		Available											
11		Available											
12	1	Finished	/home/nmr/nmr-staff-data	test	1	CGD6	benzene-d6	N PROTON-JWQ	16 scans, -2 to +15 ppm	H-1 on the Bruker 400	00:01:20	nmr	15:28 Mon Apr 24 2017
13		Available											
14		Available											
15	1	Failed	/home/nmr/nmr-staff-data	test	2	CDCB	chloroform-d	N PROTON-JWQ	16 scans, -2 to +15 ppm	H-1 on the Bruker 400	00:00:20	nmr	

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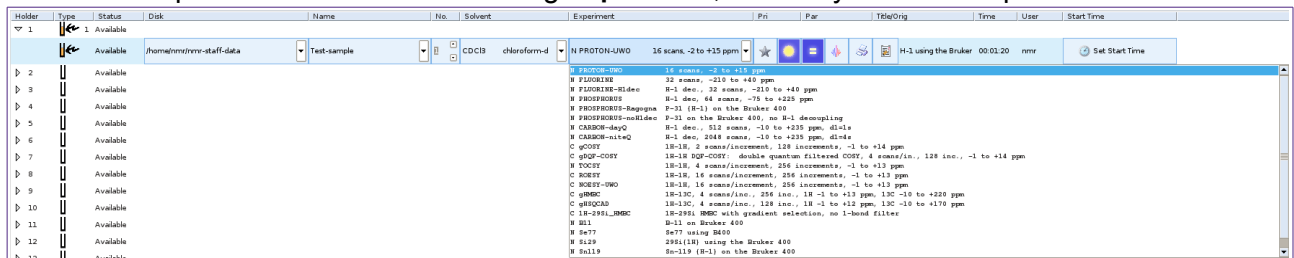
- 4) The active experiment will be outlined in blue. From the drop-down list under the heading **Disk**, select the folder with your name.




- 5) In the field under the heading **Name**, input your desired sample name.
 6) In the field under heading **No.**, click on the up button to pick a desired experiment number (typically 1).
 7) From the drop-down list under the heading **Solvent**, choose your solvent. There are both deuterated and non-deuterated solvents listed, so be sure to choose the correct one for your sample.



- 8) From the drop-down list under the heading **Experiment**, choose your NMR experiment.





- 9) (If desired) If you would like to modify NMR parameters (the default NMR parameters for the available experiments have been summarized in the previous section):
 a) Click on  to see and change NMR parameters.

NS	16	Number of scans
D1	1 [sec]	Relaxation delay (s)
O1P	6.500 [ppm]	Centre of spectral width (PPM)
SW	16.9802 [ppm]	Spectral width (ppm)
O2P	6.175 [ppm]	Indirect dimension centre of spectral width (2D only, PPM)
1SW	16.9802 [ppm]	Indirect dimension spectral width (2D only, PPM)

OK

- b) modify any NMR parameters that you wish
 c) click OK

- 10) (If desired) If you would like to submit your sample to the night queue:

- a) click on 
 b) the sun will change to , signifying that your sample or experiment is in the night queue.

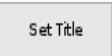
- 11) (If desired) If you would like to have your raw NMR data zipped and e-mailed to you OR to add a title that will be stored with your NMR data:

- a) click on , which will bring up the following window:


Mail Data/Notify to:

Set Title Set & Copy Title

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- b) to have the data e-mailed to you, input an e-mail address next to **Mail Data/Notify To**
- c) to modify the title, change or add to the text in the white box
- d) click 

12) (*If desired*) If you would like to add additional experiments to your sample:

- a) in the row of action buttons, click 
- b) repeat steps 7 – 11

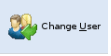
13) Click on the sample row (i.e. the top row for your sample) and click .

14) If you receive a message that one of your NMR experiments exceeds the day queue limits:

- a) select the NMR experiment and click 
- b) either click on  and reduce the ns, d1 or 1td (for 2D only) to reduce the experiment time to be less than 30 minutes OR click on  to change it to night queue.

- c) click 

15) (*If desired*) If you have additional samples to submit, repeat all steps in sections 2.1 and 2.2.

16) When all your samples have been submitted, click .

3. VIEWING COMPLETED NMR SPECTRA

- 1) If you are not logged in, log into your group's account.
 - 2) In the bottom section of the software, find your sample and experiments. The NMR experiments will be listed in reverse chronological order (i.e. more recently completed experiment first), but can be sorted by clicking on any of the displayed headings.
 - 3) Double click on the NMR spectrum that you would like to view; it will automatically be processed and displayed in TopSpin.
-