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We use this protocol and it's working

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Disclaimer

This protocol is part of the knowledge base content of **NAN: The Network for Advanced NMR** (<https://usnan.nmrhub.org/>)

Specific filenames, paths, and parameter values apply to spectrometers in the NMR facility of the Complex Carbohydrate Research Center (CCRC) at the University of Georgia.

Abstract

This is a protocol for running the standard Bruker pulseprogram '*zgpgpr*', a 1D proton experiment with water signal suppression by presaturation for biomolecular samples in 90% H_2O /10% D_2O . Alternative water suppression sequences (e.g. 'watergate' - *zgpgpwg*, 'excitation sculpting' - *zgesgp*) can also be used but are not described here.

This is the first step in collecting a series of NMR experiments for a typical ^{15}N and/or ^{13}C enriched protein sample and assumes additional pulse sequences (e.g. 15N_HSQC, 13C_HSQC, 3D HNC0, 3D HNCA, etc.) will be acquired later. As an introductory protocol, it includes steps for Locking, Tuning and Shimming your sample.

It also provides a short description of the Topspin BioTop module that can be used to optimize parameters and facilitate setup of multiple 2D and 3D pulse sequences (see guidelines).

Note: specific parameter values illustrated below may differ depending on the facility and spectrometer.

Guidelines

This protocol assumes a sample is properly loaded into the magnet. Potential **users must be trained** in the proper handling of samples for spectrometers at the CCRC NMR facility.

See protocols "Loading_a_sample_manually.nan" or "Loading_a_sample_with_SampleJet.nan" for reference.

To adequately suppress the large H_2O signal, shimming must be optimal. See protocol "Topshim GUI: routine sample shimming" for details.



Before start

A sample must be inserted in the magnet either locally by the user after training (see Guidelines) or by facility staff if running remotely.

Familiarize yourself with the general workflow for NMR study of a protein sample is outlined in protocol "Aquisition Setup Workflow, Solution NMR Structural Biology"

These protocols rely on first loading starting parameters within Topspin, then adjusting parameters for your sample. In most cases, just calibrating the proton pulse is sufficient.

By loading the starting parameter file '**presat_bio.par**' for this protocol configures **three channels 1H, 13C, and 15N**, although it is only a 1D proton observe experiment. This facilitates tuning tuning all channels.

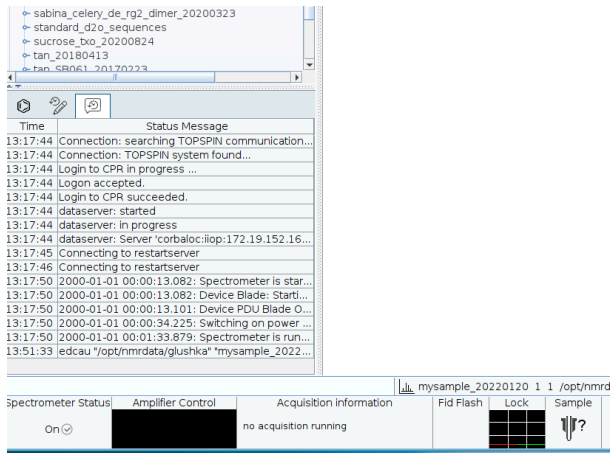
To facilitate optimization of other parameters, Topspin has a **BioTop** module, which can be used to calibrate and define pulse widths, offsets, and spectral widths. This is especially useful when setting up multiple pulse sequences in one session.

Launching BioTop and executing its optimization routines will store the calibrated parameter values, and they can be retrieved for each pulse sequence within your dataset folder you plan to run. Thus, it will update the default parameters with consistent values.

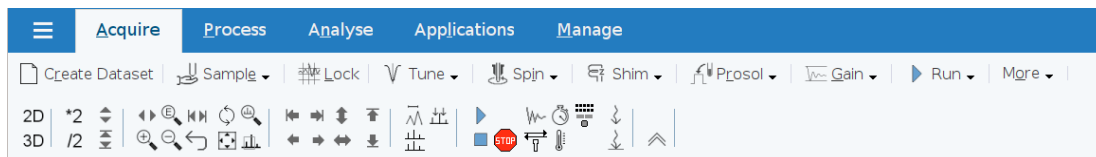
Initial setup

- 1 Click on Probe Temperature at bottom of Topspin window to open variable temperature control and set desired sample temperature.

- 1.1 Open status message - this echoes all topspin processes:



- 1.2 **Lock on solvent:** click on Lock in Acquire sub-menu, or type 'lock' on the command line.



From the list, select H2O+D2O and click OK. Wait till status confirms locking was successful.

- 2 **Create Dataset:**

Click on Acquire -> 'Create Dataset' button to open dataset entry box or type 'new'.

If you are starting with a new dataset, fill out the NAME for dataset (single string, e.g datasetName123) and start with EXPNO 1; choose appropriate directory.

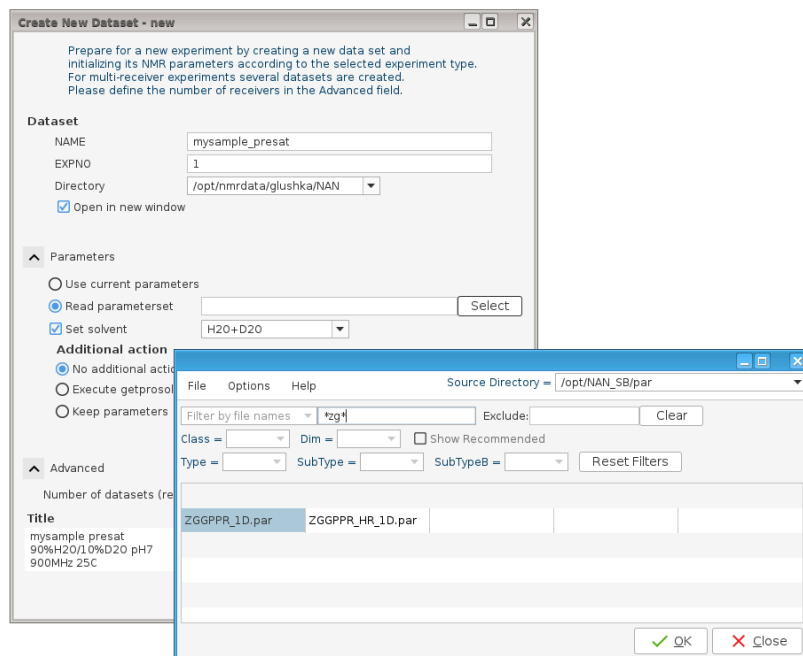
If you are adding to an existing dataset, the name and directory fields will be kept and the EXPNO will be automatically incremented by +1.

- 2.1 **Select parameter set to load initial values.**

Check 'Read parameterset' box, and click Select.

To load standard NAN parameter sets, change the Source directory at upper right corner of the window to Source = /opt/NAN_SB/par

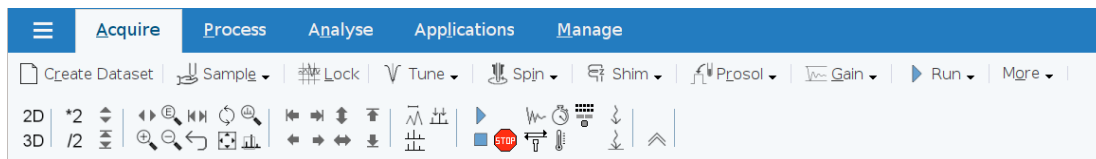
Click 'Select' to bring up list of parameter sets and select **presat_bio.par**.



Click OK at bottom of window to create the experiment directory.

It will be the active experiment in the acquisition window and will be listed in your data browser.

- 3 **Tune probe channels:** loading 'presat_bio.par' enables all three channels, ^1H , ^{13}C and ^{15}N , so click on Acquire-> Tune or type '**atma**' to execute automatic tuning for these nuclei.



Finer manual adjustment can be done by typing '**atmm**' or selecting it from the tune pulldown menu.

This opens a control window and allows changing the tune and match channels with the mouse.

- 4 **Shim the sample:**



Retrieve a recent good quality starting shim set: Type **rsh**, then select 'H2O_5mm' or 'H2O_shigemi' depending on the sample tube.

Note

Different spectrometer probes and different nmr sample tubes require different shimming procedures.

All spectrometers EXCEPT 'br800':

- Standard 5mm tubes: Click on Acquire->Shim or type '**topshim**' to execute routine shimming which adjusts Z1-Z5 shims only.
- Shigemi tubes: type '**topshim shigemi**'.

br800 spectrometer with 1.7 mm probe only:

- Type '**topshim ordmax=4**'; additional adjustment of Z1 may be required, best done with GS command and lineshape evaluation.

A more rigorous shimming procedure is to perform a full 3D shim: Type '**topshim 3D**' (NOT for br800).

This routine is more time-consuming, and only is recommended when acquiring 3D experiments, or experiments where suppression of H2O residual signal is critical, such as NOESY.

TopShim can also be run in GUI mode by invoking '**topshim gui**'. Additional details and variations of shimming routines can be found in the protocol "Topshim gui: routine sample shimming".

Pulse calibration: use calibo1p1 (step 5.1) or BioTop (step 5.2)

- 5 On a given spectrometer, the parameters loaded from the parameter sets in the /opt/NAN/par library will have the correct power levels and pulse widths, and are generally suitable for routine use with typical protein samples.

However, the proton pulse width varies from sample to sample and must ALWAYS be calibrated - see steps 5.1 and 5.2

In addition to that, 1H offset **01** typically has to be set on the H₂O resonance for H₂O signal suppression. Since H₂O signal frequency varies with temperature, **01** offset needs to be calibrated for each sample as well.

The most useful tools for such calibrations are the **BioTop** utility and **calibo1p1** AU macro.



BioTop is a module that organizes calibrated and defined parameters for a sample and propagates them to all the experiments that will be setup and run for that sample. For details see the protocol 'BioTop : calibration and acquisition setup' and attached Bruker manual 'biotop.pdf'. It is most useful for setting up a series of pulse sequences on an isotopically enriched sample, since it can also calibrate ^{13}C and ^{15}N pulse widths.

calibo1p1 is an AU macro that only calibrates P1 90° pulse width and the O1 offset for aqueous samples.


5.1 Calibrate 1H pulse and offset with calibo1p1.

Create a new experiment with **edc**. You can use a 'dummy' experiment number, like 9999.

Type **calibo1p1** to start the calibration routine. When it finishes, it will acquire a test 1D (with excitation-sculpting, zgesgp) with the calibrated values. Take note of the calibrated values **P1**, **PLdB1**, and **O1** in the acquisition parameters '**ased**' view.

Return to the original experiment.

You can also manually enter the calibrations from **calibo1p1** into the **BioTop** optimization tab for future use (see next step)

 [go to step #6](#)

5.2 Using BioTop for pulse calibrations

Type '**biotop**' to open the BioTop window. Here you can enter the relevant sample information, such as molecule type, molecular size, isotope labeling, and lock solvent. Other entries are mostly optional or descriptive. You can also import previous sample information and calibrations from a different dataset.

It is assumed you have set temperature, lock, tune and shim separately (see steps 1-4) so leave the corresponding boxes unchecked.



BioTop <2>

Sample information Optimization Experiments About Preferences

Sample

Molecule name:

Description:

Molecule type:

Sequence: (optional)

PDB structure: (optional)

Assignment import: (optional)

Molecular size [kDa]:

Labeling:

Solvent:

Tube:

General operations

Temperature [K]: Set: ☐

Lock automatically: ☐

Tune/Match automatically: ☐

Shim automatically: ☐

Click on Optimization tab to go to the second window:



BioTop <2>

Sample information Optimization Experiments About Preferences

¹H Parameters

90 degree pulse: 27.5 [μs] Powerlevel: -14.7712 [dB] ☐ Auto opt.

90 degree on water: 11.8429 [dB] ☐ Auto opt.

Water offset: 4229.01 [Hz] ☐ Auto opt.

SW: 16 [ppm]

Amide offset: 8.3 [ppm] Amide SW: 5 [ppm]

¹⁵N Parameters

90 degree pulse: 42 [μs] Powerlevel: 1000 [dB] ☐ Auto opt.

Offset: 117 [ppm] SW: 35 [ppm] ☐ Auto opt.

¹³C Parameters

90 degree pulse: 26.5 [μs] Powerlevel: -0 [dB] ☐ Auto opt.

Offset Carbonyl: 173.5 [ppm] SW Carbonyl: 14 [ppm] ☐ Auto opt.

Offset C-alpha: 53.2 [ppm] SW C-alpha: 32 [ppm] ☐ Auto opt.

Offset C-aliphatic: 43 [ppm] SW C-aliphatic: 80 [ppm]

Start optimization Cancel Close Report

For samples at natural abundance (no isotope enrichment) select only ¹H parameters: 90 degree pulse, 90 degree on water, and water offset.

For isotope enriched samples select the ¹⁵N and ¹³C 90 degree pulses as well at this stage.

You can also include ¹⁵N and ¹³C spectral width optimizations when working with a particular sample for the first time. For details see the protocol 'BioTop: calibration and acquisition setup'.

Click on '**Start Optimization**'. When optimization finishes the corresponding parameters will be updated with the new values in the 'Optimization' tab. Note that this does not update the experiment parameters yet.

Load pulse calibrations: use getprosol (steps 6.1) or bioTop (step 6.2)

- 6 The parameters within the experiment need to be updated with the calibrated values.

This can be done with either of the two methods

1. **getprosol** - load calibrations from **pulsecal** of **calibo1p1**
2. **btpprep** - load calibrations stored in the 'Optimization' tab of **BioTop**



btprep is a command-line component of the BioTop utility.

6.1 Load 1H pulse width with **getprosol**

To load default pulse calibrations, in the TopSpin menu click on Acquire -> Prosol, or type '**getprosol**'.

This loads default pulse lengths and power levels from the spectrometer's 'edprosol' table, which contains values predetermined by the facility using a standard sample.

To load the 1H 90° pulse width calibrated with calibo1p1 in step 6.2 type

getprosol 1H [calibrated P1 value] [power level for P1 in dB]

For example, if the calibrated P1 is **9.9 us** at power level PL1dB of **-13.14 dB**, type **getprosol 1H 9.9 -13.14**

This will also update other dependent 1H parameters (power levels for presaturation, decoupling and TOCSY mixing sequences, shaped and spin-lock pulses, etc.) if used in the pulse program. Note that the power level argument is required.

➡ go to step #7

6.2 Load calibrated parameters with **btprep**

Type **btprep** at the TopSpin command line.

This is mainly equivalent to executing **getprosol** (see step 7.1), but using the all calibrated pulse values from the 'Optimization' tab of BioTop.

In addition that, **btprep** updates other parameters according to the code in a corresponding *bt_<pp>.xml* BioTop description file, if present, where <pp> stands for pulse program filename. In this particular case the file name is *zggppr*, with the corresponding description file *bt_zggppr.xml*, and the additional updated parameters are offset **01**, and 1H spectral width **SW**. For additional information about BioTop description files see the protocol 'BioTop: calibration and acquisition setup'.

Inspect parameters

- 7 Although routine parameters are correct at this stage, it is recommended to inspect them for errors, apply changes if needed.

Select the 'Acqparms' tab and click on the 'pulse' icon, or **type 'ased'** to brings up the list of pulse program specific parameters. (A more complete list of parameters can be viewed by clicking on the '**A**' icon, or typing **eda**.) NOTE: default values will depend on the spectrometer used.



Probe: CP TX0 900SA N/C-H-D-05 Z

General

Channel f1

Gradient channel

General

PULPROG: zgpgpr ... E Pulse program for acquisition

TD: 32768 Time domain size

SWH [Hz, ppm]: 12500.00 13.8921 Sweep width in acquisition direction

AQ [sec]: 1.3107200 Acquisition time

RG: 64 Receiver gain

DW [μsec]: 40.000 Dwell time

DE [μsec]: 18.00 Pre-scan-delay

D1 [sec]: 1.000000000 Relaxation delay: 1-5 * T1

d12 [sec]: 0.000020000 Delay for power switching

D16 [sec]: 0.000200000 Delay for homospoil/gradient recovery

DS: 8 Number of dummy scans

NS: 32 1 * n, total number of scans: NS * TD0

TD0: 1 Number of averages in 1D

Channel f1

SF01 [MHz]: 899.7942292 Frequency of ch. 1

O1 [Hz, ppm]: 4229.20 4.700 Frequency of ch. 1

NUC1: 1H Edit... Nucleus for channel 1

P1 [μsec]: 10.710 F1 channel - 90 degree high power pul

PLW1 [W, dB]: 20.627 -13.14 F1 channel - power level for pulse (defa

PLW9 [W, dB]: 9.464e-05 40.24 F1 channel - power level for presaturati

Gradient channel

GPNAM 1: SMSQ10.100 ... E SMSQ10.100

GPZ1 [%]: 50.00 50%

P16 [μsec]: 1000.000 Homospoil/gradient pulse

7.1 Key parameters - make additional changes as needed.

- **SW:** Proton spectral width. Initially can be set large (~20 ppm) for new samples. Adjust to allow 1-2 ppm baseline at both ends of the spectrum for baseline correction. NOTE: changing SWH will change AQ.
- **O1:** frequency offset; center of spectral window: this should be on the water peak (around 4.7ppm, depending on temperature). Can be calibrated with **BioTop** or **calibo1p1**, but may require manual optimization.
- **TD** or **AQ:** Acquisition time of ~100-200ms sufficient for proteins. For small molecules or when using DSS for reference, increase to ~1s for better spectral resolution.
- **PLW9[W,dB]:** The right column is the level of **attenuation** (Note: higher number, lower power) used for the pre saturation pulses and defaults to ~40-55 dB depending on the spectrometer. It is also updated by **getprosol** based on the calibrated 1H 90° pulse. Increasing the **PLdB9** value will reduce the presaturation power (e.g. suitable for lower H2O/D2O ratio like 99.9% D2O)

DO NOT reduce the value below the recommended range.

NOTE: **PLW9** refers to the power in watts, **PLdB9** refers to the attenuated power in dB.

- **D1:** increase for more accurate integration values (1-2 seconds is normal)
- **DS:** dummy scans that are not recorded for steady state equilibration; at least 4
- **NS:** increase for increased signal to noise (S/N increases as \sqrt{NS})



Acquire data and evaluate spectrum

8 Adjust receiver gain.
Click on 'Gain' in the main topspin Acquire menu or type '**rga**'.

8.1 Click on 'Run' in Topspin Acquire menu, or type '**zg**'.

If you are collecting many scans, you can check the data while it is still acquiring.
Type '**tr**' for immediate write to disk, or '**tr #**' where # is the number of scans to write, then wait a few seconds and execute next substep.

8.2 Click on 'Proc.Spectrum' on the Topspin Process menu.
This will execute an automated processing macro.

Alternatively, type '**efp**', then '**apk0**' to execute exponential line broadening, Fourier transform, followed by an automated phase adjustment. See Bruker TopSpin manuals for details.

8.3 Evaluate the degree of solvent saturation and the bandwidth of the signal suppression.
You can adjust **PLW9[W,dB]** in the acqpars window (pldb9 on the command line) to reduce the bandwidth of the presaturation. Generally look for the minimum power needed to bring the residual solvent signal within 1-5 times the height of the largest sample signal.