

bioTop

- bioTop
User Manual
Version 004



Copyright © by Bruker Corporation

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form, or by any means without the prior consent of the publisher. Product names used are trademarks or registered trademarks of their respective holders.

© August 22, 2018 Bruker Corporation

Document Number:

P/N: H169690

Contents

1	Introduction	5
2	Quick Start Guide	7
2.1	Prerequisites	7
2.2	Starting	7
2.3	The Sample Information Tab	8
2.4	The Optimizations Tab	9
2.5	The Experiments Tab	10
3	Performed Optimizations	11
3.1	Proton 90 Degree Pulse	11
3.2	Water Offset	11
3.3	90 Degree Water-Selective Pulse	11
3.4	90 degree pulse for Carbon or Nitrogen	11
3.5	Nitrogen Offset and Spectral Width	11
3.6	Carbonyl Offset and Spectral Width	12
3.7	Alpha Carbon Offset and Spectral Width	12
4	Automated Processing	13
5	Adding User Defined Experiments	15
6	bioTop Input Formats	19
6.1	Experiment List	19
6.1.1	Experiment Parameters	19
6.1.1.1	Name	19
6.1.1.2	Parset	19
6.1.1.3	Desc	19
6.1.1.4	ExcludeSolvent	20
6.1.1.5	RequireLabeling	20
6.1.1.6	Pulprog	20
6.2	Experiment Description Files	20
6.2.1	Mappings	20
6.2.2	UserEdit	21
6.2.3	preAQ	21
6.2.3.1	cmd	21
6.2.3.2	pycode	21
6.3	Template Files for ProteinDynamicsCenter	21
7	Command Line Usage	23
7.1	Experiment Setup	23
7.2	Processing	23
8	References	25
9	Contact	27

1 Introduction

bioTop is a graphical user interface to simplify and automate setup, acquisition and processing of biomolecular NMR experiments. bioTop makes use of TopSpin parameter sets, the prosol table and a number of fully automated optimizations of commonly used and shared parameters. The main goal is to make biomolecular NMR easily accessible as well as to eliminate common sources of error when setting up more complex experiments.

The level of automation is set by the user. Options range from experiment selection to spectrum (including parameter optimizations and processing) in full automation to adjustments of individual parameters by experienced users. bioTop works with regular TopSpin datasets so all the flexibility is kept if needed.

Last but not least, user defined experiments can be added graphically, either starting from scratch or from an existing experiment description. When adding user defined experiments, prosol compatibility is not a requirement (but simplifies the process).

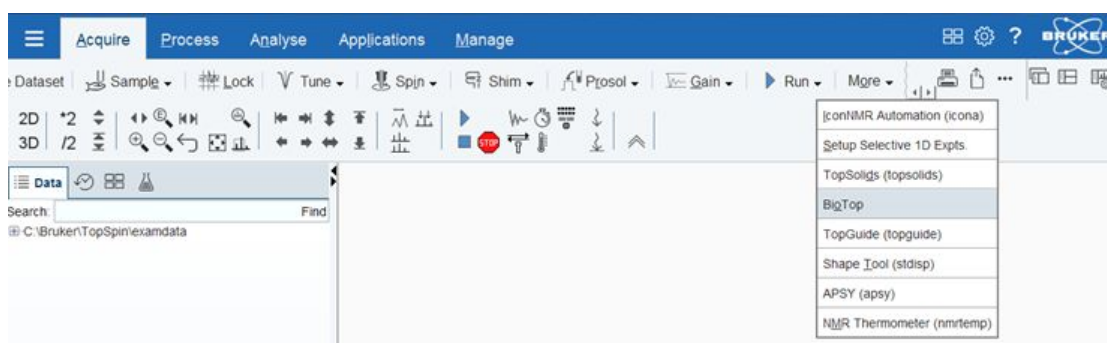
2 Quick Start Guide

2.1 Prerequisites

- Topspin 3.5.6 or above / TopSpin 3.6 / TopSpin 4.x
- Three channel (H/C/N) console and probe
- Populated **prosol** Table
- **Expinstall** for high-resolution NMR
- Activated Command Spooling (will prompt to activate it otherwise)

2.2 Starting

Just execute **biotop** from the command line or select **More | bioTop** from the **Acquire** menu:



2.3 The Sample Information Tab

The screenshot shows the BioTop software window with the 'Sample information' tab selected. The 'Sample' section contains the following fields:

- Molecule name: Ubiquitin
- Description: 0.5mM in d3-NaAc Buffer, 10% D2O/ 90% H2O
- Molecule type: Protein
- Molecular size [kDa]: 8.5
- Labeling: 13C/15N
- Solvent: H2O+D2O
- Tube: 5mm

The 'General operations' section includes:

- Temperature [K]: 298.0, Set:
- Lock automatically:
- Tune/Match automatically:
- Shim automatically:

Buttons for 'Close' and 'Import...' are located at the bottom of the window.

Provide all listed details of your sample.

Molecular size, Labeling and Solvent will be used for the choice of optimizations and filtering of the available experiments, the tube type will be used for the shimming if selected.

Select all desired operations. These are executed when the first optimization is started.

2.4 The Optimizations Tab

The screenshot shows the BioTop software window with the Optimization tab selected. The window contains the following parameters and options:

Section	Parameter	Value	Unit	Auto opt.
¹ H Parameters	90 degree pulse:	9.23	[μs]	<input type="checkbox"/>
	Powerlevel:	-7.29998	[dB]	<input type="checkbox"/>
	90 degree on water:	29.6	[dB]	<input type="checkbox"/>
	Water offset:	2351.64	[Hz]	<input type="checkbox"/>
	SW:	16	[ppm]	
¹⁵ N Parameters	90 degree pulse:	34.9300	[μs]	<input type="checkbox"/>
	Powerlevel:	-19.9000	[dB]	<input type="checkbox"/>
	Offset:	117.9	[ppm]	<input type="checkbox"/>
¹³ C Parameters	90 degree pulse:	11	[μs]	<input type="checkbox"/>
	Powerlevel:	-22.0000	[dB]	<input type="checkbox"/>
	Offset Carbonyl:	172.9	[ppm]	<input type="checkbox"/>
	SW Carbonyl:	11.3	[ppm]	<input type="checkbox"/>
	Offset C-alpha:	53.7	[ppm]	<input type="checkbox"/>
	SW C-alpha:	24.5	[ppm]	<input type="checkbox"/>
	Offset C-aliphatic:	43	[ppm]	
	SW C-aliphatic:	80	[ppm]	

At the bottom of the window, there are four buttons: Start optimization, Cancel, Close, and Report.

Select all necessary optimizations or change parameters manually, if known.

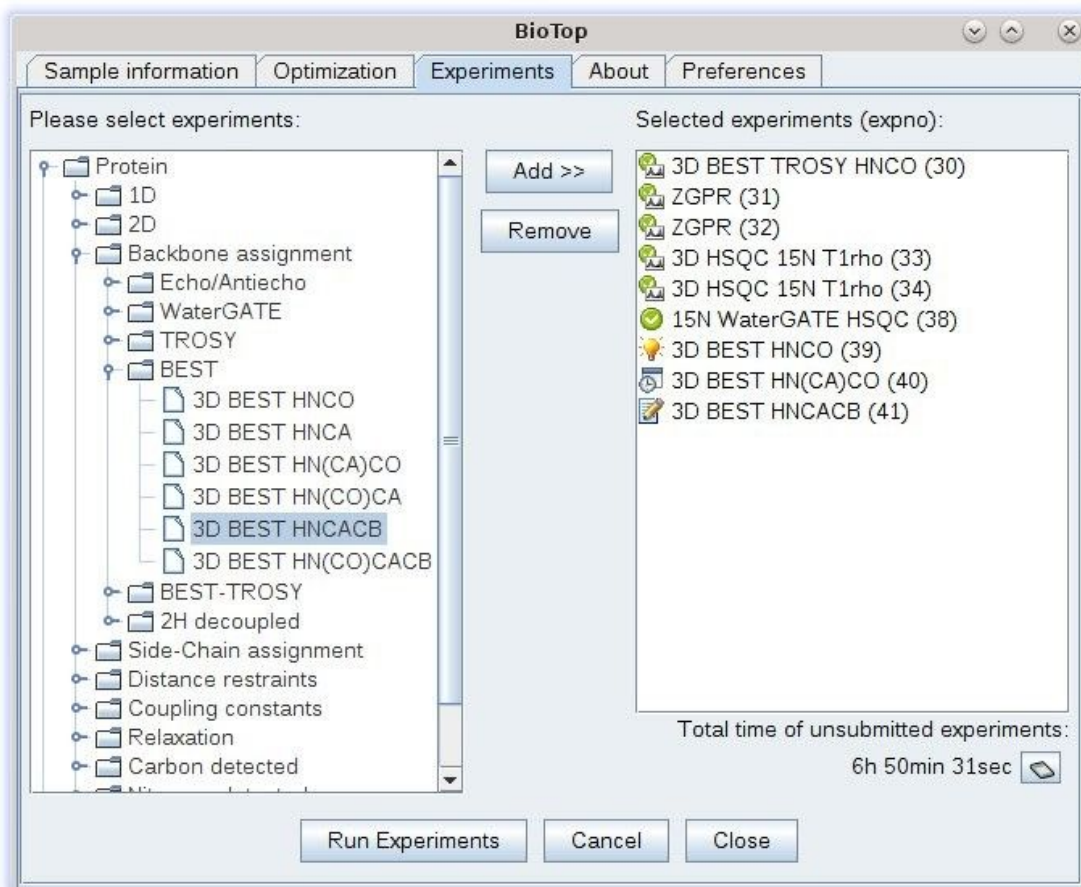


This step can be omitted completely, in that case optimizations will be selected based on experiments selected in the next step.

The **Start optimization** button will run the selected optimizations and the general operations (temperature, shimming...) previously selected.

The **Report** button generates and displays a PDF report including sample information and optimized parameters.

2.5 The Experiments Tab



Highlight experiments in the left hand tree and click the **Add** button to add them to the experiment queue. Right click experiments on the right hand experiment queue to edit or show details and parameters. Experiments not suitable for the current solvent and/or labeling will be greyed out, but can still be selected.

The **Run Experiments** button will send the experiments to the TopSpin command spooler. If any parameters used in the selected experiments have not yet been optimized, a popup will appear, prompting if these optimizations should be done automatically before starting the experiments.

Once finished, a processing option will be available in the right click menu on the right hand side.

3 Performed Optimizations

Whenever optimizations are started, all general operations, which are checked on the sample tab, will be executed first (set temperature, tune, shim). Afterwards, all selected optimizations will be executed in a fixed order:

- Proton 90 degree pulse
- Water offset
- 90 degree water-selective pulse
- 90 degree pulse for ¹³C or ¹⁵N (has to be manually selected)
- Nitrogen offset and spectral width
- Carbonyl offset and spectral width
- Alpha carbon offset and spectral width

All optimizations on nitrogen or carbon will use the proton parameters determined previously.

3.1 Proton 90 Degree Pulse

The proton pulse will be determined by executing the command **pulsecal**.

3.2 Water Offset

The water offset will be determined by using the au program `bt_o1calib`, which will determine the water offset by applying a continuous presaturation field at 50 Hz RF field strength and optimize the offset in real time.

3.3 90 Degree Water-Selective Pulse

The 90 degree water selective pulse will be determined by performing a parameter optimization (**popt**) on a selective WaterGATE experiment (**zggpwg**). The power for the optimization ranges from -1 to +2 dB with respect to the power calculated from the 90 degree hard pulse.

3.4 90 degree pulse for Carbon or Nitrogen

1D SOFAST-HMQC² type Experiments in which the t1 evolution period has been replaced by a single 270 degree pulse which will be varied using **popt**.

3.5 Nitrogen Offset and Spectral Width

The nitrogen offset and spectral width is determined from a short 2D proton nitrogen correlation experiment. This can be either a SOFAST-HMQC², Fast HSQC³, BEST-TROSY⁴ or TROSY⁵ experiment depending on the molecular size (> 20 kD) and whether the molecule is deuterated or not.

The resulting spectrum is processed, phase corrected and peak picked. If the resulting spectral width is larger than 42 or smaller than 25 ppm, a fall-back value of 35 ppm is used with an offset of 117 ppm.

3.6 Carbonyl Offset and Spectral Width

This optimization is performed on a 2D proton carbon plane of an HNCO⁶ (HSQC based, BEST⁷, BEST-TROSY⁴ or TROSY⁵) and the analysis is done the same way as for nitrogen.

If the resulting spectral width is larger than 20 or smaller than 19 ppm, a fall-back value of 14 ppm is used with an offset of 173.5 ppm.

3.7 Alpha Carbon Offset and Spectral Width

This optimization is performed on a 2D proton carbon plane of an HNCA⁶ (HSQC based, BEST⁷, BEST-TROSY⁴ or TROSY⁵) and the analysis is done the same way as for nitrogen.

If the resulting spectral width is larger than 40 or smaller than 24 ppm, a fall-back value of 32 ppm is used with an offset of 53.2 ppm.

4 Automated Processing

bioTop currently supports automated processing up to 3D experiments, including pseudo3D and NUS. Automated processing includes processing, phase correction and strip transformation for proton detected experiments.

In case of 3D experiments, 2D F2F3 projections will be used to determine the phase correction. In case of HNCACB type experiments, if the “-DLABEL_CB” flag is not set, the F1F3 projection is used to avoid peak cancellation in the F2F3 plane due to opposite signs of CA and CB signals which would be projected on top of each other.

For non-uniformly sampled 3D experiments, the full 3D will be processed without reconstructing the missing data points using “ftnd 0 nusft”. The resulting 3D is projected to a 2D plane and used for phase correction and strip limits determination after Hilbert Transformation to reconstruct the missing imaginary parts. The final processing uses the reconstruction settings from the dataset (by default mdd for 3D and cs for 2D experiments).

Pseudo3D experiments will be processed in F3 and F2, the number of points in F1 (“1 SI”) will be set to the number of planes acquired. If a corresponding ProteinDynamicsCenter (PDC) template is available, PDC will be launched if installed and a standard analysis is executed.

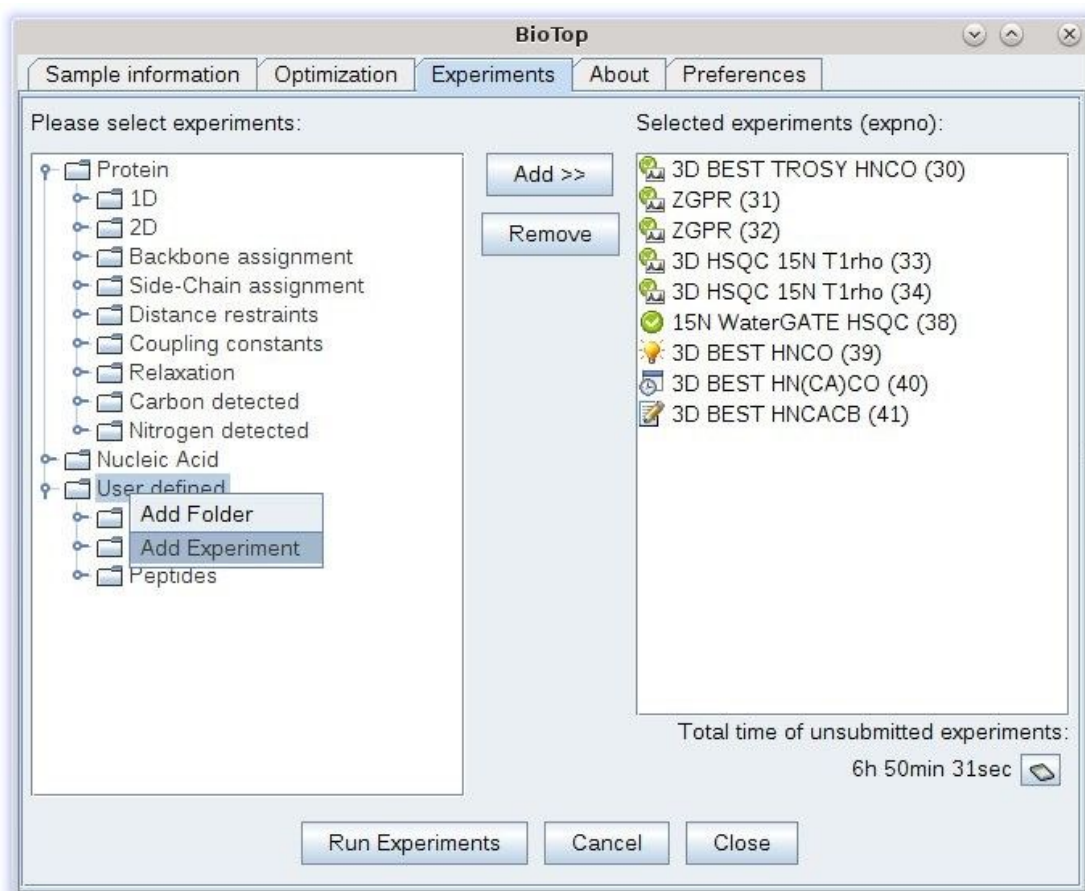
Experiments that require splitting of the raw data (e.g. IPAP for virtual decoupling or determination of coupling constants) will be split to the current expno+10000 (when free) automatically, using the appropriate **split** or **splitcomb** command.

5 Adding User Defined Experiments

bioTop supports user defined experiments. This can be done in two flavours:

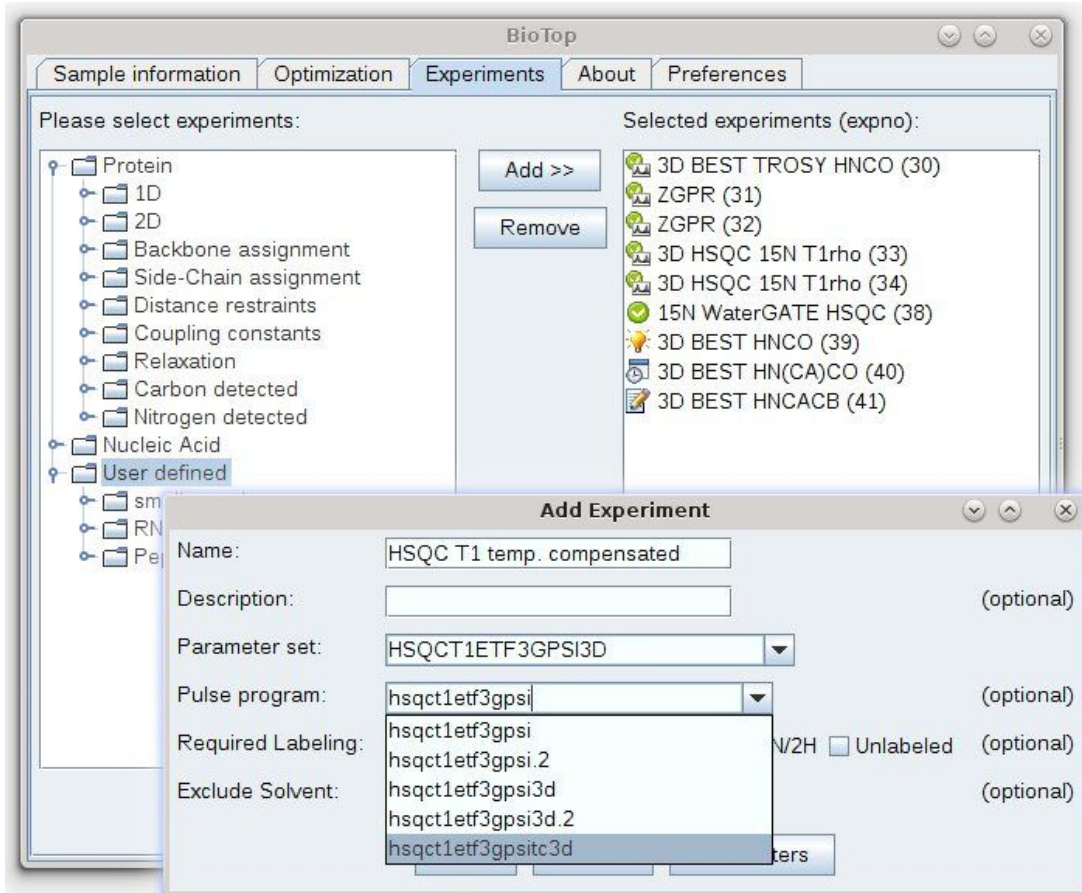
- Re-adding existing experiments to the user defined section (e.g. to resort or create your own pre-sets of multiple experiments)
- Adding custom parameter sets and pulse programs or just pulse programs based on existing parameter sets

Starting from TopSpin 3.5p17, a graphical user interface is included to add user defined experiments. To access it, just right click on the user defined experiments folder:

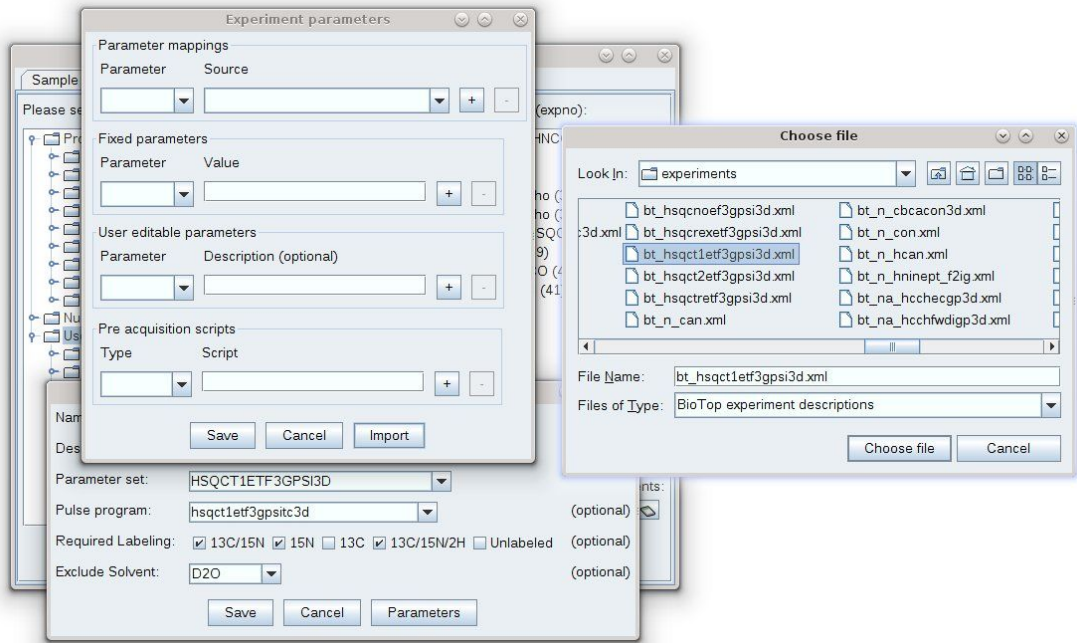


Adding User Defined Experiments

An experiment consists of at least a name, a parameter set to start with and eventually a pulseprogram that deviates from the parameter set if necessary:

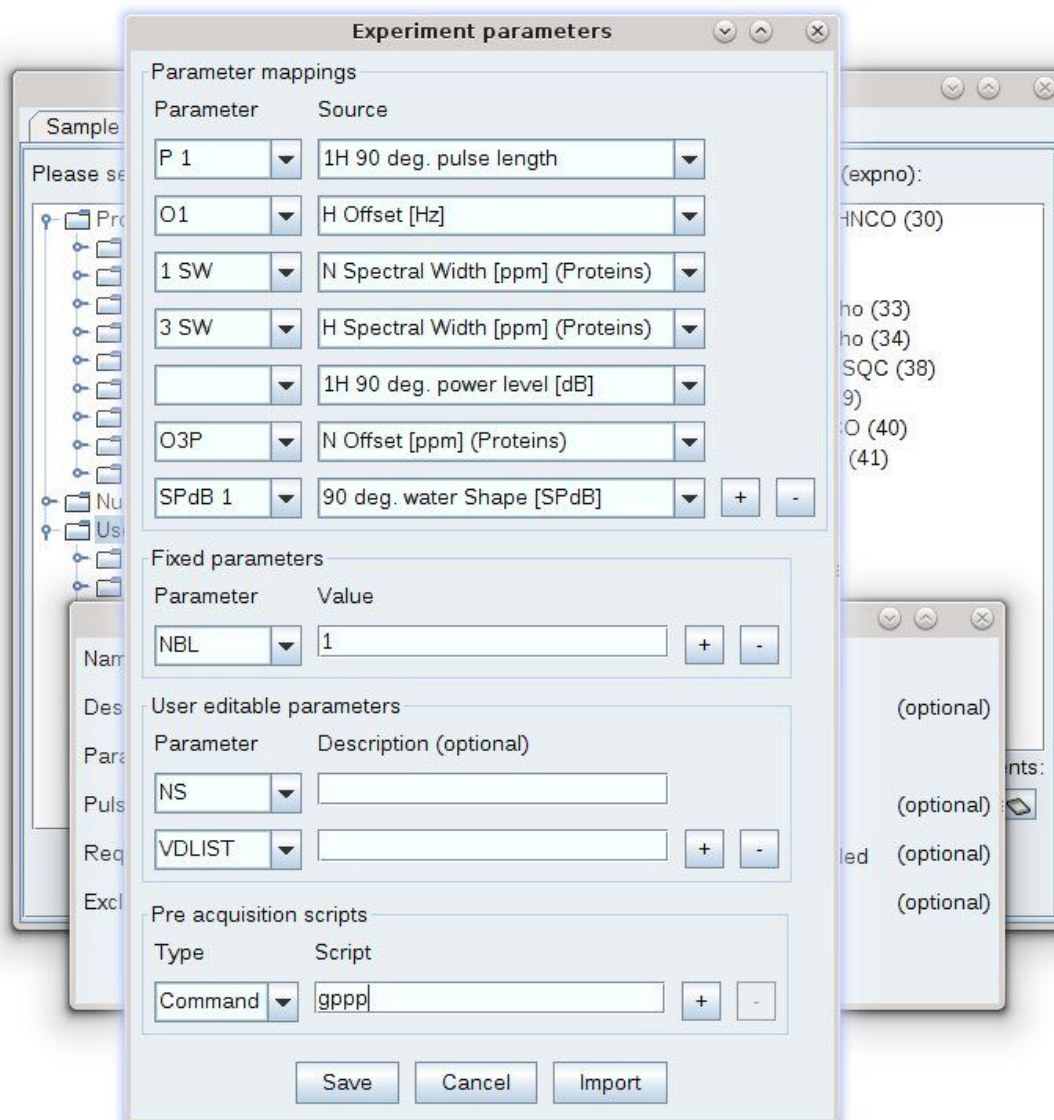


At this point, the experiment can already be used; however the setup consists of only **rpar** and **getprosol**. To add individual parameter mappings or parameters to be added by the user, click the Parameters button. For simplicity, you can start by importing an existing experiment description of a similar experiment. Experiment descriptions are named *bt_<pulse_program>.xml*:



Once this is done, make final adjustments and add setup scripts if necessary, e.g. **gppp** to set gradients according to the pulse program comments, if starting from a different parameter set:

Adding User Defined Experiments



A user defined experiment list can be found in `<user_home>/topspin-<computer_name>/biotop/bt_Experiments.xml`, user defined experiment descriptions can be found in `<user_home>/topspin-<computer_name>/biotop/experiments`.

If these files or folders do not exist, they will be created on the first launch of bioTop.



You cannot overwrite default experiments. If a pulse program (and therefore the experiment description) exists with the exact same name in the default folder, this one will be used.

6 bioTop Input Formats

bioTop mainly uses three types of input formats:

- An experiment list, containing all experiments, including the required solvent, labelling...
- An experiment description for individual experiments (optional)
- Template files for ProteinDynamicsCenter analysis (only for certain relaxation measurements)

6.1 Experiment List

The default experiment list can be found in the *exp/stan/nmr/py/biotop* subfolder named *bt_Experiments.xml*.

This is an example of a few experiment entries (descriptions have been shortened for better readability):

```
<?xml version="1.0" ?>
<Experiments>
  <Protein>
    <oneD alias="1D">
      <Experiment name="PROTON" parset="PROTON" desc="" ExcludeSolvent="H2O+D20"/>
      <Experiment name="ZGPR" parset="ZGPR" desc="1D using presaturation"/>
      <Experiment name="ZGGPWG" parset="ZGGPWG" desc="..."/>
      <Experiment name="ZGCPGPPR" parset="ZGGPWG" desc="..." Pulprog="zgcpgppr"/>
      <Experiment name="ZGGPW5" parset="ZGPR" desc="1D W5..." Pulprog="zggpw5"/>
    </oneD>
    <twoD alias="2D">
      <Experiment name="15N FHSQC" parset="FHSQCF3GPPH" desc="15N Fast HSQC"
        ExcludeSolvent="D20" RequireLabeling="15N;13C/15N;13C/15N/2H"/>
      <Experiment name="15N WaterGATE HSQC" parset="HSQCFPF3GPPHWG" desc="..."
        ExcludeSolvent="D20" RequireLabeling="15N;13C/15N;13C/15N/2H"/>
    </twoD>
  </Protein>
</Experiments>
```

All enclosing tags have to be valid standard XML but can have aliases for the Experiments list e.g. `<oneD alias="1D">` will show up as „1D“ in the Experiment tree. The individual experiments have to be enclosed by an “Experiment” tag. Required Parameters are name and parset, optional ones are desc, ExcludeSolvent, RequireLabeling and Pulprog.

6.1.1 Experiment Parameters

6.1.1.1 Name

The name will show up in the experiment tree as well as the experiments list. It does not have to match the parameter set or pulse program name.

6.1.1.2 Parset

The parameter set to be loaded. This one has to be available for the standard **rpar** command.

6.1.1.3 Desc

An experiment description. This one will be shown as mouse-over in the experiment tree. This can be used to further specify experimental details not covered by the name.

6.1.1.4 ExcludeSolvent

A semi-colon separated list of solvents which are not suitable for the experiment (e.g. because they require solvent suppression). Experiments not matching the solvent will be grayed out in the experiment list.

6.1.1.5 RequireLabeling

A semi-colon separated list of labeling schemes which are suitable for the experiment. Experiments not matching the labeling will be grayed out in the experiment list.

6.1.1.6 Pulprog

This can be used to set up an experiment when there is no parameter set available. If Pulprog is set, the parameterset defined by parset is loaded and the pulse program is changed afterwards.

6.2 Experiment Description Files

Individual experiments are described by files in the *exp/stan/nmr/py/biotop/experiments* folder. These files are linked to pulse programs, the name will be *bt_<pulprog>.xml*. You can see an examples (*bt_zggpw5.xml*) here:

```
<?xml version="1.0" ?>
<BioKit>
  <Mappings>
    <Row parameter="P 1" source="Pulses/Row:nucleus=1H:pulseLength"/>
    <Row parameter="PLdB 1" source="Pulses/Row:nucleus=1H:powerLevelDB"/>
    <Row parameter="O1" source="Offsets/Row:nucleus=H:ohz"/>
  </Mappings>
  <UserEdit>
    <Row parameter="D 19"/>
  </UserEdit>
  <preAQ>
    <Row cmd="gppp"/>
    <Row pycode="PUTPAR('D 19', str(float(GETPAR('DW'))*2)+'u')"/>
  </preAQ>
</BioKit>
```

So far there are three main sections which are *Mappings*, *UserEdit* and *preAQ*. All of these, as well as the file itself are optional. If no experiment description is present, the setup will include a **getprosol** using the proton pulse, but nothing more. The parameters available to the user via the simplified interface will be TD, NS and DS.

6.2.1 Mappings

These are definitions which optimized parameters will be transferred to which experimental parameters. The source consists of the section (e.g. Pulses, SpectralWidth and Offset) followed by a Row Identifier (e.g. nucleus=1H) and the parameter to be actually used (e.g. pulseLength, ohz, oppm, sw...) alternatively instead of source, value="..." can be used for a hard coded value. Parameter is the TopSpin parameter this value will be transferred to.



This one is in the notation of the TopSpin python interface e.g. ("D 1") instead of "d1" on the command line).

6.2.2 UserEdit

These are the parameters which are available to the user in the simplified bioTop parameter editor. An optional desc="..." statement can be used to overwrite the comment from the pulse program.

6.2.3 preAQ

A list of scripts that will be executed after the experiment, setup before the acquisition. Currently two statements are supported:

6.2.3.1 cmd

This can be any command that would also work from the TopSpin command line. In the above example, a command called **gppp** will be executed, which is a python script that sets all gradients to the values specified in the comments of the pulse program.

6.2.3.2 pycode

A single line of python code. This can be used to do calculations using parameters from the dataset. In the above example, the binomial solvent suppression delay d19 is set to twice the dwell time in microseconds.

6.3 Template Files for ProteinDynamicsCenter

A set of PDC template files are shipped in the *exp/stan/nmr/py/biotope/pdctemplates* folder. Currently included are T1, T2, T1ρ, hetNOE, CPMG relaxation dispersion and CEST. These will be used to do an automated PDC analysis for supported experiments.

7 Command Line Usage

The better part of bioTop can be accessed from the command line:

7.1 Experiment Setup

Once an optimization has been performed in a data folder, the command **btprep** can be used in all expnos to transfer optimized parameters to the current dataset (e.g. after reading a parameter set using **rpar**). Experiments added this way will not be added to the GUI.

7.2 Processing

The command **btproc** can be used from the command line. The only difference compared to the GUI will be the handling of experiments that require splitting of the raw data. When executed from the command line, **btproc** will always search for a free expno in increments of 10000, no matter whether the current dataset has been split before, expnos are not reused.

8 References

1. Piotto M., Saudek V., Sklenár V. "Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions.", *J. Biomol. NMR.* 1992, 2(6):661-665.
2. Schanda P., Kupce E., Brutscher B. „SOFAST-HMQC experiments for recording two-dimensional heteronuclear correlation spectra of proteins within a few seconds.“, *J. Biomol. NMR.* 2005, 33(4), 199-211.
3. Mori S., Abeygunawardana C., Johnson M.O., van Zijl P.C. "Improved sensitivity of HSQC spectra of exchanging protons at short interscan delays using a new fast HSQC (FHSQC) detection scheme that avoids water saturation.", *J. Magn. Reson. B,* 1995, 108(1), 94-98.
4. Solyom Z., Schwarten M., Geist L., Konrat R., Willbold D., Brutscher B. "BEST-TROSY experiments for time-efficient sequential resonance assignment of large disordered proteins.", *J Biomol. NMR.* 2013, 55(4), 311-321.
5. Pervushin K., Riek R., Wider G., Wüthrich K. "Attenuated T2 relaxation by mutual cancellation of dipole-dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution". *Proc. Natl. Acad. Sci. USA* 1997, 94, 12366-12371.
6. Grzesiek S., A. Bax A., "Improved 3D triple-resonance NMR techniques applied to a 31 kDa protein.", *J. Magn. Reson.* 1992, 96, 432 – 440.
7. Lescop E., Schanda P., Brutscher B. „A set of BEST triple-resonance experiments for time-optimized protein resonance assignment.“, *J. Magn. Reson.* 2007, 187(1), 163-169.

9 Contact

Manufacturer

Bruker BioSpin GmbH
Silberstreifen 4
D-76287 Rheinstetten
Germany
<http://www.bruker.com>

WEEE DE43181702

NMR Hotlines

Contact our NMR service centers.

Bruker BioSpin NMR provides dedicated hotlines and service centers, so that our specialists can respond as quickly as possible to all your service requests, applications questions, software or technical needs.

Please select the NMR service center or hotline you wish to contact from our list available at:

<https://www.bruker.com/service/information-communication/helpdesk.html>

Phone: +49 721-5161-6155

E-mail: nmr-support@bruker.com





Bruker Corporation

info@bruker.com
www.bruker.com

Order No: H169690