



LSU DEPARTMENT OF CHEMISTRY  
NMR FACILITY

# TOPSPIN 3.2 USER GUIDE

## Introduction

This short manual is meant to be used to collect and process NMR data using the Bruker Spectrometers in room CMB309 with TopSpin 3.2. Use our brief TopSpin 2.0 Guide if you are using the spectrometer in room B20 of Choppin Hall.

In this manual, TopSpin commands will be in bold and italicized. TopSpin commands must be followed by enter or return and this is assumed in what follows.

## Starting TopSpin

After logging in to your Linux Work Station using your account login name and password, you can start TopSpin in one of two ways:

1. Click on the TopSpin icon on your desktop.
2. Open a Terminal/Shell window and type *topspin*.

Once TopSpin is up and running, you will see the TopSpin user interface that looks similar to Fig. 1 below. It might look a little different for you depending on how your preferences were set.

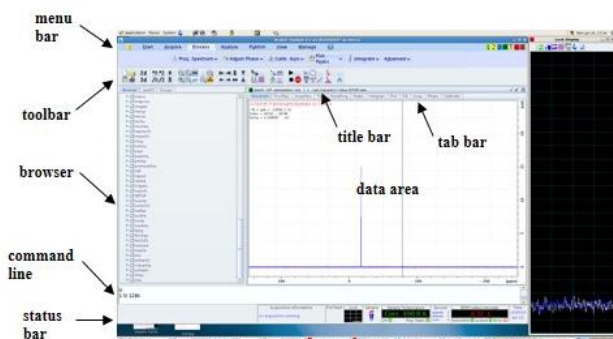


Figure 1 TopSpin Graphical User Interface

## Sample Preparation

Compared to other techniques, NMR is a relatively insensitive technique and requires concentrated samples. A typical NMR sample is a few milli-mollars (mM) in concentration. For small molecules, 2-3 mg of sample in 0.5 ml solvent is enough to get a  $^1\text{H}$  NMR spectrum with good signal to noise ratio in 1 minute. You will need 10-20 mg of sample to acquire a good  $^{13}\text{C}$  NMR spectrum in one hour.

NMR samples should be prepared in deuterated solvents, if possible. For biomolecular NMR, you will need 5-10% of  $\text{D}_2\text{O}$  in your sample. You will also need to add TMS, DSS or some other reference standard in your NMR sample for chemical shift referencing.

In addition to having a sufficiently concentrated sample in some deuterated solvent, there are other issues you need to pay attention to for successful NMR data acquisition. Use clean, dry and medium to high quality NMR tubes. Make sure your NMR sample is free of insoluble materials, filter it always. Use the same sample volume (about 0.6 ml or 5 cm for 5mm NMR tubes and 4ml or 5 cm for 10 mm NMR tubes). Adjust the sample depth using the sample depth gauge (figure 2. below) and wipe the sample tube clean before putting it on top of the magnet.

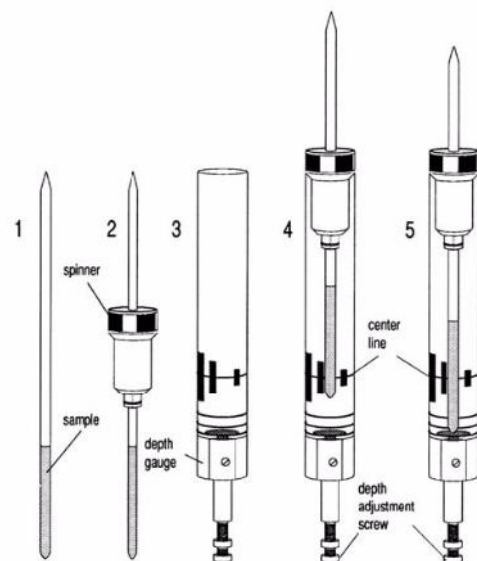


Figure 2 Put the NMR tube in a spinner to the sample depth gauge and center your sample about the marked center line.

All three of our AVIII spectrometers in CMB309 are equipped with SampleXpress Lite sample changer with a capacity of 16 samples. The 16 positions on the sample changer are marked 1-16 and you can choose any of the available spots for your sample. ***Please make sure there is no sample inside the magnet before you put a sample on the position immediately on top of the magnet bore.*** To insert your sample in to the magnet, put your sample and spinner on to one of the available slots on the sample changer (SampleXpress) and type *sx #* on the TopSpin command line (where # is the position your sample is

located on the sample changer). For example, if your sample is in position 6 on the sample changer you will type **sx 6** followed by enter.

### Tuning and Matching the Probe

**Tuning** involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.)

**Matching** involves ensuring that the maximum amount of the power arriving at the probe base is transmitted up to the coil which lies towards the top of the probe. This ensures that minimum amount of the power arriving at the probe base is reflected back towards the amplifiers (and consequently wasted).

Two of our AVIII spectrometers (Nanobay-400 and Prodigy-500) are equipped with probes with Automatic Tuning and Matching (ATM) Accessory but the three channel 400 MHz spectrometer has a probe without ATM. The tuning and matching procedure is different for probes with and without ATM and follow the appropriate procedure for the probe you are using.

### Probes equipped with ATM

#### Automatic tuning

1. Create a new data set and read appropriate parameter file for the experiment you want to run by typing **rpar "parameter-set" all**. For example, if you want to run a 1D proton NMR you would type **rpar PROTON all**. This will define the *observe* and *decouple* nuclei. Parameter set names are case sensitive. The parameter set names for most routine NMR experiments are listed on the table below. The last column in the table shows the parameters you might want to modify to suit your needs.
2. Type **atma**. The display will switch automatically to the acquisition window and displays the wobble curve. Tuning and matching are performed automatically. If multiple frequencies are used in a parameter set such as C13CPD, C13DEPT, P31CPD etc., ATMA will start adjusting the lowest frequency first and will switch in the order of increasing frequency automatically.

### Manual tuning

Automatic tuning works if the probe has been tuned to that particular frequency before and the tuning and matching values have been saved. If you are trying to tune to a frequency not used before or if **atma** fails, you can tune the probe manually as follows:

1. Type **atmm**. The ATM control window appears and the display will switch automatically to the acquisition window and displays the wobble curve.

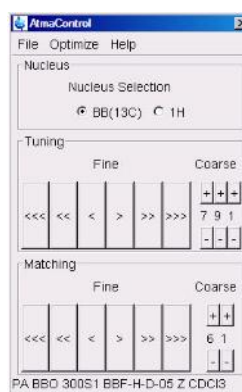


Figure 3 Type **atmm** to open the manual tuning interface.

2. Click on the **'Tuning'** buttons in the ATM control window to move the wobble curve in to the center of the display
3. Click on the **'Matching'** buttons in the ATM control window to adjust the dip of the wobble curve to the lowest position

NOTE: Since the Tuning and Matching adjustment interact with each other, a repeat of steps 2 and 3 are necessary for a perfect tune and match. If multiple frequencies are used in a parameter set, such as C13CPD, use the **'Nucleus Selection'** radio buttons in the ATM control window to switch to another nucleus and repeat steps 2 and 3 (Fig. 4).

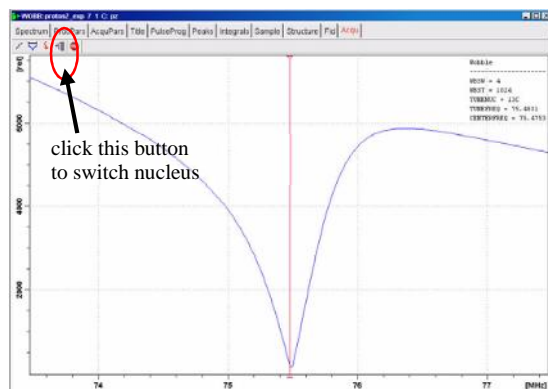


Figure 4: Wobble signal for properly tuned and matched probe.

**Table 1. Experiment, parameter file and pulse program names of routine NMR experiments.**

NMR Experiment	Parameter Set (case sensitive)	Pulse Program	Parameters you may need to modify
<b>Conventional 1D Experiments</b>			
1D $^1\text{H}$	PROTON	zg30	d1, ns, o1p, sw
1D $^{13}\text{C}\{^1\text{H}\}$	C13CPD	zggg30	d1, ns, o1p, sw
1D DEPT-135	C13DEPT135	dept135	d1, ns, o1p, sw
1D DEPT-90	C13DEPT90	dept90	d1, ns, o1p, sw
<b>Conventional 2D <math>^1\text{H}</math>-<math>^1\text{H}</math> Experiments</b>			
ge-2D $^1\text{H}$ - $^1\text{H}$ COSY (magnitude)	COSYGPSW	cosygppqf	d1, ns, o1p,1 sw, 2 sw
ge-2D $^1\text{H}$ - $^1\text{H}$ NOESY (phase sensitive)	NOESYPHSW	noesygpph	d1, ns, o1p,1 sw, 2 sw, d8
ge-2D $^1\text{H}$ - $^1\text{H}$ ROESY (phase sensitive)	ROESYPHSW	roesyph	d1, ns, o1p,1 sw, 2 sw, p15
ge-2D $^1\text{H}$ - $^1\text{H}$ TOCSY (phase sensitive)	MLEVPHSW	mlevph	d1, ns, o1p,1 sw, 2 sw, d9
<b>2D Inverse <math>^1\text{H}</math>-<math>^{13}\text{C}</math> Experiments</b>			
ge-2D $^1\text{H}$ - $^{13}\text{C}$ HMQC (magnitude)	HMQCGP	hmqcgp	d1, ns, o1p,1 sw, 2 sw
ge-2D $^1\text{H}$ - $^{13}\text{C}$ HSQC (phase sensitive)	HSQCGP	hsqcetgpsi2	d1, ns, o1p,1 sw, 2 sw
ge-2D $^1\text{H}$ - $^{13}\text{C}$ HSQC (phase sensitive)	HSQCEDETG	hsqcetgcp	d1, ns, o1p,1 sw, 2 sw
ge-2D $^1\text{H}$ - $^{13}\text{C}$ HMBC (magnitude)	HMB CGP	hmbcgpndqf	D6, ns, o1p, o2p, 1 sw, 2 sw
<b>Selective 1D Experiments</b>			
Selective 1D NOESY	SELNOGP	selnogp	ns,spoffs2, p12, sp2,d8
Selective 1D ROESY	SELROGP	selrogp	ns,spoffs2, p12, sp2,p15
Selective 1D TOCSY	SELMIGP	selmigp	ns,spoffs2, p12, sp2,d9
<b><math>^{31}\text{P}</math> Experiments</b>			
$^1\text{H}$ -decoupled $^{31}\text{P}$	P31CPD	zggg30	ns, sw, o1p
$^1\text{H}$ -coupled $^{31}\text{P}$	P31	zg30	ns, sw, o1p
$^{31}\text{P}$ -decoupled $^1\text{H}$ spectrum	PROP31DEC	zggd30	ns, o2p
2D $^1\text{H}$ - $^{31}\text{P}$ HMBC	HMB CGP	hmbcgpndqf	ns, o2p,1 sw, 2 sw, 1 td, d6

## Tuning and Matching Probes without ATM Accessory

1. Create a new data set.
2. Type **wobb**. Tuning and Matching rods are color-coded. For example in our TBI probe  $^1\text{H}$  tune and

match rods are gold and  $^{13}\text{C}$  tune and match rods are blue.

3. Adjust the Tuning rod marked **T** underneath the probe to move the wobble curve in to the center of the display.

4. Adjust the Matching rod marked **M** underneath the probe to adjust the dip of the wobble curve to the lowest position.

NOTE: Since the Tuning and Matching adjustment interact with each other, a repeat of steps 3 and 4 are necessary for a perfect tune and match. If multiple frequencies are used in a parameter set and to switch to other frequencies, follow steps 5 through 6 below and repeat steps 3 and 4.

5. Click on the nucleus selection button in the acquisition window to switch nucleus.

6. Tune and Match.

## Field-Frequency Locking

To display the lock signal type **lockdisp** on the Topspin command line. This opens a window in which the lock trace appears. The most convenient way to lock is to use the TopSpin command **lock**. To start the lock-in procedure, type **lock** and select the appropriate solvent from the menu. Alternatively, enter the solvent name together with the lock command, e.g., **lock cdc13**. Once lock-in is achieved, the lock gain is set so that the lock signal is visible in the lock window. At this point the message "lock: finished" appears in the status line at the bottom of the TopSpin window. If lock-in can be achieved but the lock signal oscillates due to saturation, reduce the lock power manually to stop the oscillation.

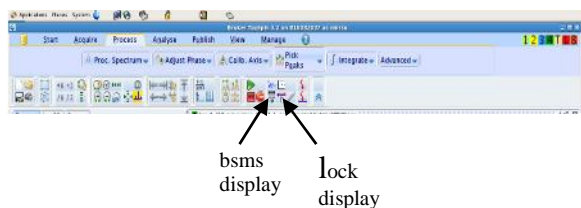


Figure 5: You can open the lock and bsms display windows either from the command line using **bsmsdisp** and **lockdisp** commands or by clicking the corresponding buttons on the toolbar.

## Shimming the Magnet

The purpose of shimming is to maximize the magnetic field homogeneity, which depends on probehead and sample geometry. In general, it is

necessary to shim the magnetic field after each sample change. Optimal shim settings may vary substantially from probehead to probehead. However, provided the probehead is always positioned at the same position in the magnet and the sample is always positioned the same with respect to the receiver coil, the shim values for a given probehead will be fairly reproducible. Thus, shimming time can be greatly reduced if the shim settings for each probehead are stored as a shim file on the computer. To save your shim file type *wsh* and type a name for your shim then click on **write**. You can load this shimset at any time in the future by typing *rsh* and choosing the file name.

### Shimming on the Lock Signal

When the spectrometer is locked, the vertical offset of the lock trace on the graphics display corresponds to the amplitude of the lock substance signal, assuming constant lock DC, gain, and power levels. The lock level, then, serves as useful guide for basic shim adjustment. The goal in shimming on the lock signal is to adjust the shims so that the lock trace appears as high on the graphics display as possible. This lock level corresponds to the highest possible lock substance signal amplitude.

### Shimming using TopShim

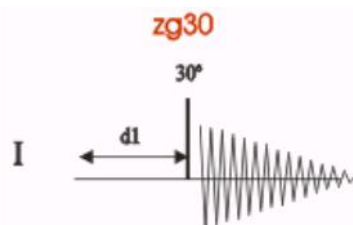
TopShim is a tool designed for easy and automatic shimming. Type *topshim* on the TopSpin command line to do automatic shimming using this tool. Topshim works faster if your starting shims are reasonably close to optimum. If you have saved a good shim file, read it using the command *rsh* before you run *topshim*. Topshim might fail if there is convection in your sample. You can issue the topshim command with additional arguments to overcome problems associated with convection and or to select special features you might be interested in. To optimize your shimming for lineshape and compensate for convection use the *topshim* command as follows:

*topshim ls convcomp*

## One-Dimensional (1D) Proton experiment

### Introduction

The following section describes a procedure for the acquisition and processing of a one-dimensional (1D)  $^1\text{H}$  NMR spectrum using the standard Bruker parameter set **PROTON**. The procedure for acquiring 1D  $^{13}\text{C}$ ,  $^{31}\text{P}$  or any other nucleus is the same except that you will have to read appropriate parameter set. The pulse sequence **zg30**, shown below, is used for acquiring the 1D data and it consists of the recycling delay (d1), the radio-frequency (RF) pulse (p1), and the acquisition time during which the signal is recorded. The pulse angle is shown to be 30 degrees. The two parameters, d1 and p1 (30° pulse is defined as  $0.3 \cdot p1$  in the pulse sequence), correspond to the length of the recycle delay, and the length of the 90° RF pulse, respectively



In most cases, d1 is typically a few seconds while p1 is typically a few microseconds in length.

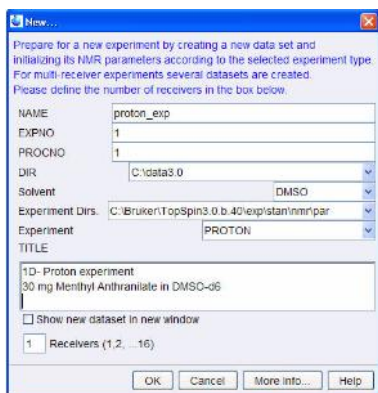
### Experiment setup

1. Click on the 'Start' tab in the TopSpin Menu bar



2. Select "Create Dataset" by clicking on it.





3. Enter the name you want for your file in the “Name” field and any notes about your sample/experiment on the “Title” field.

4. Click on Ok

5. Put your sample on a spinner and adjust depth using sample depth gauge. Make sure the sample depth gauge is set for 3-10 mm tubes.

6. Place the sample and spinner on one of the available slots on the sample changer and note the slot number.

7. Insert your sample into the magnet by issuing the command **sx #** where # is the slot number where your sample is at. For example, if your sample is in position 6, you will type **sx 6**.

8. Select parameter set for 1H NMR experiment by typing **rpar PROTON all**.

9. Read probe/solvent dependent parameters using the command **getprosol**.

10. Type **lock** and select your solvent from the list of solvent and click OK to lock the field.



11. Tune the probe. If you are using the Nanobay or Prodigy-500, type **atma** to perform automatic tuning and matching. If you are using the AV-3, follow the instruction for manual tuning on page 5.

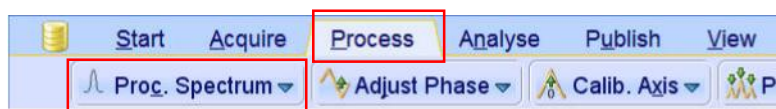
12. Shim the magnet using the command **topshim**

13. Executes the command **rga** to adjust the receiver gain.

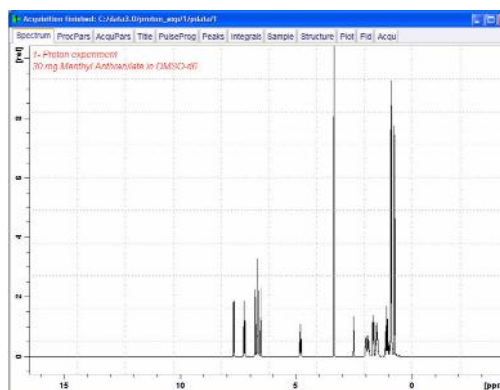
14. Executes the command **zg** to start acquisition.

## Data Processing

Click on the ‘Process’ tab in the TopSpin Menu bar and then click on “Proc. Spectrum” tab.



This executes a processing program including commands such as an exponential window function ‘em’, Fourier transformation ‘ft’, an automatic phase correction ‘apk’ and a baseline correction ‘abs’. Other options are available by clicking on the down arrow inside the ‘Proc. Spectrum’ button.



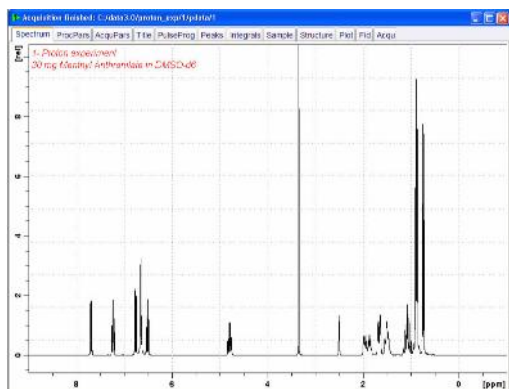
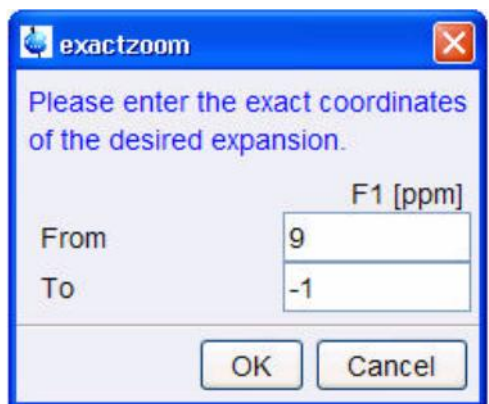
## Optimizing the Spectral width

To display a specific region of your spectrum hold the left mouse key and drag your mouse over the region of interest. If you want an exact chemical shift range, click on the “Exact Zoom” tab (shown below) and specify the range you wish to display.



Exact Zoom Tab

For example, if you want to display the region of your spectral window from 9 ppm to -1 ppm put 9 and -1 in the **From** and **To** fields and click OK and the selected region will be displayed.



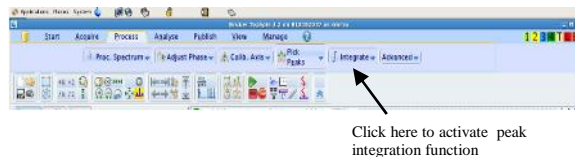
If you want collect the spectrum with just the region of interest, you can click on the sw selection button as shown below.



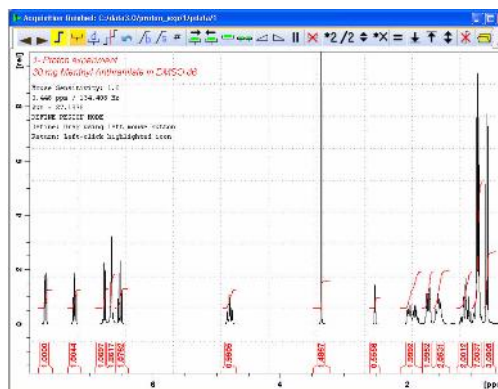
With your region of interest displayed, click on the button shown above to set sw and o1p.

Rerun your experiment and processed your file as described above. You can integrate your peaks using the Peak Integration button as shown below.

This enters the manual Integration mode. Other options are available by clicking on the down arrow inside the **'Integrate'** button.



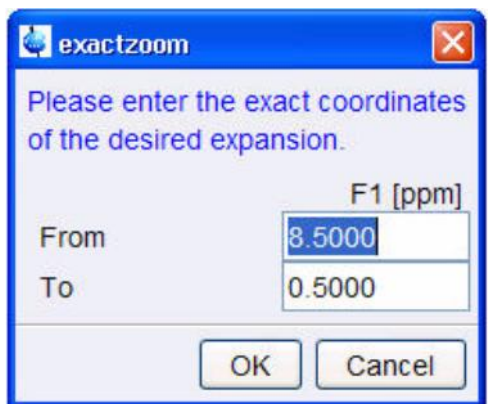
Set the cursor line, starting at the left of the spectrum, to the left of the first peak to be integrated, click the left mouse button and drag the cursor line to the right of the peak, then release the mouse button. Repeat this process to integrate all peaks of interest.



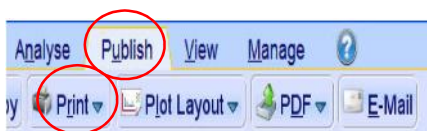
Make sure to click on to **save** button to save the integration regions.

### Plotting the 1D Proton spectra

1. Expand the spectrum (all peaks in display)
2. Click on the Exact Zoon Tab if you want to print a specific region.
3. Type the ppm values you want. For example, **From = 8.5 To = 0.5** then click **OK**.



You can print your spectrum to a printer by clicking on the **Print** button (click **Publish** then select Print) as shown below.



You can also export your spectrum a PDF file or image file. If you wish to do so, click on th File button and select **Export** from the options you have under the file menu. Write a file name you want on the popup window and choose an extension from the list of extension you see on the screen based on the file type you want. For expmample, if you want to save your spectrum as a png image you would do: **my\_file\_name.png**.



## Summary

The following is a procedure you can follow to collect routine NMR data using TopSpin 3.2. Read other sections of this Guide if any part of this summary is not clear.

1. Open TopSpin by clicking on the TopSpin icon in your Desktop.
2. Put your sample in a spinner and adjust its position using the one sample depth gauge in the lab. Put the sample and spinner on the sample changer (SampleXpressLite). Insert your sample into the magnet by typing *sx #* where # is the number on the sample changer where the sample is located at.
3. Create a new dataset using *edc* or *ctrl-N*.
4. Read appropriate parameter set by typing *rpar PARAMETERSET all* (eg. *rpar PROTON all* for <sup>1</sup>H NMR). Type *getprosol* to read probe/solvent dependent parameters.
5. Type *lock* and select your solvent from the list to lock the file.
6. Read a good shim file (if you have saved one) using *rsh* command then shim using *topshim*.
7. Tune the probe using the *atma* command (Nanobay and Prodigy) or manually (AVIII-3), as appropriate.
8. Type *rga* to set the receiver gain.
9. Type *zg* to start data acquisition.
10. After the acquisition has completed or after you have used the *halt* command or you typed *tr*, process the FID using the *efp* command.
11. Do manual or automatic phase correction (using *apk* command) as needed. Use *abs* to do baseline correction.
12. Print your spectrum using the Print button.
13. If you were spinning your sample, turn it off (open BSMS window and click on **Spin** button) and eject your sample using the command *sx ej*. WAIT UNTILL THE SAMPLE CHANGER DOME LIGHT CHANGES GREEN BEFORE YOU PICK UP YOUR SAMPLE.