UC Davis NMR Facility

UC DAVIS NMR FACILITY
BRUKER SHORT MANUAL
FOR TOPSPIN 3.X

VERSION 2
October, 2015

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Brief Topspin 3.2 User Guide for Bruker NMR Spectrometers
Avance III 800, Avance III 600

DISCLAIMER

This document is intended to be a brief, bare-bones user's guide for NMR data collection using the Avance-III Bruker NMR spectrometers managed by the UC Davis NMR Facility. For detailed help with both routine and advanced NMR experiments, please consult Bruker's User Guides, which can be found within the Resources tab on our website, nmr.ucdavis.edu.

CONVENSIONS

Keyboard input is shown as **boldface** type in this manual. Note that in TS the "enter" key must be used after the command is typed; this is assumed through-out this manual and "enter" key strokes are not given explicitly. Commands in TS are typed in on the TS command line near the bottom of the TS window; again this is assumed and will not generally be stated explicitly herein. LMB, MMB, and RMB are used to indicate actions of the left, middle, and right mouse button respectively. On a PC the mouse wheel acts as the MMB. Click, and double click refer to pressing the LMB.

GENERAL PROCEDURE

You will find that the general procedure for acquiring NMR data on all NMR spectrometers is essentially the same. The general procedure is as follows:

1	Sample Preparation
2	Login and Startup
2	Setup Initial Parameters
3	Insert Sample
4	Lock onto Solvent
5	Tune the Probe
6	Shim
7	Check Acquisition parameters
8	Check Receiver Gain
9	Acquire
10	Process Data
11	Remove Sample
12	Logout, Sign Logbook

Experiment: Routine Proton NMR
Instrument: 800 Medsci, 600 Medsci, 400 Chem

SAMPLE PREPARATION

1) Dissolve your sample in an appropriate deuterated NMR solvent. Make sure there is no un-dissolved material. If there is, you will need to either centrifuge or filter your sample to remove crystals/debris.

TIP: use a small amount of KimWipe in a Pasteur Pipette to quickly filter out any undissolved material

2) Transfer about 600 uL of solvent into a clean NMR tube. We recommend high-quality NMR tubes - rated 600 MHz or higher, but economy tubes will be OK for routine work at lower fields (400 MHz and below). **Your sample height should be about 5 cm.**

LOGIN AND STARTUP

1) **Log In:** Log into your user account.
   a) User ID: ad3\KerberosID
   b) password: YourKerberosPassword

NOTE: If you are using the instrument for the first time, you may need to do a couple things to set up your Topspin account properly. See Setting up your account for details.

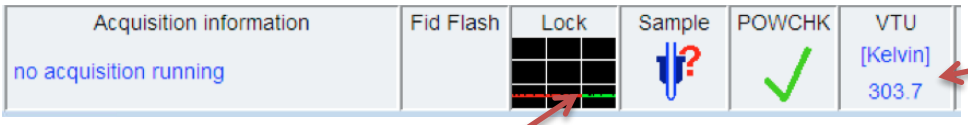
2) **Launch Topspin:** Launch Topspin 3.2 (or 3.5) software using the icon on the desktop

3) **Navigate to your data directory** in the file browser on the left. Example file tree: C:\Bruker\Topspin3.2\data\UserName

4) **Open the Lock Panel:** Double-click on the Lock panel to launch the Lock display, or type **lockdisp** on the command line

NOTE: Reading in a standard shim set (step 5) is not always necessary. Usually you can get away with skipping this step, but if you end up having trouble locking or shimming, try reading in the standard shim set

5) **Read shims:** Read in an optimal shim set by using the Read Shims command **rsh**. On the Topspin command line, type in **rsh bbo** or **rsh cptci** depending on the probe you are using (see table below). The NMR Facility staff will constantly update the **bbo** and **cptci** shim files.

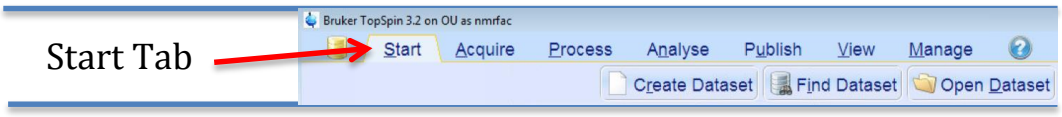


Temperature Control
edte

Lock Panel
lockdisp

Standard Shim Files:

800 MHz Medsci:	cptci
600 MHz Medsci:	cptci
500 MHz Medsci:	bbo
400 MHz Chemistry:	bbo



CREATE DATASET / SET INITIAL PARAMETERS

IMPORTANT: There are two main philosophies on how to set up your initial parameters. 1 – Copy parameters from an old data set into a new one, or 2 – Read in a generic parameter set. Both methods are described below.

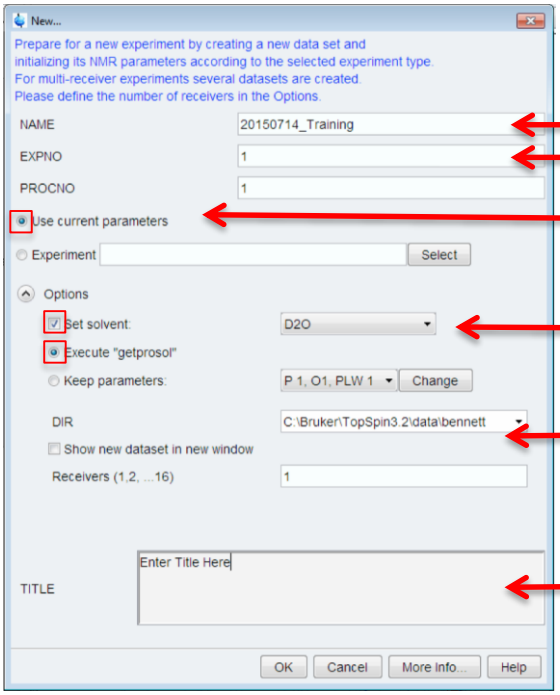
Option 1: Copy Parameters From an Old Data Set (Suggested Option)

1) Use the file browser on the left to load an old experiment into the workspace. For example, double click on your most recent Proton NMR experiment. Make sure the spectrum has loaded into the viewing window.
2) From this old experiment, create a new experiment by typing **edc** or **new** on the command line, or by hitting the Create Dataset button. Make sure the Options tab is opened to display all setup options
3) Hit OK. You now have initial Proton acquisition parameters, which are identical to the experiment from which you copied them. To check and edit these parameters, type **ased** on the command line

TIP: If you use the naming format YYYYMMDD_SampleInfo, your file tree will be organized by date. Many users find this very helpful!

Create a new data set using **edc** or using Create Dataset button under the Start tab.

When you type **edc**, you are copying the major experimental parameters from an old experiment and pasting into a new data set.



Provide new experiment name
Experiment number

Select Use Current Parameters

Set Solvent (select from drop down)
Select Execute "getprosol"

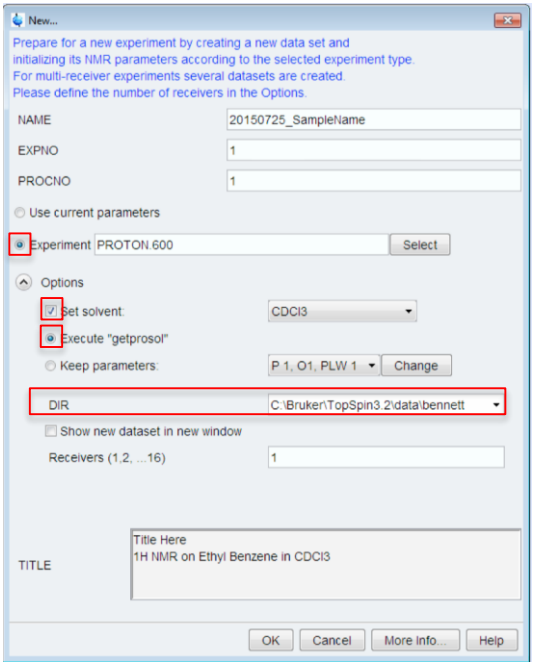
Make sure you are using your data directory!

Enter a title here. You can change the title information at any time by typing **edti** on the command line.

4) If you are not satisfied with your parameters, or unsure if your previous acquisition and processing parameters were adequate, you can always read in a generic Proton parameter set after you have created your dataset with steps 1-3. To read in routine Proton parameters, see Using Parameter Sets on the next page.

Option 2: Use a Standard Parameter Set

1) Type **new** on the command line, or select the Create New Dataset button under the Start tab.
2) Provide experiment name. Common example: YYYYMMDD_SampleInfo. Do not use spaces or special characters.
3) Enter experiment number. Often users start with EXPNO 1
4) Select "Experiment" option, and enter the following in the experiment selector
    a. For 800 MHz, enter PROTON.800
    b. For 600 MHz, enter PROTON.600
    c. For 500 MHz, enter PROTON.500
    d. For 400 MHz, enter PROTON.400
5) Under Options tab, set your solvent
6) Select execute "getprosol" option
7) Make sure you are in your user directory. The DIR should look like **C:\Bruker\Topspin3.2\data\username**
8) Enter a title. There are no restrictions here, type whatever you want.
9) Hit OK. You now have initial parameters from a generic Proton parameter set



TIP: **edc** will remember your last choice. For example, if you loaded PROTON.800 experiment in the **edc** window, this same choice will be selected if you use **edc** again to create another experiment. In many cases, you will want to select "Use current parameters" instead of "Experiment".

USING PARAMETER SETS

You can also load standard parameter sets for initial acquisition parameters. For a standard Proton experiment, load parameters by using the **rpar** command:

rpar PROTON.800 all; getprosol	Instrument: 800 MHz
rpar PROTON.600 all; getprosol	Instrument: 600 MHz
rpar PROTON.500 all; getprosol	Instrument: 500 MHz
rpar PROTON.400 all; getprosol	Instrument: 400 MHz

INSERT YOUR SAMPLE

1) Place your sample into the blue spinner
2) Adjust the sample height using the depth gauge
3) Remove fingerprints and debris from the NMR tube using a Kimwipe
4) Remove the protective black cap from the magnet, if present
5) Type **ej** on the command line to start the eject gas flow
6) Carefully place your sample into the magnet. It should float on a bed of air
7) Type **ij** to insert your sample. Wait until the spinner icon on the bottom menu indicates that your sample has been inserted correctly.

LOCK, TUNE, SHIM

1) LOCK: Type **lock** on the command line or hit the Lock button, then select your solvent from the list.

2) TUNE: Type **atma** to automatically tune and match the Proton channel, or type **atmm** to control the match and tune stepper motors manually. If you use **atmm**, be sure to save the probe position by selecting File – Save Position, and then File – Exit.

CAUTION: **atma** or **atmm** will only tune the nucleus that is open from your current experiment. Example, if you have a 1H experiment loaded, only the 1H channel will be tuned. Don't forget to tune 13C if you plan to do a 13C experiment later!

3) SHIM: The shimming procedure varies slightly depending on the instrument and solvent you are using. From the command line enter the indicated command depending on the instrument and solvent:

Instrument	Solvent Type	Topshim Command
800	Organic	topshim tuneb convcomp
800	Aqueous	topshim tuneb
600	Organic	topshim tuneb convcomp ordmax=4
600	Aqueous	topshim tuneb ordmax=4
400	Organic	topshim tuneb

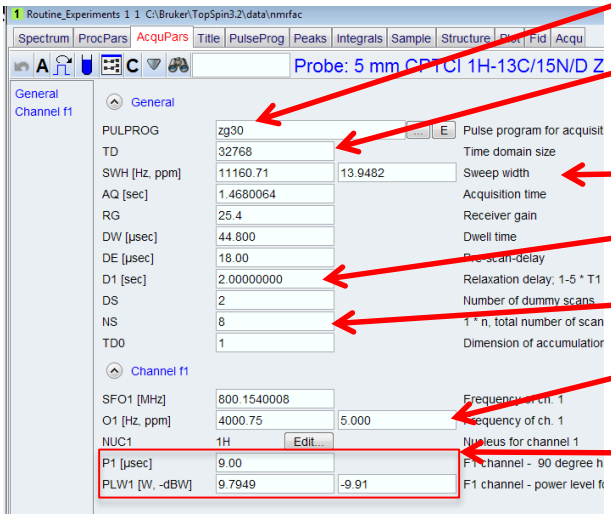
NOTE: On the 800, **topshim** seems to miss the Z1 shim by about 15-20 units when the solvent is either H2O/D2O or D2O. For this reason, for optimal lineshape results on aqueous samples you may have to first perform **topshim**, and then manually add 15-20 units to the Z1 shim using the BSMS panel.

When performing 2H gradient shimming on the 600, it is advised to use the ordmax=4 option, because topshim usually misses Z5, resulting in a broad hump near the base of your peaks. If you have a 90/10 H2O/D2O sample, you do not need to use this option.

CHECK ACQUISITION PARAMETERS

1) Type **ased** on the command line. This will display the important acquisition parameters in a condensed table. To access all of the acquisition parameters, type **eda** on the command line.

2) Modify acquisition parameters if desired.



Pulse Program. zg30 means 30° hard pulse, and acquire

Number of points collected. Usually 32k or 64k

Sweep Width, in Hz and in ppm

Relaxation Delay, usually 2 seconds for zg30

Number of scans

Carrier Frequency in ppm. Center of your spectrum

90° pulse length and power. The **getprosol** command will populate these values for you.

You can also use the command **pulsecal**, which will find the accurate 90° pulse

CHECK RECEIVER GAIN

1) Type **rga** on the command line to automatically set the receiver gain, or hit the Gain button

ACQUIRE YOUR DATA

1) Type **zg** on the command line, or select the Go button.

Process Tab

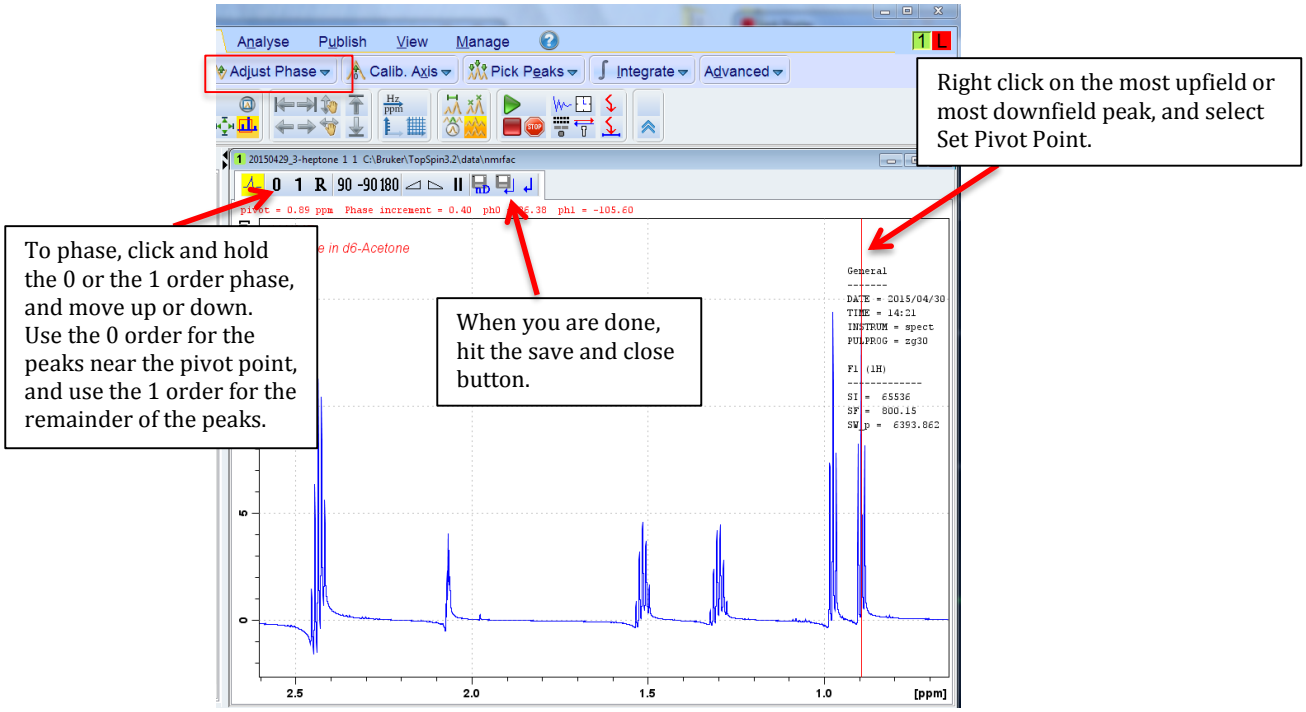
INITIAL DATA PROCESSING

1) Navigate to the Process tab
2) Perform a weighted Fourier transform by typing **efp** on the command line. Check the exponential weighting factor by typing **lb.** Typical weighting factors for 1H NMR are 0 to 0.3 Hz.
3) Perform automatic phasing of your data by typing **apk** on the command line
4) View your full spectral window by typing **.all** on the command line, or by using the navigation buttons.

NOTE: You can also select the Proc Spectrum button, which will perform **efp**, then **apk**, and finally an automatic baseline correction **abs**. You can change the exponential weighting factor by typing **lb** into the command line. Typically, one uses 0.1 to 0.3 Hz exponential weighting.

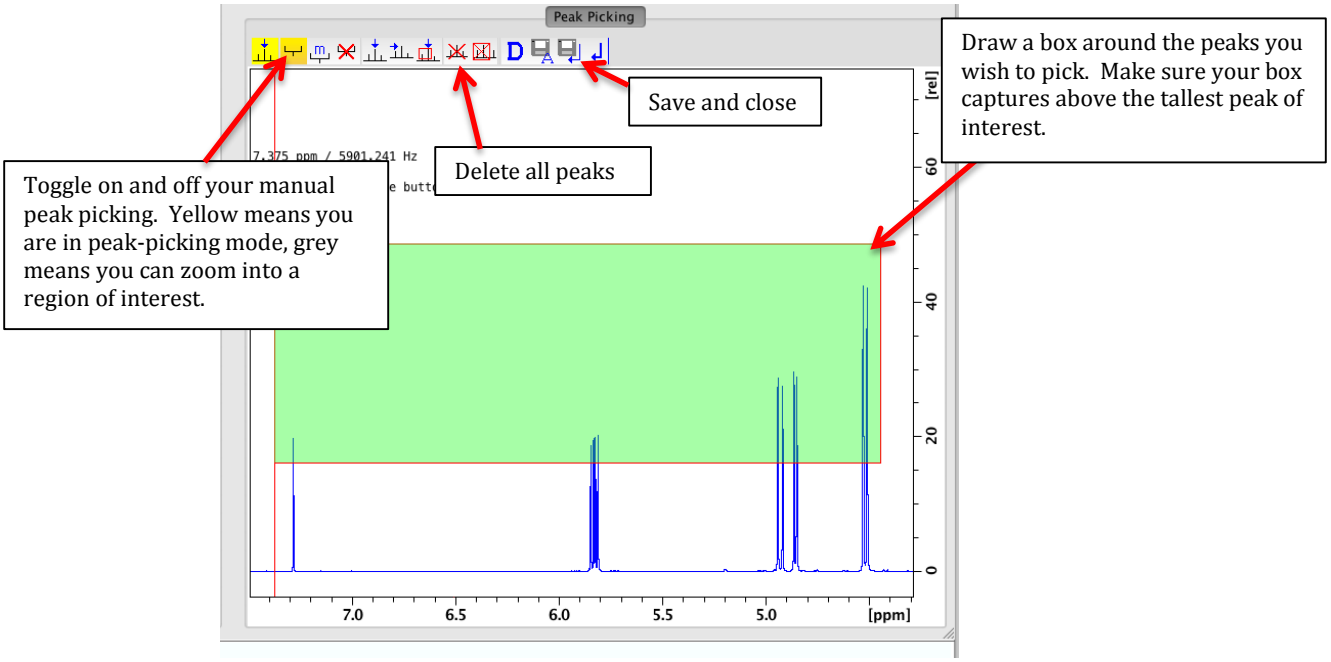
MANUAL PHASE ADJUSTMENT

1) Often times, automatic phasing will not do an adequate job. To perform automatic phasing, select the Adjust Phase button, or type **.ph** on the command line
2) Set the pivot point to either the most upfield or the most downfield resonance: right-click on the peak, and select Set Pivot Point
3) Use the 0 order to phase the peaks near the Pivot Point, and the 1 order to phase the other peaks.



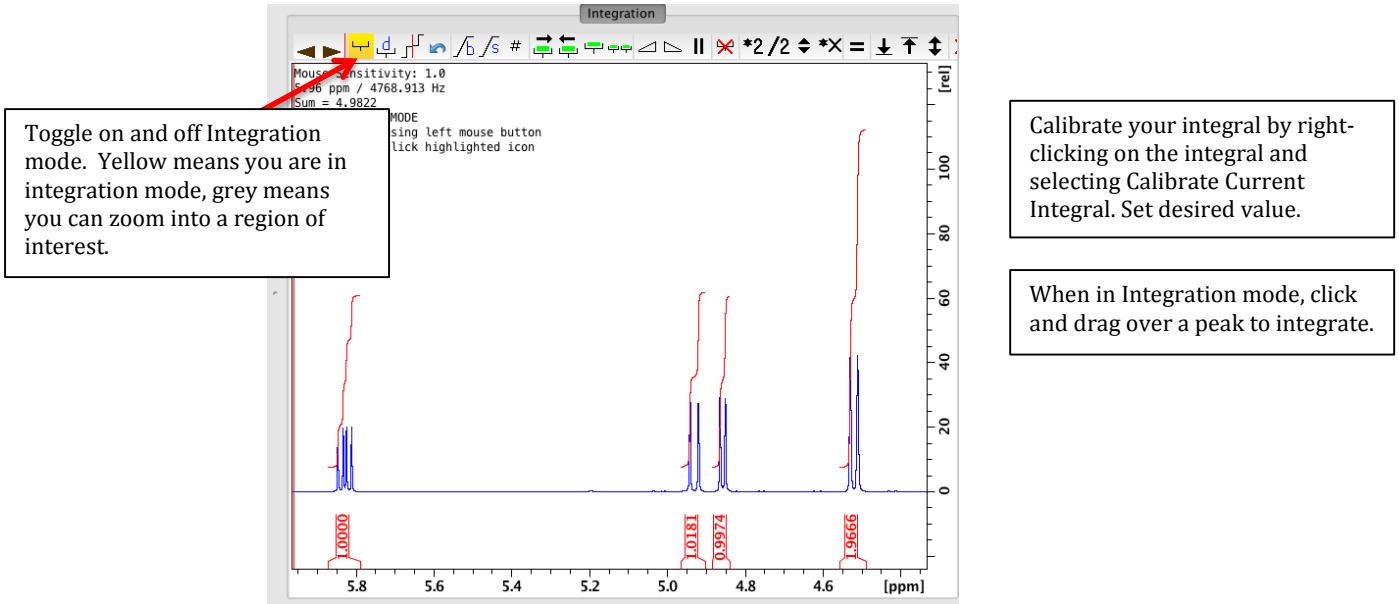
PEAK PICKING

1) Perform peak picking by typing **.pp** on the command line, or select the Peak Picking button under the Process tab.
2) Draw a box around the peaks you wish to pick. Make sure you catch the top of the peak.



INTEGRATION

1) Perform integration by typing **.int** on the command line, or select the Integrate button under the Process tab.



Exit Procedure

REMOVE YOUR SAMPLE

1) Type **ej** on the command line
2) Remove your sample, and place the black cap onto the bore of the magnet
3) Type **ij** to stop the air flow. Do not insert any blank sample
4) Place the empty spinner in the sample holder for the next user

COPY YOUR DATA TO KONA

> Before uploading your data to KONA, please first delete your processed data. Your raw data will be untouched, and you can re-process your data at any time.

1) Open up a Windows Explorer and navigate to the Shared drive, S:\\Share$
2) Open up a second Windows Explorer, and navigate to your Bruker data directory: C:\\Bruker\Topspin3.2\data\username
3) Find the experiment names in your user directory that you wish to copy to KONA
4) Copy, or drag and drop to your data to your KONA folder

LOGOUT AND SIGN LOGBOOK

1) Close Topspin 3.2 and any other applications
2) Log out of your account using the Start Menu
3) Sign the logbook
4) Fill out the billing slips.

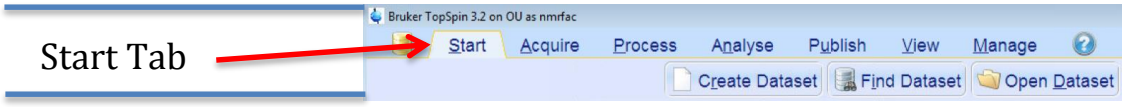
Experiment: Routine Carbon NMR
Instrument: 800 Medsci, 600 Medsci, 400 Chem

Before collecting 13C NMR, it is suggested you first collect a routine 1H experiment. Follow the procedures starting on page 2. After you have collected your Proton NMR experiment, and saved your data, you can proceed with 13C NMR setup and acquisition.

Note, for 13C NMR you will need at least 10 mg to obtain decent signal to noise in a reasonable timeframe. Dissolve your sample in an appropriate deuterated NMR solvent. Make sure there is no un-dissolved material. If there is, you will need to either centrifuge or filter your sample to remove crystals/debris.

SAMPLE PREPARATION
LOGIN AND STARTUP
INSERT YOUR SAMPLE
LOCK, SHIM

If you have not already collected a Proton NMR experiment, follow procedures in the Routine Proton guide. Make sure you have tuned 13C in addition to 1H.



CREATE DATASET / SET INITIAL PARAMETERS

1) From your Proton experiment, create a new identical experiment in EXPNO 2 by typing **edc** or **new** on the command line, or by hitting the Create Dataset button. Make sure the Options tab is opened to display all setup options. Just keep the settings the same as from when you created your Proton experiment -- your goal here is simply to create a place for your data, not to load any parameters.
2) Load standard CARBON acquisition and processing parameters by loading the relevant parameter set and executing getprosol:

CARBON-13 PARAMETER SETS	
rpar CARBON.800 all; getprosol	Instrument: 800 MHz
rpar CARBON.600 all; getprosol	Instrument: 600 MHz
rpar CARBON.500 all; getprosol	Instrument: 500 MHz
rpar CARBON.400 all; getprosol	Instrument: 400 MHz

TUNE

1) You previously tuned the 1H coil, but not the 13C coil. Type **atma** or **atmm** on the command line. Note, when you use **atma** or **atmm**, Topspin knows which nuclei you are using based on your acquisition parameters. For example, when the CARBON experiment is loaded, **atma** will tune both 1H and 13C coils.

CHECK ACQUISITION PARAMETERS

2) Type **ased** on the command line to check the condensed version of your acquisition parameters. Pay attention to the following parameters:

Parameter	Description	Suggested Value
ns	Number of scans	512 is default. Depends on your sample concentration
ds	Dummy scans	4
sw	Spectral Width	240 ppm
td	Number of acquired points	32k
d1	relaxation delay	1 second for most cases
o1p	13C Offset Frequency	110 ppm, or center of your spectrum
o2p	1H Decoupler Offset Freq	5 ppm, or center of your Proton spectrum

3) Check your experiment time by typing **expt** on the command line, or by hitting the Time button. Adjust your acquisition parameters if necessary 

ACQUIRE YOUR DATA

1) To start data acquisition, type **zg** on the command line, or hit Go.

   Note, you can perform a Fourier transform during acquisition so that you don't have to wait until the experiment has completed to observe your data. To perform a Fourier transform while your data is acquiring, you must first transfer the FID so that it can be processed. Type **tr** on the command line. After a couple additional scans your data will be transferred and ready for processing. Type **efp** to view your transferred data.

2) If you are satisfied with your signal to noise before your experiment has completed, you can stop data acquisition by typing **stop** (more harsh) or **halt** (less harsh) on the command line, or by hitting the Stop or Halt buttons. If you use **halt**, you can continue acquisition later.

Process Tab

INITIAL DATA PROCESSING

1) Check your SI (number of points used during Fourier transform) by typing **si** on the command line. Make sure that **si** is at least the number of td points, in this case 32k.
2) Check your line broadening function for the Fourier transform by typing **lb** on the command line. Set **lb** to 0.5 or 1 Hz.
3) Perform Weighted Fourier transform by typing **efp** on the command line
4) Perform automatic phasing of your data by typing **apk** on the command line
5) View your full spectral window by typing **.all** on the command line, or by using the navigation buttons.

MANUAL PHASE ADJUSTMENT

Often, **apk** does not do an adequate job with phasing your data, especially if there is probe ring-down or background signal (evident as a broad hump in your 13C spectrum). You can manually adjust the phasing of your C13 spectrum in the same manor as for H1 phase adjustment. See Manual Phase Adjustment section in the Proton guide for details. Briefly, first phase the upfield peaks using ph **0**, then phase the downfield peaks using ph **1**. You may need to iterate back and fourth between **0** and **1** for best results.

PEAK PICKING

Perform 13C Peak Picking in the same manor as for your 1H experiment. See Peak Picking in the Proton guide for details.

PLOTTING

To create a hard plot, navigate to the Publish tab. Here you can print a paper copy by hitting the Plot button, or electronic PDF copy with the PDF button.

SAVE YOUR DATA
TRANSFER YOUR DATA TO KONA
REMOVE YOUR SAMPLE
LOGOUT AND SIGN LOGBOOK

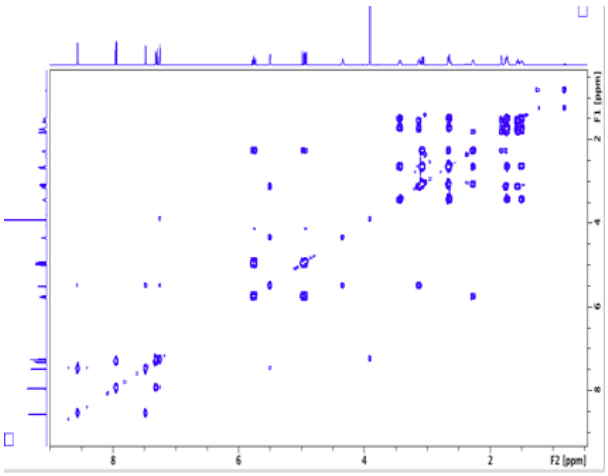
Follow instructions in the Routine Proton user guide for how to save and archive your data, and for proper exit procedures.

Experiment:	Routine 2D NMR Experiments: COSY, HSQC, HMBC, H2BC
Instrument:	Bruker 800 and 600 (MedSci), Bruker 400 (Chem)

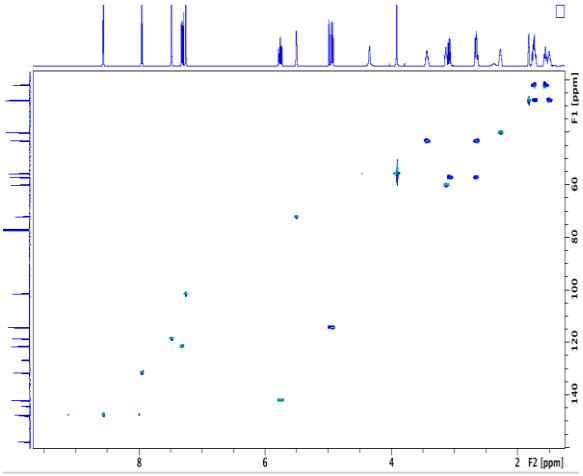
Before you start:

Tuning:
It is extremely important that you tune and match the probe for all nuclei used in your experiments before acquiring any 2D NMR experiment. These 2D pulse programs rely on accurate 90 and 180 pulses, and if the probehead is not tuned for your specific sample, these pulses will not be correct. One of the most common mistakes is for users to first load a Proton experiment, correctly lock, tune, shim, acquire, and then load a Carbon or 2D experiment and forget to tune the 13C channel. Be sure to execute **atmm** or **atma** after you have loaded any experiment using the X-channel,

90 Pulses:
In most cases, assuming you have tuned the probe, the default pulselengths will work for your sample. You can make sure you have loaded the default pulselengths and powers by executing the command **getprosol**. However, sometimes even if the probe is tuned properly, the default pulse lengths will not be accurate. Thus, you may benefit from identifying accurate 90 and 180 pulselengths before data acquisition. The most efficient way to calibrate pulselengths specifically for your sample is to execute the command **pulsecal.** The **pulsecal** command will automatically determine your 90 and 180 pulselengths, and then input them as acquisition parameters in your NMR experiment. This procedure typically only takes about 30 seconds, so it is highly recommended!

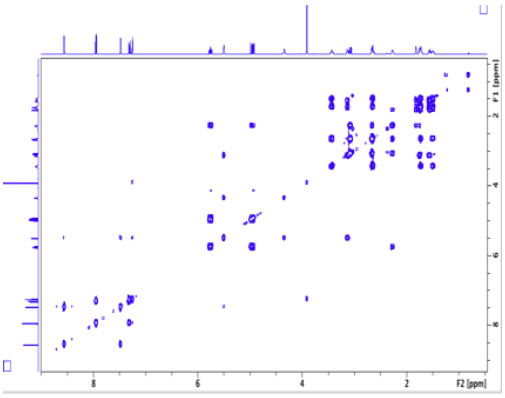


COSY



HSQC

Experiment: (HH) COSY:



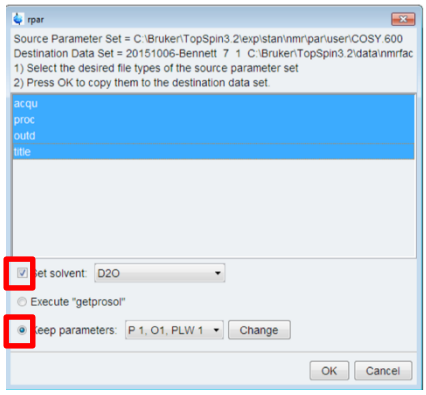
Gained Information: The COrrelation SpectroscopY (COSY) NMR experiment is a proton-detected 2D experiment that shows you protons that are J-coupled. You will see your 1H spectrum on both axis, and you will see a cross peak for any protons that are J-coupled.

Acquisition Procedure:

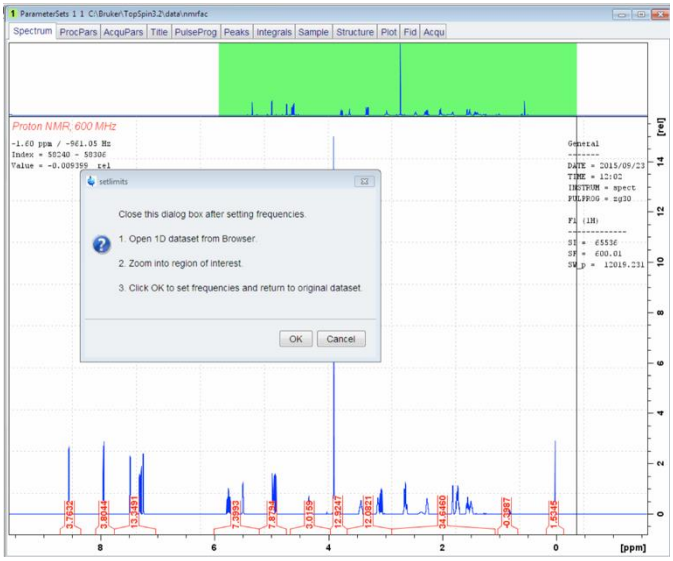
1. Set up a routine Proton NMR experiment. Be sure to lock, tune the probe, shim.
2. Calibrate your 90 and 180 pulses
   - Type **pulsecal** in the command line. This will find and set your 90 and 180 pulses for your current sample. This process takes about 30 seconds
   - A window will pop up with your **pulsecal** results. Make note of your 90 pulse and power level. For example, 90 pulse 8.59 microseconds at power level -9.91 dB (800 MHz)
3. Acquire your routine 1D Proton spectrum.
   - Process your data as normal, and inspect if you need to change any acquisition parameters. If you made any changes, re-acquire your Proton spectrum and re-process your data.
4. Load Gradient COSY experiment
   - Create a new experiment using **edc** or **iexpno.** If using **edc**, select the "use current parameters" and "Keep parameters" options. You simply want to copy your Proton parameters into a new experiment

   - Read in COSY parameters by loading the relevant parameter set using the **rpar** command
   - Select the "Keep parameters" option. This way your pulselengths will be correct, assuming you executed **pulsecal** in step 2.

800 Avance III Medsci	**rpar COSY.800**
600 Avance III Messci	**rpar COSY.600**
400 Avance III Chem	**rpar COSY.400**



5. Set your spectral width using Set Limits (Acquire tab)



Using SetLimits:

Step 1: Hit the SetLimits button under the Acquire tab

Step 2: Load your 1D Proton spectrum. Leave the dialog box open for now

Step 3: Click and drag to zoom into region of interest

Step 4: Hit OK. Your spectral width and carrier frequency in both your F2 and F1 Proton dimensions should now be set based on your selection

6. Check the following acquisition parameters. You can see these parameters under AcqPars. Use the command **eda** to see all acquisition parameters, or **ased** to see condensed acquisition parameters. You can also edit each parameter manually by typing the parameter into the command line.

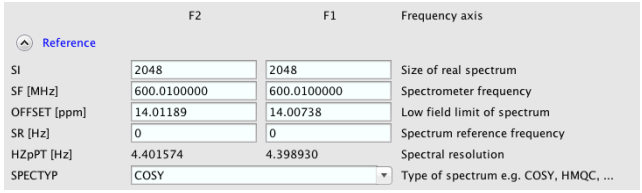
Name	Parameter	Suggested Value
Spectral Width	**sw**	14 ppm, or set with Set Limits
Offset Frequency	**o1p**	Center of spectrum (use Set Limits)
Number of Points	**td**	2048 in F2, 256 points in F1. Check your acquisition time **aq**, shoot for about 0.2 sec
Acquisition time	**aq**	Change number of points in F2 so that **aq** in F2 is about 0.2 seconds.
Relaxation Delay	**d1**	1.5 or 2 seconds
Number of Scans	**ns**	minimum 1 scan. Depends on your sample concentration
Dummy Scans	**ds**	For best results use 16 dummy scans

- Check your acquisition time by typing **expt** on the command line, and edit your acquisition parameters if needed.

7. Acquire your data
   - Type **rga** on the command line or hit the Gain button to automatically set your receiver gain
   - Type **zg** on the command line, or hit the Go button to begin data acquisition

Processing Procedure:

1. Check your processing parameters under ProcPar: type **edp** to edit processing parameters

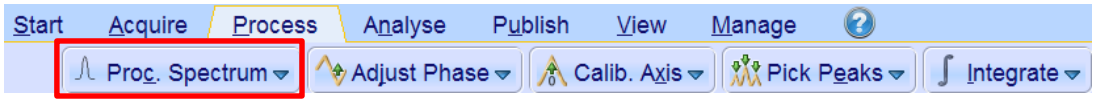


	F2	F1	Frequency axis
Reference			
SI	2048	2048	Size of real spectrum
SF [MHz]	600.0100000	600.0100000	Spectrometer frequency
OFFSET [ppm]	14.01189	14.00738	Low field limit of spectrum
SR [Hz]	0	0	Spectrum reference frequency
HZpPT [Hz]	4.401574	4.398930	Spectral resolution
SPECTYP	COSY		Type of spectrum e.g. COSY, HMQC, ...

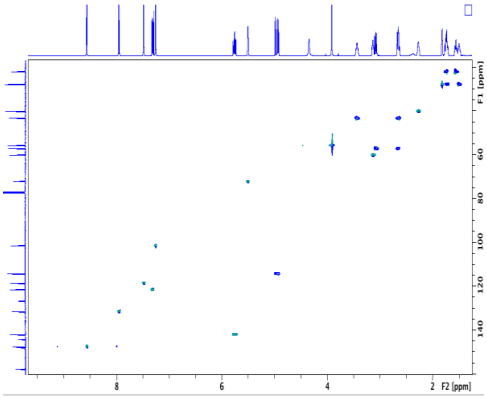
Processing Parameters
Set number of points **si** based on your number of points used in acquisition **td**. For example, if you acquired 2048 in F2 and 256 points in F1, then set SI to 2048 and 2048. You should always process using binary numbers (ie, 512, 1024, 2048, 4096, etc)

Set SPECTYP to COSY

2. Under the Process tab hit the Proc Spectrum button, or type **xfb** on the command line



Experiment: (HC) HSQC:



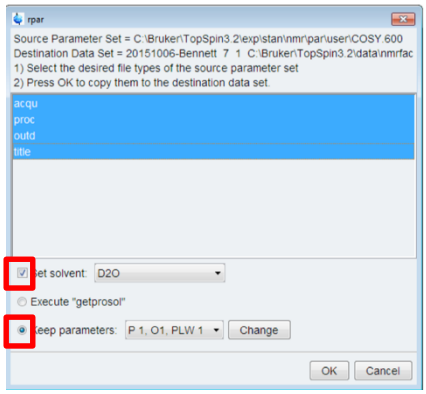
Gained Information: The Heteronuclear Single Quantum Correlation (HSQC) NMR experiment is a proton-detected 2D experiment that shows you 1H / 13C connectivity. You will see your 1H spectrum as your F2 axis and your 13C spectrum as your F1 axis, and you will see a cross peak for any 1H connected to a 13C that is one bond away. The HSQC is often faster to acquire and more informative than the 1D 13C DEPT experiment, and the same information is obtained.

Acquisition Procedure:

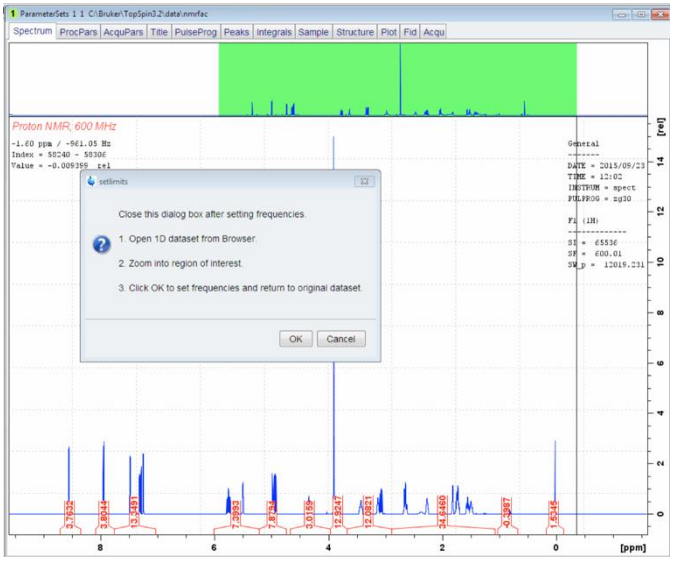
1. Set up a routine Proton NMR experiment. Be sure to lock, tune the probe, shim.
2. Calibrate your 90 and 180 pulses
   - Type **pulsecal** in the command line. This will find and set your 90 and 180 pulses for your current sample. This process takes about 30 seconds
   - A window will pop up with your **pulsecal** results. Make note of your 90 pulse and power level.
3. Acquire your routine 1D Proton spectrum.
   - Process your data as normal, and inspect if you need to change any acquisition parameters. If you made any changes, re-acquire your Proton spectrum and re-process your data.
4. Setup and Acquire a Routine Carbon experiment. Be sure to tune the 13C coil
   - Create a new experiment using edc or iexpno, and acquire 13C data as normal.
5. Load the HSQC experiment parameters
   - Load your 1D Proton experiment into the workspace (type **re 1** if your 1H spectrum is in expno 1)
   - Create a new experiment using **edc.** Select the "use current parameters" and "Keep

     parameters" options. You simply want to copy your Proton parameters into a new experiment
   - Read in HSQC parameters by loading the relevant parameter set using the **rpar** command
   - Select the "Keep parameters" option. This way your pulselengths will be correct, assuming you executed **pulsecal** in step 2.

800 Avance III Medsci	**rpar HSQC.800**
600 Avance III Messci	**rpar HSQC.600**
400 Avance III Chem	**rpar HSQC.400**



6. Set your 1H and 13C spectral widths using Set Limits (Acquire tab)



Using SetLimits:

Step 1: Hit the SetLimits button under the Acquire tab

Step 2: Load your 1D Proton spectrum. Leave the dialog box open for now

Step 3: Click and drag to zoom into region of interest

Step 4: Hit OK. Your spectral width and carrier frequency in both your direct dimension (F2) is now set based on your selection

Step 5: Repeat steps 1 – 4 but this time load your 1D Carbon spectrum. This will set your F1 dimension based on your selection.

7. Check the following acquisition parameters. You can see these parameters under AcqPars. Use the command **eda** to see all acquisition parameters, or **ased** to see condensed acquisition parameters. You can also edit each parameter manually by typing the parameter into the command line.

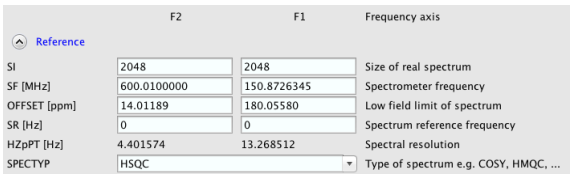
Name	Parameter	Suggested Value
Spectral Width	**sw**	14 ppm in F2 and 200 ppm in F1, or set with Set Limits
Offset Frequency	**o1p, o2p**	Center of spectrum (use Set Limits)
Number of Points	**td**	2048 in F2, 400 points in F1. Check your acquisition time **aq**, shoot for about 0.2 sec
Acquisition time	**aq**	Change number of points in F2 so that **aq** in F2 is about 0.2 seconds.
Relaxation Delay	**d1**	1.5 seconds
Number of Scans	**ns**	minimum 1 scan. Depends on your sample concentration
Dummy Scans	**ds**	For best results use 16 or 32 dummy scans

- Check your acquisition time by typing **expt** on the command line, and edit your acquisition parameters if needed.

8. Acquire your data
   - Type **rga** on the command line or hit the Gain button to automatically set your receiver gain
   - Type **zg** on the command line, or hit the Go button to begin data acquisition

Processing Procedure:

1. Check your processing parameters under ProcPar: type **edp** to edit processing parameters

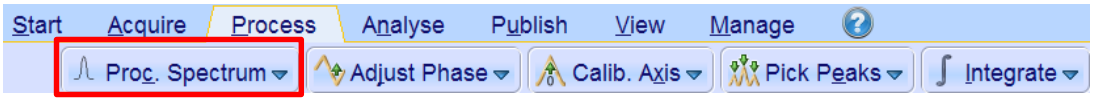


	F2	F1	Frequency axis
Reference			
SI	2048	2048	Size of real spectrum
SF [MHz]	600.0100000	150.8726345	Spectrometer frequency
OFFSET [ppm]	14.01189	180.05580	Low field limit of spectrum
SR [Hz]	0	0	Spectrum reference frequency
HZpPT [Hz]	4.401574	13.268512	Spectral resolution
SPECTYP	HSQC		Type of spectrum e.g. COSY, HMQC, ...

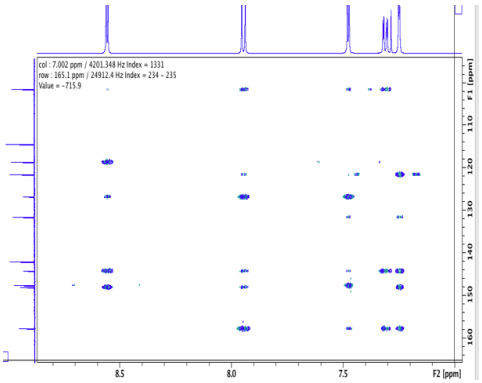
Processing Parameters
Set number of points **si** based on your number of points used in acquisition **td**. For example, if you acquired 2048 in F2 and 400 points in F1, then set SI to 2048 and 2048. You should use the next highest binary numbers (ie, 512, 1024, 2048, 4096, etc)

Set SPECTYP to HSQC

2. Under the Process tab hit the Proc Spectrum button, or type **xfb** on the command line
3. For advanced 2D Data processing, including phasing and referencing 2D data, see the Advanced Processing guide.



Experiment: (HC) HMBC:



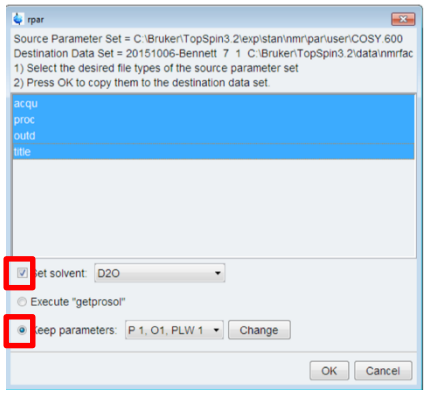
Gained Information: The Heteronuclear Multiple Bond Correlation (HMBC) NMR experiment is a proton-detected 2D experiment that shows you 1H / 13C multiple-bond connectivity. You will see your 1H spectrum as your F2 axis and your 13C spectrum as your F1 axis, and you will see a cross peak for any 1H / 13C pair that are multiple bonds away, typically 3 to 5 bonds.

Acquisition Procedure:

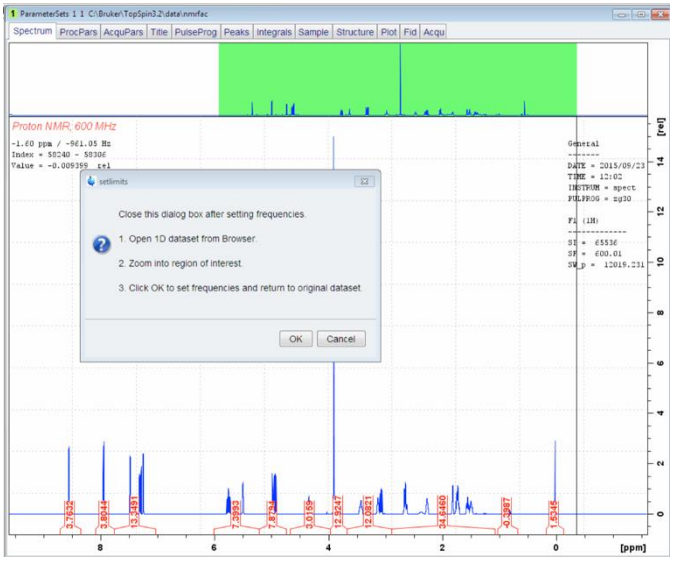
2. Set up a routine Proton NMR experiment. Be sure to lock, tune the probe, shim.
3. Calibrate your 90 and 180 pulses
    - Type **pulsecal** in the command line. This will find and set your 90 and 180 pulses for your current sample. This process takes about 30 seconds
    - A window will pop up with your **pulsecal** results. Make note of your 90 pulse and power level.
4. Acquire your routine 1D Proton spectrum.
    - Process your data as normal, and inspect if you need to change any acquisition parameters. If you made any changes, re-acquire your Proton spectrum and re-process your data.
5. Setup and Acquire a Routine Carbon experiment. Be sure to tune the 13C coil
    - Create a new experiment using **edc** or **iexpno**, and acquire 13C data as normal.
6. Load the HSQC experiment parameters
    - Load your 1D Proton experiment into the workspace (type **re 1** if your 1H spectrum is in expno 1)
    - Create a new experiment using **edc.** Select the "use current parameters" and "Keep

parameters" options. You simply want to copy your Proton parameters into a new experiment
    - Read in HMBC parameters by loading the relevant parameter set using the **rpar** command
    - Select the "Keep parameters" option. This way your pulselengths will be correct, assuming you executed **pulsecal** in step 2.

800 Avance III Medsci	**rpar HMBC.800**
600 Avance III Messci	**rpar HMBC.600**
400 Avance III Chem	**rpar HMBC.400**



7. Set your 1H and 13C spectral widths using Set Limits (Acquire tab)



Using SetLimits:

Step 1: Hit the SetLimits button under the Acquire tab

Step 2: Load your 1D Proton spectrum. Leave the dialog box open for now

Step 3: Click and drag to zoom into region of interest

Step 4: Hit OK. Your spectral width and carrier frequency in both your direct dimension (F2) is now set based on your selection

Step 5: Repeat steps 1 – 4 but this time load your 1D Carbon spectrum. This will set your F1 dimension based on your selection.

8. Check the following acquisition parameters. You can see these parameters under AcqPars. Use the command **eda** to see all acquisition parameters, or **ased** to see condensed acquisition parameters. You can also edit each parameter manually by typing the parameter into the command line.

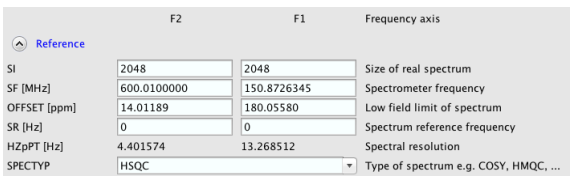
Name	Parameter	Suggested Value
Spectral Width	**sw**	14 ppm in F2 and 200 ppm in F1, or set with Set Limits
Offset Frequency	**o1p, o2p**	Center of spectrum (use Set Limits)
Number of Points	**td**	2048 in F2, 400 points in F1. Check your acquisition time **aq**, shoot for about 0.2 seconds
Acquisition time	**aq**	Change number of points in F2 so that **aq** in F2 is about 0.2 seconds.
Relaxation Delay	**d1**	1.5 seconds
Number of Scans	**ns**	4 scans if at high sample concentration (> 25 mg/mL).
Dummy Scans	**ds**	For best results use 16 or 32 dummy scans

- Check your acquisition time by typing **expt** on the command line, and edit your acquisition parameters if needed.

9. Acquire your data
   - Type **rga** on the command line or hit the Gain button to automatically set your receiver gain
   - Type **zg** on the command line, or hit the Go button to begin data acquisition

Processing Procedure:

1. Check your processing parameters under ProcPar: type **edp** to edit processing parameters

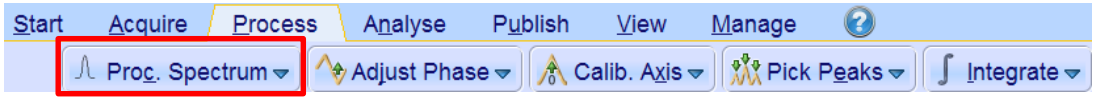


	F2	F1	Frequency axis
Reference			
SI	2048	2048	Size of real spectrum
SF [MHz]	600.0100000	150.8726345	Spectrometer frequency
OFFSET [ppm]	14.01189	180.05580	Low field limit of spectrum
SR [Hz]	0	0	Spectrum reference frequency
HZpPT [Hz]	4.401574	13.268512	Spectral resolution
SPECTYP	HSQC		Type of spectrum e.g. COSY, HMQC, ...

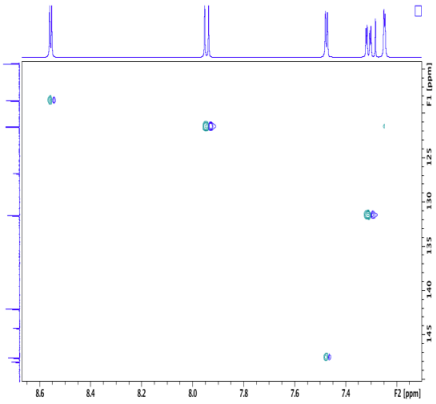
Processing Parameters
Set number of points **si** based on your number of points used in acquisition **td**. For example, if you acquired 2048 in F2 and 400 points in F1, then set SI to 2048 and 2048. You should use the next highest binary numbers (ie, 512, 1024, 2048, 4096, etc)

Set SPECTYP to HSQC

2. Under the Process tab hit the Proc Spectrum button, or type **xfb** on the command line
3. For advanced 2D Data processing, including phasing and referencing 2D data, see the Advanced Processing guide.



Experiment: (HC) H2BC:



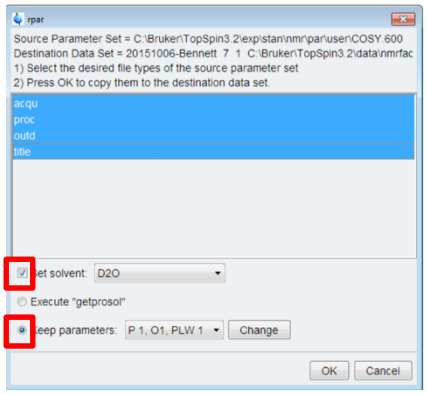
Gained Information: The Heteronuclear 2-Bond Correlation (H2BC) NMR experiment is a proton-detected 2D experiment that shows you 1H / 13C two-bond connectivity, but only for carbons with attached protons. You will see your 1H spectrum as your F2 axis and your 13C spectrum as your F1 axis, and you will see a cross peak for any 1H / 13C pair that are separated by two bonds if the carbon also has attached protons. This experiment often clears up ambiguity in the HMBC data

Acquisition Procedure:

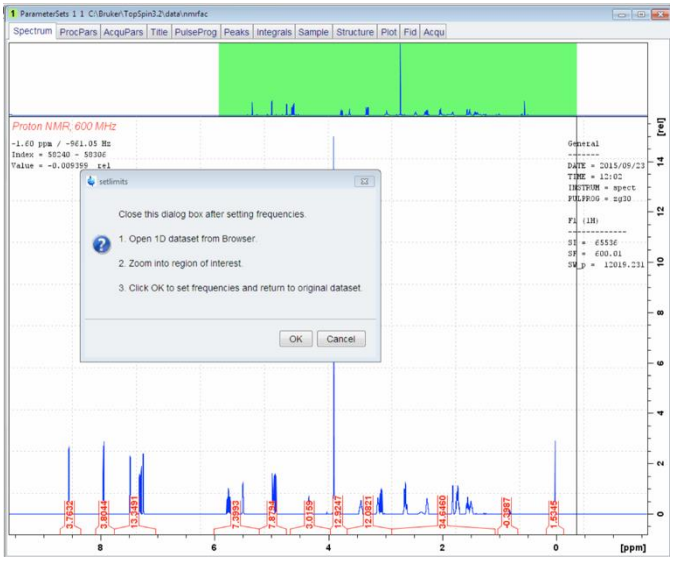
1. Set up a routine Proton NMR experiment. Be sure to lock, tune the probe, shim.
2. Calibrate your 90 and 180 pulses
   - Type **pulsecal** in the command line. This will find and set your 90 and 180 pulses for your current sample. This process takes about 30 seconds
   - A window will pop up with your **pulsecal** results. Make note of your 90 pulse and power level.
3. Acquire your routine 1D Proton spectrum.
   - Process your data as normal, and inspect if you need to change any acquisition parameters. If you made any changes, re-acquire your Proton spectrum and re-process your data.
4. Setup and Acquire a Routine Carbon experiment. Be sure to tune the 13C coil
   - Create a new experiment using **edc** or **iexpno**, and acquire 13C data as normal.
5. Load the H2BC experiment parameters
   - Load your 1D Proton experiment into the workspace (double click in the file browser, or type **re 1** on the command line if your 1H spectrum is in expno 1)
   - Create a new experiment using **edc.** Select the "use current parameters" and "Keep

   parameters" options. You simply want to copy your Proton parameters into a new experiment
   - Read in H2BC parameters by loading the relevant parameter set using the **rpar** command
   - Select the "Keep parameters" option. This way your pulselengths will be correct, assuming you executed **pulsecal** in step 2.

800 Avance III Medsci	**rpar H2BC.800**
600 Avance III Messci	**rpar H2BC.600**
400 Avance III Chem	**rpar H2BC.400**



6. Set your 1H and 13C spectral widths using Set Limits (Acquire tab)



Using SetLimits:

Step 1: Hit the SetLimits button under the Acquire tab

Step 2: Load your 1D Proton spectrum. Leave the dialog box open for now

Step 3: Click and drag to zoom into region of interest

Step 4: Hit OK. Your spectral width and carrier frequency in both your direct dimension (F2) is now set based on your selection

Step 5: Repeat steps 1 – 4 but this time load your 1D Carbon spectrum. This will set your F1 dimension based on your selection.

7. Check the following acquisition parameters. You can see these parameters under AcqPars. Use the command **eda** to see all acquisition parameters, or **ased** to see condensed acquisition parameters. You can also edit each parameter manually by typing the parameter into the command line.

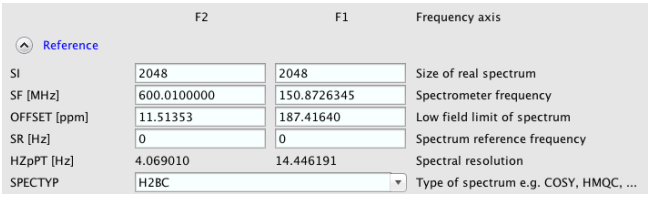
Name	Parameter	Suggested Value
Spectral Width	**sw**	14 ppm in F2 and 200 ppm in F1, or set with Set Limits
Offset Frequency	**o1p, o2p**	Center of spectrum (use Set Limits)
Number of Points	**td**	2048 in F2, 256 points in F1. Check your acquisition time **aq**, shoot for about 0.2 sec
Acquisition time	**aq**	Change number of points in F2 so that **aq** in F2 is about 0.2 seconds.
Relaxation Delay	**d1**	1.5 seconds
Number of Scans	**ns**	Minimum 2 scans, but use as many as possible in allowed time.
Dummy Scans	**ds**	For best results use 16 or 32 dummy scans

- Check your acquisition time by typing **expt** on the command line, and edit your acquisition parameters if needed.

8. Acquire your data
   - Type **rga** on the command line or hit the Gain button to automatically set your receiver gain
   - Type **zg** on the command line, or hit the Go button to begin data acquisition

Processing Procedure:

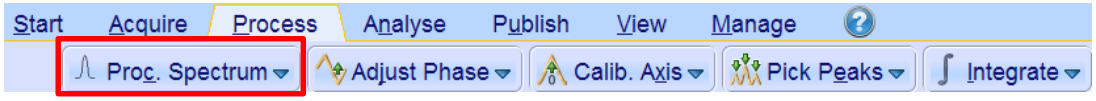
1. Check your processing parameters under ProcPar: type **edp** to edit processing parameters



Processing Parameters
Set number of points **si** based on your number of points used in acquisition **td**. For example, if you acquired 2048 in F2 and 400 points in F1, then set SI to 2048 and 2048. You should use the next highest binary numbers (ie, 512, 1024, 2048, 4096, etc)

Set SPECTYP to H2BC

2. Under the Process tab hit the Proc Spectrum button, or type **xfb** on the command line
3. For advanced 2D Data processing, including phasing and referencing 2D data, see the Advanced Processing guide.



Experiment: Advanced 1D Experiments: T1, T2, Homonuclear Decoupling, 1D NOE
Instrument: Bruker 800 and 600 (MedSci), Bruker 400 (Chem)

Experiment: Measuring 1H T1 relaxation times: T1 Inversion Recovery experiment

Gained Information: This experiment will give you T1 relaxation times for all resonances in your Proton spectrum. This information is especially important if you wish to collect quantitative NMR spectra, where you will need to wait at least 5x T1 of your slowest relaxing peak.

Acquisition Procedure:

1. Collect a routine 1H NMR spectrum. Be sure to lock, tune the probe, shim.
    - Process your 1H NMR spectrum, and make sure you can view all of your peaks. Optimize proton acquisition parameters if necessary including spectral width, number of scans, carrier frequency, and acquisition time.
2. Create a new experiment with optimized parameters from Experiment 1
    - Say you collected 1H in experiment 1. Copy these experimental parameters over to the next available experiment (experiment 2) by typing **iexpno** on the command line. Alternatively, type **edc**, enter an experiment number of choice, select use current parameters, hit OK. Experiment 2 should be identical to Experiment 1.
3. Load the T1 Inversion Recovery parameters using an **rpar** (Read Parameters)
    - Type the following into the Topspin command line, depending on which instrument you are using:

800 Avance III Medsci	**rpar T1.800**
600 Avance III Messci	**rpar T1.600**
400 Avance III Chem	**rpar T1.400**

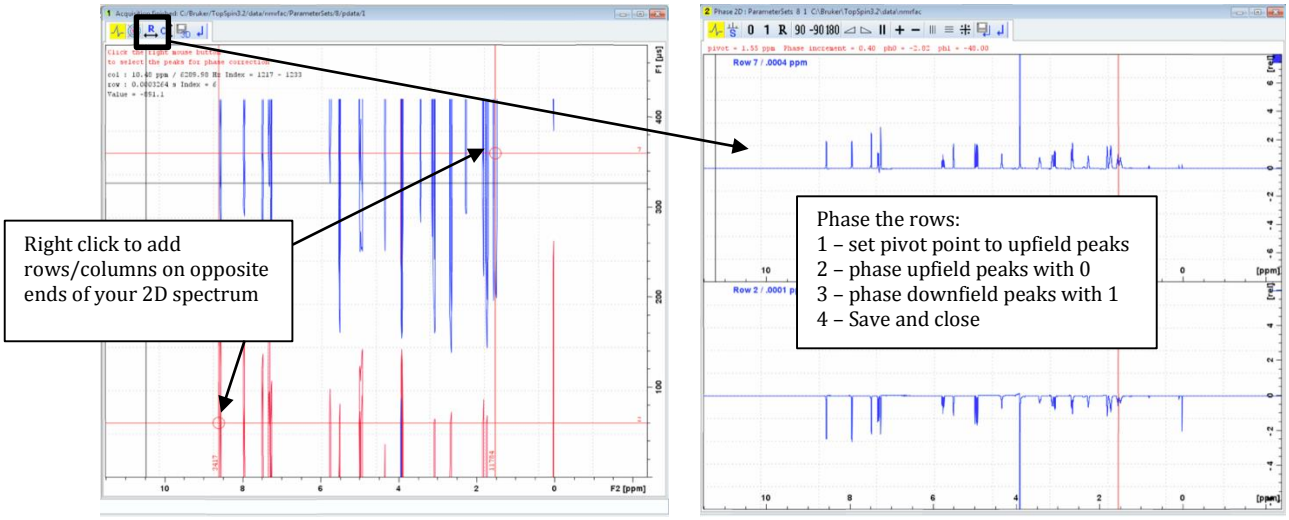
    - Select Set Solvent and Execute getprosol
4. Calibrate your 90 and 180 pulses
    - Type **pulsecal** in the command line. This will find and set your 90 and 180 pulses for your current sample. This process takes about 30 seconds, and you will see a window when it is done listing your calibrated 90 degree pulses at relevant powers.
5. Set your spectral width using the SetLimits option (See Using SetLimits in the 2D guide for help)
    - Under the Acquire tab, hit the SetLimits button
    - With the dialog box still open, navigate to and open your Proton spectrum from Step 1.
    - Click and drag to set your spectral width approximately 1 ppm on either side of your most upfield and most downfield peaks.
    - Hit OK, and you will see a message stating that your spectral width SW and your offset frequency O1P has been changed.
6. Check your acquisition paramters:
    - Type **ased** or **eda** on the command line, and check the following acquisition parameters:

Name	Parameter	Suggested Value
Spectral Width	**sw**	14 ppm, or set with Set Limits
Offset Frequency	**o1p**	Center of spectrum (use Set Limits)
Number of Points	**td**	32k in F2, 8 points in F1. Must match number of points in vdlist
Relaxation Delay	**d1**	use 5 times T1. Default is 20 seconds
Number of Scans	**ns**	Use at least 2 scans
Dummy Scans	**ds**	For best results use 4, but you can set this to 2 or 0 if you just want a crude estimate
Variable Delay List	**vdlist**	T1_8 Edit or load the delay list. Must match TD in F1.

7. Set Receiver Gain using **rga** or with the Gain button
8. Acquire your data by typing **zg** or by selecting the Go button.

Data Processing Procedure for T1 Experiments:

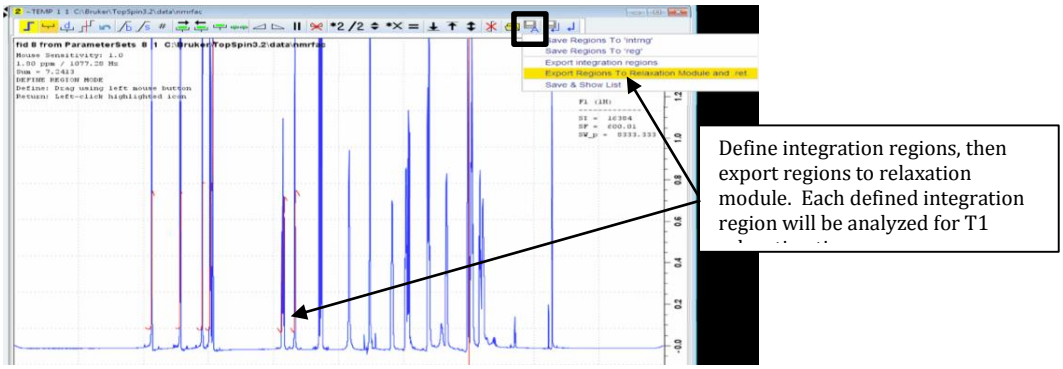
1. Navigate to the Process tab
2. Check your processing parameters (type **edp,** or select ProcPars).
    - You should set **si** equal to **td** in both F1 and F2. For example, **si** should be set to 32k in F2 and 8 in F1, matching the number of acquired points (**td**) in both dimensions. Note that even if you are not using a power of 2 for **td** in F1, you must process out to the next highest power of 2 for F1, (**si** = 8, 16, 32, 64, etc).
    - Set SPECTYP to PSEUDO2D.
3. Perform 2D Fourier Transform
    - Type **xf2** on the command line
4. Phase your 2D spectrum, and perform automatic baseline correction
    - Enter phasing mode by selecting the Adjust Phase button, or by typing **.ph**
    - Add two columns representing your first and last data points by right clicking and selecting Add. Repeat the process for another row.
    - Select the Rows icon to phase the rows 
    - Set the pivot point near the most upfield peaks, and use the Ph **0** button to phase the long **vdlist** delay times as positive peaks (protons are completely relaxed), and the shortest **vdlist** delay times as negative (protons not relaxed).
    - Phase downfield peaks using Ph **1** phase adjustment
    - When satisfied, safe and return to your 2D display.
    - Perform automatic baseline correction for 2D dataset by typing **abs2** on the cmd line



5. Perform Fourier transform of your longest variable delay spectrum
    - Type **rser #** where **#** is the longest delay in your **vdlist**. In this example, type **rser 8.** You should see a TEMP 1D data file, which is one of your acquired FIDs in your psudo2d experiment.

    - Perform normal Fourier transform and automatic phasing with **efp** and **apk**

6. Set your integration regions (use for peak area determination of T1)
    - Enter integration mode, and integrate all resonances for which you wish to calculate T1
    - Export your integral regions to the Relaxation module using Save Regions As -> Export Regions to Relaxation Module and .ret
    - Close out of this Temporary 1D mode and return to your 2D data set.



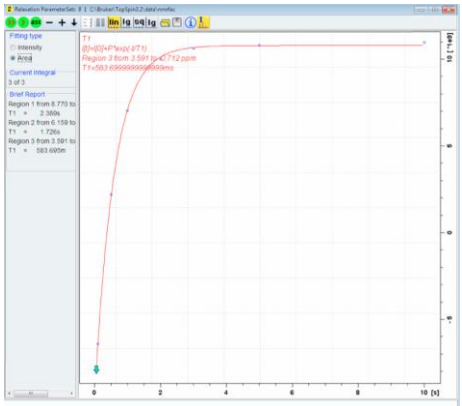
7. Pick your peaks (use for peak intensity determination of T1)
    - Repeat step 5, then enter peak picking mode
    - Use manual peak picking to select peaks you wish to analyze for T1 relaxation times.
    - Export your peak list to the Relaxation module using Save Regions As -> Export Regions and biggest peak within region to Relaxation Module and .ret
    - Close out of this Temporary 1D mode and return to your 2D data set.
8. Enter Relaxation Module for T1 Extraction
    - Navigate to the Analyze tab, select T1/T2 Module, and select T1T2

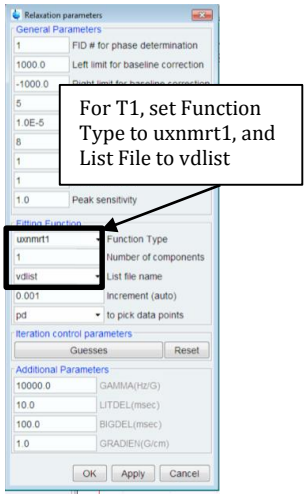
    - Select Relaxation button. Note, you have already defined ranges using the integrations or peak picking in Step 6 / Step 7.

    - Check the Settings, and select **uxnmrt1** and **vdlist** as Function Type and List Name
        - Select either Area (if you would like to use integrations) or Intensity (if you would like to use your picked peaks)

9. Fit your data and extract T1 for each region
    - Select that "Calculate Fits for All Peaks" icon.

    - Observe the results of the fit for each selected peak by cycling through your peaks using the + or – options.





Experiment: Measuring 1H T2 relaxation times: CPMG experiment

Gained Information: Measure T2 relaxation time for each of your proton resonances in your spectrum.

Procedure to Acquire Data:

1. Collect a routine 1H NMR spectrum. Be sure to lock, tune the probe, shim.
    - Process your 1H NMR spectrum, and make sure you can view all of your peaks. Optimize proton acquisition parameters if necessary including spectral width, number of scans, carrier frequency, and acquisition time.
2. Create a new experiment with optimized parameters from Experiment 1
    - Say you collected 1H in experiment 1. Copy these experimental parameters over to the next available experiment (experiment 2) by typing **iexpno** on the command line. Alternatively, type **edc**, enter an experiment number of choice, select use current parameters, hit OK.
3. Load the T2 CPMG acquisition and processing parameters using an **rpar**
    - Type the following into the VnmrJ command line, depending on which instrument you are using:

800 Avance III Medsci	**rpar T2.800**
600 Avance III Messci	**rpar T2.600**
400 Avance III Chem	**rpar T2.400**

    - Select Set Solvent and Execute getprosol
4. Calibrate your 90 and 180 pulses
    - Type **pulsecal** in the command line. This will find your 90 and 180 pulses for your current sample.
5. Set your spectral width using the Set Limits option (See Using SetLimits in the COSY guide for help)
    - Under the Acquire tab, hit the SetLimits button
    - With the dialog box still open, load your Proton spectrum from Step 1 into the workspace.
    - Click and drag to set your spectral width approximately 1 ppm on either side of your most upfield and most downfield peaks.
    - Hit OK, and you will see a message stating that your spectral width SW and your offset frequency O1P has been changed.
6. Check your acquisition parameters:
    - Type **ased** or **eda** on the command line, and check the following acquisition parameters:

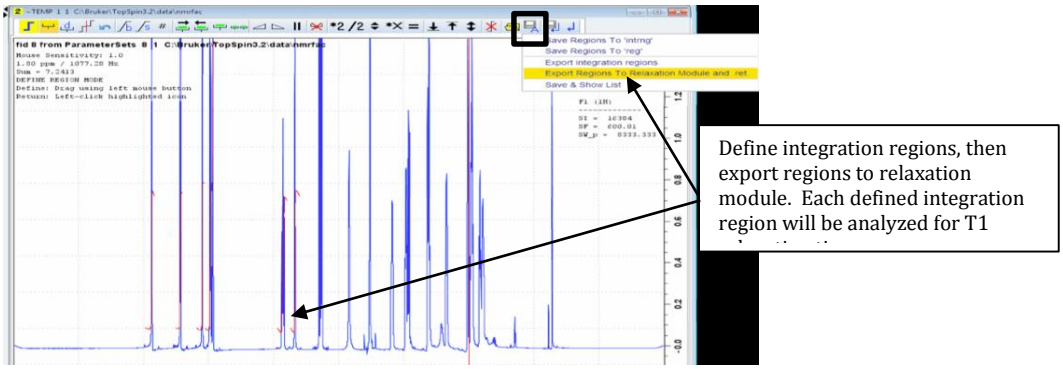
Name	Parameter	Suggested Value
Spectral Width	**sw**	14 ppm, or set with Set Limits
Offset Frequency	**o1p**	Center of spectrum (use Set Limits)
Number of Points	**td**	32k in F2, 8 points in F1. Must match number of points in vdlist
Relaxation Delay	**d1**	use 5 times T1. Default is 20 seconds
Number of Scans	**ns**	Use at least 2 scans
Dummy Scans	**ds**	For best results use 4, but you can set this to 2 or 0 if you just want a crude estimate
Echo time	**d20**	0.5 ms in most cases
Variable Delay List	**vclist**	T2_8 This list has 8 variable counters, so TD in F1 should be set to 8.

7. Set Receiver Gain using rga or with the Gain button
8. Acquire your data by typing **zg** or by selecting the Go button.

Data Processing Procedure for T2 Experiments:

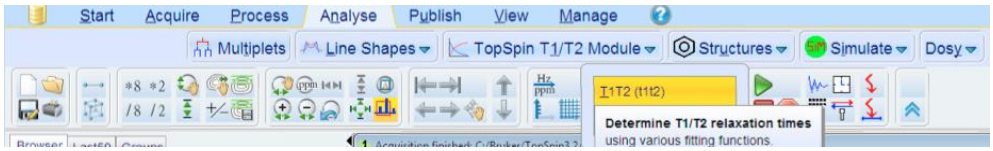
Note: this is similar to T1 Processing, with minor variations.

1. Navigate to the Process tab
2. Check your processing parameters (type **edp,** or select ProcPars).
   - You should set **si** equal to **td** in both F1 and F2. For example, **si** should be set to 16k in F2 and 8 in F1, matching the number of acquired points (**td**) in both dimensions. Note that even if you are not using a power of 2 for **td** in F1, you must process out to the next highest power of 2 for F1, (**si** = 8, 16, 32, 64, etc).
   - Check that SPECTYP is set to PSEUDO2D.
3. Perform 2D Fourier Transform
   - Type **xf2** on the command line
4. Phase your 2D spectrum, and perform automatic baseline correction
   - Enter phasing mode by selecting the Adjust Phase button, or by typing **.ph**
   - Add two columns representing your first and last data points by right clicking and selecting Add.
   - Select the Rows icon to phase the rows 
   - Set the pivot point near the most upfield peaks, and phase all peaks positive using Ph0 and Ph1.
   - When satisfied, save and return to your 2D display.
   - Perform automatic baseline correction for 2D dataset by typing **abs2** on the cmd line
5. Perform Fourier transform of your shortest echo time experiment in your **vclist**
   - Type **rser #** where **#** is the smallest value in your **vclist**. In this example, type **rser 1.** You should see a TEMP 1D data file, which is one of your acquired FIDs in your psudo2d experiment.
   - Perform normal Fourier transform and automatic phasing with **efp** and **apk**
6. Set your integration regions (use for peak area determination of T2)
   - Enter integration mode, and integrate all resonances for which you wish to calculate T2
   - Export your integral regions to the Relaxation module using Save Regions As -> Export Regions to Relaxation Module and .ret
   - Close out of this Temporary 1D mode and return to your 2D data set.



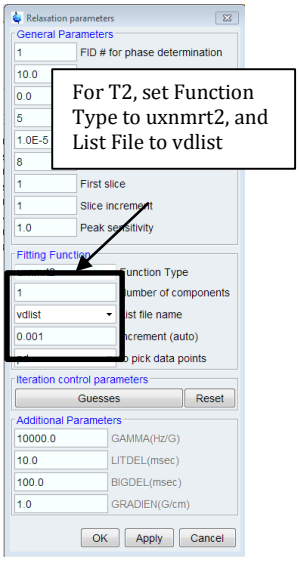
7. Pick your peaks (use for peak intensity determination of T2)
   - Repeat step 5, then enter peak picking mode
   - Use manual peak picking to select peaks you wish to analyze for T2 relaxation times.
   - Export your peak list to the Relaxation module using Save Regions As -> Export Regions and biggest peak within region to Relaxation Module and .ret
   - Close out of this Temporary 1D mode and return to your 2D data set.
8. Convert your T2 couter list vclist into a time list vdlist
   - Type **t2convert** on the command line, and hit OK through any messages. This macro will calculate the total echo time for each of your items in the vclist and save as a new **vdlist**. You later will use this calculated **vdlist** to fit your T2 decays

9. Enter Relaxation Module for T2 Extraction
    - Navigate to the Analyze tab, select T1/T2 Module, and select T1T2



    - Select Relaxation button. Note, you have already defined ranges using the integrations or peak picking in Step 6 / Step 7.

    - Check the Settings, and select **uxnmrt2** and **vdlist** as Function Type and List Name. Note, this will only work properly if you have executed the **t2convert** macro (Step 8)
    - Select either Area (if you would like to use integrations) or Intensity (if you would like to use your picked peaks)



10. Fit your data and extract T2 for each region
    - Select that “Calculate Fits for All Peaks” icon.

    - Observe the results of the fit for each selected peak by cycling through your peaks using the + or – options.

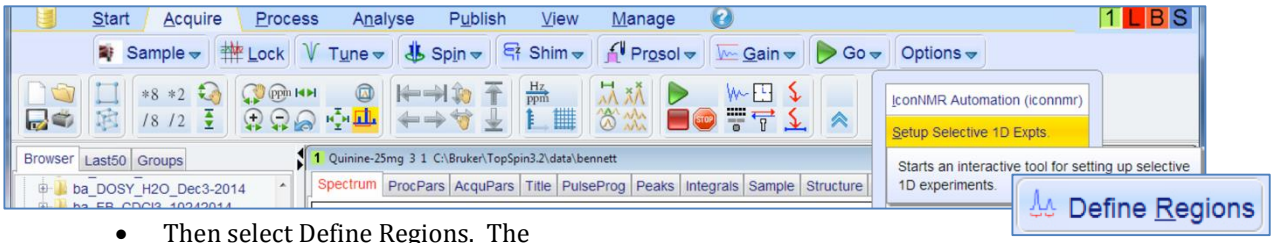
Experiment: 1D Selective NOE Experiment

Gained Information: The selective NOE experiment is commonly used to determine stereochemistry; one selectively excites a proton resonances and observes NOE transfer to nearby protons. Typically, one observes NOE peaks for proton resonances that are in a relatively rigid environment that are within 5 Angstroms of each other.

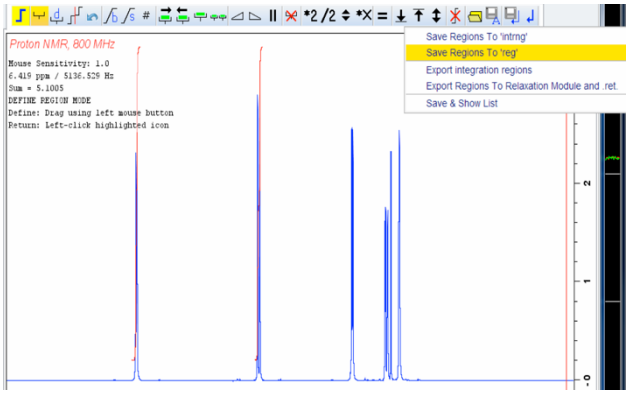
Assuming you are only trying to identify NOEs for a select few resonances, this experiment is often much faster than the 2D NOESY. With any NOE experiment, you need to keep in mind that NOE depends on the molecular tumbling rate, so molecular weight is an important factor in choosing both your experiment and your acquisition parameters. NOE will be positive for small molecules (under 600 Daltons), go through a zero for medium-sized molecules (700 to 1500 Da), and become negative for larger molecules (greater than 1500 Da). For medium-sized molecules, you should try the Selective ROESY experiment, since ROE is always non-zero. You should choose your NOE mixing time based on your molecular weight. As a starting point, for small molecules try a mixing time of 0.5 seconds, medium sized molecules try 0.3 seconds, and large molecules try 0.1 seconds.

Acquisition Procedure:

1. Set up routine Proton NMR acquisition. Be sure to lock, tune the probe, shim.
    - Optimize proton acquisition parameters if necessary including spectral width, number of scans, relaxation delay, carrier frequency, and acquisition time.
2. Calibrate your 90 and 180 pulses
    - Type **pulsecal** in the command line. This will find and set your 90 and 180 pulses for your current sample. This process takes about 30 seconds, and you will see a window when it is done listing your calibrated 90 degree pulses at relevant powers.
3. Acquire your routine 1D Proton spectrum.
    - Process your data as normal, and inspect if you need to change any acquisition parameters. If you made any changes, re-acquire your Proton data and re-process your data.
4. Setup Selective 1D Gradient NOESY experiment: Define Regions
    - Under the Acquire tab, select Options / Setup Selective 1D Expts.



    - Then select Define Regions. The Integration window should show up.
    - Define your regions for selective 1D NOE experiments by setting integrals for each resonance for which you intend to collect 1D NOE data. If you select 5 regions, you will end up collecting 5 1D NOE experiments.
    - Select Save As -> Save Regions to 'reg'.
    - Now select Save and Return. Your regions are now defined.

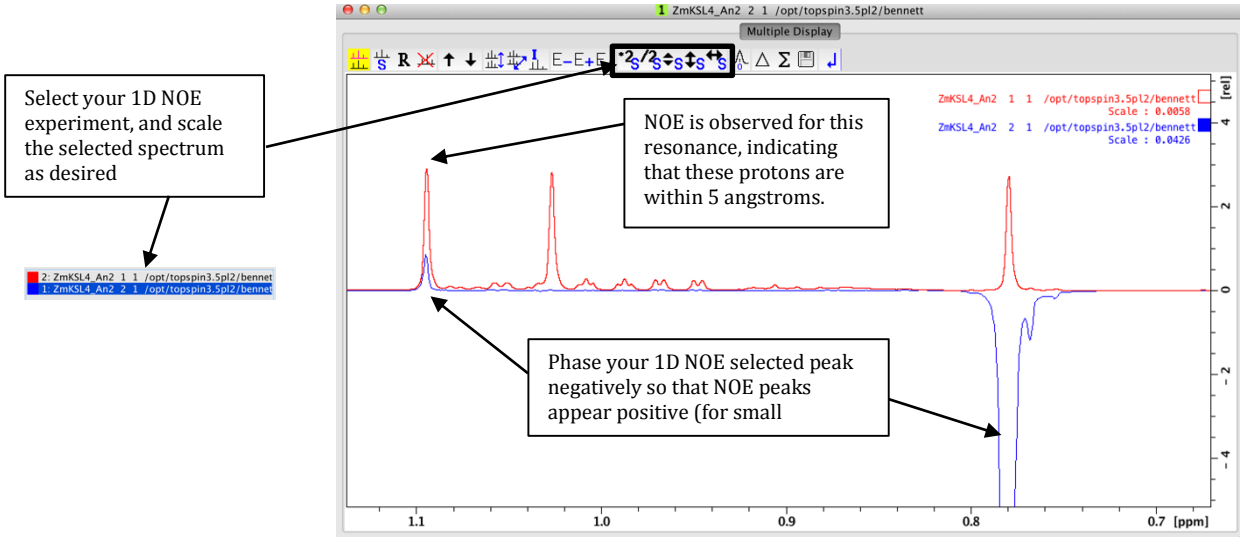


5. Setup Selective 1D Gradient NOESY experiment: Create Datasets
    - After you have defined regions, select the Create Datasets button
    - Select the **Selective Gradient NOESY** experiment
    - Set NOE mixing time. For small molecules (under 600 Da) try 0.5 seconds. For medium-sized molecules try 0.3 seconds, and for large molecules try 0.1 seconds.
    - Choose desired number of scans. Use at least 32, but the more the better.

6. Acquire your data:
    - After you have chosen prompted parameters, select Acquire. This will create a new experiment and collect 1D NOE for each of your chosen regions.
    - Feel free to adjust any acquisition parameters and re-acquire your data (for example number of scans, spectral width)

Processing Procedure:

1. Initial Data Processing: perform Fourier transform with **efp**.
2. Phase your data:
    - Enter manual phasing mode by selecting the Adjust Phase in the Process tab
    - Move the pivot point to the large peak, and use the **0** order phase correction to phase your large peak negative. Then use the Ph **1** to phase any NOE peaks positive.
3. Perform baseline correction with **abs**
4. Compare your 1D NOE spectra with your routine Proton spectrum using Multiple Displays:
    - Type **.md** on the command line, or use the Multiple Display button 
    - Add your 1D Proton spectrum to the display, either by double-clicking on your 1H spectrum, or by reading in the proper experiment number with the command **re #** where is the experiment number in which you collected your routine proton experiment. For example, if your Proton spectrum was collected in experiment 1, type **re 1** on the command line to load this spectrum into the multiple display window.
    - Select your 1D NOE data set on the left, and scale the data as you see appropriate.



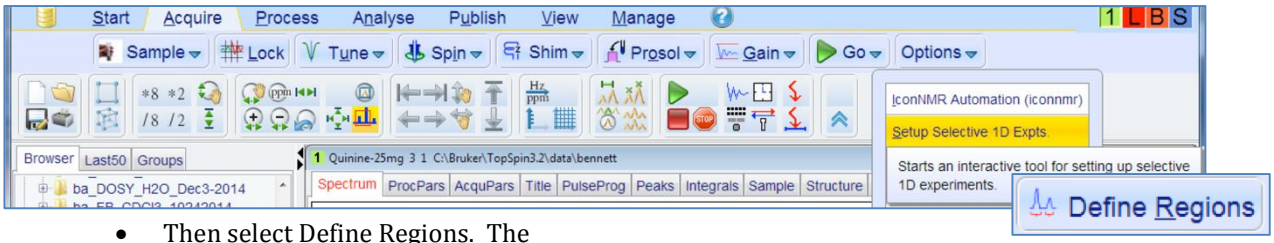
5. Interpret your data: Analyze your stacked spectrum for indications of NOE. Most peaks will appear as a null or will be antiphase, meaning no NOE transfer occurs. If you see multiple peaks with NOE transfer, you can estimate their proximity to the irradiated proton as close, medium, or far, where far is near the edge of NOE range (5 Angstroms).

Experiment: 1D Selective Homonuclear Decoupling

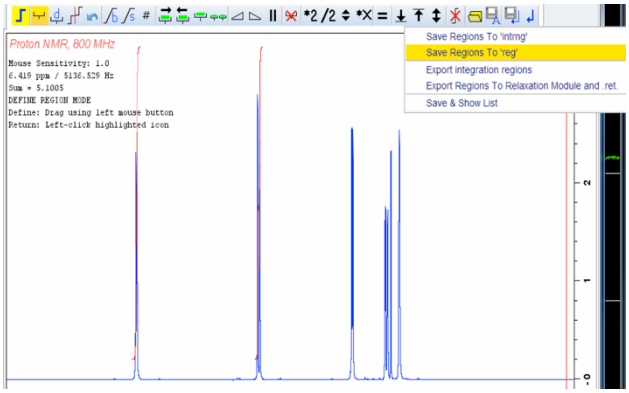
Gained Information: The Selective 1D Homonuclear decoupling experiment allows you to acquire a routine Proton spectrum while selectively decoupling a chosen resonance to observe the effect on other resonances. For example, the proton spectrum of Ethanol has a CH3 that appears as a triplet due to the CH2, while the CH2 appears as a quartet due to the CH3. If you selectively decouple the CH3 peak during acquisition, your CH2 peak will collapse into a singlet.

Acquisition Procedure:

1. Set up routine Proton NMR acquisition. Be sure to lock, tune the probe, shim.
    - Optimize proton acquisition parameters if necessary including spectral width, number of scans, relaxation delay, carrier frequency, and acquisition time.
2. Calibrate your 90 and 180 pulses
    - Type **pulsecal** in the command line. This will find and set your 90 and 180 pulses for your current sample. This process takes about 30 seconds, and you will see a window when it is done listing your calibrated 90 degree pulses at relevant powers.
3. Acquire your routine 1D Proton spectrum.
    - Process your data as normal, and inspect if you need to change any acquisition parameters. If you made any changes, re-acquire your Proton data and re-process your data.
4. Setup 1D Homonuclear Decoupling experiment: Define Regions
    - Under the Acquire tab, select Options / Setup Selective 1D Expts.



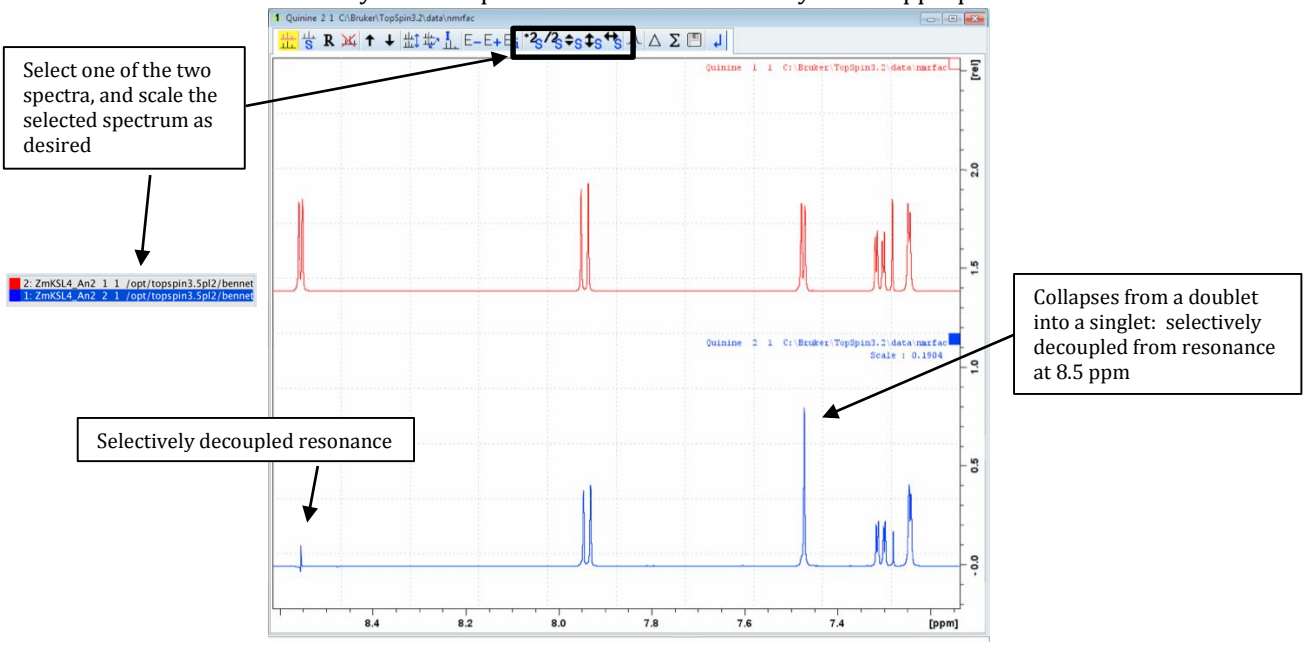
    - Then select Define Regions. The Integration window should show up.
    - Define your regions for 1D Homonuclear decoupling using the Integration mode: select which resonance or resonances you wish to decouple.
    - Select Save As -> Save Regions to 'reg'.
    - Now select Save and Return. Your regions are now defined.



5. Setup 1D Homonuclear Decoupling experiment: Create Datasets
    - After you have defined regions, select the Create Datasets button
    - Select the **1D Homonuclear Decoupling** experiment
    - Choose desired number of scans. Use at least 32, but the more the better.
6. Acquire your data:
    - After you have chosen prompted parameters, select Acquire. This will create a new experiment and collect 1D Homonuclear decoupling spectra for each of your chosen regions.

Processing Procedure:

1. Initial Data Processing: perform Fourier transform with **efp** and phase with **apk**.
2. Perform baseline correction with **abs.**
   - Often the abs command will auto-find integration regions, but will do so not to your liking. In this case, it may be helpful to delete all integrals. To do so, enter integration mode .**int** and select the Delete All Integrals option, then hit Save and Close
3. Compare your 1D NOE spectra with your routine Proton spectrum using Multiple Displays:
   - Type **.md** on the command line, or use the Multiple Display button 
   - Add your 1D Proton spectrum to the display, either by double-clicking on your 1H spectrum, or by reading in the proper experiment number with the command **re #** where is the experiment number in which you collected your routine proton experiment. For example, if your Proton spectrum was collected in experiment 1, type **re 1** on the command line to load this spectrum into the multiple display window.
   - You can choose to either superimpose or stack your spectra with: 
   - Select one of your two spectra and scale the data as you see appropriate.

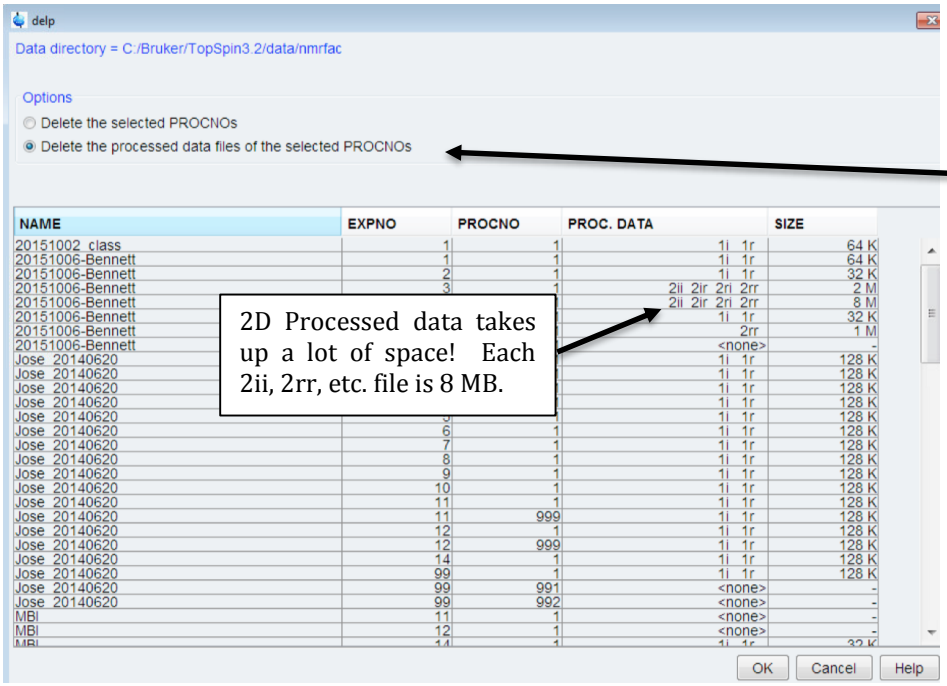


4. Interpret your data: Analyze your stacked spectrum for indications of 1H / 1H decoupling. The resonance that is decoupled will be nulled or severely distorted, while any resonances which are J-coupled to the selected peak will now appear as if no J-coupling is present.

Disk Utilization: Deleting Processed Data

Raw data does not take up much file space, however processed data, especially 2D datasets, can be extremely large files; a couple overnight 2D experiments can easily amount to 300 MB of processed data. Uploading all of this to KONA not only takes a long time, it is also unnecessary.

After having a look at your data in Topspin, please consider deleting the processed data from the host computer before uplading to KONA.



Step 1: Type **delp** from the topspin command line

Step 2: Select "Delete the processed data files…" option

Step 3: Highlight all processed datasets that you wish to remove. Use Shift or Control to select multiple files

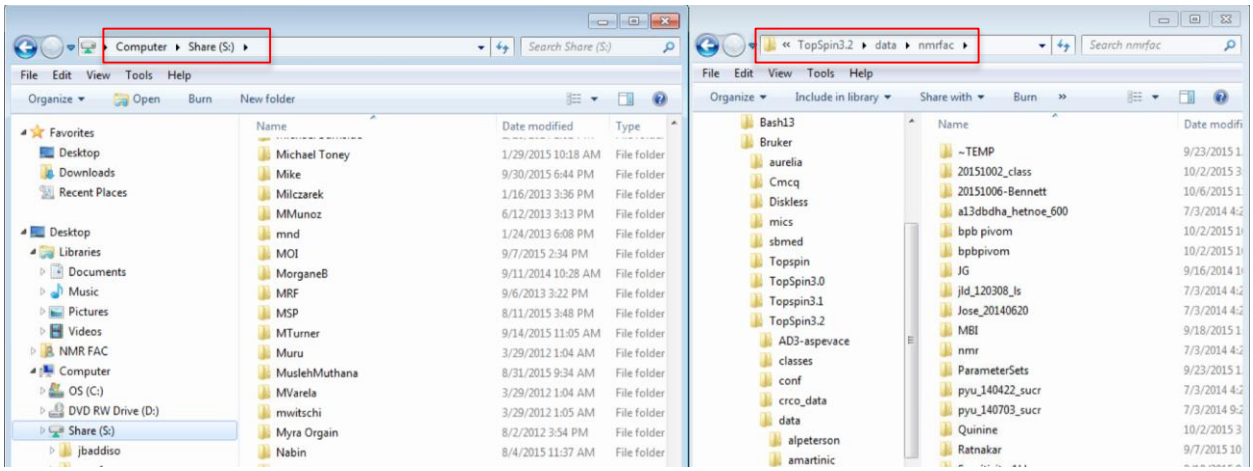
Step 4: Hit OK. Your raw data will be untouched, and you can re-process your data at any time.

Uploading your data to KONA:

You are strongly encouraged to archive all your data using the FTP server (KONA) as soon as each of your NMR runs is completed. Please avoid using USB flash drives because of potential malware/viruses, and please consider the environment when choosing to print. To upload your data to KONA from the Bruker 800 and 600 NMR spectrometers (Windows 7 operating systems), do the following:

Before uploading your data to KONA, please first delete your processed data. Your raw data will be untouched, and you can re-process your data at any time.

1) Open up a Windows Explorer and navigate to the Shared drive, S:\\Share$
2) Open up a second Windows Explorer, and navigate to your Bruker data directory: C:\\Bruker\Topspin3.2\data\username
3) Find the experiment names in your user directory that you wish to copy to KONA
4) Copy, or drag and drop to your data to your KONA folder



Retrieving your data from KONA:

KONA is an FTP server that can be accessed from any campus IP address. For best results, you should connect via Ethernet using DHCP. You should also be able to connect via mooblenetx and eduroam wifi, however there have been some problems with this method. You should also be able to connect using your lab wifi assuming your wifi router is connected to the campus network via Ethernet.

1) Download and install some FTP client onto your computer, depending on your operating system. Cyberduck and FileZilla are common choices for both windows and mac users.
2) Launch the software, and enter the following information:

Protocol:	sftp
Server:	kona.ucdavis.edu
Username:	nmrftp
Port:	22
Password:	Ask for password.

3) Navigate to your directory, and copy your data to your computer.

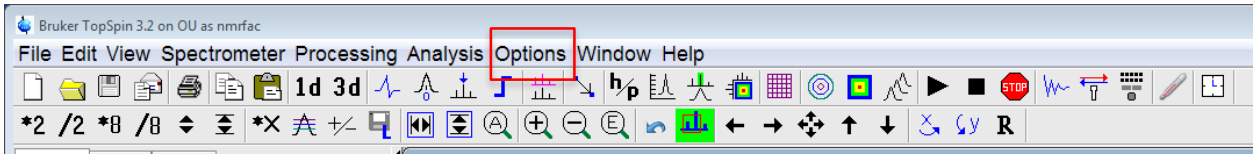
Setting up your Topspin 3.2 Account for the First Time

If you are using a spectrometer for the first time, the Topspin interface / layout for your account may not be optimal. To correctly set up your Topspin 3.X interface, take the following steps:

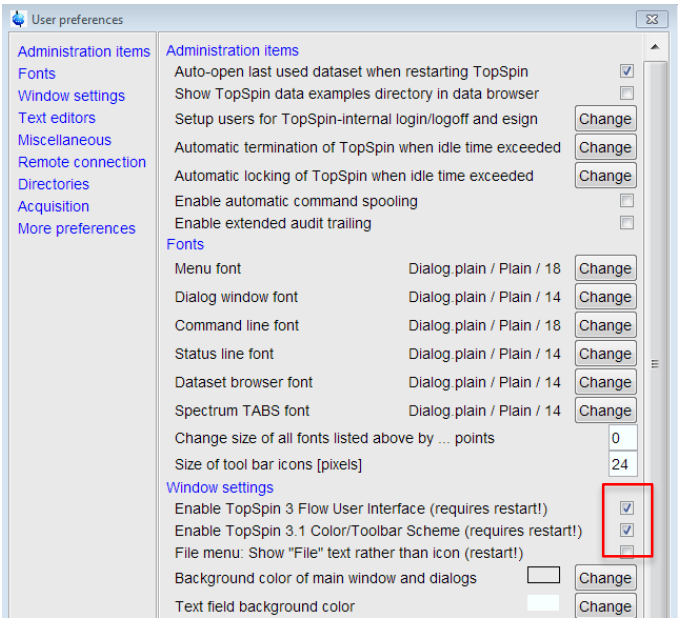
Enable Topspin 3 Workflow:

When you open up Topspin 3.2 for the first time, your interface/layout will be similar to the old version of topspin: Topspin 1.3. This is the same layout you will see on the 500 and 400 Bruker DRX instruments at Medsci. The newer Topspin 3.2 layout is much better, and it is highly recommended that you enable the new workflow:

Step 1: Select Options -> Preferences

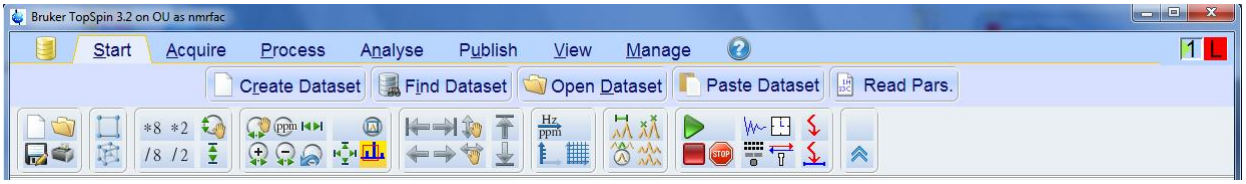


Step 2: Enable Topspin 3 Flow and Topspin 3.1 Color/Toolbar Scheme



Check these two options, hit Apply, Close, then close and restart Topspin 3.2

Step 3: Apply your changes, and close and re-open Topspin 3.2. Your interface should now look like this:

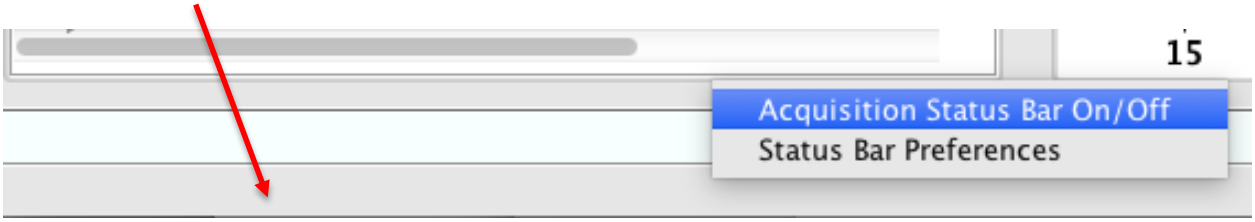


If you would like to revert back to the Topspin 1.3 workflow, go to Manage -> Preferences, and de-select the Topspin 3 options. Apply your changes, close and re-open Topspin.

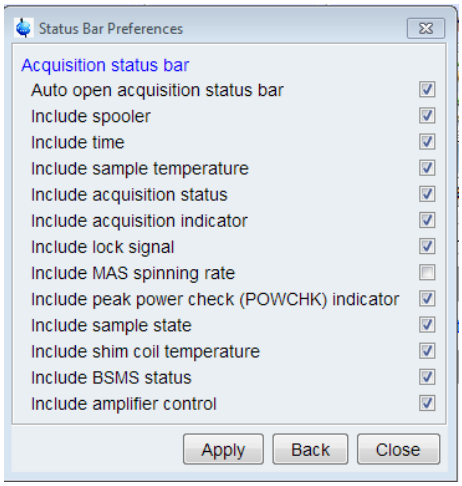
Enable Acquisition Status Bar:

The Acquisition Status Bar is the region at the bottom of your Topspin software that provides various status information including lock status, temperature, sample status, and generally what the spectrometer is currently doing (ie shimming, acquiring data, etc.) To enable and configure the acquisition status bar, take the following steps:

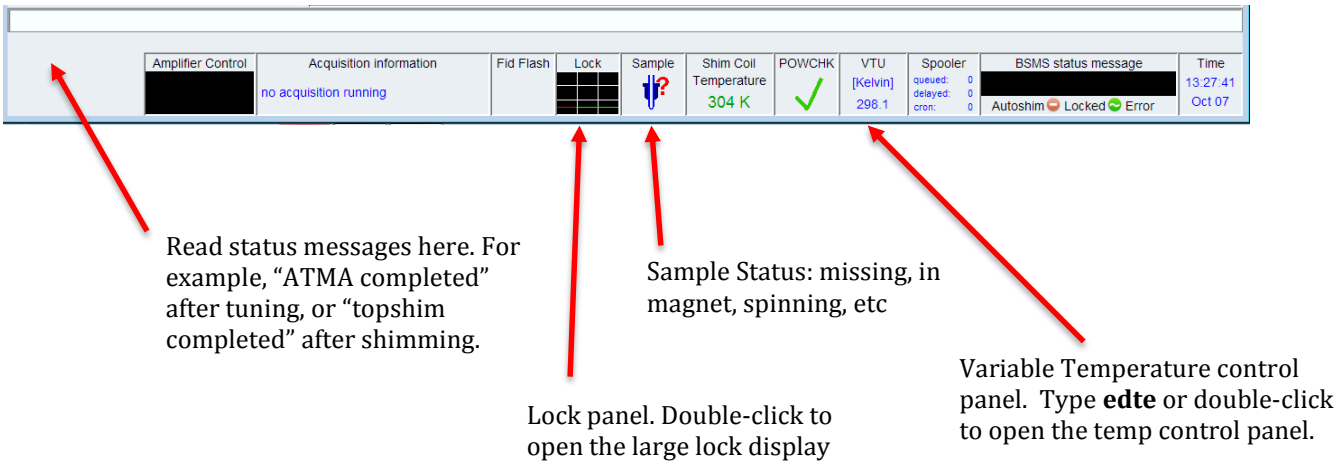
Step 1: Right click on the grey space just below the command line, and select Acquisition Status Bar On/Off



Step 2: When setting Status Bar Preferences, select all options except MAS spinning rate, hit Apply and Close.



Your Acquisition status bar should now be displayed. If it is not displayed after setting your preferences, you may need to right-click again and turn it off, then back on, or you may need to close and re-open topspin.



List and Brief Description of Important Commands

Command	Short description
abs	automatic baseline straighten
apk	automatic phasing of 1D spectra
apk2d	automatic phasing of 2D spectra
ased	displays data acquisition parameters (short list)
atma	automatic tuning and matching, automatic option
atmm	automatic tuning and matching, manual option.
eda	displays all data acquistion parameters
edc	create new dataset from old dataset
edp	displays all data processing parameters
edte	start-up and display temperature control window
efp	em + ft + pk
em	exponential multiplication
expt	check experiment time
ft	fourier transform
halt	halts data acquisition (data saved)
lb	line broadening value (for em)
lock	displays lock solvent list and then locks on chosen solvent
new	create new dataset
ns	number of scans
o1p	set o1 offset (carrier frequency, center of spectrum) in ppm
o2p	set o2 carrier frequency (non-observe nucleus, ie 1H for decoupling, or 13C in HSQC)
pk	applies last phase correction
pps	pick peaks and display
rg	receiver gain
rpar	read parameter file
rsh	read shim file
stop	stops data acquisition (data not saved) or tuning
sw	sweepwidth in ppm
td	number of acquired data points
wobb	starts tune display
xf2	fourier transform of data in 1 dimension, intensity in the other dimension
xfb	two dimensional fourier transform
zg	starts data acquisition
.md	enter multiple display mode
.int	enter integration mode
.ph	enter manual phasing mode
.pp	enter peak picking mode
.all	zoom out to display full spectrum
.cal	calibrate your 1D or 2D axis using the curser