TopSolids

● Assisted Solid State NMR

User Manual

Version 001

Innovation with Integrity

NMR

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Contents

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1   About This Manual

This manual enables safe and efficient handling of the device.

This manual is an integral part of the device, and must be kept in close proximity to the device where it is permanently accessible to personnel. In addition, instructions concerning labor protection laws, operator regulations tools and supplies must be available and adhered to.

Before starting any work, personnel must read the manual thoroughly and understand its contents. Compliance with all specified safety and operating instructions, as well as local work safety regulations, are vital to ensure safe operation.

The figures shown in this manual are designed to be general and informative and may not represent the specific Bruker model, component or software/firmware version you are working with. Options and accessories may or may not be illustrated in each figure.

1.1   Policy Statement

It is Bruker's policy to improve products as new techniques and components become available. Bruker reserves the right to change specifications at any time.

Every effort has been made to avoid errors in text and figure presentation in this publication. In order to produce useful and appropriate documentation, we welcome your comments on this publication. Field Service Engineers are advised to check regularly with Bruker for updated information.

Bruker is committed to providing customers with inventive, high-quality, environmentally-sound products and services.

1.2   Symbols and Conventions

Safety instructions in this manual and labels of devices are marked with symbols.

The safety instructions are introduced using indicative words which express the extent of the hazard.

In order to avoid accidents, personal injury or damage to property, always observe safety instructions and proceed with care.

⚠ **DANGER**
DANGER: Indicates a hazardous situation that, if not avoided, will result in death or serious injury. This signal word is limited to the most extreme situations.
This is the consequence of not following the warning.
1. This is the safety condition.
▶ This is the safety instruction.

⚠ **WARNING**

WARNING: Indicates a hazardous situation that, if not avoided, could result in death or serious injury.

This is the consequence of not following the warning.

1. This is the safety condition.
   ▶ This is the safety instruction.

⚠ **CAUTION**

CAUTION: Indicates a hazardous situation that, if not avoided, could result in minor or moderate injury.

This is the consequence of not following the warning.

1. This is the safety condition.
   ▶ This is the safety instruction.

NOTICE

NOTICE: Indicates information considered important, but not hazard-related (e.g. messages relating to property damage).

This is the consequence of not following the notice.

1. This is a safety condition.
   ▶ This is a safety instruction.

SAFETY INSTRUCTIONS

SAFETY INSTRUCTIONS are used for control flow and shutdowns in the event of an error or emergency.

This is the consequence of not following the safety instructions.

1. This is a safety condition.
   ▶ This is a safety instruction.

ℹ This symbol highlights useful tips and recommendations as well as information designed to ensure efficient and smooth operation.

1.3   Font and Format Conventions

Type of Information	Font	Examples
Shell Command, Commands, "All that you can enter"	Arial bold	Type or enter **fromjdx zg**
Button, Tab, Pane and Menu Names "All that you can click"	Arial bold, initial letters capitalized	Use the **Export To File** button. Click **OK**. Click **Processing**…
Windows, Dialog Windows, Pop-up Windows Names	Arial, initial letters capitalized	The Stacked Plot Edit dialog will be displayed.
Path, File, Dataset and Experiment Names Data Path Variables Table Column Names Field Names (within Dialog Windows)	Arial Italics	*$tshome/exp/stan/nmr/lists* *expno, procno,*
Parameters	Arial in Capital Letters	VCLIST
Program Code Pulse and AU Program Names Macros Functions Arguments Variables	Courier	`go=2` `au_zgte` `edmac` `CalcExpTime()` `XAU(prog, arg)` `disk2, user2`
AU Macro	Courier in Capital Letters	`REXPNO`

Table 1.1: Font and Format Conventions

2   Introduction

TopSolids is an interactive user interface that is especially designed to assist in the delicate setup of probe hardware and difficult experiments dedicated to solid state NMR in structural biology and material science. It is readily accessible to users from a broad diversity of background and experience.

TopSolids generates a project structure within TopSpin™ and organizes the available experiments automatically. It offers a standard library of state of the art multinuclear multidimensional experiments designed for 4 mm and 3.2 mm probes.

Based on specific spectrometer and probe configurations, which are detected automatically in the background, a fully automated measurement of ^{1}H and ^{13}C RF hard pulses as well as spectral referencing is rapidly realized. The user is guided through the magic-angle adjustment. Probe shimming can be done fully automated in the 'z' direction. Both outcomes are evaluated by TopSolids to guarantee for best performance of the probe.

Afterwards, TopSolids guides the user successively through the optimization process needed to acquire multidimensional experiments, either on standard samples or directly on the sample of interest. Here, different flow bar modules for different applications are available.

All optimized parameters and settings are stored in a parameter data bank within the project and can be reviewed in form of a PDF report at any time to assist fast publication. Furthermore, this data bank can be imported to a later project to avoid re-optimization.

To use TopSolids you need to have installed at least TopSpin 3.6 or TopSpin 4.

This Manual describes the overall software setup to guarantee a working TopSolids interface to create your personal project, to set up the general spectrometer environment for your experiments, and finally to show you how to successfully run the desired correlation spectra on your sample of interest.

This Manual is written primarily for Bruker AVANCE III/HD and AVANCE NEO instruments.

> **i** Please note that the figures shown in this manual are designed to be general and informative and may not represent the specific version you are working with.

2.1   Disclaimer

Any hardware mentioned in this manual should be used only for their intended purpose as described in their respective manual. Use of hardware for any purpose other than that for which it is intended is taken only at the users own risk and invalidates any and all manufacturer warranties.

> **i** Service or maintenance work on the units must be carried out by qualified personnel. Only those persons schooled in the operation of the units should operate the units.

Read the appropriate user manuals before operating any of the units mentioned. Pay particular attention to any safety related information.

2.2   Safety Issues

In order to work safely in laboratories with NMR spectrometers all users have to follow the safety regulations for magnetic, electrical, cryogenic and chemical safety. For detailed information please refer to the safety instructions in the Beginners Guide manual provided on the TopSpin DVD or in the TopSpin **Help** → **Manuals** submenu.

3 Expected Expertise

3.1 General Requirements

Although TopSolids is mainly addressed to users, who are not familiar with the field of solid-state NMR, we recommend the user to have a certain expertise of the following scope:

- TopSpin (acquisition, processing).
- Rotor - handling, sample changing, temperature (especially when handling biological samples), etc.

  Video Tutorial "*How to fill a rotor*" available at:

  http://www.theresonance.com/2014/categories/material-science/nmr-tutorial-mas-rotor-filling

- Video Tutorials on how to run different TopSolids steps are available on Bruker's YouTube channel: *https://www.youtube.com/user/brukertv/videos*
- MAS unit & MA adjustment.
- Probe tuning, matching & shimming.

In case you miss any of the points mentioned above, please read this manual and its corresponding instructions very carefully.

In TopSpin, the **Help** menu contains a list of available manuals. Especially the following ones can be supportive when using the TopSolids software:

- **General**
  - *User Manual*
- **Beginners Guides**
- **Acquisition** – **User Guides**
  - *1D and 2D Step-by-Step-Basic : '* edhead*'*
- **Acquisition - Application Manuals**
  - *Solids Introduction*
  - *Solids*
- **Acquisition & Processing References**
  - *Proc. Commands & Parameters*
  - *Edprosol Manual*
- **Technical Manuals**
  - *Temperature Regulation*

3.2 PROSOL for TopSolids

TopSolids fetches some of the experimental parameters from PROSOL. This chapter describes the basic usage of PROSOL and its entries, focusing on those parameters which are used by TopSolids and which, therefore, need to be set by the user.

This is a step that only has to be made once for every probe. It is recommended to take the time needed to set up the PROSOL table for the probe you want to use to ensure a proper TopSolids workflow.

Expected Expertise

 Please note that there is a video tutorial available at our Bruker YouTube channel: *https://www.youtube.com/watch?v=rGkdUR0mW2I*

An overall description of PROSOL can be found in the *Edprosol Manual* (see chapter *General Requirements [▷ 11]*).

3.2.1 Introduction

PROSOL (**Pro**be and **Solv**ent related parameter settings) is a general tool to store and get probe and solvent related parameters. Basically, it provides tables of experimental parameters like pulse widths and corresponding power levels etc., which are suitable for the corresponding experiment, will provide good results, and are within probe specifications. The reference to the solvent stems from the fact that in liquid state NMR the solvent determines the quality factor (Q) of the probe which in turn determines the pulse width etc. at a given power level. For liquid state NMR probes, therefore, usually separate PROSOL tables exist. For solid state NMR probes usually a single table is sufficient, because the main impact on the probe's Q is provided by the rotor material. For lossy samples, like proteins dispersed in a buffer solution, however, the sample's impact on Q often cannot be neglected and a distinct PROSOL table is needed or – as is done by TopSoldis – a fine-tuning of parameters on the respective sample of interest is performed.

3.2.2 Basic Usage of PROSOL

The PROSOL table can be edited by typing the command **edprosol** at the TopSpin command prompt or by clicking the corresponding menu entry:

Manage | Spectrometer | Experiments/Parameters | Probe/Solvent Depending Params (edprosol)

This will open the PROSOL tables and allows setting or changing of parameters.

The command **getprosol** will read the PROSOL table for the solvent set in the TopSpin parameter `solvent`. Within the TopSolids tool the command **getprosol** is executed automatically.

3.2.2.1 The Command edprosol and the PROSOL Tables

The command **edprosol** opens the PROSOL tables. By default the parameters are displayed for the currently defined probe, the solvent '**generic**', and the '**observe nucleus**' and the '**decoupling nucleus**' which are defined in the routing (**edasp**) for F1 and F2, respectively. The parameters for '**generic**' will be used for any solvent including '**none**' unless a dedicated PROSOL table exists or is created for a certain solvent. Thus, for solid state NMR, '**generic**' is fine.

The **edprosol** interface is shown in the figure below. In the upper part of this window the probe is shown on the left side, the solvent on the right side. The **observe** and the **decouple nucleus** are shown in the center. Each of these settings may be changed by the user if required. The lower part of the **edprosol** window has four tabs:

- **90 deg Pulses**
- **Square Pulses**
- **Shape Pulses**
- **Others**

The tab **90 deg Pulse** displays the 90° pulse width and the corresponding power for each nucleus within the probe's tuning range for observe and decouple.
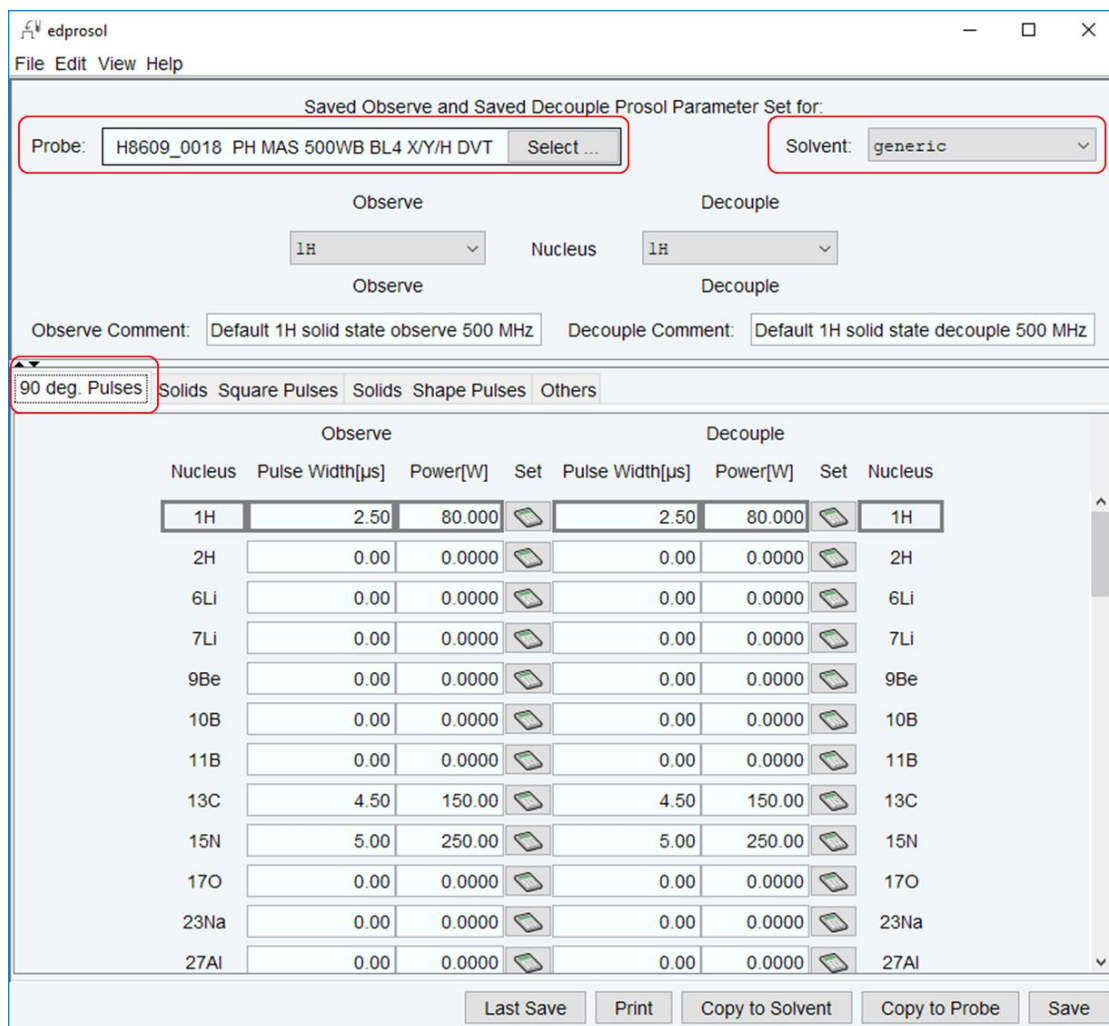
Figure 3.1: The edprosol Interface.

> *Interface of edprosol. In the top left corner, the actually used **Probe** has to be set. In the top right corner the **Solvent** has to be set to **generic**. In the bottom part four tabs can be activated and edited.*

When starting from scratch (no PROSOL tables were created before), both the pulse width and the power level in Watts will be set to zero. If a PROSOL table already exists, the stored parameter values will be shown. The power units can be changed from dB to Watt via the **edprosol** menu: **View | View Mode for Power Power / Watt.**

Based on the entries made in this tab, other parameters will be calculated in the following tabs.

The tab **Solids Square Pulses** displays a set of predefined parameters like 90° and 180° hard pulses, soft square pulses for selective excitation, etc. This is shown in the following figure:
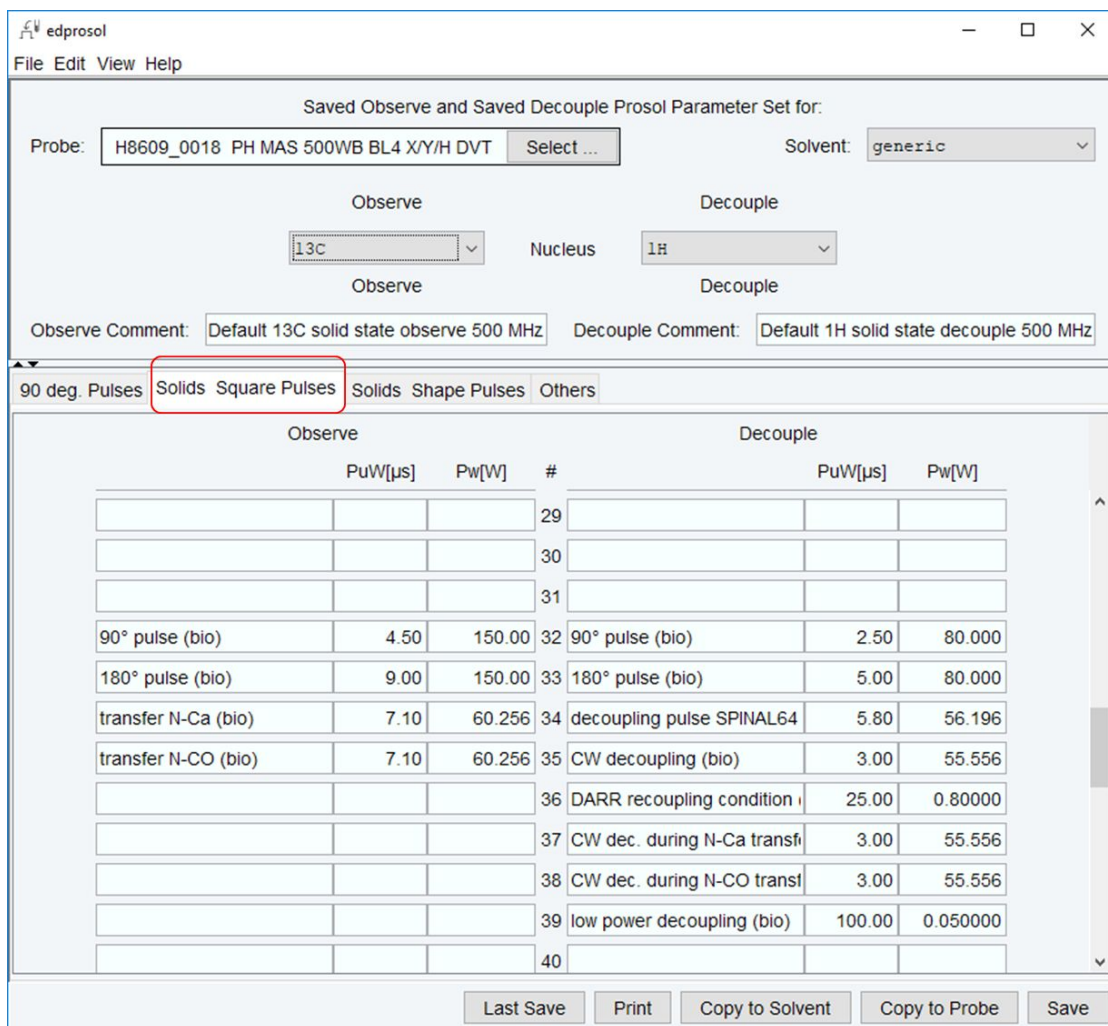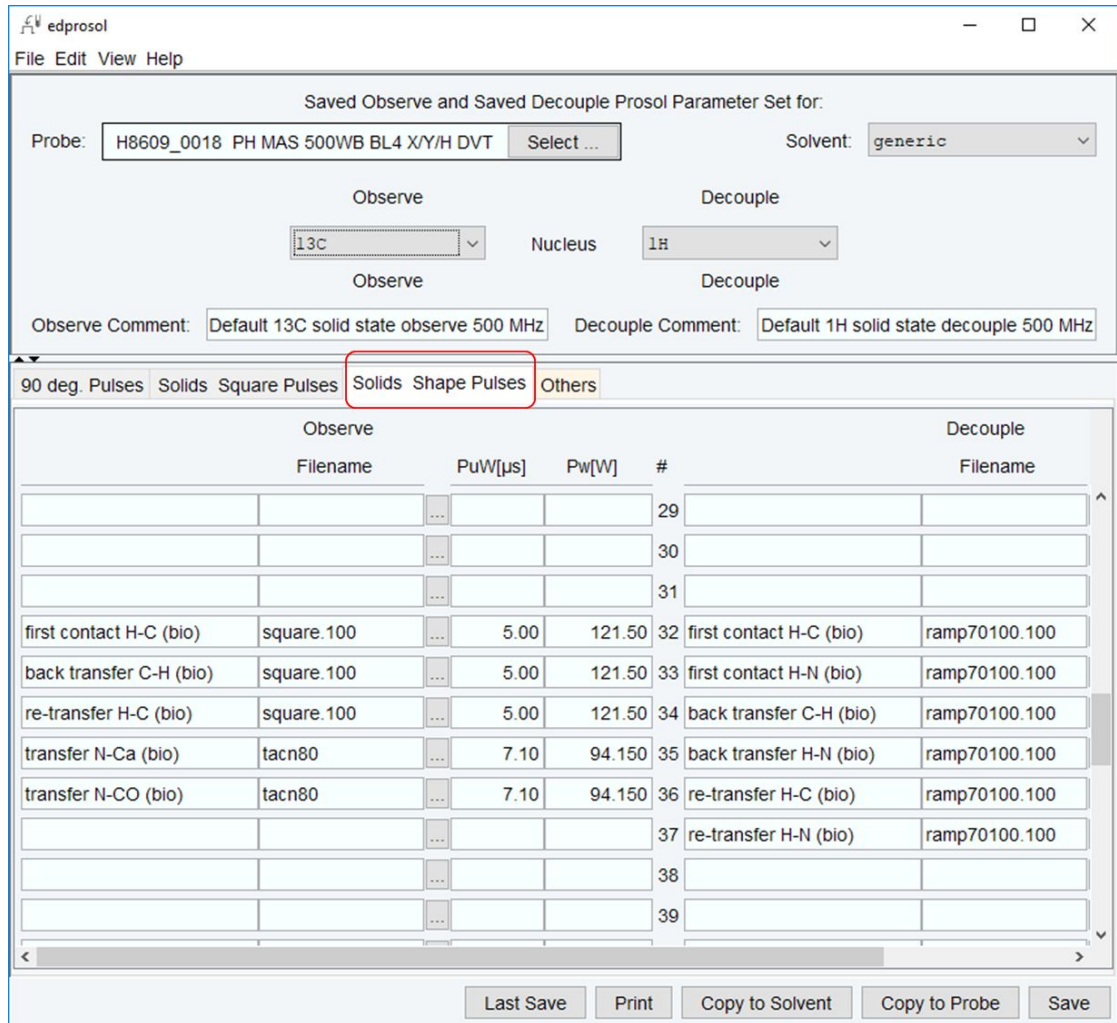
Figure 3.2: Solids Square Pulse Tab in edprosol.

> *Solids Square Pulse Tab in edprosol. If no pulse calculation was performed, any power in Watts is zero and the entries are marked in red.*

The tab **Solids Shape Pulses** displays a set of predefined parameters like shaped contact pulses, shaped selective pulses, etc. This is shown in the figure below. Like for the square pulses, a default pulse width is set as well as a shape file, but all power levels in Watts are set to zero and marked in red when starting from scratch.

Figure 3.3: Solids Shape Pulses Tab in edprosol.

Solids Shape Pulses Tab in edprosol. If no pulse calculation was performed, any power in Watts is zero and the entries are marked in red.

The tab **Others** displays a set of predefined parameters that depend on the probe as well as on the selected nucleus, as shown in the Figure below.
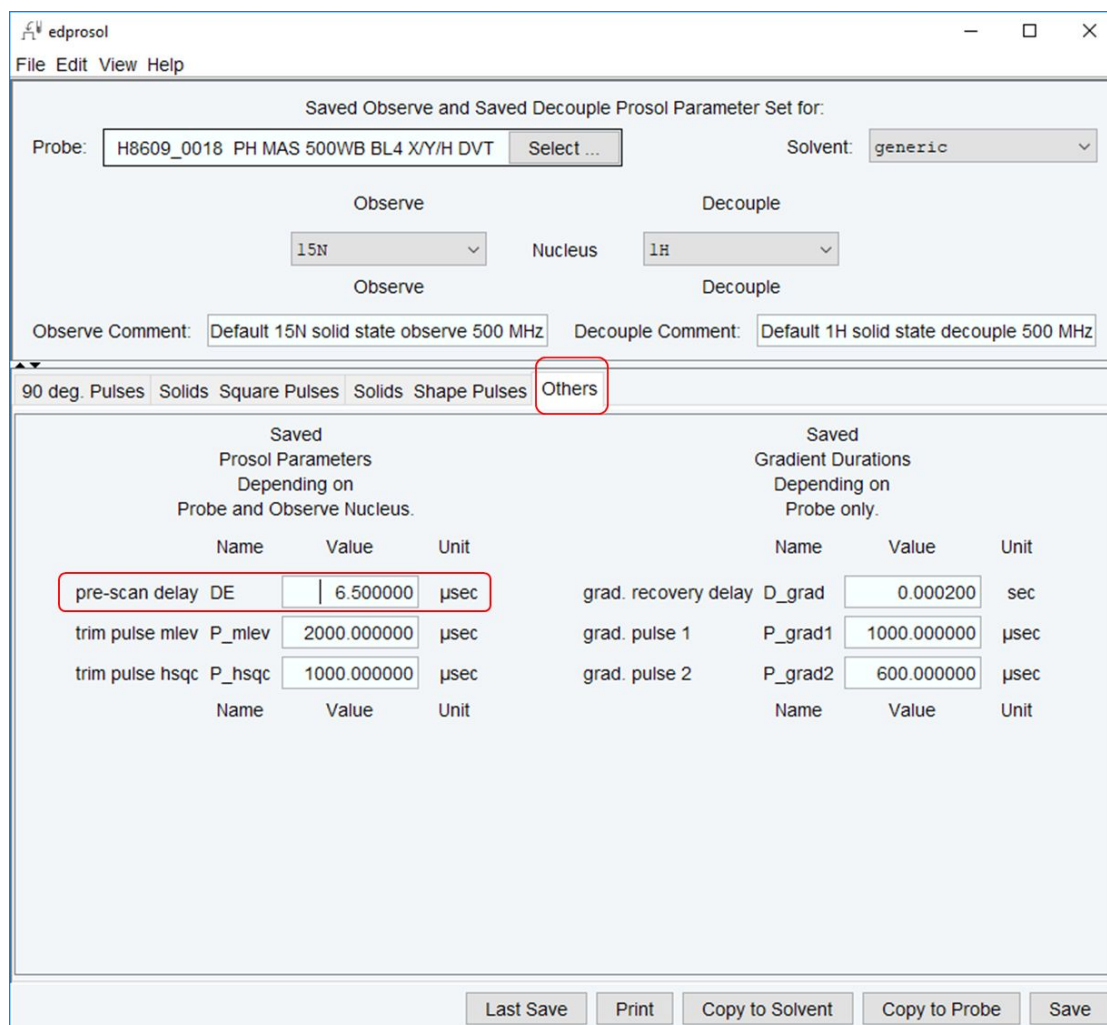

Figure 3.4: The Others tab in edprosol

> ℹ️ Sometimes it might be needed to increase the pre-scan delay slightly for certain nuclei (e.g. 15N detection) in case so called probe ringing occurs. This is reflected in a strong spike at the beginning of an FID. If this spike is too strong an error message will occur saying "TRX warning: ADC overflow during last scan!". When this problem occurs, perform the **edprosol** and increase the value of the pre-scan delay for the respective nucleus to e.g. 10 or 12 μs.

3.2.2.2   Filling in the PROSOL Table

The basic idea of the PROSOL table is to calculate all power levels according to the given flip angle and width for each respective pulse. The 90° pulse as defined in the tab **90 deg Pulses** is used as the master variable for each nucleus. The discrimination of **observe** and **decouple** pulses is kept to keep the tables similar to that of liquid state NMR probes. Usually, there is no difference in the pulse widths and power level for **observe** and **decouple** for solid state NMR probes. It is, therefore, recommended to use identical parameter values for both.

Usually, the 90° pulse and corresponding power level for a nucleus and probe is known from previous experiments, e.g. from the acceptance tests during the probe's initial installation or from the latest experiments performed. The entries do not need to be made for all nuclei, but only the ones you would like to use in combination with TopSolids, as shown in the Figure below. Next to the desired X nuclei of choice, the PROSOL tables for the following nuclei need to be updated:

- observe/ decouple: ^{1}H, ^{13}C, ^{79}Br (and for protein applications) ^{15}N.

> **i** The user has to make sure that the RF field and power values stay within the probe specifications.

Figure 3.5: Filling in the PROSOL Table.

> Filling in the PROSOL table. The 90° pulse width and power level of a nucleus are used as the master variables. A subsequent **Calculate all pulses of the set** are performed when clicking on the calculator icon.

By clicking on the **Set** button right to the pulse width and power level (calculator icon) a window will open, which allows to calculate pulses (square and shapes) of the set. The user may choose to calculate all pulses or to just recalculate the calculated ones. The first option is useful for the initial calculation or whenever any pulse should be set according to the 90°

pulse settings. The latter option is useful if some user calibration should be kept rather than overwritten. This is explained in more detail in the Chapter *Manipulating PROSOL Parameters [▷ 20]*.

Once the calculation has been done, the tables for square and shape pulses will show proper power levels. The PROSOL table should be saved after each entry. This can be done by clicking on the save button in the lower right corner of the **edprosol** window or by using the menu **File | Save**. If the PROSOL table has not been saved, the user will be prompted to do so before the next 90° pulse width and power level may be entered (see figure below).
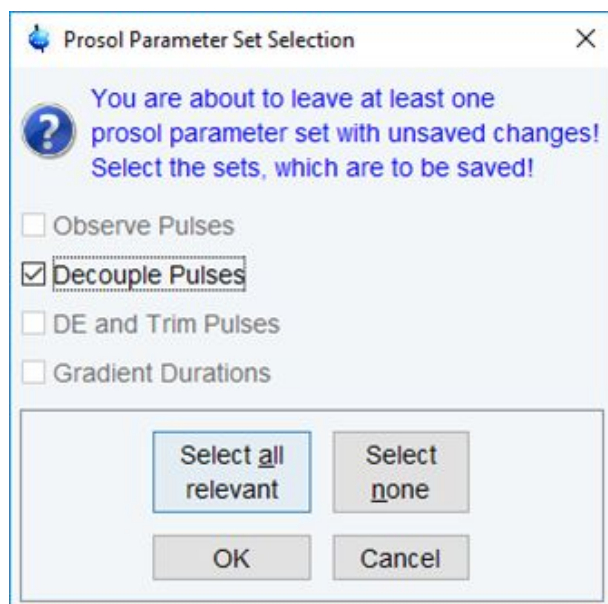

Figure 3.6: Prompt to save latest changes before continuing. Choose "Select all relevant" before clicking "OK".

> **i** To be able to change the PROSOL table entries the TopSpin password is requested.

TopSolids makes use of a subset of the PROSOL parameters. The most important parameters are explicitly defined in the entries 32 and following of the tabs **Solids Square Pulses** and **Solids Shape Pulses**, respectively (the next two figures).

Figure 3.7: Dedicated Square Pulses for ^{13}C (observe) and ^{1}H (decouple) parameters used in TopSolids Experiments.

Dedicated **Square Pulses** for TopSolids experiments are given in entries 32 and following. The power level for the given pulse width and flip angle was calculated from the entries made for the 90°pulses.
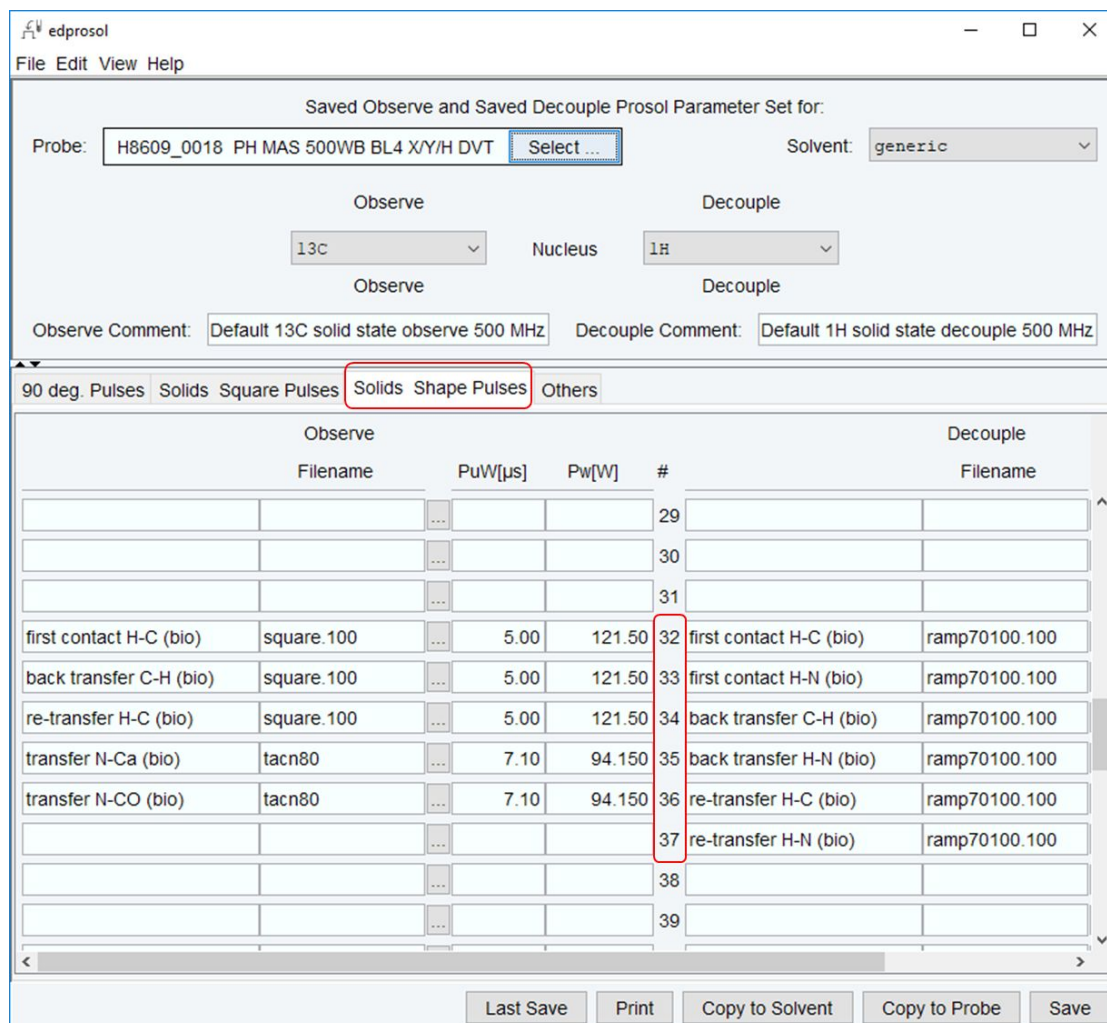
Figure 3.8: Dedicated Shape for ^{13}C (observe) and ^{1}H (decouple) parameters used in TopSolids Experiments.

> Dedicated **Shape Pulses** for TopSolids experiments are given in entries 32 and following. The power level for the given pulse width and shape file was calculated from the entries made for the 90°pulses.

3.2.3   Manipulating PROSOL Parameters

Entries of the PROSOL tables can be changed by the user. With respect to TopSolids this usually is not necessary, because the default parameter settings are chosen such that they are reasonable for solid state NMR experiments and optimization of the parameters is done subsequently by TopSolids.

There may, however, be some instances in which the user may want to change parameters:

- The experiment or probe may require pulse widths (or RF fields) different from the default ones entered in the **90 deg Pulses** Tab. In this case for dedicated square or shape parameters the user may enter the desired pulse width and PROSOL will calculate the corresponding power level accordingly. The user has to make sure that the RF field and power values stay within probe specifications!

- Previous optimization results may exist for some parameters. In this case the user may want to enter both the pulse width and the corresponding power level. In this case it is mandatory to enter the pulse width first and the power setting afterwards. When a power level is entered for a square pulse or a shape pulse, PROSOL will not recalculate the corresponding pulse width. Rather, it will remain unchanged and the power level will be marked in red to indicate that the corresponding pulse is no longer a calculated one.

- Care has to be taken in this case not to overwrite these optimized pulses by changing the 90°pulse width and calculating the pulses of the set. The user should use the option *Recalculate only calculated pulses!* as shown in *Figure 3.5 [▷ 17]*

3.2.4 Miscellaneous

3.2.4.1 Forcing PROSOL to Read Default Descriptions and Default Parameters

PROSOL will read and display existing tables. If these were created in TopSpin 3.2pl5 or below the TopSolids related parameters may not show up. In this case the user may force PROSOL to read the default description by accessing the corresponding entry in the **Edit** menu. The same holds true for the default parameter settings.

> **i** Reading default parameters will set all 90° pulse widths and power levels to zero and will do the same for all square pulse and shape pulse power levels. The user should note or print the previous PROSOL table and enter the proper 90° pulse width and power levels.

3.3 TopSpin

This is a brief introduction only. For detailed information please refer to the safety instructions in the Beginners Guide manual provided on the TopSpin DVD or in the TopSpin **Help** menu (see chapter *General Requirements [▷ 11]*).

3.3.1 Acquisition Functions

During the use of TopSolids there may be need to interact with TopSpin. You will find the most important acquisition functions summarized in the icon bar below:
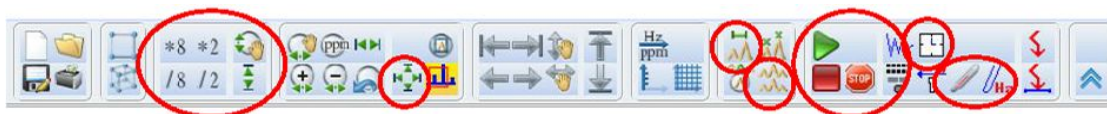

*Figure 3.9: Icon Bar of the TopSpin 3.x **Acquisition** Tab with TopSolids Relevant Functions Indicated.*

The top symbols from left to right with **bold** indicating important functions during the use of TopSolids are:

- New Dataset
- Open old Dataset
- Switch to last 2D
- **Enlarge spectrum by factor of 8**
- **Enlarge spectrum by factor of 2**
- **Enlarge spectrum manually**
- **Zoom in manually**
- Define exact zoom region in ppm

- Show full spectrum X-scale
- Toggle/Change the interactive zoom mode
- Move to left end of spectrum
- Move to right end of spectrum
- Move baseline up/down
- Move baseline to window center
- Change X-scale from Hz to ppm and vice versa
- **Measure distance in hz/ppm of two points in the spectrum**
- Prepare a frequency list
- **Start the acquisition**
- **Show the FID live window**
- **Calculate the expected running time**
- Set the SFO1 to the cursor position

The bottom symbols from left to right with **bold** indicating important functions during the use of TopSolids are:

- Save
- Print
- Switch to last 3D
- **Scale down spectrum by factor of 8**
- **Scale down spectrum by factor of 2**
- **Reset the intensity scale**
- Zoom in (by moving the mouse)
- Zoom out (by moving the mouse)
- Return to last saved zoom region
- **Show whole Spectrum**
- Toggle to keep the zoom region then changing datasets
- Move left in spectrum
- Move right in spectrum
- Move spectrum by moving the mouse
- Reset baseline to bottom of window
- Toggle Y-axis units
- Toggle grid display
- Toggle spectrum overview
- **Switch to spectrum overlay mode**
- **Halt an acquisition (data until halt is saved)**
- **Stop an acquisition (all data is lost)**
- **Open the BSMSDISPLAY**
- Open the Lock Display
- **Open the Temperature Display (EDTE window)**
- **Open the MAS display**
- Set the left/right limits of the display to the spectrum width and center the SFO1
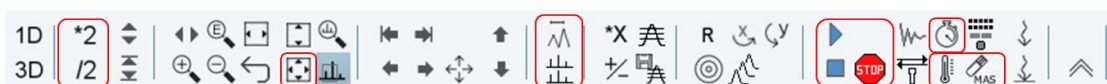- Close the lower icon bar

*Figure 3.10: Icon Bar of the TopSpin 4.x **Acquisition** Tab. Though icons have been altered, functionalities are the same as described above.*

3.3.2 Processing Functions

Beside acquisition, TopSpin provides processing functions to reference and process acquired data. If you select the tab **Process** shown in the figure below, you will find the processing functions (most important for TopSolids in **bold**) summarized in a flow bar:

- **Process the spectrum**
- **Adjust the phase manually**
- Baseline correction
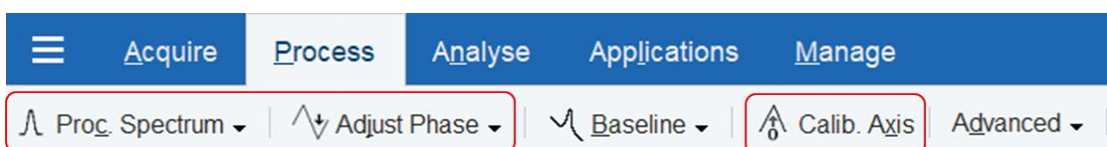- **Calibrate the ppm axis**
- Advanced functions

*Figure 3.11: Flowbar of the TopSpin **Process** Tab..*

Most of these functions are carried out by TopSolids automatically, but if it is necessary to reprocess data, the necessary functions can be found here.

3.3.2.1 Spectral Referencing

Spectral Referencing is an essential step for evaluating and comparing NMR results.

When executing the Probe Setup module, TopSolids is automatically referencing the spectrometer's B_0 field to neat TMS using the standard sample adamantane [*1 [▷ 79]*]. Nevertheless, two approaches for manual spectral referencing are described below in case further or different referencing may be needed.

The important parameter for spectral calibration is called **sr**, which stands for **S**pectral **R**eference. There are two possibilities for referencing:

Before you do measurements:

- Adjust the B_0 field value such that the **sr** value becomes zero.

> **i** Please note to do this after the step of **Shimming and Spectral Referencing** has been performed. Otherwise your referencing will be overwritten by TopSolids.

At any time:

- Calibrate the spectrum of a standard sample and use the resulting **sr** value for all spectra. Note: A non-zero sr value for one nucleus (e.g. ^{13}C) needs to be converted for any other nucleus (e.g. ^1H, ^{15}N – see *SR Value Calculation [▷ 78]*).

Following the first approach, it is best to calibrate the field directly after shimming the probe. Therefore, insert the reference sample, start the **gs** mode and:

- Open the **BSMS** display (**bsmsdisp**) and choose the tab **Lock/Level** (see chapter *BSMS Display [▷ 25]*).
- Modify the **field** value carefully in units of 1 until the reference peak is shifted to the desired chemical shift e.g. the left (low field) peak of the adamantane spectrum is shifted to 38.48 ppm for referencing to neat TMS.
- Stop the acquisition. Use a **sr** value of zero for all experiments.

Following the second approach you can calibrate the reference spectrum, e.g. on adamantane at any time:

- Go to the reference spectrum.
- Zoom into the region of the respective peak.
- Go to the tab **Process** and click on **Calib. Axis**.
- Do a left-click on the center of the peak and reference it to the needed value, e.g. 38.48 ppm for referencing to neat TMS. After referencing, click the arrow button to leave the menu.
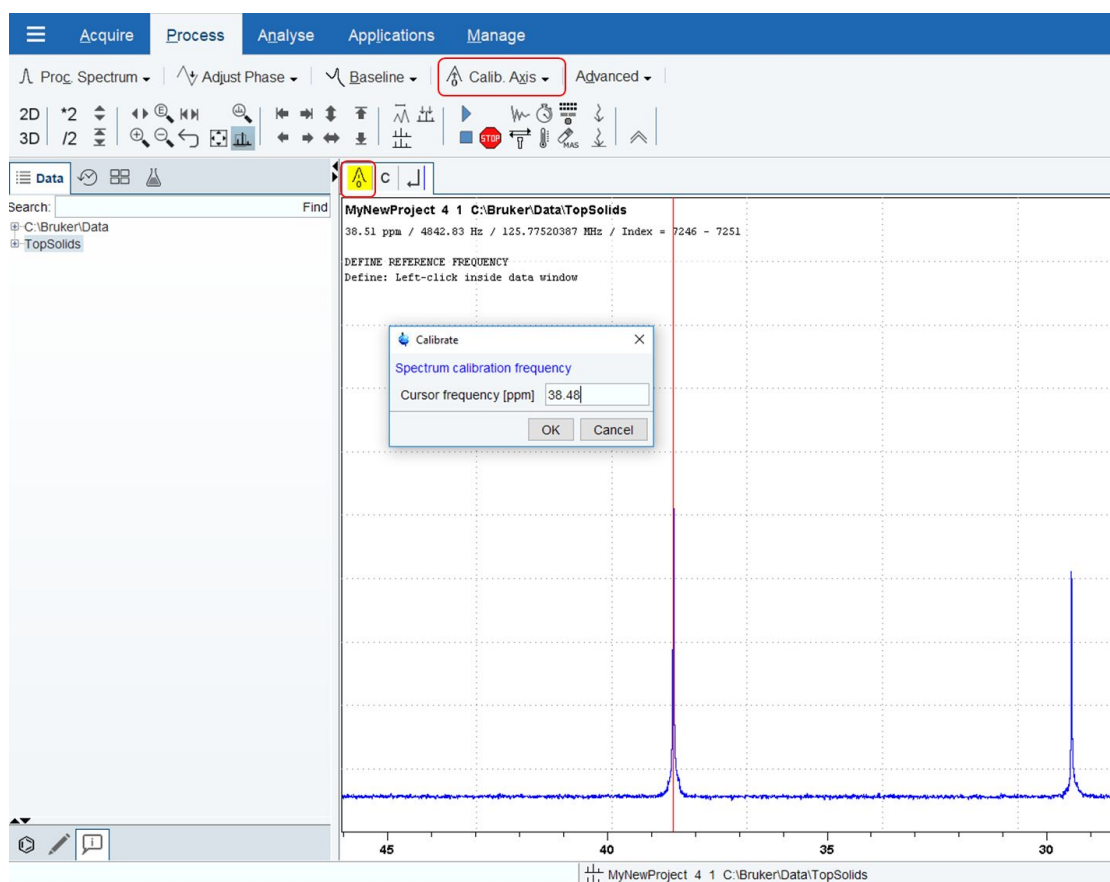

Figure 3.12: Calibration of the Left Peak of an Adamantane ^{13}C Spectrum to 38.48 ppm.

- The resulting **sr** value can be used for referencing of ^{13}C dimensions and for recalculation of **sr** values for other nuclei (see *SR Value Calculation [▷ 78]*).
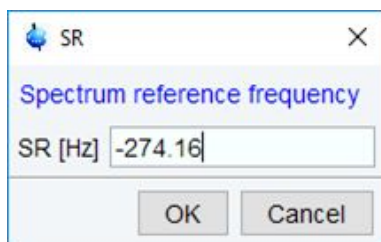
Figure 3.13: SR Value Used for Spectral Referencing.

3.3.3 BSMS Display

This is a brief introduction only. For detailed information please refer to the safety instructions in the Beginners Guide provided on the TopSpin DVD or in the TopSpin **Help** menu (see chapter *General Requirements [▷ 11]*).

The BSMS (**B**ruker **S**mart **M**agnet control **S**ystem) provides an overview of the most important features and states of your shim system and magnet. It can be opened by using the button explained in the chapter *Acquisition Functions [▷ 21]* or by typing '**bsmsdisp**' into the TopSpin command line.
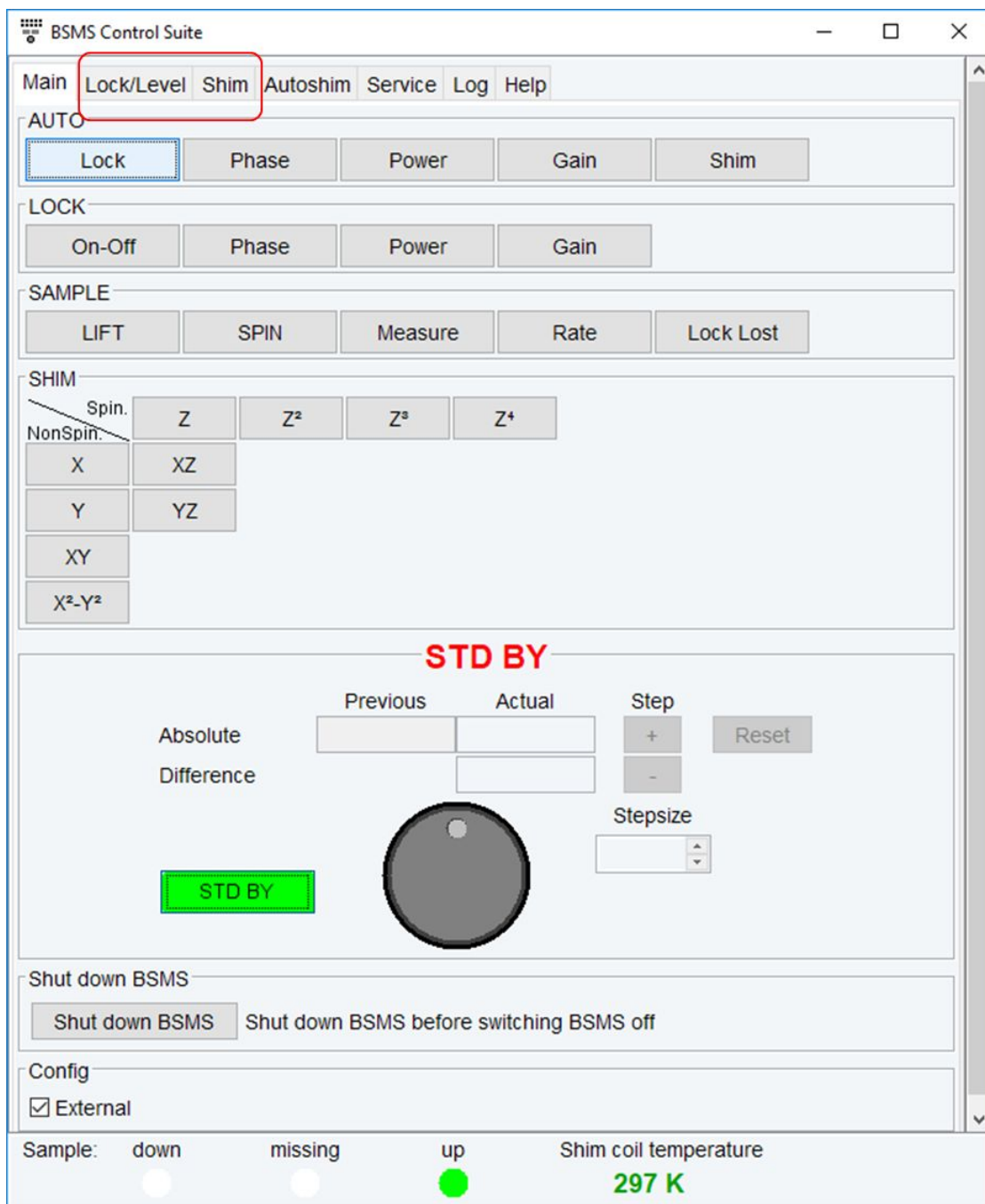
Figure 3.14: The BSMS Interface.

> Interface for the BSMS. The tabs **Shim** and **Lock/Level**, are especially important for TopSolids.

If the magnetic B_0 field needs to be adjusted, the field setting can be accessed in the tab **LOCK/LEVEL** (figure below, left). Here, you can modify the magnets base field, to match the [1]H-frequency to the standard frequency used for referencing.

During the shim procedure you might switch to the tab **SHIM** to manually adjust the shim (figure below, right). Here, especially the buttons for Z, X and Y shim adjustment are important.

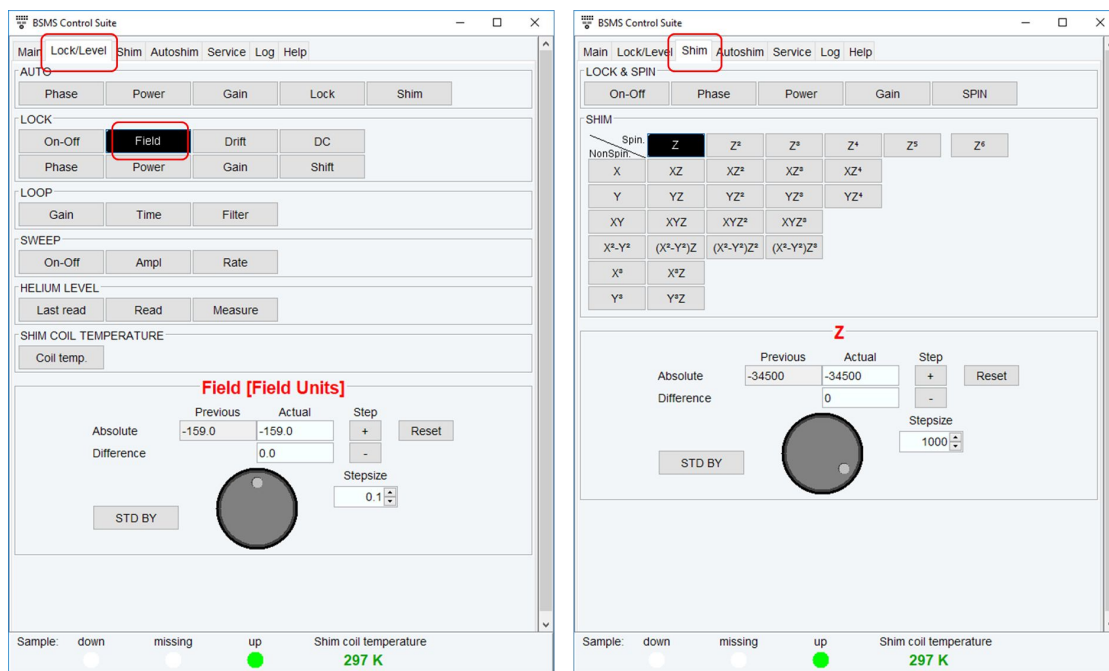Further information can be found in the shimming procedure description as well.

Figure 3.15: BSMS Tabs **Lock/Level** *(left) and* **Shim** *(right).*

3.3.4 Temperature Control - edte

The **edte** or temperature control window displays the state of the spectrometer VT functions (previous figure).

Since TopSolids is designed i.a. for proteins, most often the biological samples will be temperature sensitive and VT control will be needed during the experiments.

If the hardware and the MAS probe are connected correctly, you only need to switch on the cooling with the '**On**' button (green).

Afterwards, define the desired target temperature using the '**Set**' button.

During an MAS experiment, the gas flow should be between 500 and 1500 l/h. Do not turn the gas flow too high, since it can interfere with the MAS drive flow causing rotor imbalances.

The standby gas flow is usually set to 200 to 500 l/h. Do not hesitate to ask your supervisor or colleagues for experienced values.

Finally, if a cooling unit (BCU) is used, the cooling target power can be adapted. Under normal operation use either '**medium**' or '**strong**' power for the BCU Chiller.

The remaining tabs of the **edte** window can be used for setting up monitor displays and log-files. These functions are not critical for the operation and are normally set by the NMR supervisor, if necessary.

For detailed explanations, please refer to the Temperature Manual in TopSpin or the Solid State Experiments (see chapter *General Requirements [▶ 11]*).
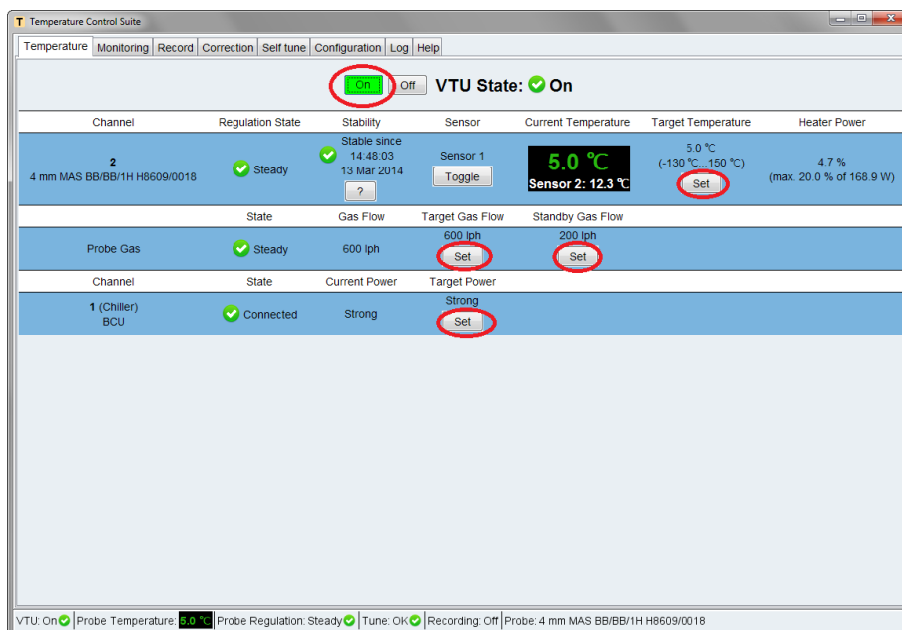
Figure 3.16: Temperature Control Suite (edte).

> *Temperature Control Suite (edte). Important settings to be adjusted are encircled in red.*

3.3.5   MAS

This is a brief introduction only. For detailed information please refer to the manuals provided on the TopSpin DVD or in the TopSpin **Help** menu (see chapter *General Requirements [▷ 11]*).

The figures below shows the MAS II and MAS III displays to control and change the MAS rate while using TopSpin. Though the displays look a little bit different, the functions described in the following are the same. During the TopSolids operation only the **Main** tab is used. The configuration should be done by the NMR supervisor, who knows the super user password to change the TopSpin configuration.

If you are asked by TopSolids to change the MAS rate or to change a sample, you need to call the MAS display by typing e.g. **masdisp** into the TopSpin command line.

The display will show the actual MAS rate in green numbers. Below the control item to change the spinning speed can be found. The new desired spinning frequency can be entered (in Hz) and will be sent to the MAS unit by pushing **ENTER** on the computer keyboard.

If the sample needs to be exchanged, first push the **HALT** or **STOP** command. When all bearing and drive pressure are at zero, the sample can be ejected using the **EJECT** button. After having changed the rotors, push the **INSERT** button, set the desired spin rate and push **GO**.

Furthermore, there is a control for **Frame Cooling**. Here, the gas flow to cool the shim coils and probe can be adjusted, if connected to the probe. During VT operation it is highly recommended to use frame cooling and set it to values between 20 and 60%.

Figure 3.17: MAS II Pneumatic Unit Control Display.

*MAS II Pneumatic Unit Control display. In green, the actual spinning rate is shown, which can be changed by entering a new value in the field below. Make sure that the correct probe is selected, else change it, either in the tab **Config** (note: the SU password must be entered) or directly at the MAS II unit at the spectrometer.*
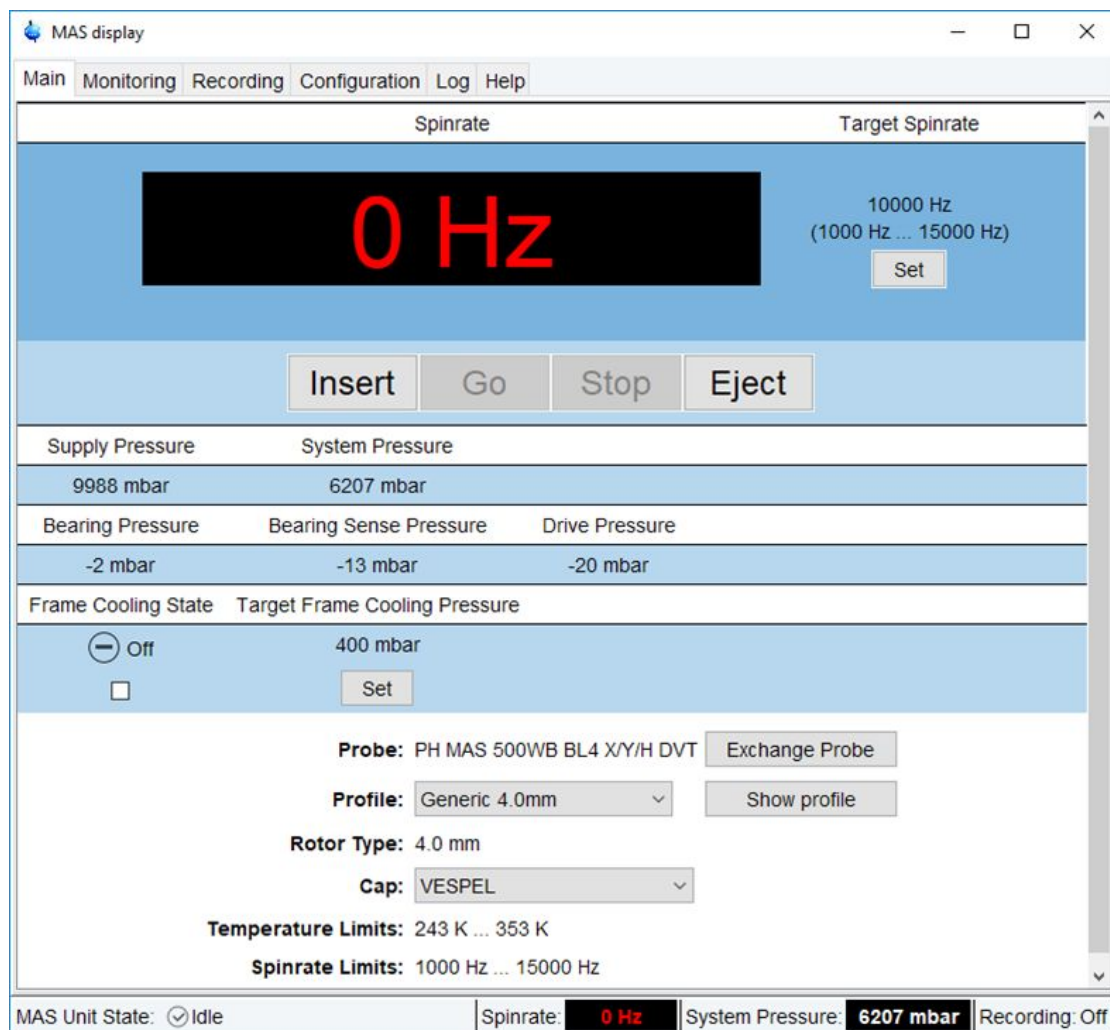
Figure 3.18: MAS III Pneumatic Unit Control Display.

3.4 Probes

This chapter will give only a short introduction. For detailed information, please refer to manuals provided on the TopSpin DVD or in the TopSpin **Help** menu (see chapter *General Requirements [▷ 11]*) as well as probe specific forms.

3.4.1 Tuning and Matching

Concerning tuning and matching, there are mainly two different types of probes: those that use color-coded knobs for each channel (first figure below) and those with labels (second figure below).

In the latter case, tuning knobs are always thicker than those for matching. Furthermore, the tuning knob on the X channel is often not labeled, which can cause confusion with the knob for magic angle adjustment. Nevertheless, both knobs can be distinguished quite easy: While the X channel tuning knob is thick and sticks out quite a lot, the MAS knob always is a short and thin one. Further information about adjusting the magic angle is explained in the following chapter.

Once having changed a probe, make sure to select the correct one at the spectrometer.

Latest probes are equipped with a *pics* cable to communicate with TopSpin automatically. If your probe does not have this cable, take special care about the correct communication between probe and TopSpin by running an **edprpbe**. However, this is also an integral part of the TopSolids workflow (see Chapter *Preparation Module [▶ 37]*) to take care about correct communication.

Maybe, you need to check the PROSOL parameters (**edprosol**). Further information can be found in the chapter *PROSOL for TopSolids [▶ 11]*.
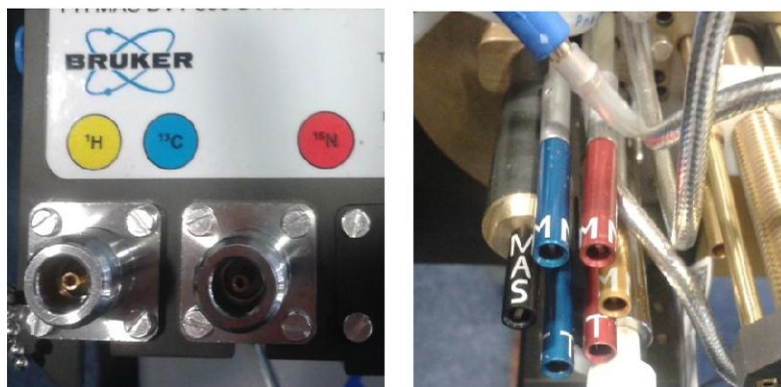
Figure 3.19: Probe with Color-Coded Knobs for Each Channel.

> *Probe with color-coded knobs for each channel. As can be seen in the left figure, the ^{1}H channel is coded in yellow, ^{13}C in blue and ^{15}N in red. On the right the respective tuning (T) and matching (M) knobs can be distinguished easily. Furthermore, the screw for adjusting the magic angle is seen in black (MAS).*
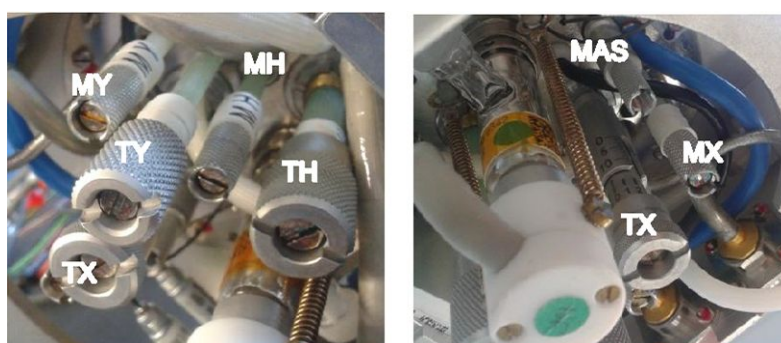

Figure 3.20: Probe with Labeled Knobss.

> *Probe with labeled knobs. As can be seen in the left figure, the tuning knobs are labeled with T, matching knobs with M, the ^{1}H channel as H, the second channel as X and the third channel as Y. For the X channel, only the Tuning knob is visible. On the right figure, both tuning and matching knobs for the X channel are depicted as well as the knob to adjust the magic angle (MAS). Usually TX is the only knob that is not labeled, which often causes confusion with the MAS knob. Note: The MAS knob is always shortest and thin, while the TX knob is thick and sticks out of the probe quite a lot.*

3.4.2 Magic Angle Adjustment

Setting the magic angle (MA) is not 'magic'. Typically, it is set using KBr spinning at a spinning frequency of 5 kHz. It is important making sure the rotation rate is stable. After the probe was tuned and matched, a reference spectrum has to be recorded to compare the line-width of the center band with the 5th spinning sideband using the command **peakw**. If these

differ less than 10 %, the magic angle setting is sufficient for ^{13}C CPMAS experiments. For more information, please refer to the **Solids** manual (see chapter *General Requirements [▶ 11]*).

On wide bore (WB) probes, the MA setting should not change over time, but it is still best to check and make sure once in a while.

On standard bore (SB) probes, however, checking the MA is crucial whenever the probe is being installed.

Depending on the year a SB probe was produced (before or after 2010), the MA adjustment knob must either always be turned clockwise or always be turned counterclockwise into the MA position (as seen from underneath the probe) in order to ensure reproducible accuracy of the MA after a sample exchange.

The good news is that TopSolids is guiding the user through the angle setting completely, including dedicated SB probe routines as well (see Chapter *Probe Setup [▶ 40]*). However, to understand the background better, in the following there are some details given.

The correct rotation direction for probes produced before 2010 (figure below) is counterclockwise. The MA knob ends in a pin that is visible when viewing the probe bottom from the side (figure below, **B**, **C**). The visible length of this pin defines the *MA position* (pin touches the probe frame) and the *rotor eject position* (pin does not touch the probe frame), respectively. It is important that you convince yourself that the pin is firmly touching the probe frame to ensure that the MA position is retained (figure below, **B**).
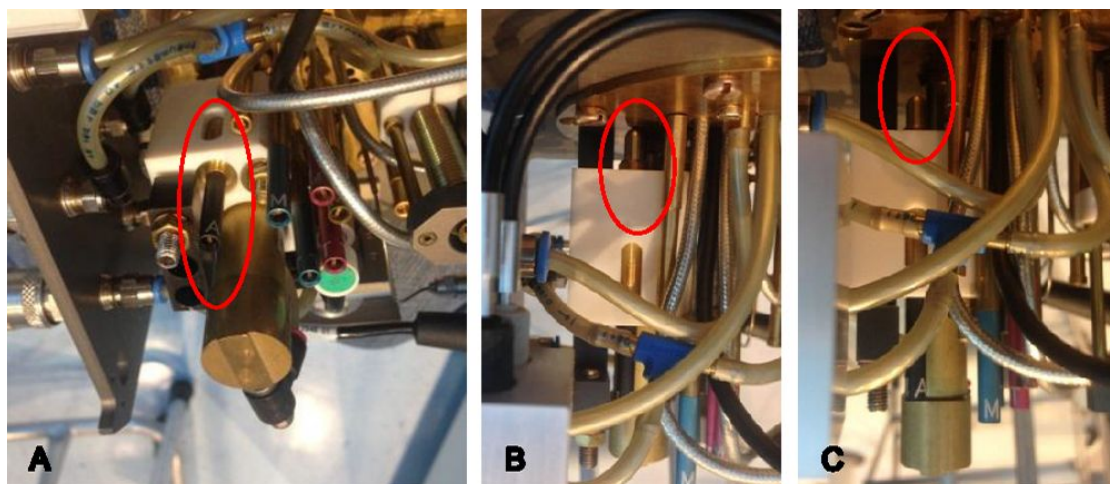

Figure 3.21: SB Probe Produced Before 2010.

SB probe produced before 2010. (A) Bottom view showing the black knob for MA adjustment (encircled in red). This knob should always be turned counterclockwise. The visible length of the pin defines the MA position. (B) Side view of the probe showing the 'MA position' with the adjustable pin touching the probe frame, meaning that the stator is tilted correctly. (C) Side view of the probe showing the 'rotor eject position', where the pin does not touch the probe frame because the rotor has been ejected and hence the stator is not tilted.

If you have to turn the knob in the opposite direction, e.g. because you overshot while setting the MA, you need to toggle the stator by running a sample exchange cycle (MAS Halt, Eject, and Insert) afterwards in order to ensure proper alignment. Then continue to adjust the MA in incremental steps towards the correct MA position using the correct rotation direction.

In the modern design of SB probes produced after 2010 (figure below), the MA knob has to be turned clockwise in order to adjust the MA with accuracy. Here you will not see a pin, but a pneumatic cylinder that is surrounded by a metallic rectangular block (see figure below, **B**).

The block touches the probe frame while maintaining the 'MA position', and moves away from the frame when the stator is in the 'rotor eject position'. It is important to check that the block is firmly touching the probe frame to ensure that the MA position is adopted.

If the MA knob has been turned too far, turn it back slightly and toggle the stator by a sample exchange cycle before continuing.
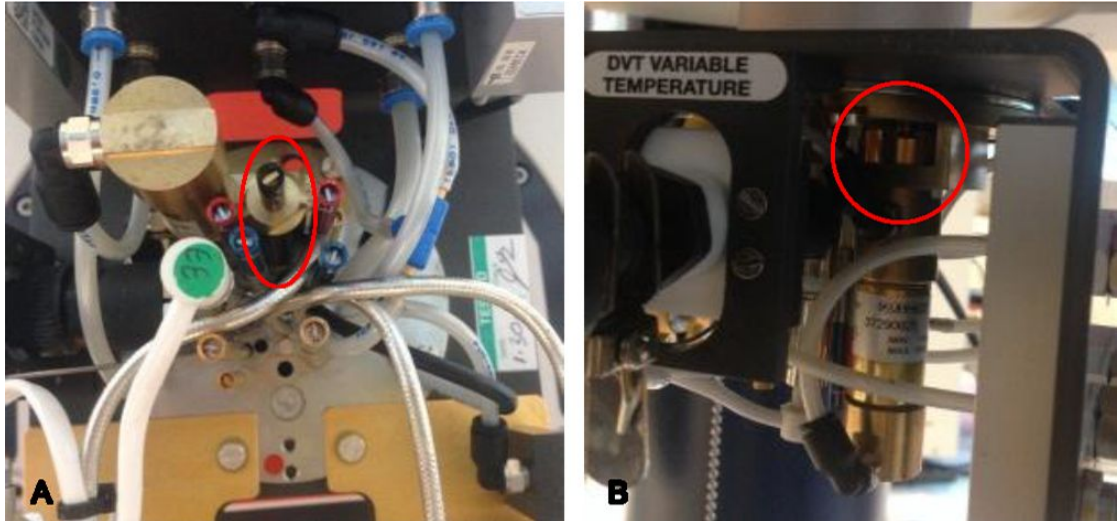

Figure 3.22: SB Probe Produced After 2010.

SB probe produced after 2010. (A) Bottom view showing the black knob for MA adjustment (encircled in red). This knob should always be turned clockwise. (B) Side view of the probe showing the rectangular block surrounding the pneumatic cylinder touching the plate of the probe, indicating that the stator is tilted towards the 'MA position'. In the 'rotor eject position', the rectangular block at the cylinder is lifted off the probe frame.

4   TopSolids – Step by Step

Depending on your personal level of knowledge, we recommend reading this manual carefully. If there is further information available, there will be a link directing you to a detailed description of a topic.

> **i**   Before starting step by step, please note that tutorial videos are available at *www.theresonance.com* as well as the Bruker YouTube channel.

4.1   General Information

To use TopSolids you need to have installed at least TopSpin 3.5 patch level 6.

Make sure that you executed an **expinstall** with the **Solid State System** field activated (see following figure) after running a **cf**. Otherwise, an error message will occur when pushing any TopSolids button, saying *Command not implemented*'.
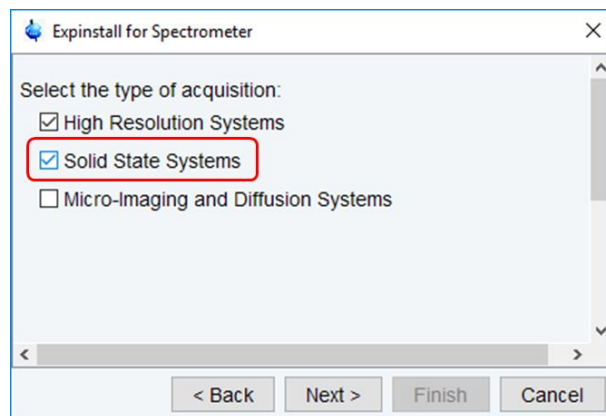
*Figure 4.1: Activating Solid State Systems when Executing **expinstall**.*

Whenever you click on a **Cancel** button using TopSolids, the respective automation step will be stopped, but can be repeated at any time.

> **i**   In case an automation is not stopping, enter **kill** in the TopSpin command line and select the respective program. Note that all TopSolids programs start with the prefix 'SOL_'.

4.1.1   How to Start TopSolids?

TopSolids is integrated in the acquisition options of TopSpin.

First, open any data set in the data path you want to store your TopSolids project at. Then, to open TopSolids either type **topsolids** into the command line or go to the **Acquire Tab** and in the pulldown menu **More** select **TopSolids**.
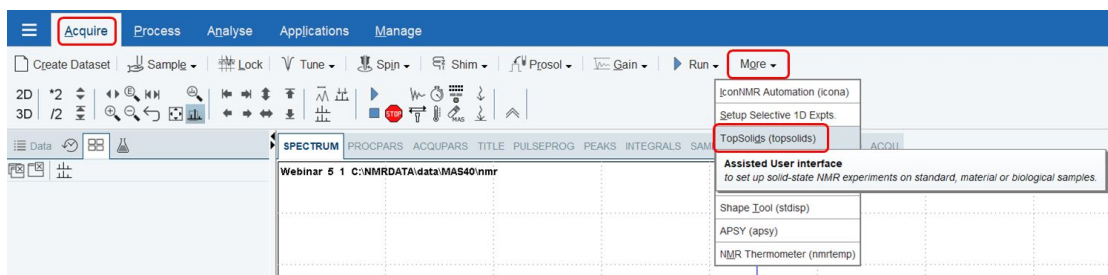
TopSolids – Step by Step

Figure 4.2: The TopSolids Menu Location.

The TopSolids Preparation flow bar will open. By pushing the **Exit** button, you can return to the **Acquire** menu at any time.
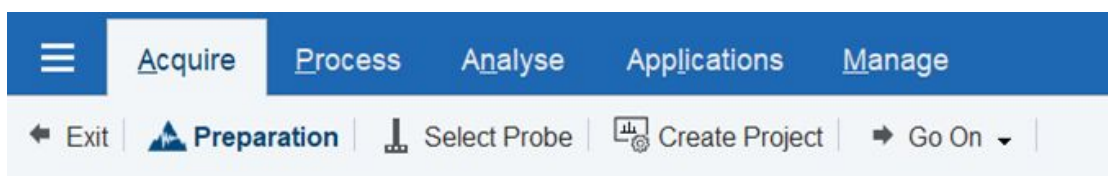

*Figure 4.3: The TopSolids Preparation Flow Bar and the **Exit** Button.*

To recall a previous TopSolids session simply load any data set of the respective project and open TopSolids as described above. A dialog window will ask you whether to return to this previous session (type **y**) or to start a new one (type **n**). If you want to recall, the program will open the flow bar module that has been used last. Otherwise, to start a new project, the Preparation module will be launched
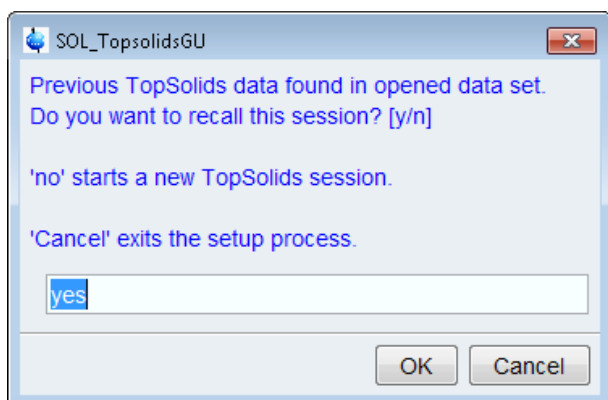
Figure 4.4: Recalling the Previous TopSolids Session or Creating a New Project

4.1.2 Description of the Flow Bar Modules

The TopSolids interface is comprised of different modules:

- *Preparation [▶ 37]*
- *Probe Setup [▶ 40]*
- *Applications [▶ 44]*
  Proteins [▶ 45]:

Since TopSolids is designed in flow bars, it is highly recommended to perform all steps within one flow bar consecutively. However, switching between or skipping flow bars completely is possible. Further information is given in the respective module sections.

> **i** In case there are parameters missing, TopSolids will start the optimization automatically or will inform you about it.

Please note that all biological multidimensional experiments are ^{13}C-detected as indicated by "13C". For applications on material samples, any X nucleus the hardware can detect can be selected.

> **i** TopSolids will use PROSOL parameters as initial values if the Standard Setup has not been executed. Thus, ensure to have an updated PROSOL table for the probe you are using and the correct probe installed (see also *PROSOL for TopSolids [▷ 11]* and *Probes [▷ 30]*).

4.2 The Preparation Flow Bar

The first TopSolids module is the Preparation flow bar, consisting of **Select Probe** and **Create Project.**
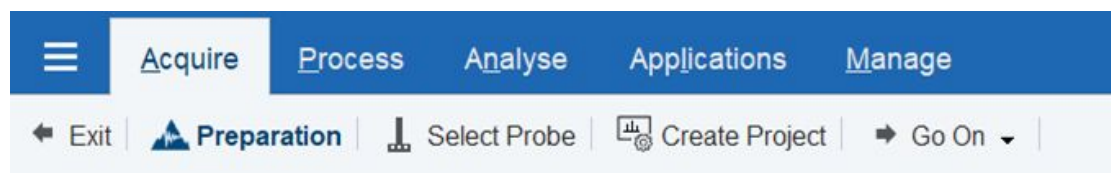
Figure 4.5: The Preparation Flow Bar

The first button in any TopSolids module is always called **Exit**, directing back to the **Acquire** tab.

The last step of any TopSolids module comprises the **Go on** tab. Here, either a PDF parameter report can be generated, or other flow bar modules can be selected. Only reasonable modules that built upon the active one will be shown.

> **i** The parameter report can be generated at any time. A reapplication of the button **Create Report PDF** is overwriting the existing PDF.
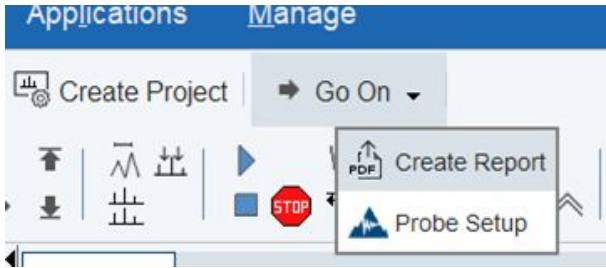
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*Figure 4.6: The **Go on** menu of **Preparation** module to create a PDF report or to continue with the **Probe Setup** module.*

4.2.1   Select Probe

To ensure a proper communication between hard- and software, the mounted probe needs to be defined. Therefore, click on **Select Probe**. The TopSpin interface **edprobe** will open. If your probe is connected with the console via a PICS cable, the probe will be detected automatically. Otherwise please choose the respective probe from the list and click on **Set as current** before leaving the window via **Close**.
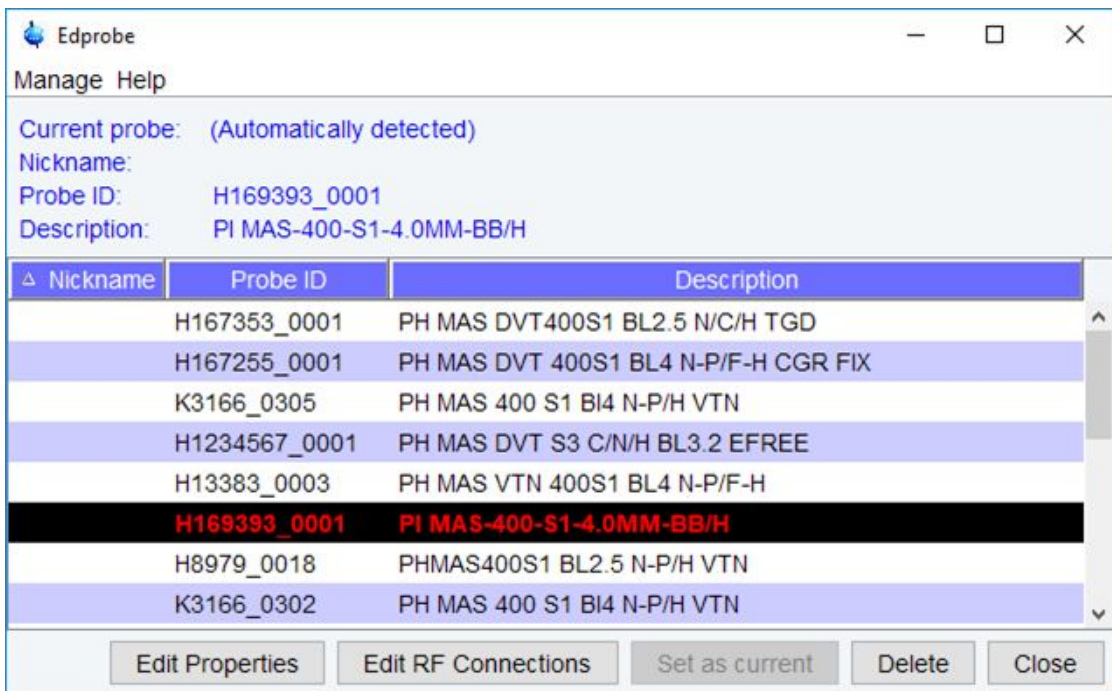
*Figure 4.7: Select probe via **edprobe** interface.*

4.2.2   TopSolids - Create Project

After having selected the actual probe, the TopSolids interface can be started to create a new project.
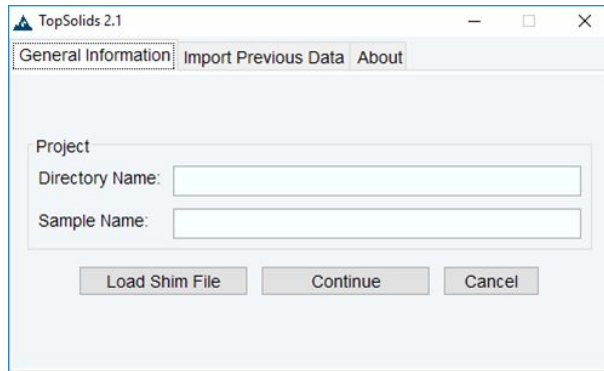
Figure 4.8: TopSolids Interface

> **i** The TopSolids interface always appears on top of any other dialog window. For better navigation of further dialog windows, it is best to move the interface to any corner of the screen.

The TopSolids interface consists of the three tabs **General Information**, **Import Previous Data** and **About** and can be understood as a little flowbar itself with working from left to right.

In the General Information tab, select a **Directory Name** and **Sample Name** for your project.

> **i** TopSolids offers an automated shim procedure which is based on a *Z* shim optimization. Nevertheless, starting with reasonable values is always better than starting from scratch. Thus, if available, load the latest shim file saved for the current probe in the TopSolids interface.

Finally, with clicking the **Continue** button, TopSolids will create the new project and automatically open the **Import Previous Data** tab to indicate it is done.
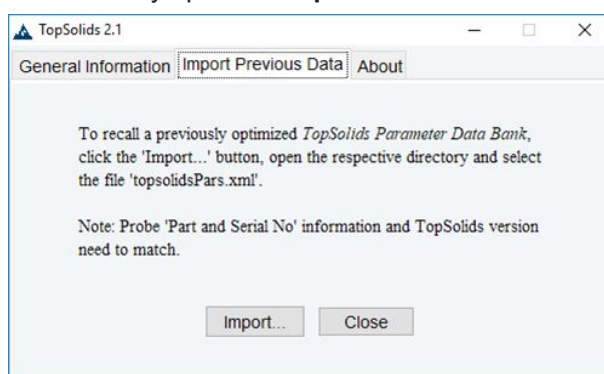
Figure 4.9: Import Previous Data or Close the Interface.

If you have a previous TopSolids project based on the same probe and created with the same TopSolids version, you can import the previously optimized parameters, which are stored in a data bank named *topsolidsPars.xml*, to avoid re-optimization. Please note that this is an optional and not a mandatory step. To do so, click on **Import**, browse to the respective directory and select the file named *topsolidsPars.xml*.
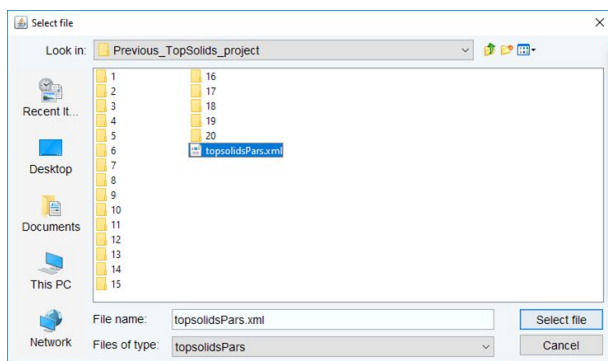
Figure 4.10: How to Import a Previous Parameter Databank

If you are done, you can leave the interface by clicking the **Close** button. Nevertheless, if you have any question, it is recommended to open the **About** tab, where you can find contact information and a link to this manual. Furthermore, the current version of TopSolids is shown here.
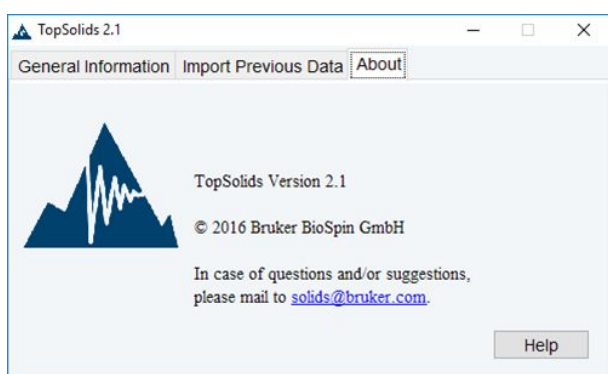

Figure 4.11: Contact Information and Link to this Manual.

4.3   The Probe Setup Flow Bar

After the successful creation of a target directory, the probe may require to be adjusted and miscellaneous parameters may need to be optimized to achieve best possible results. This can be true, even if you copied a parameter data bank - depending on the elapsed time.

The flow bar includes the adjustment of the magic angle position, determination of the most important 90° hard pulses, namely on ^{1}H and ^{13}C, spectral referencing and last, but not least probe shimming.

All steps in this module are done on the standard samples KBr (MA adjustment) and adamantane, which are part of the Bruker solid-state NMR sample kit (H147454-01).
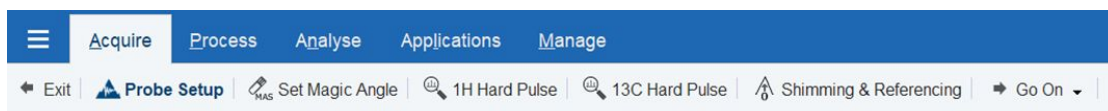

Figure 4.12: The Probe Setup Flow Bar

> TopSolids will use PROSOL parameters as initial values. Thus, ensure to have an updated PROSOL table for the probe you are using and the correct probe installed (see also *PROSOL for TopSolids [▸ 11]*).

4.3.1 Set Magic Angle Position

Though the magic angle has to be set manually, evaluation of the position is done automatically in TopSolids. Afterwards you will be informed, whether the adjustment is within Bruker specification for probes, which is maximum 10% difference between line widths at half height of the center peak compared to the fifth left spinning sideband signal (see *Magic Angle Adjustment [▶ 31]*). In case the difference is higher, you can either continue at own risk or try to adjust the position again.

In case you are working with a standard bore probe, TopSolids will initiate an angle-stability test afterwards. Therefore, the MAS stator needs to be flipped once by ejecting and re-inserting the rotor. TopSolids will give detailed instructions and will automatically check the angle quality afterwards.

After every setup is finished, a dialog window will inform you about the results.

After a setup has been done, the optimized values are shown in an information dialog. Push **Close** to finish this setup.
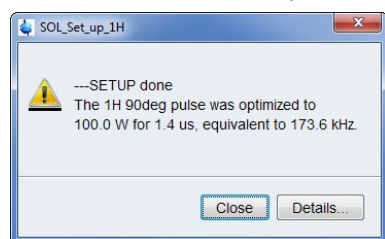
Figure 4.13: Setup Done Information Dialog

4.3.2 90 Degree Pulse Optimization for 1H and 13C

The 90° hard pulses for ^{1}H and ^{13}C are determined in a program called `pulsecal`, which is started in the background. This may take some seconds.

In both experiments the spectral referencing (to neat TMS[1] is automatically performed in the background (see *Spectral Referencing [▶ 23]* for further information).

See also

▤ References [▶ 79]

4.3.3 Probe Shimming

During this setup, the spectral referencing (to neat TMS [*1 [▶ 79]*]) is automatically performed in the background as well (see *Spectral Referencing [▶ 23]* for further information).

Shimming can be done automatically by calling the automated shim procedure. If you are using a probe which was not shimmed before, we recommend doing a rough manual shimming first and repeating the whole Setup again afterwards.

The option 'manual shimming' is done as follows:

• A dialog window will open, informing you about the easiest way to shim a solid state NMR probe. As for the MA adjustment, the acquisition mode is **gs** to directly see changes in the FID (or the displayed spectrum if selected).

---

[1] Morcombe CR, & Zilm KW (2003) Chemical shift referencing in MAS solid state NMR. J Magn Reson 162:479-486.
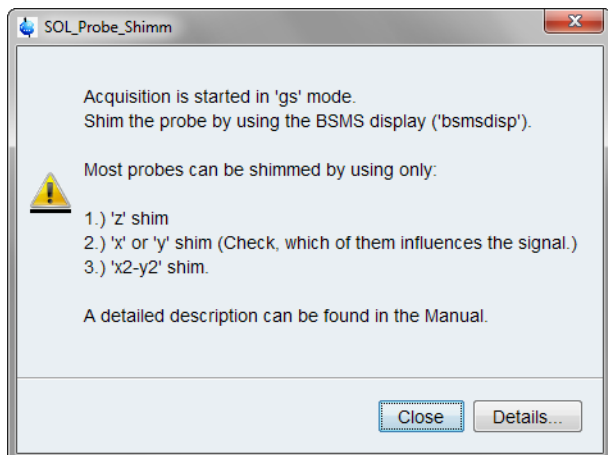
Figure 4.14: Instructions for Manual Shimming.

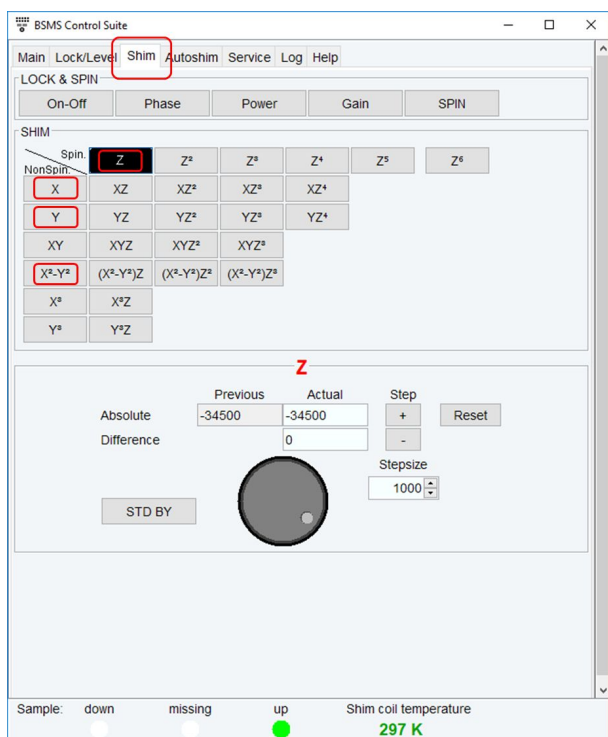- In the **BSMS display** (`bsmsdisp`) go to the tab **Shim**.

Figure 4.15: BSMS Display with the Shim Tab Activated.

To turn off the **Standby Mode** push the **STD BY** button. Important shims can be modified afterwards (encircled in red in the figure above).

- Click on each of the shim units (**Z**, **X**, **Y**,..) and put their **Actual value** to zero to start with a zero shim.
- Start with modifying the Z shim in a step size of **100** points. The aim is to optimize the intensity of the carbon adamantane line to be maximal. Adjust the Z shim to positive or negative values and observe the line width and intensity until you found the best setting:
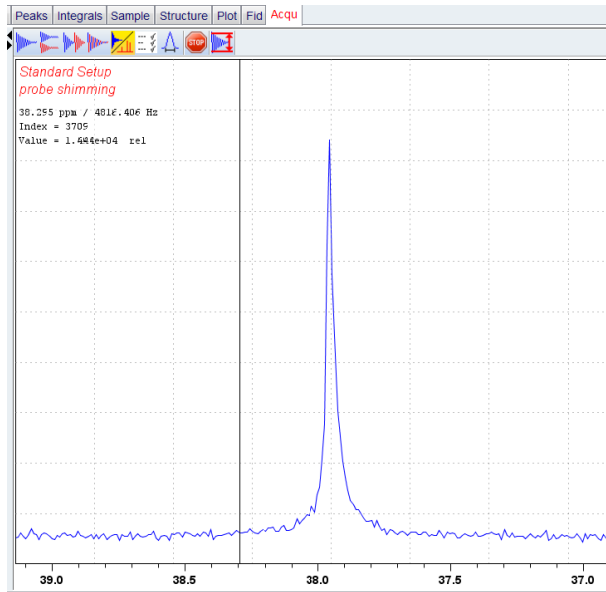
Figure 4.16: Peak already Shimmed on Z.

- Next select the **X** or **Y** shim. You need to change the step size to **1000** units, since these shims have less effect on the line shape. Find out, which of the two shims does have an effect on the line shape. You only need to shim the respective one.
- Finally, you need to select the **X²-Y²** shim to fine tune the line width and shape.
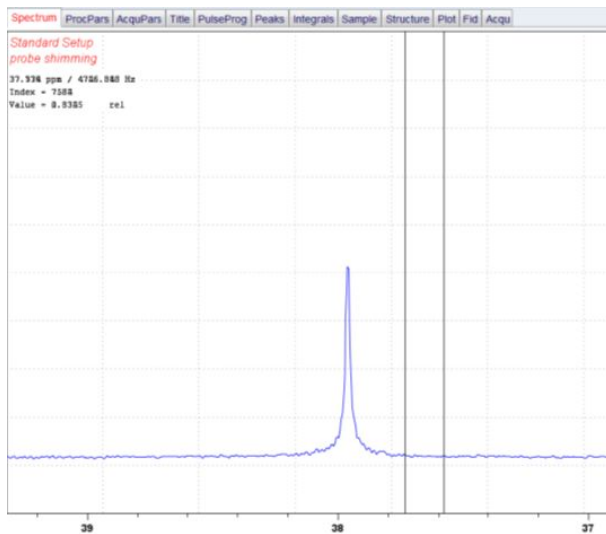

Figure 4.17: Left (low field) Adamantane Peak after Shimming.

- Once you are finished, you can save this shim for the next time by typing **wshim**. Name the shim file appropriately.
- Once the **gs** mode is stopped, TopSoilds will automatically evaluate the quality of the shim and will write the line widths at half height to the data set title.

Afterwards you can select the TopSolids module of interest or repeat the Probe Shimming, this time using the automated shimming routine to refine the shims.

4.4 Applications Flow Bar

This is a new flow bar that is accessible as soon as the step "Probe Shimming and Referencing" in the probe setup module has been performed.
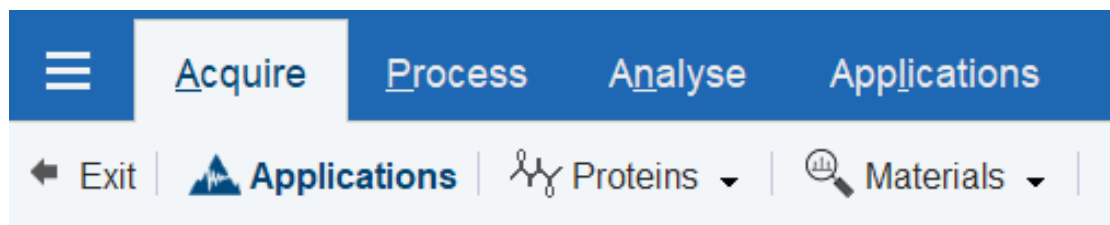

Figure 4.18: Applications module.

If the referencing has not been done, an error message will inform about what needs to be done. This step is needed especially for protein applications as they work with fixed offsets set in ppm, which requires a referenced magnetic field.
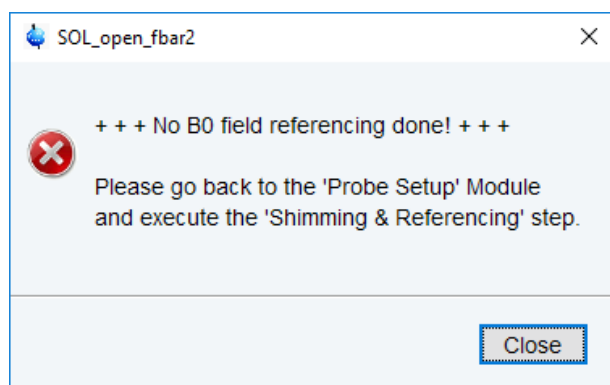

Figure 4.19: If field referencing has not been done, the Applications flow bar is locked.

The Applications flow bar is dividing applications meant for protein ("Proteins") and non-protein ("Materials") samples.
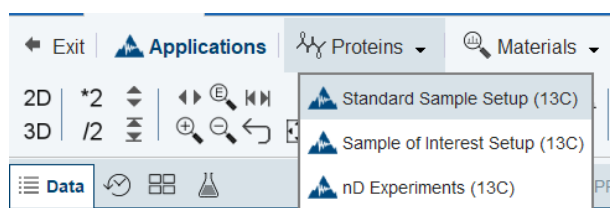
Figure 4.20: Sub-modules within protein applications.

While navigation between different sub-modules is possible at any time using the "Go On" button in the respective flow bars, navigation back to the probe setup is not intended. If this is wanted, a new TopSolids project can be created.
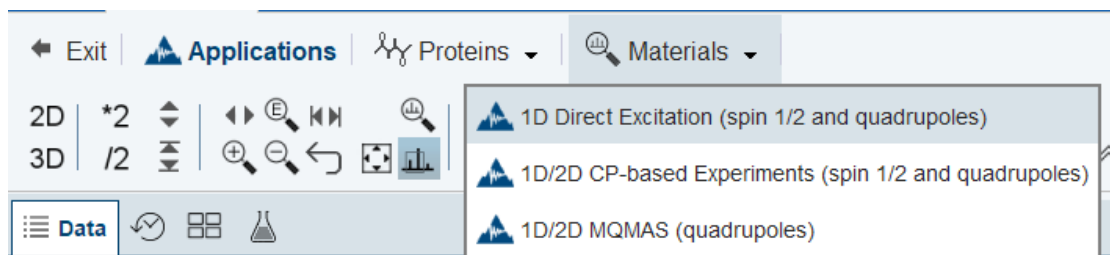
Figure 4.21: Sub-modules within the material applications.

4.4.1   Proteins

4.4.1.1   Standard Sample Setup (13C) Flow Bar

If you know, the biological sample of interest is giving a good signal-to-noise ratio and the probe is calibrated well, you can continue with the module *Sample of Interest (13C). [▷ 46]*

However, in most cases parameters should be optimized on standard samples first. This ensures best results in shortest time and avoids degradation of the sample by wrong power settings and overheating. It is recommended to execute the Standard Sample Setup consecutively.

> **i** If you do not know, which parameters are needed for the planned multidimensional experiment you are interested in, go directly to the module *nD Experiments (13C) Flow Bar [▷ 47]* and select the respective experiment. TopSolids will automatically start the optimization of missing parameters.

The **Standard Sample Setup (13C)** flow bar consists of three different parts, as well as the **Exit** button and the **Go on** tab, namely the optimization of the ^{15}N hard pulse, cross polarization transfers (H to C and H to N) and double cross polarization steps (N to Ca and N to CO).
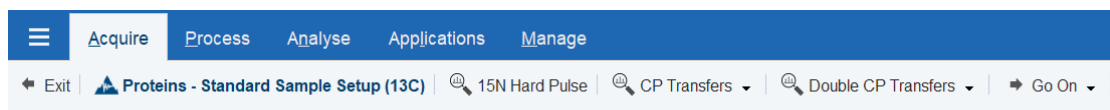

Figure 4.22: Standard Sample Setup (13C) Flow Bar.

All optimization steps are done on [^{13}C, ^{15}N]-labeled glycine, except for the NCO double CP transfer, since there is no protein backbone N-CO chemical bond in any single amino acid. Here, use a short peptide, e.g. fMLF, or a standard protein, such as ubiquitin, SH3, GB1 or similar.

> **i** Ensure each sample is labeled [^{13}C, ^{15}N]!

4.4.1.2   The Sample of Interest Setup (13C) Flow Bar

It is recommended to execute the 'Standard Sample Setup (13C)', if you know, the biological sample of interest is giving a bad signal-to-noise ratio (see *Standard Sample Setup (13C) Flow Bar [▶ 45]*).

---

i   If you do not know, which parameters are needed for the planned multidimensional experiment you are interested in, go directly to the module *nD Experiments (13C) Flow Bar [▶ 47]* and select the respective experiment. TopSolids will automatically start the optimization of missing parameters.

Since magic-angle spinning and pulsing cause heating effects, it is recommended to connect a temperature control system (e.g. a BCU) to the probe. For further reading refer to *Temperature Control - edte [▶ 27]*.

The **Sample of Interest (13C)** flow bar consists of four major steps: 90° pulse optimization, CP optimization, double CP optimization and optional optimizations. Different from the previous flow bars, this module does not need to be executed consecutively, since TopSolids will automatically optimize missing parameters. Nevertheless, the consecutive execution is possible, of course.
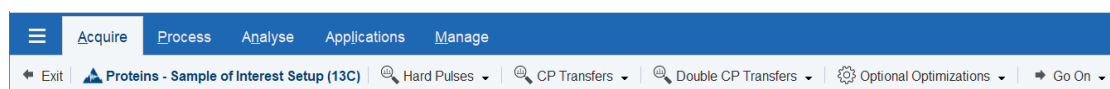

Figure 4.23: Sample of Interest Setup (^{13}C) Flow Bar.

For optimization of the hard pulses, existing values are taken into account, if available. Note, that all hard pulses are optimized in CP experiments only, since direct polarization experiments are rarely possible in the majority of protein samples.

If you want to execute H-N experiments only, the ^{1}H 90° pulse can be optimized in an HN CP experiment, or else the standard HC CP should be used. Here only calculated values will be used as CP parameters, since an optimum value is not necessarily needed for determining the hard pulses.
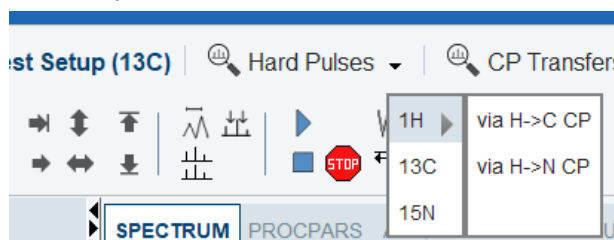

Figure 4.24: Options for Optimizing the ^{1}H Hard Pulse.

However, for multidimensional experiments optimized CP parameters are essential of course. In the tab **CP Optimization**, the HC and HN CP transfers can be optimized. Double CP transfers between N and C are determined in the tab **Double CP Optimization**. Here, not only N to Ca or CO transfers can be determined, but the opposite direction, from Ca or CO to N, as well. Usually, the values do not differ much, but it is worth the optimization, because the optimum NC transfer range is quite small (e.g. 0.5 dB difference can lead to 50% less efficiency).

The tab **Optional Optimizations** is newly introduced, containing the automated determination of spectral widths for the complete ^{13}C, ^{13}Ca, ^{13}CO and ^{15}N protein backbone regions, as well as the optimization of ^{1}H decoupling parameters during acquisition. As the name implies, these optimizations are recommended, but not mandatory.
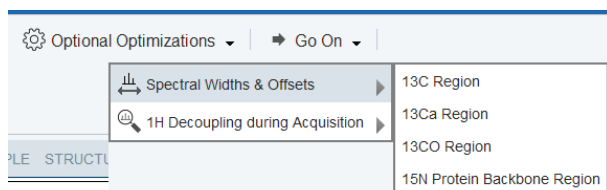
Figure 4.25: Optional Optimizations.

Once optimized, the values can be used for any multidimensional experiment for protein applications. This is especially important for spectral widths in indirect dimensions, since unnecessarily large spectral widths dramatically increase the experimental time. Further information is given in the *Appendix [▶ 61]*.

4.4.1.3   nD Experiments (13C) Flow Bar

Since magic-angle spinning and pulsing cause heating effects, it is recommended to connect a temperature control system (e.g. a BCU) to the probe. For further reading refer to *Temperature Control - edte [▶ 27]*.

In this module, you can choose between a diversity of ^{13}C-detected experiments to gain information about the protein backbone of your sample of interest. The flow bar is divided into *Intraresidue Assignment*, *Interresidue Assignment* and *Distance Measurements*.

Some general information about protein assignment is given in the *Appendix [▶ 61]*.
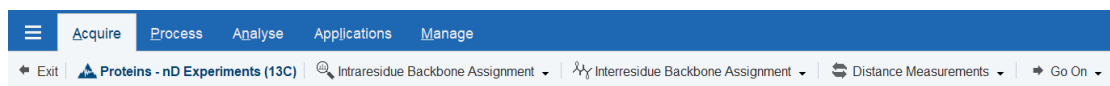
Figure 4.26: The nD Experiments (^{13}C) Flow Bar.

While for distance measurements only 2D experiments are available, the assignment tabs are further divided by dimensionality, from 2D up to 4D experiments.

> **i** If you want to measure 3D or 4D experiments, make sure to have sufficient signal-to-noise, else the required measurement time to obtain appropriate spectra will be excessively long. If you cannot measure reasonable 2D PDSD/DARR and NCa experiments within one day each, we recommend not measuring higher dimensional experiments on that sample.

There is no need for consecutive execution. Rather, choose a dimensionality and select an experiment. If any parameter is missing, TopSolids will start the optimization automatically.

In case no optimization is missing, all multidimensional experiments will be queued and acquired in the order of setup.

> **i** If a parameter for an experiment is missing, but another experiment is acquired at the same time, you need to wait for the acquisition to finish, before this parameter can be optimized. TopSolids will inform you, if this is the case.

To avoid unwanted side band backfolding artifacts, a rotor-synchronized spectral width can be set in all indirect dimensions. Depending on the needed spectral width it will be either a multiple or a divisor of the MAS frequency. Further information is given in the *Appendix [▷ 61]*.

ℹ The longer the acquisition time in an indirect dimension, the longer the whole experimental time will be. Furthermore, keep in mind that high power decoupling is turned on during acquisition, causing sample heating! Further information is given in the *Appendix [▷ 61]*.

In each setup, the experimental time will be displayed. In case you want to make changes, click **Cancel** to repeat the time-determining steps.
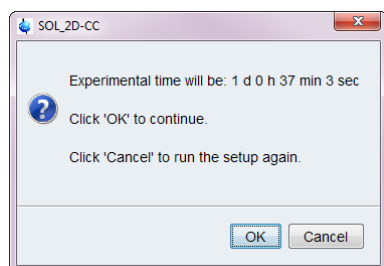
Figure 4.27: Experiment Information Dialog

If you agree with the duration of the experiment click **OK**.

The tab Intraresidue Backbone Assignment comprises 2D PDSD [*2 [▷ 79]*]/ DARR [*3 [▷ 79]*], 2D NCa [*4 [▷ 79]*] and 2D/ 3D NCaCx [*4 [▷ 79]*] experiments.

DARR and PDSD are recoupling techniques that use proton-driven spin diffusion to ob-tain homonuclear ^{13}C-^{13}C correlations in a time-dependent manner.

The NCa experiment is providing a specific heteronuclear cross polarization4 from ^{15}N to ^{13}C-alpha within the same amino acid residue, thus giving important intraresidue information for assigning a protein. To discriminate residues of the same type of amino acid and correlations with similar chemical shifts, a homonuclear ^{13}C-alpha to ^{13}Cx transfer is introduced in the NCaCx double CP experiment, resulting in additional intraresidue information about the side chains of residues. The C-C transfer can be done either by PDSD or DARR.

In the tab Interresidue Backbone Assignment 2D PDSD/ DARR, 2D NCO, 2D/ 3D NCOCx and 3D/ 4D CONCaCx and CaNCOCx experiments [*4 [▷ 79]*] can be found:
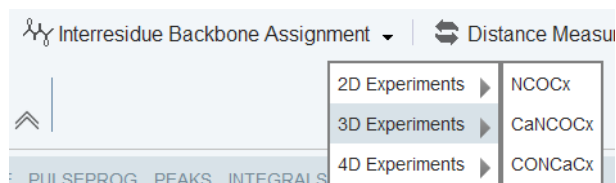
Figure 4.28: Experiments for Interresidue Backbone Assignment.

While DARR/ PDSD experiments provide further homonuclear ^{13}C-^{13}C correlations using a longer mixing time, all other assignment experiments include either specific double CP steps [*4 [▷ 79]*] from ^{1}H to ^{15}N to ^{13}C or even triple CP transfers from ^{1}H to ^{13}C to ^{15}N and back to ^{13}C, comprising sequential protein backbone correlations.

The NCO double CP is providing a specific heteronuclear cross polarization from the backbone-^{15}N of amino acid residue 'i' to the backbone-^{13}CO of the residue 'i-1', thus giving backward sequential information. To discriminate residues of the same type of amino acid

and correlations with similar chemical shifts, a homonuclear ^{13}CO to ^{13}Cx transfer is introduced in the NCOCx double CP experiment, resulting in additional backward sequential information about the side chains of residue 'i-1'. As in the NCaCx experiment, the C-C transfer can be done either by PDSD or DARR.

Based on three heteronuclear cross polarization steps (^{1}H to ^{13}Ca to ^{15}N to ^{13}CO-Cx) the CaNCOCx sequence provides backward sequential ('i' to 'i-1') information. On the other hand, the CONCaCx experiment, based on three heteronuclear cross polarization steps as well (^{1}H to ^{13}CO to ^{15}N to ^{13}Ca-Cx), provides forward sequential ('i' to 'i+1') and side chain ('i+1') information.

ℹ️ During all specific N-C double CP transfers, ^{1}H-decoupling is turned on. Usually, high power decoupling is used, but can be customized in this step. If you are unsure what to use, the suggested value can be accepted as appropriate.

Experiments for acquiring Distance Measurements are 2D PDSD/ DARR, ChhC and NhhC.
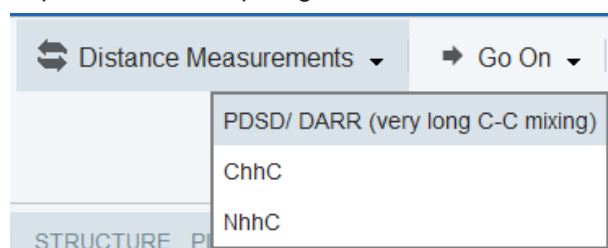
Figure 4.29: Experiments for Distance Measurements.

The DARR/ PDSD recoupling technique can not only be used for protein backbone assignment, but can provide spatial information as well, when choosing a ^{13}C-^{13}C mixing time > 500 ms. Well suited for these experiments are protein samples with sparse ^{13}C-labeling, which reduces dipolar truncation [*6-10 [▷ 79]*].

The ChhC [*5 [▷ 79]*] experiment is based on a fast polarization transfer between the highly abundant ^{1}H spins. Through ^{1}H-^{1}H mixing ^{13}C-^{13}C correlations are obtained through space. The ^{1}H-^{1}H mixing specifies the time the magnetization is allowed to spread through space to transfer spatial information (~ 350 μs which represent ~ 6 Å). The strongest ^{13}C-^{13}C correlations represent the shortest (nearest-neighbor) ^{1}H-^{1}H distances. Thus, the spectra comprise direct information about the spatial arrangement of the detected nuclei. To ensure that the polarization transfer belongs to only CH_x groups, the CP contact times before and after the ^{1}H-^{1}H mixing have to be selected short.

ℹ️ For ChhC experiments, only carbons directly attached to at least one proton are observed. Thus, the CO-region does not give any signal. Therefore, the offset is set to the CH_x region to choose a smaller SW.

The NhhC [*5 [▷ 79]*] experiment is, as the ChhC, based on a fast polarization transfer between the highly abundant ^{1}H spins. Through ^{1}H-^{1}H mixing ^{15}N-^{13}C correlations are obtained through space. The ^{1}H-^{1}H mixing specifies the time the magnetization is allowed to spread through space to transfer spatial information. The strongest ^{15}N-^{13}C correlations represent the shortest (nearest-neighbor) ^{1}H-^{1}H distances. Thus, the spectra comprise direct information about the spatial arrangement of the detected nuclei. To ensure that the polarization transfer belongs to only NH and CH_x groups, the CP contact times before and after the ^{1}H-^{1}H mixing have to be chosen short.

4.4.2 Materials

4.4.2.1 Direct Excitation Flow Bar

This module is intended to determine the 90° pulse of any X nucleus, which is supported by the probe. Dependent on the X nucleus, the 90° pulse calibration can be done as a hard or a soft pulse.
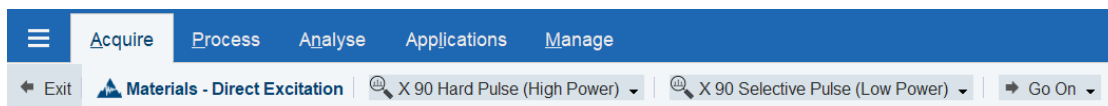

Figure 4.30: Direct Excitation flow bar for material samples.

> **i** Acquisition of quadrupolar nuclei is possible using TopSolids.

The X nucleus can be selected via a pull-down menu at the beginning of each flow bar step. TopSolids is automatically checking if the selected nucleus can be measured with the mounted probe.
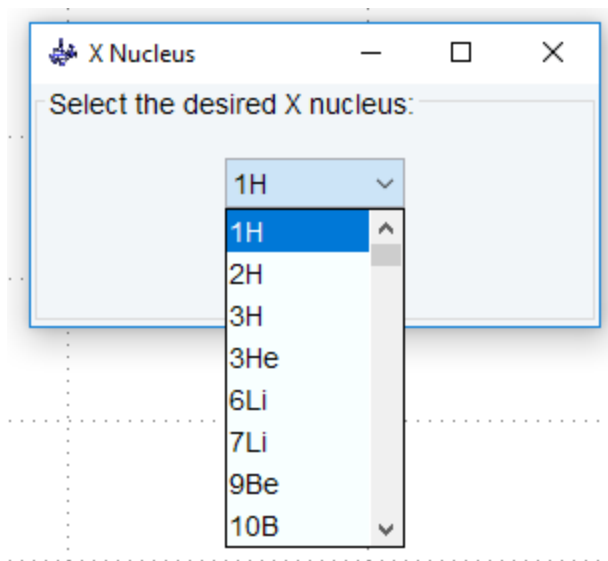
Figure 4.31: Select the X nucleus of choice from pull-down menu.

For the optimization we recommend samples with sufficient signal-to-noise to make the calibration more reliable. If your sample of interest is giving good signal-to-noise, no extra standard sample is needed for optimization, else the use of a standard sample is highly recommended.
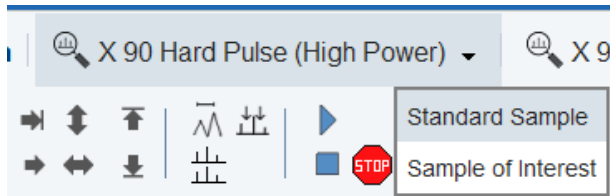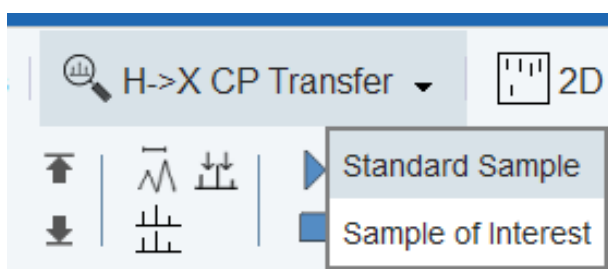
Figure 4.32: Choose between a standard sample or the sample of interest, dependent on availability and signal-to-noise of the sample.

Dependent on the X nucleus, not only a differentiation between high and low power for the hard pulse can be made, but also for 1H decoupling during acquisition. Therefore, the value in the Parameter Check needs to be adjusted to e.g. ¼ MAS rate for low power or e.g. 100 kHz for high power decoupling. TopSolids will calculate the respective values in Watts and set the parameters correspondingly.
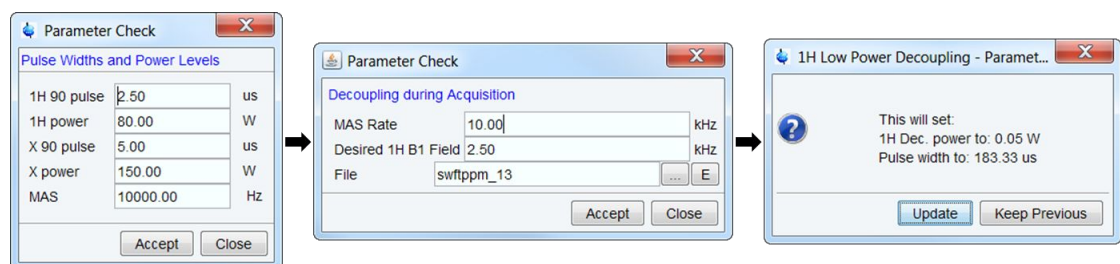
Figure 4.33: During the setup you need to confirm found parameters for 1H and X 90° pulses as well as the MAS rate in Hz. As soon as the values are (adjusted if needed and) accepted, a low power decoupling field will be suggested. If there is need for high power 1H decoupling during acquisition on X, the value can be adjusted. TopSolids will calculate the new power level in Watts, but will only adjust it when the button 'Update' is clicked.

All optimized values are stored in the TopSolids parameter data bank, readily accessible for further automated setups, e.g. a 2D HetCor experiment, using the consecutive flow bar, which can be opened via the **Go On** pull down menu.
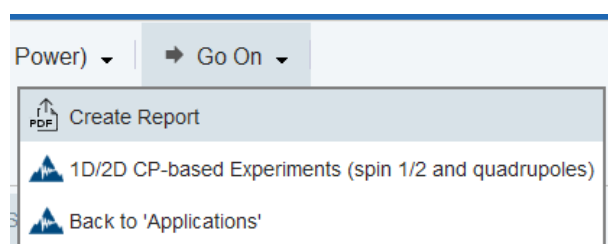
*Figure 4.34: Using the **Go on** button, the '1D/2D CP-based Experiments' flow bar can be accessed easily.*

4.4.2.2   CP-based Experiemtns Flow Bar

Using this module, the H-X CP transfer can be optimized and if wanted a 2-dimensional heteronuclear (H-X) correlation experiment (HetCor) can be set up and acquired.
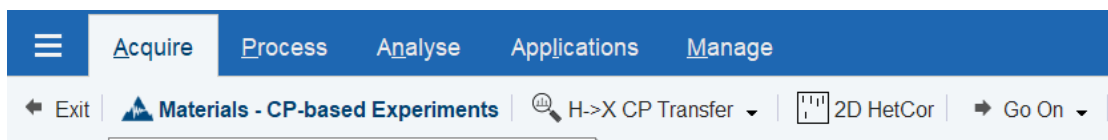
Figure 4.35: CP-based Experiments flow bar

TopSolids checks if the X 90° pulse has been optimized already and will start optimization automatically if it is missing using the *Direct Excitation [▶ 50]* module. For the optimization we recommend samples with sufficient signal-to-noise to make the calibration more reliable. If your sample of interest is giving good signal-to-noise, no extra standard sample is needed for optimization, else the use of a standard sample is highly recommended.

Figure 4.36: Choose between a standard sample or the sample of interest, dependent on availabiliy and signal-to-noise of the sample.

During the CP transfer optimization, TopSolids is testing, whether the selected X nucleus is a quadrupolar nucleus. In this case the spinlock on the X nucleus is set accordingly to about ¼ of the MAS rate automatically. If the X nucleus is a spin-½, TopSolids will suggest the highest possible Hartmann-Hahn condition, which is still in agreement with the probe's power limits.
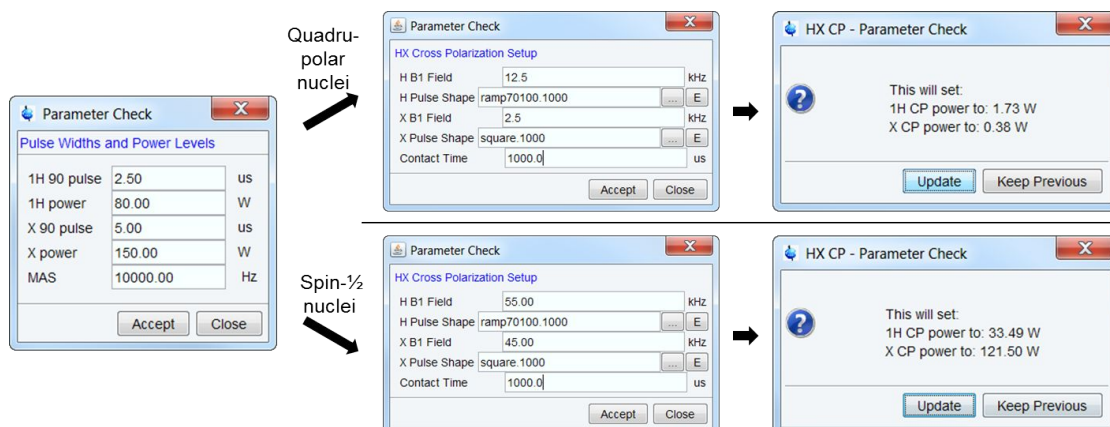
Figure 4.37: Dependent on the X nucleus of choice TopSolids will suggest the best Hartmann-Hahn condition.

Based on the H-X CP parameters, a '2D HetCor' experiment can be set up in the final step. TopSolids will guide through the setup of all important 2D parameters giving suggestions. Before the start of the experiment, the experimental time will be displayed and in case changes are needed, TopSolids will go back to the parameter adjustments that influence the experimental time. As soon as the 2D HetCor experiment is finished TopSolids will perform a basic processing automatically, which includes phasing.

Afterwards, it is possible to go back to the *Applications [▶ 44]* module to continue with further experiments or to print important parameters in a PDF report, all using the **Go On** menu.
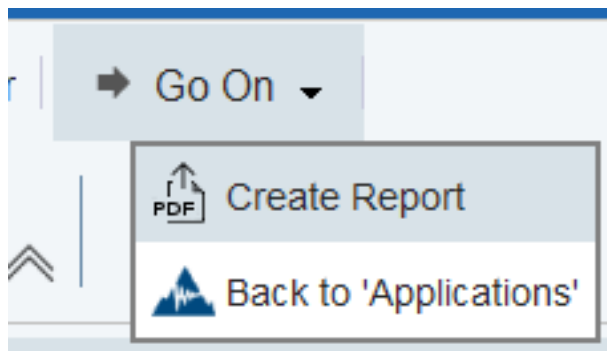

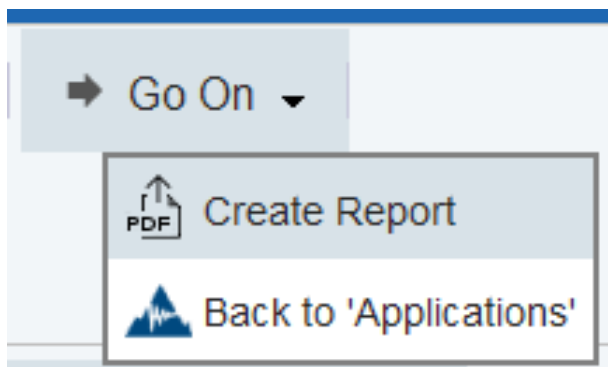*Figure 4.38: **Go on** pull-down menu.*

4.4.2.3 MQMAS Experiment Flow Bar

Another feature in TopSolids is the setup and acquisition of 2D MQMAS experiments using this module. The flow bar contains of four steps: X 90° pulse optimization (if has not been done already), 1D setup for 2D MQMAS, optimization of the spectral width and the setup of the 2D MQMAS experiment itself. A consecutive execution of the flow bar is ensuring for best results, except for the first step, if this has been done already using the *Direct Excitations flow bar [▶ 50]*.
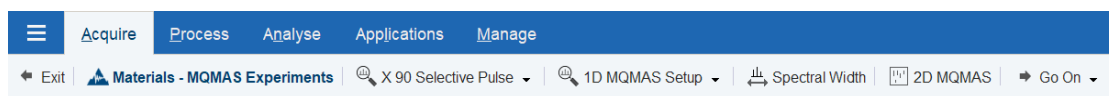
Figure 4.39: The Materials MQMAS Experiments Flow Bar.

While optimization of the X 90° and special MQMAS pulses can be performed either on a standard sample first or directly on the sample of interest, optimization of the spectral width and the setup of the 2D MQMAS experiment itself can only be performed on the sample of interest directly.
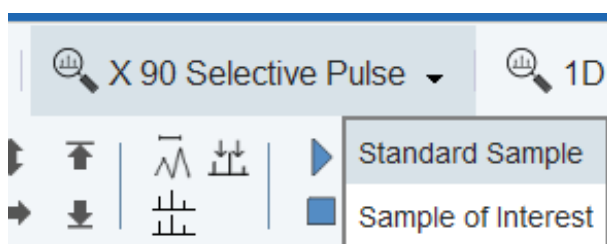
Figure 4.40: Choose between a standard sample or the sample of interest, dependent on availability and signal-to-noise of the sample.

For the setup of the 2D experiment user interaction is only needed for entering the desired number of scans and the relaxation delay of the sample. TopSolids will take care that only multiples of the phase cycle are used as number of scans though. Furthermore, it will automatically synchronize the optimized minimum spectral width in the indirect F1 dimension with the MAS rate before starting the experiment.

After the 2D MQMAS experiment has been successfully acquired, basic processing of the data is carried out automatically. As a result an already sheared and correctly phased 2D spectrum is displayed in TopSpin.

Afterwards, it is possible to go back to the *Applications [▶ 44]* module to continue with further experiments or to print important parameters in a PDF report, all using the **Go On** menu.

*Figure 4.41: **Go on** pull-down menu.*

4.5 Processing

A basic processing is done already automatically for all biological multidimensional experiments. 2D experiments are processed and phased with the TopSpin commands **xfb** and **apk2D**. 3D and 4D experiments are processed with the command **ftnd 0**. Phasing is done using the command **btproc**.

The MQMAS experiments need special processing, which is described in *MQMAS Experiment Flow Bar [▶ 53]*.

Detailed information about data processing in TopSpin is given in the Manual *Proc. Commands & Parameters* (Chapter 2.1).

Nevertheless, a brief overview is listed below:

1D data

- F1 - first and only direction
- Processed with `ft`, `ef`, `gf`, `efp`, `gfp`, `trf`*

2D data

- F2 - first direction (acquisition or direct direction)
- F1 - second direction (indirect direction)
- Processing commands like `xfb` and `xtrf` work in both, F2 and F1
- Processing commands like `xf2` and `abs2` work in the F2
- Processing commands like `xf1` and `abs1` work in F1

3D data

- F3 - first direction (acquisition or direct direction)
- F2 - second direction (indirect direction)
- F1 - third direction (indirect direction)
- Processing command `ftnd` works in all, F3, F2, and F1
  - Allowed values for the directions to be processed are:
    0 : all directions, in the order defined by AQSEQ
    321, 312, 231, 213, 132, 123 : all directions in specified order
    3, 2, or 1: F3, F2 or F1, respectively.
- Processing commands like `tf3` and `tabs3` work in F3
- Processing commands like `tf2` and `tabs2` work in F2
- Processing commands like `tf1` and *tabs1* work in F1

4D data

- F4 - first direction (acquisition or direct direction)
- F3 - second direction (indirect direction)
- F2 - third direction (indirect direction)
- F1 - fourth direction (indirect direction)
- Processing command `ftnd` works in all, F4, F3, F2, and F1
  - Allowed values for the directions to be processed are:
    0 : all directions, in the order defined by AQSEQ
    4321, 4312, 4231, 4213, 4132, 4123 : all directions in specified order
    4, 3, 2, or 1: F4, F3, F2 or F1, respectively.

5   Troubleshooting

- Error Message: '*Command not implemented*'
  - Execute an **expinstall** with the **Solid State System** field activated (see figure below).
- After a wrong entry of the MAS rate (left figure), take care to enter the MAS rate in Hz, not kHz (right figure).
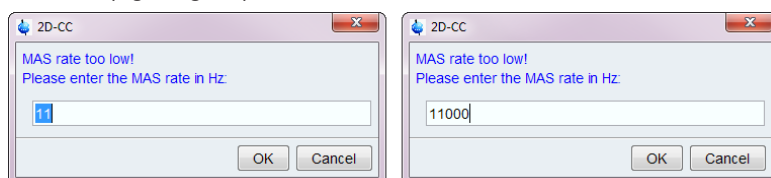
Figure 5.1: Error: MAS Rate Too Low.

- If PROSOL is not filled in correctly, the program `pulsecal` that is used in *Set Magic Angle Position [▷ 41]* and *90 Degree Pulse Optimization for 1H and 13C [▷ 41]* will not be able to fine tune 90° ^{1}H and ^{13}C pulse lengths of your probe in respect to the used samples. In this case you will see error messages and you should refer to *Filling in the PROSOL Table [▷ 16]* in order to check the PROSOL table.
- If an error message occurs, e.g. *No peak found*, repeat the whole setup step once again. Sometimes, automated peak picking cannot find any peak. If necessary, increase the number of scans. If you are working with the **Probe Setup** or **Protein Standard Sample Setup** flow bars, please ensure to use exactly the samples TopSolid is asking for.
- If an error message occurs, e.g. *DRU/ADC overflow…*, immediately stop the optimization using the command **kill**. Only then open the ProSol table (**edprosol**), go to the tab **Others**, select the nucleus you just observed (usually ^{15}N) and increase the pre-scan delay DE to 10 to 15 μs.
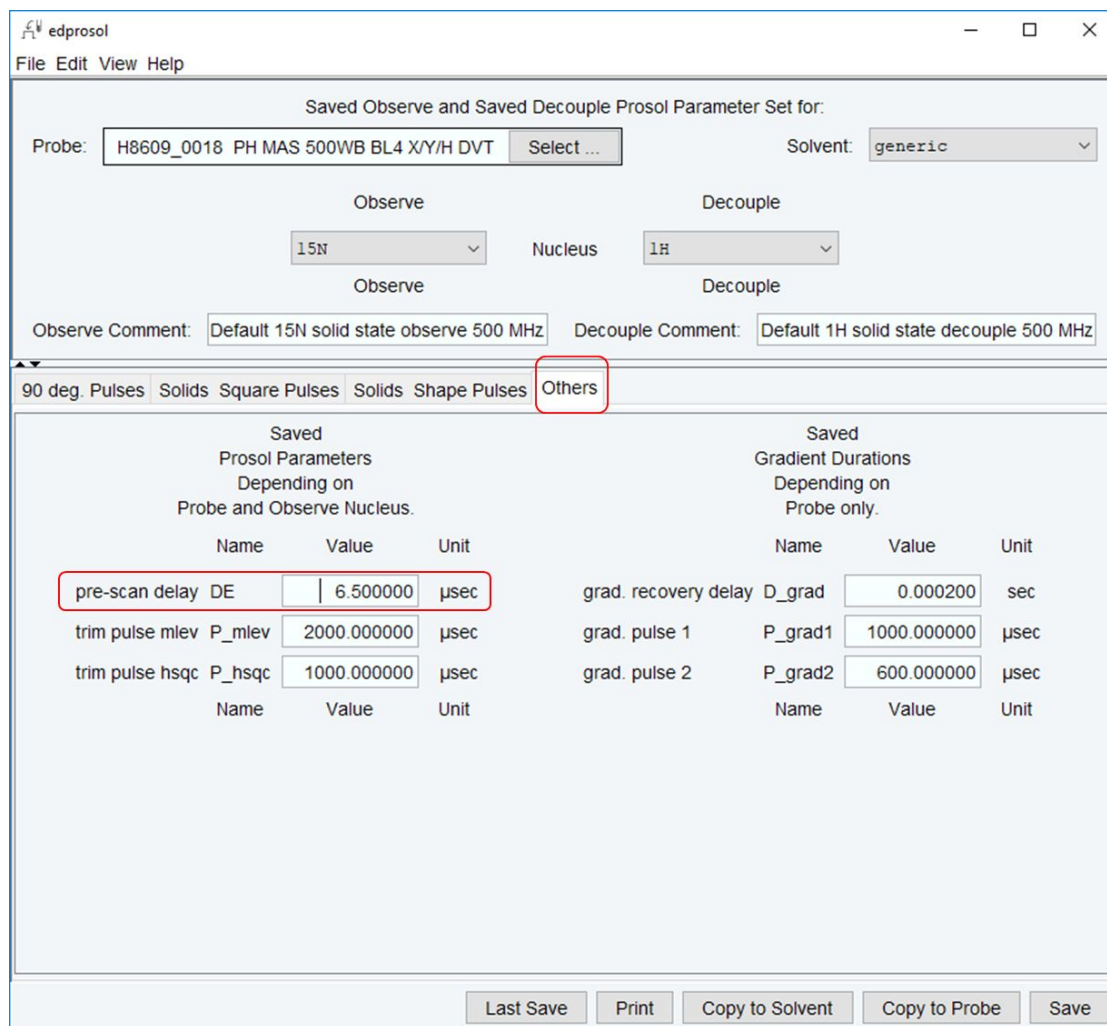
Figure 5.2: Edprosol tab Others. Increase the prescan delay DE to 12 µs for the Observe Nucleus ^{15}N.

- If so-called micro arching is observed in the FID, increase the parameter d1 and reduce the ^{1}H decoupling strength during acquisition. If the arching does not vanish, most likely your sample is too salty or, in worst case, your probe is not working properly. In the latter case, contact Bruker.
- Ensure that files in your TopSpin user libraries are not named the same as any files in the standard libraries. This is especially true for the pulse program library and the parameter set library. If you are not sure, contact the spectrometer responsible person.
- It is recommended to have a look at PROCNOs 999, 998, etc. to control, if the automatically chosen values are appropriate. If not, read next point.

The following figure is PROCNO 999 of EXPNO 5 displaying the optimization of p21, the pulse width for a 90° pulse on ^{15}N. In the title (red) it is written, that p21 has to be found as a ZERO crossing as is indicated by the cursor at ~ 6.3 µs.
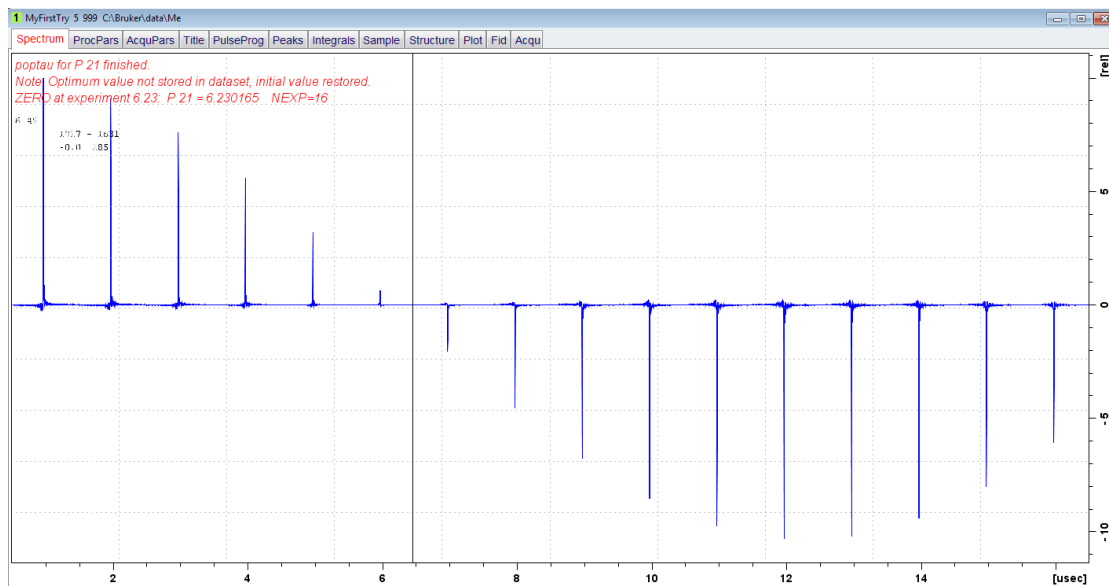
Figure 5.3: PROCNO 999 of EXPNO 5

- If the parameter optimization (**popt**) is providing bad results or cannot find any optimum at all, either repeat the setup again with a higher number of scans, or enter slightly changed starting parameters in the Parameter Check dialog. For example, if no zero crossing could be found for a pulse length, increase or decrease the starting pulse length slightly in direction of the zero crossing.

A dialog appears at the beginning of each optimization. Slightly change the respective parameter here, if **popt** cannot find any optimum.
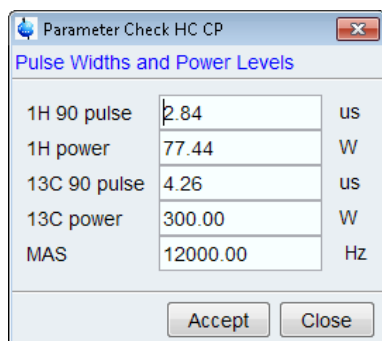
Figure 5.4: Parameter Check Dialog

6 Appendix

6.1 Protein Assignment – A Brief Introduction

Although there are a large variety of experiments for determining the tertiary structure of proteins, the desired information can generally be summarized as follows:

- Which types of amino acids do I observe?
- Which amino acid belongs to which residue of the protein sequence?
- Which parts of the protein sequence do I observe?
- What does the secondary structure look like?
- Which kind of structural elements (helix, loop, and sheet) does the protein sequence feature?
- What does the tertiary structure of the protein look like?
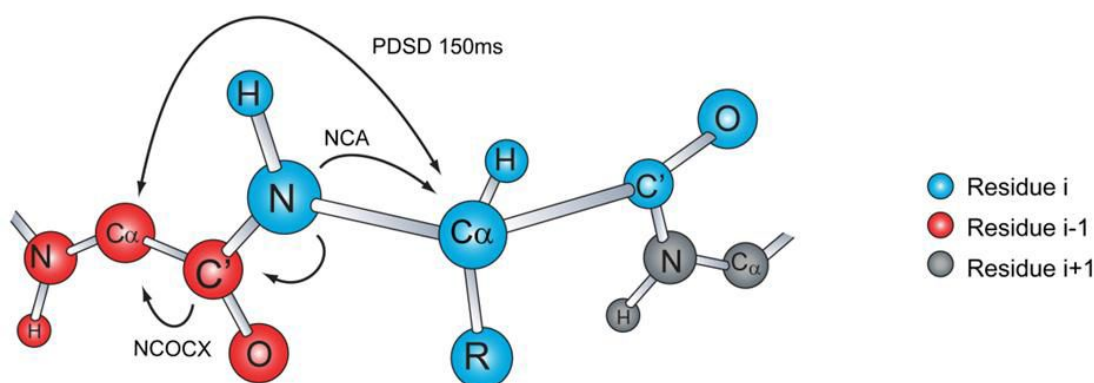
Figure 6.1: Schematic Representation of a Protein Backbone and the Correlations the Different Experiments Reveal.

> *Schematic representation of a protein backbone and the correlations the different experiments reveal. While the NCa experiment and a PDSD with a short mixing time (e.g. 20 ms; not depicted here) correlate nuclei within one amino acid only, the NCOCx and PDSD with a longer mixing time (e.g. 150 ms) correlate spins of different residues. How this can be used for the protein backbone assignment is depicted in the next figure.*

Answers to these questions can be found in different NMR correlations, as listed below:

Intraresidue:

Describes carbon-carbon or nitrogen-carbon correlations within one amino acid (also called a 'residue'). Because each type of amino acid has a specific chemical shift pattern, intra-residue correlations can give you an overview of the different types of amino acids that are observable in your sample. By comparing the chemical $C\alpha$ and $C\beta$ shifts to random coil chemical shifts (*References [▶ 79]* Ref. # 11), you get information about the secondary structure of these residues.

Experiments: e.g. PDSD/ DARR with short mixing times (20 ms), $NC\alpha$

Appendix

Interresidue:

Describes carbon-carbon or nitrogen-carbon correlations of different residues.

Experiments: e.g. PDSD/ DARR with long mixing times (150 ms), NCO.

Sequential:

Describes interresidue correlations of an amino acid 'i' to its neighbor 'i±1'. To discriminate single amino acids of one type, you need to use the amino acid sequence of your protein when assigning the correlations.

Experiments: e.g. PDSD/ DARR with long mixing times (150 ms), NCO, NCOCx.

Backward sequential:

Describes interresidue correlations of an amino acid 'i' to its neighbor 'i-1'.

Experiments: e.g. NCOCx, CaNCOCx.

Forward sequential:

Describes interresidue correlations of an amino acid 'i' to its neighbor 'i+1'.

Experiments: e.g. CONCaCx.

Long range:

Describes interresidue correlations of an amino acid 'i' to at least residue 'i±5'.

Experiments: e.g. ChhC, NhhC.

Instructions on how to proceed with the experiments and their respective information are shown below:
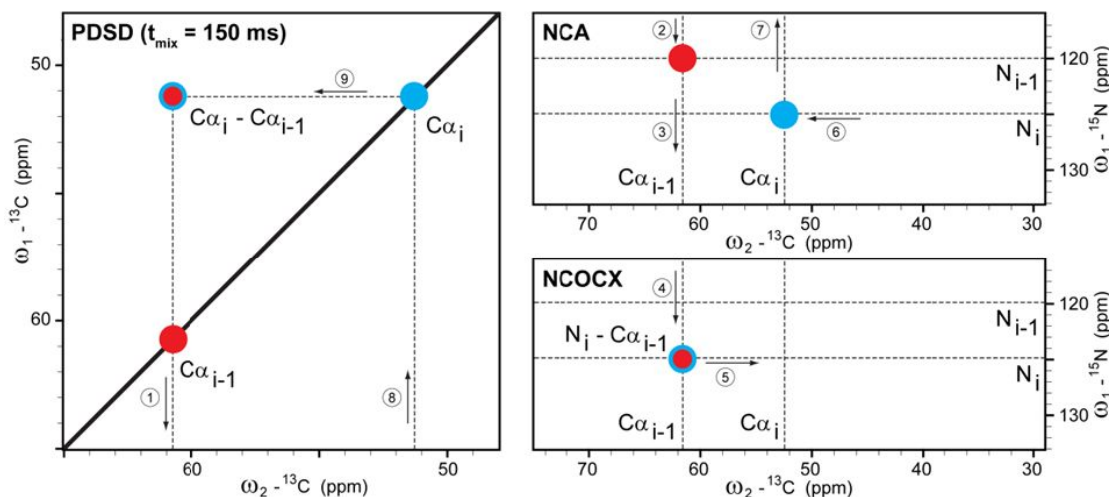
Figure 6.2: Schematic Representation of a Sequential Assignment.

Schematic representation of a sequential assignment. After having obtained chemical shifts of carbons within one amino acid in a short PDSD ($t_{mix} \approx 20$ ms, e.g. the $C\alpha_{i-1}$ in red and the $C\alpha_i$ in blue, not depicted here), a long PDSD ($t_{mix} \approx 150$ ms) will offer interresidue $^{13}C\alpha_{i-1}$ - $^{13}C\alpha_i$ correlations (red-blue dot). To prove that both carbons are really consecutive neighbors, the ^{15}N chemical shift needs to be taken into account. In an NCA, the intraresidue NCA correlation has to be assigned first. If the two observed $C\alpha$ atoms belong to sequential neighbors, a correlation peak at the chemical shifts of $^{15}N_i$ to $^{13}C\alpha_{i-1}$ is visible in the NCOCX spectrum.

6.2 Mixing Times and Their Information Content

Information about sample labeling can be found in the *References [▶ 79]* Ref. # 6-10.

Experiment	Sample Labeling Scheme	Mixing Time	Information
PDSD/DARR	u-[^{13}C]	20 ms	Intraresidue ^{13}C-^{13}C
		150 ms	Interresidue ^{13}C-^{13}C ('i' to ~'i ± 1, 2')
	sparsely [^{13}C]	100 ms	Intraresidue ^{13}C-^{13}C
		500 ms	Interresidue ^{13}C-^{13}C ('i' to ~'i ± 1, 2')
		≤ 1 s	^{13}C-^{13}C distance restraints ('i' to 'i± 5' and more)
NCaCx (via PDSD/ DARR)	u-[^{13}C, ^{15}N]/ sparsely [^{13}C], u-[^{15}N]	20 up to max. 50 ms to avoid unspecific spreading of polarization	Intraresidue ^{15}N-^{13}Ca-^{13}Cx
NCOCx (via PDSD/ DARR)	u-[^{13}C, ^{15}N]/ sparsely [^{13}C], u-[^{15}N]	20 up to max. 50 ms to avoid unspecific spreading of polarization	backward sequential ^{15}N-^{13}CO-^{13}Cx ('i' to ~'i-1')
ChhC	u-[^{13}C]/ sparsely [^{13}C]	~ 350 µs (via ^{1}H-^{1}H)	^{13}C-^{13}C distance restraints (at least 'i' to 'i± 5', ~6Å)
NhhC	u-[^{13}C, ^{15}N]/ sparsely [^{13}C], u-[^{15}N]	~ 350 µs (via ^{1}H-^{1}H)	^{15}N-^{13}C distance restraints (at least 'i' to 'i± 5')

6.3 Rotor Synchronized Spectral Widths

When recording spectra under magic-angle spinning each peak will be broken up into an isotropic signal and an accompanying set of spinning side bands (SSB). These need to be taken into account when choosing a spectral width (SW) to avoid uncontrolled SSB backfolding, so called aliasing. Since they depend on the MAS frequency (v_r), SSBs are separated from the isotropic signal by integer multiples of the MAS frequency:
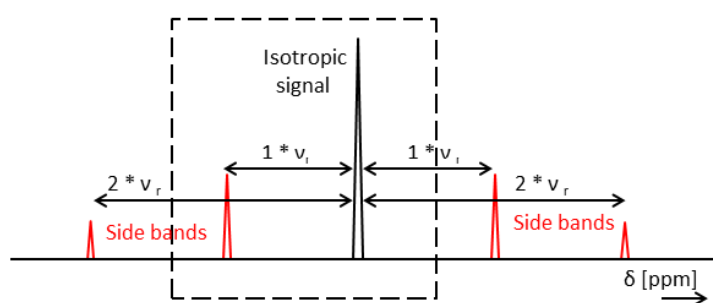
Figure 6.3: Schematic Representation of an Isotropic Peak (black) and its Corresponding Side Bands.

*Schematic representation of an isotropic peak (black) and its corresponding side bands (red), which occur at integer multiples of the MAS frequency (n * v_r) from the isotropic peak. The peaks in the dotted box are shown in the following Figures.*

When you want to record multidimensional (nD) experiments, you want to acquire as many scans as fast as possible. Since the indirect dimensions of nD experiments are incremented rows of 1D's, the experimental time can be shortened dramatically when using the smallest possible spectral widths for the 1D's. Nevertheless, the backfolding of signal from outside a chosen SW must be taken into account, which is true for most SSB.

You can avoid SSB backfolding artifacts by choosing a SW that is synchronized with the rotor frequency. This ensures that SSBs are folded on top of the isotropic peak, adding them up.

> **i**
>
> The preset acquisition mode for nD experiments in *TopSolids* is called STATES-TPPI, which uses so-called "wrap-around" backfolding of a peak. For any acquisition mode that is based on complex transformation, rotor synchronization using *TopSolids* will work. Modes, as TPPI, that are based on real transformation only, will lead to aliasing though!

The next figure illustrates the side band backfolding problem, when a SW is not rotor synchronized. The final position of the backfolded side band depends on how far past the edge of the SW the real side band lies. With wrap-around backfolding, a peak located at "SW – x" past the edge will be folded in from the opposite border of the SW and will appear at "SW – x" in the spectrum. Since the SW differs from the MAS rate, "SW – x" is different from "SW – p", which is the position of the isotropic peak. Hence, the folded SSB will appear as a separate peak in the spectrum.
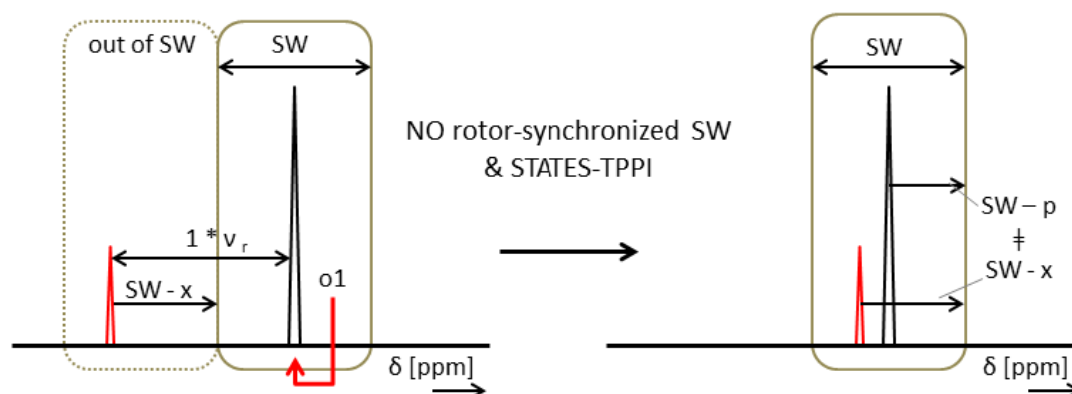
Figure 6.4: Non-synchronized SW.

> *Non-synchronized SW. On the left, an excerpt of the spectrum from the previous figure (dotted box) is shown. Choosing a SW that is not a (half-, quarter- or) integer multiple of the MAS rate (n * v_r) causes uncontrolled SSB backfolding. Under STATES-TPPI this looks like a "wrap-around" of signal that is located outside of the SW (red). In the left panel, the side band (red) lies a distance "SW – x" left of SW border. In the right panel, its backfolded signal appears within the SW at exactly the same position in relation to the right border of the SW. Because this distance does not match with the isotropic peak position ("SW – p" ppm away from the same border), the peaks cannot sum up.*

The principle of using a rotor-synchronized SW is depicted in the following figure. Now, the SW is a half, quarter or integer multiple of the MAS frequency. Because the distances of the isotropic peak to its side bands are integer multiples of the MAS frequency as well, they will always fall on the same position and sum up during backfolding.

Thus, we recommend a rotor-synchronized acquisition!
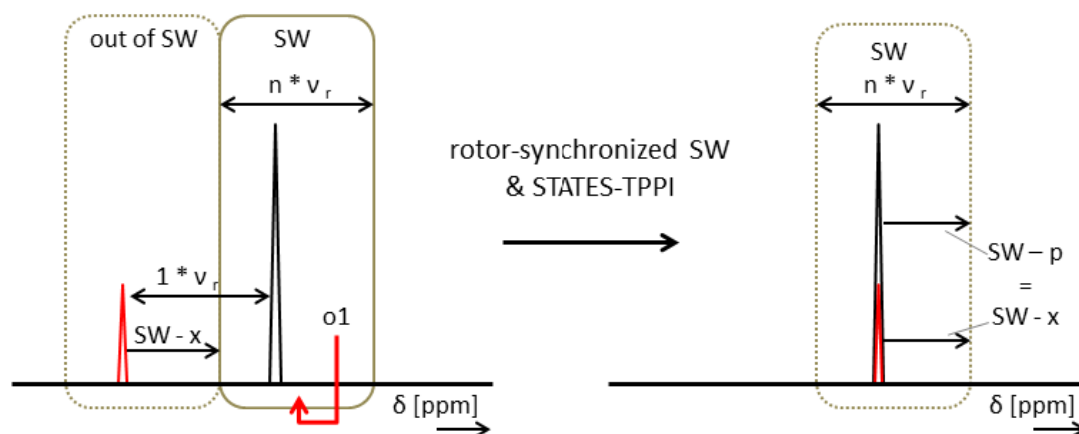
Figure 6.5: Rotor Synchronization.

> *Rotor-synchronization. On the left, an excerpt of the spectrum from an isotropic peak (dotted box) is shown. Choosing a SW that is a (half-, quarter- or) integer multiple of the MAS rate (n * v_r) avoids uncontrolled SSB backfolding, because the distance "SW – x" is now equal to "SW – p". Both, the side band and isotropic peak, lie at the same distance from the spectral border. Because backfolding under STATES-TPPI looks like a "wrap-around", the side band signal will now sum up.*

For further reading, we recommend basic NMR spectroscopy books, such as "*Multidimensional NMR in Liquids – Basic Principles and Experimental Methods*" by F. J. M. van de Ven as well as "*Protein NMR Spectroscopy: Principles and Practice*" by J. Cavanagh *et al.*.

6.4   Typical Carbon Spectral Widths

A typical peak pattern for a ^{13}C-spectrum of a biological sample is well known and can be seen in the next figure. In proteins, three different types of carbons occur: a) carbons bound to oxygen, b) carbons as part of aromatic ring systems and c) aliphatic carbons.

Quaternary carbons, as the carbonyl-^{13}Cs of a protein backbone, show a specific shift of about 175 ppm. Carbons that are part of an aromatic ring system give signal at approximately 120 ppm. The most crowded spectral region originates from aliphatic carbons in the range of 75 to 0 ppm. The more protons are bound to a carbon, the smaller the chemical shift will be. Thus, Cα's of a protein backbone (only one proton bound, except for glycine Cα, which binds two protons) give signal in the range of 75 to 40 ppm, while aliphatic side chain carbons (Cβ, Cγ, aso.) can mainly be assigned to signals from 40 to 0 ppm. Arithmetic mean values for amino acid chemical shifts can be taken from the "*Biological Magnetic Resonance Data Bank*".
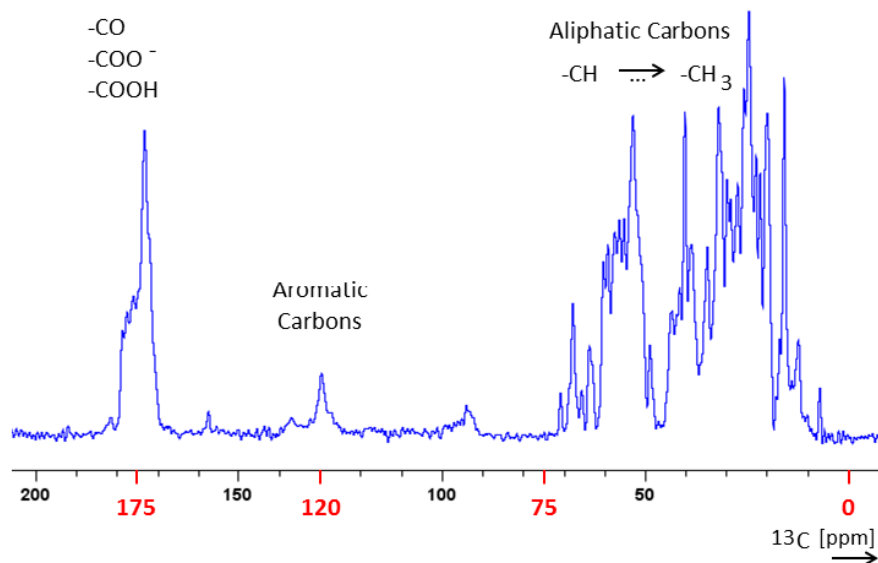
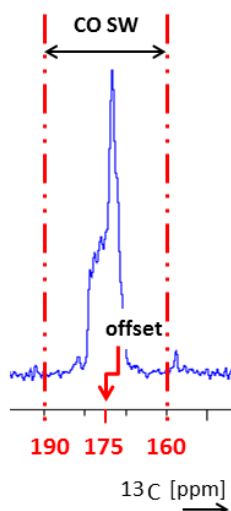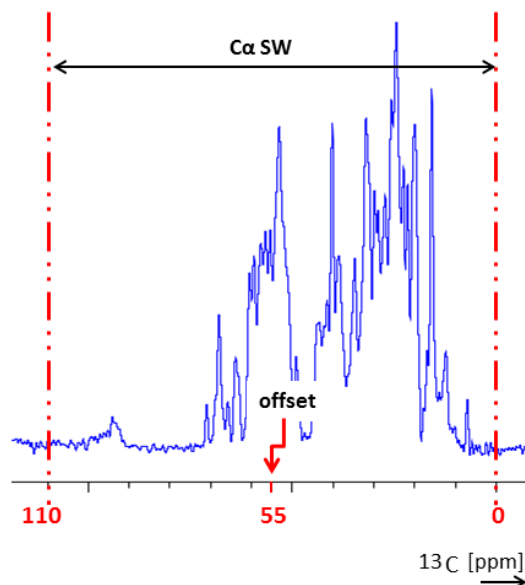*Figure 6.6: Typical Peak Pattern for a ^{13}C-detected HC CP Experiment on a Biological Sample.*

> *Typical peak pattern for a ^{13}C-detected HC CP experiment on a biological sample. Red numbers indicate the regions of ^{13}C chemical shifts that belong to specific functional groups.*

Knowledge of common ^{13}C peak patterns of proteins is essential when estimating the spectral width (SW) for indirect dimensions of multidimensional experiments. Depending on the information content and the kind of experiment you want to perform, appropriate choices for offsets (o1, o2, etc.) and SWs can dramatically shorten the experimental time.

There are some standard widths worth remembering. To cover the complete ^{13}C SW of a protein sample, a sweep of 200 ppm at a ^{13}C offset of 100 ppm is commonly used:
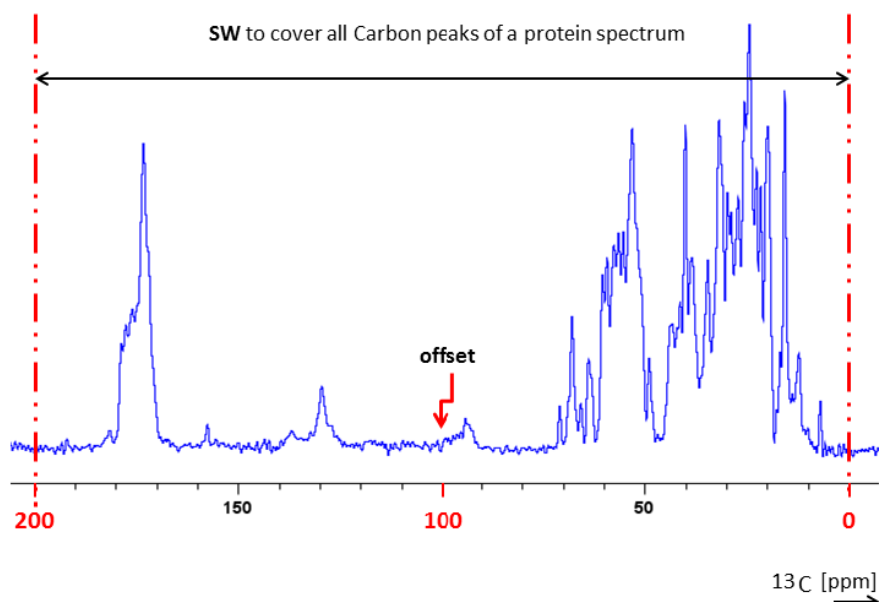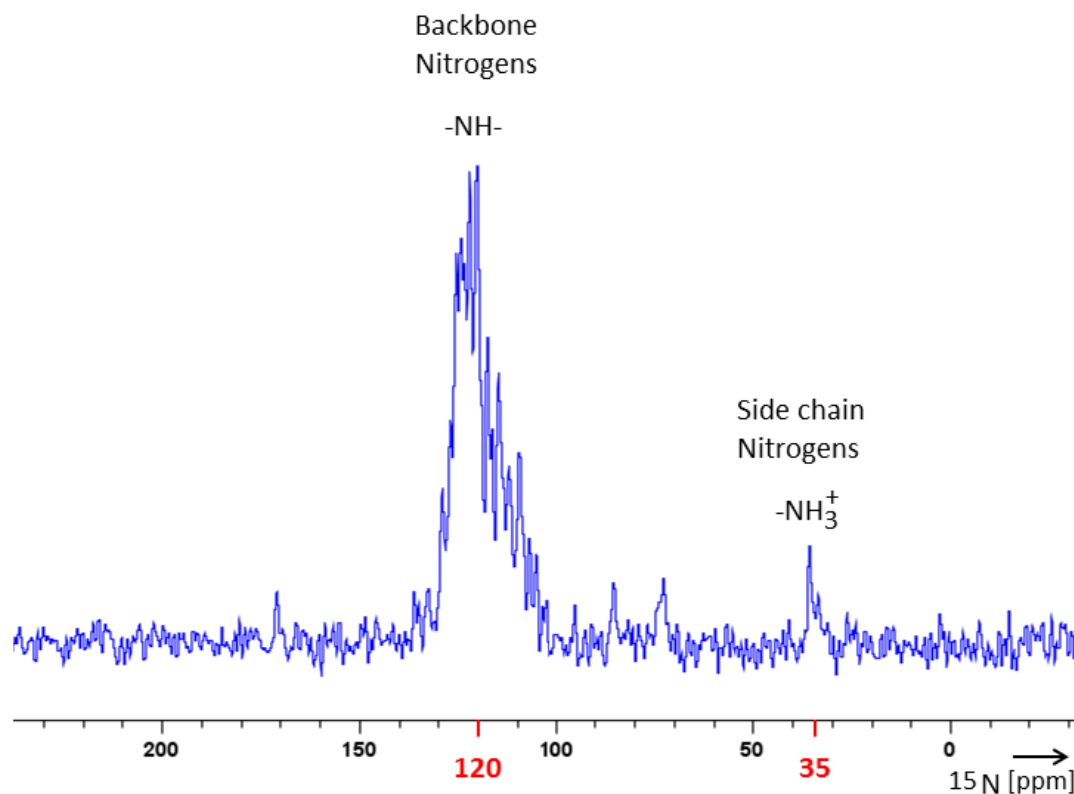

Figure 6.7: Typical Peak Pattern for a ^{13}C-detected HC CP Experiment on a Biological Sample.

> *Typical peak pattern for a ^{13}C-detected HC CP experiment on a biological sample. Red numbers indicate the SW from 200 to 0 ppm and the ^{13}C offset of 100 ppm.*

After a specific cross polarization step from ^{15}N to ^{13}CO (all dimensions that include an NCO dcp transfer), a small SW of < 30 ppm can be chosen as the ^{13}C offset during the ^{15}N to ^{13}CO CP transfer is set to 175 ppm in these experiments (figure below). Because this polarization transfer is specifically for CO's, we do not need to care about possible backfolding of the remaining carbon signals shown in the figure Typical Peak Pattern for a ^{13}C-detected HC CP Experiment on a Biological Sample above.

Figure 6.8: Typical Peak Pattern for a ^{13}C-detected Specific NCO Double CP Experiment on a Biological Sample.

> *Typical peak pattern for a ^{13}C-detected specific NCO double CP experiment on a biological sample. Red numbers indicate the SW from max. 190 to 160 ppm and the ^{13}C offset of 175 ppm.*

After a specific cross polarization step from ^{15}N to ^{13}Cα (all experiments with an NCα DCP transfer), a smaller SW than 200 ppm can be chosen as the ^{13}C offset during the ^{15}N to ^{13}Cα CP transfer is set to 55 ppm in these experiments. Because this polarization transfer is specifically for Cα's, we do not need to worry about possible backfolding of the CO and aromatic carbon signals shown in the figure *Typical Peak Pattern for a 13C-detected HC CP Experiment on a Biological Sample*. However, even though we are talking about a specific N to Cα polarization transfer only, other nearby aliphatic carbons (such as Cβ) can potentially be polarized as well. To still be on resonance for Cα (55 ppm) as well as avoiding backfolding artifacts of possible Cβ signals and to check for the specificity of the NCα, a sufficiently large SW of ~110 ppm should be selected:

Figure 6.9: Typical Peak Pattern for a ^{13}C-Detected Specific NCα Double CP Experiment on a Biological Sample.

> *Typical peak pattern for a ^{13}C-detected specific NCα double CP experiment on a biological sample. Red numbers indicate the SW from 110 to 0 ppm and the ^{13}C offset of 55 ppm.*

6.5 Typical Nitrogen Spectral Widths

The typical peak pattern for a ^{15}N-spectrum of a biological sample is well known and can be seen in the next figure. Each amino acid comprises at least one ^{15}N atom, which is part of the peptide bond in the protein backbone. These nitrogen's give the main signal in a ^{15}N spectrum at a specific chemical shift of ~135 to 110 ppm.

Furthermore, there are several amino acids that feature side chain nitrogen, which need to be taken into account when choosing the spectral width (SW) for multidimensional (nD) experiments. The most prominent ^{15}N side chain signal is that of protonated amino groups (NH_3^+; N-terminus of a protein, lysine side chain), which has a characteristic chemical shift of ~45 ppm. Depending on the buffer conditions, especially positively charged proteins can give strong $^{15}NH_3^+$ signal.

Less important, but still noteworthy are histidine, tryptophan, arginine, asparagine and glutamine, all of which include nitrogen atoms. The aromatic amines of histidine give signal at ~180 to 195 ppm. Likewise, the ^{15}N in tryptophan has a chemical shift of ~130ppm. Arginine features three nitrogen's in its side chain that give signal at ~90 and 80 ppm. The carboxyamide group in asparagine and glutamine has a chemical shift of ~112 ppm.

Thus, when choosing a SW for nD experiments, you need to pay attention to potential backfolding of possible side chain nitrogen. Therefore, check in a 1D HN CP experiment, which kind of side chain nitrogen your protein comprises.

Arithmetic mean values for amino acid chemical shifts can be taken from the "*Biological Magnetic Resonance Data Bank*".

Figure 6.10: Typical Peak Pattern for a ^{15}N-detected HN CP Experiment on a Biological Sample.

Typical peak pattern for a ^{15}N-detected HN CP experiment on a biological sample. Red numbers indicate the approximate regions of ^{15}N chemical shifts that belong to specific functional groups. Smaller peaks at ~170, 90, and 70 ppm belong to side chain nitrogen.
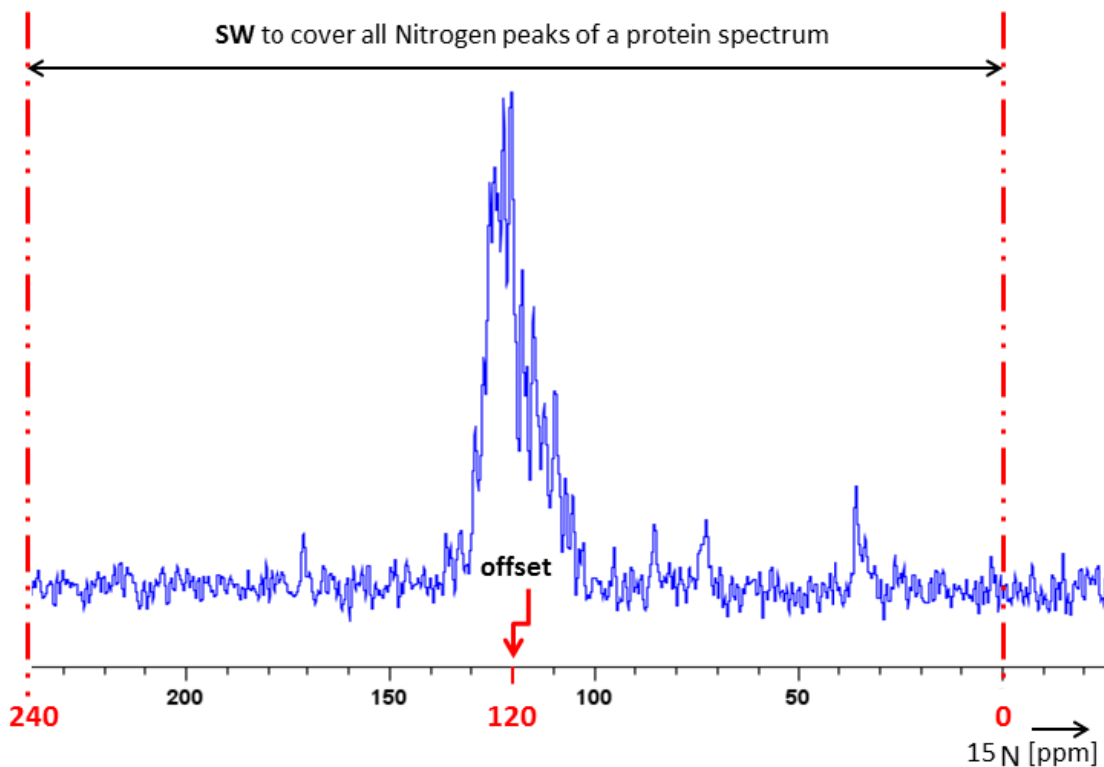
Figure 6.11: Typical Spectral Width for a ^{15}N-detected HN CP Experiment on a Biological Sample.

Typical spectral width for a ^{15}N-detected HN CP experiment on a biological sample. Red numbers indicate an overall SW from 240 to 0 ppm and the ^{15}N offset at the backbone signal at ~120 ppm. No backfolding artifacts will occur, but the SW is inappropriately large for nD experiments.
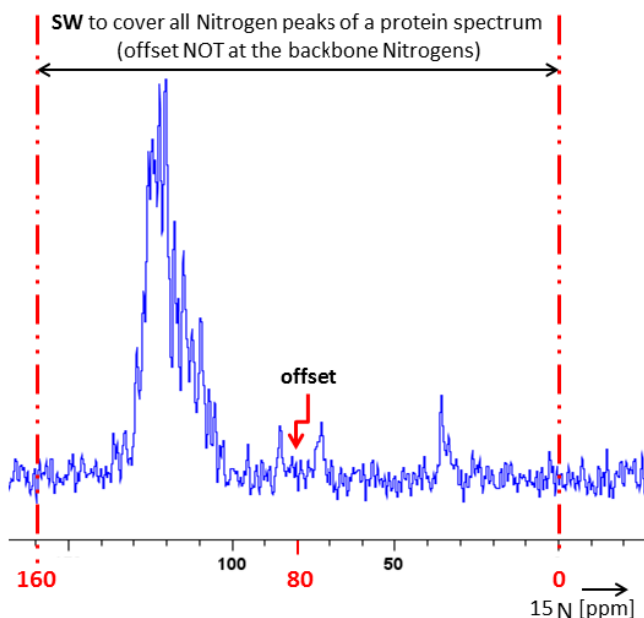
Figure 6.12: Reduced Spectral Width for a ^{15}N-detected HN CP Experiment on a Biological Sample.

Reduced spectral width for a ^{15}N-detected HN CP experiment on a biological sample. Red numbers indicate an overall SW from 160 to 0 ppm and the ^{15}N offset off resonance from the backbone signal at ~80 ppm. Here, the NH_3^+ signal does not cause backfolding artifacts, even though the spectral width is reduced.
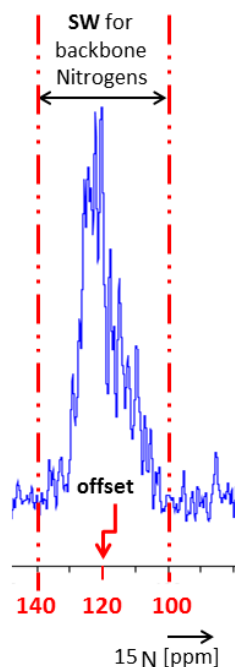
Figure 6.13: Small Spectral Width for a ^{15}N-detected HN CP Experiment on a Biological Sample.

Small spectral width for a ^{15}N-detected HN CP experiment on a biological sample. Red numbers indicate an overall SW from 140 to 100 ppm and the ^{15}N resonance offset from the backbone signal at ~120 ppm. By choosing a small SW, backfolding artifacts of probable side chain ^{15}N signal can occur. To avoid this, check in a 1D HN CP experiment, which SW would be appropriate.

6.6   How to Choose an Appropriate Acquisition Time

The acquisition time (aq) defines the time during which the signal of the observed nucleus is recorded. Generally speaking, the aq should be set such that the signal in the FID (free induction decay) drops below the noise (see figure below).

> **i** Usually high power decoupling is turned on during acquisition, which by overheating can damage both, your sample as well as the probe, especially if it is not an '*Efree*'. Thus, keep the aq as short as possible to prevent any damage! Furthermore, Bruker pulse programs include protection that stops acquisition times above 50 ms.
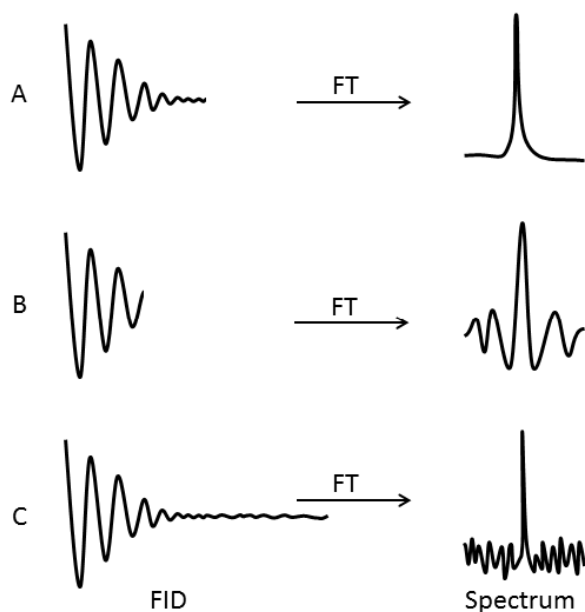
Figure 6.14: Different Acquisition Times Lead to Different Spectra.

Different acquisition times lead to different spectra. (A) An appropriate aq lasts until the signal of the FID has dropped below the noise. After Fourier Transformation (FT), this spectrum gives the best S/N ratio. (B) If the FID is cut short due to a small aq, the spectrum shows truncation artifacts at the bottom of the peak. (C) On the other hand, choosing an unnecessarily large aq will result in extra noise in the spectrum.

For 1D experiments, only relaxation and the heating effects of high power decoupling limit the acquisition time. When acquiring ^{13}C experiments on biological samples, the signal typically lasts 10 to 20 ms. Hence, the acquisition time for ^{13}C is mostly shorter than 20 ms. ^{15}N is less abundant in proteins than ^{13}C. Hence, the signal typically survives 5 to 12 ms and acquisition times for ^{15}N are usually not longer than 12 ms.

> **i** To estimate appropriate acquisition times, have a look at the FIDs of the 1D experiments performed on your sample (see optimized experiments in the chapter *The Sample of Interest Setup (13C) Flow Bar [▶ 46]*).

When setting up multidimensional (nD) experiments, you always have to make compromises between different spectral parameters that are linked to each other.

Direct dimension:

The number of points that are acquired (TD) to generate the FID signal depends on the duration between two TD points (dw, also known as 'dwell time' or 'sampling rate') as well as the total acquisition time (aq):

- Equation (1): TD = aq / dw
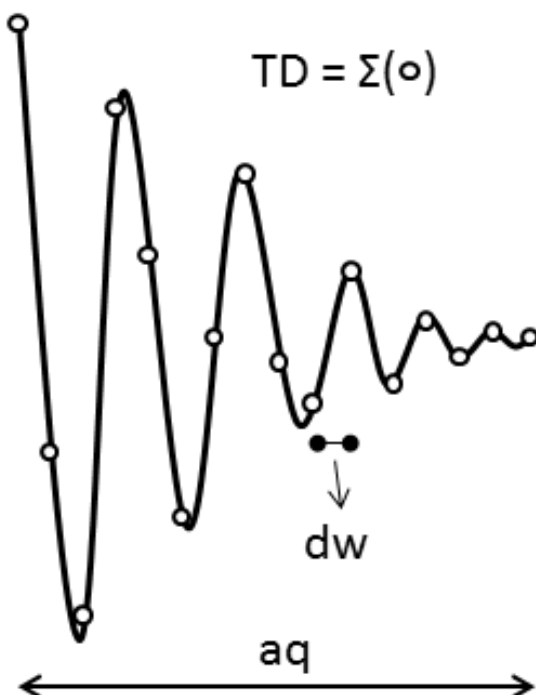
Figure 6.15: Schematic Illustration of Important Acquisition Parameters.

Schematic illustration of important acquisition parameters acquisition time (aq), sampling rate (dw) and the number of FID points (TD).

The Bruker dwell time is the reciprocal of half the spectral width in Hz (SWH):

- Equation (2): dw = 1 / (2 * SWH)

Solving Eq. 1 for the acquisition time and inserting the spectral width shows that the larger the SWH is, the shorter the aq will be:

- Equation (3): aq = TD * dw
- Equation (4): aq = ½TD / SWH

> **i** When you enter a spectral width, the acquisition time and the sampling rate will be changed, but the number of TD points stays the same. On the other hand, when entering an acquisition time, the spectral width and the sampling rate will stay the same, but the number of TD points will be modified.

Indirect dimensions:

As mentioned before, the indirect dimensions of nD experiments are incremented rows of 1D's (figure below). Each incremented row is recorded with a given number of scans (ns). Therefore, the indirect dimensions can prolong nD experiments dramatically, while the direct dimension does not.
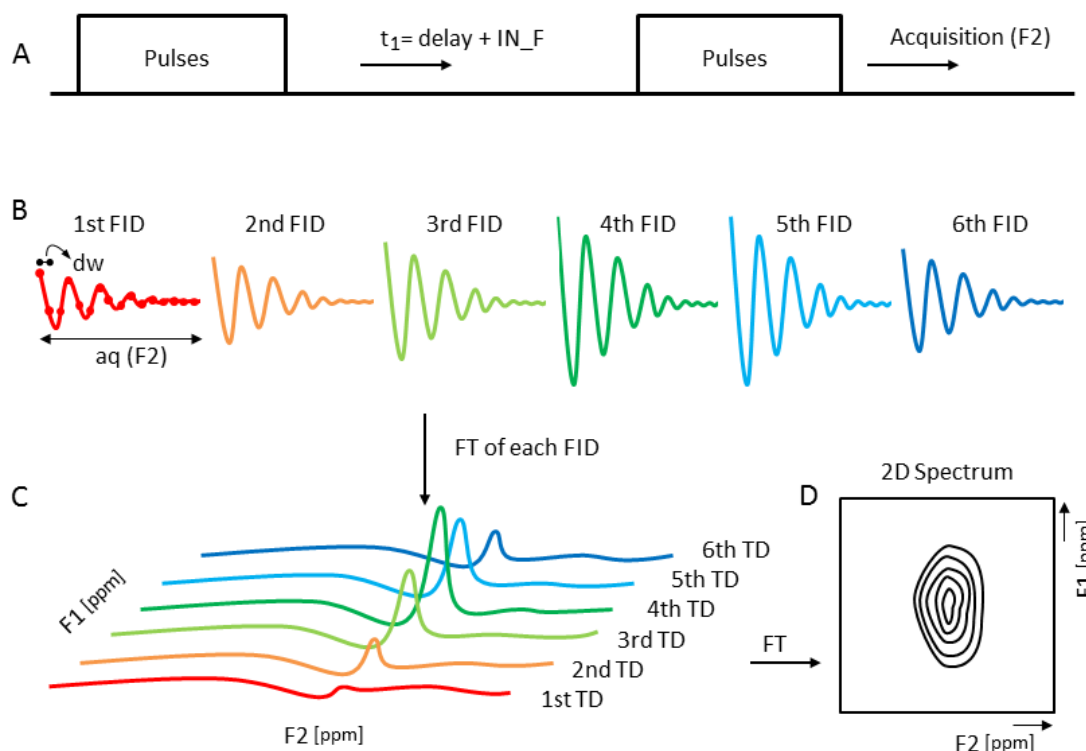
Figure 6.16: Schematic Illustration of Important Acquisition Parameters in Multidimensional (here: 2D) Experiments.

> Schematic illustration of important acquisition parameters in multidimensional (here: 2D) experiments. (A) Each nD experiment consists of numerous pulses, followed by the incremented F1 delay (t_1) for the indirect dimension. Afterwards, another sequence of pulses follows, accompanied by the acquisition of the direct F2 dimension (B). As in the previous figure, each single FID (varying colors) has the length of one acquisition time (aq) and consists of TD points, which are sampled with an interval of the dwell time (dw). After each FID, the pulse sequence (A) is revisited and recorded again, but with a t_1 that is incremented by IN_F, the sampling rate of the indirect dimension. The experiment is repeated until t1 equals the acquisition time of the indirect dimension. (C) The indirect dimension is displayed as incremented rows of 1Ds by Fourier transforming (FT) each single FID (see color code). (D) Schematic spectrum of a 2D experiment.

In the indirect dimensions, the sampling rate is the increment (IN_F), which is the reciprocal of SWH:

- Equation (5): $IN\_F = 1 / SWH_{F1}$

Similar to Eq. 1, the number of TD points in the indirect dimension (TD_{F1}) depends on the acquisition time and the increment:

- Equation (6): $TD_{F1} = 2 * aq_{F1} / IN\_F$

The relationship between aq and SWH is still the same as in the direct dimension:

- Equation (7): $aq_{F1} = \frac{1}{2}TD_{F1} / SWH_{F1}$

More importantly, the number of TD points is directly proportional to the acquisition time:

- Equation (8): $aq_{F1} = \frac{1}{2}TD_{F1} * IN\_F$

Since TD_{F1} defines how often the whole experiment has to be repeated (to reach the final aq_{F1} by incrementing t_1), it is important to keep the acquisition times in the indirect dimensions as short as possible.

6.7 Field Strength Calculation

There are different ways of expressing pulses in solid state NMR. To apply a pulse, *TopSpin* needs to know its length ('p' in µs) and power ('pl' in W or dB). When speaking about spectroscopy though, it is worth knowing how to convert these parameters (length and power) of a pulse into its frequency, also called field strength, in kHz. This is especially important when choosing Hartmann-Hahn matching conditions for Cross Polarization steps.

First, you need to define the length and power of a 90° pulse by optimizing:

For Example:

p_{90}= 2.5 µs

plw_{90}= 30 W.

In TopSpin 3.0 and higher versions, 1 W corresponds to 0 dB. Thus:

$$pldB_{90} = 10 \times \log_{10} \frac{1W}{plw_{90}} + 0 \; dB$$

$$pldB_{90} = 10 \times \log_{10} \frac{1W}{30W} + 0 \; dB$$

$$pldB_{90} = -14.77 \; dB$$

Frequency is nothing more than the number of occurrences of a repeating event per time unit. Therefore, the frequency of a 90° pulse is simply the reciprocal of four times its pulse length:
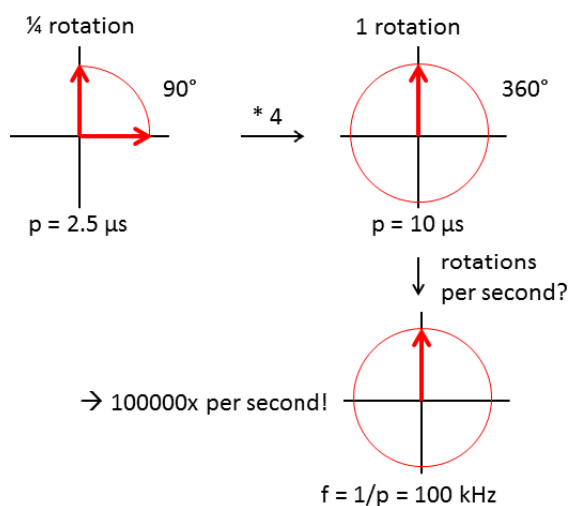
Figure 6.17: Converting the Length of a 90° Pulse p_{90} to its Frequency.

> To convert the length of a 90° pulse $p_{90}$to its frequency, the length for a full rotation (p_{360}) is needed. Its reciprocal depicts the frequency, also called 'field strength'.

Coming back to the example, this means:

$$p_{360} = 4 \times p_{90} = 4 \times 2.5 \; µs = 0.00001 \; s$$

$$v = \frac{1}{0.00001\ s} = 100000\ Hz = 100\ kHz$$

Thus, when applying this 90° pulse, field strength of 100 kHz is used with a power of 30 W.

The other way around works the same: If you want to use e.g. only 90 kHz for a 90° pulse, you need to recalculate the pulse length:

$$v = 90\ kHz = 90000\ Hz$$

$$p_{360} = \frac{1}{90000\ Hz} = 0.0000111\ s = 11.1\ \mu s$$

$$p_{90} = \frac{p_{360}}{4} = \frac{11.1\ \mu s}{4} = 2.8\ \mu s$$

Now, the pulse length should be 2.8 µs instead of 2.5 µs. At the same time, the power level needs to be adjusted. The difference in dB (= attenuation value) can be calculated by typing **calcpowlev** into the TopSpin command line and following the instructions:
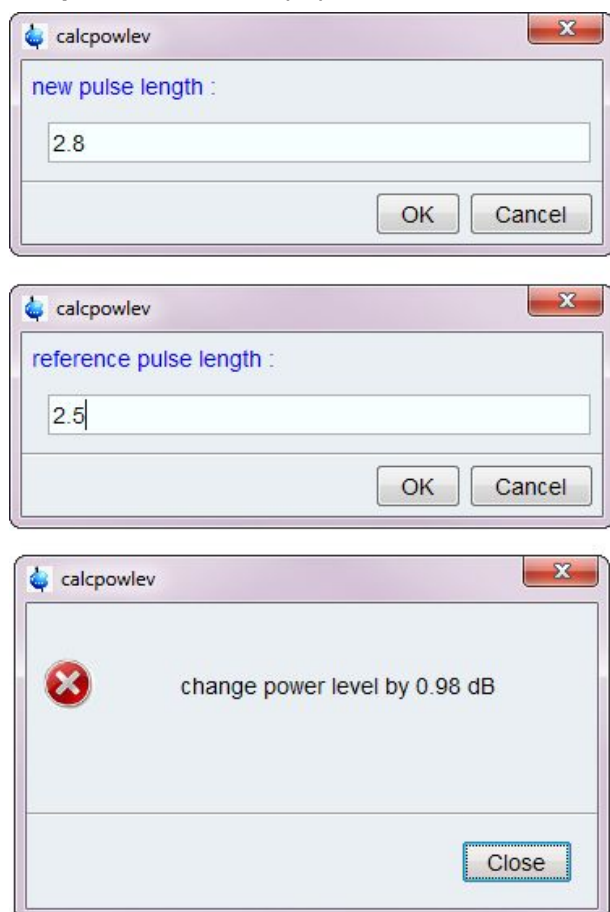
Figure 6.18: The AU Program calcpowlev.

The AU program calcpowlev. Example for using calcpowlev to calculate the difference in dB when changing a pulse length from 2.5 µs (100 kHz) to 2.8 µs (90 kHz).

Similarly, the AU program **pulse** can be used (next figure).

---

i   The AU program **pulse** uses the value of p1 together with the associated power level pl1 as a "reference 90 degree pulse". Thus, the program assumes that the reference pulse p1 and power level pl1 of the selected data set are appropriate for a ninety degree pulse!

It can be used to calculate the attenuation value for a given pulse length or nutation frequency, or vice versa. The pulse length, frequency, or attenuation value may be entered on the command line followed by the appropriate unit.

The (calculated) attenuation is rounded to the next lower integer value and the corresponding pulse length is re-calculated.
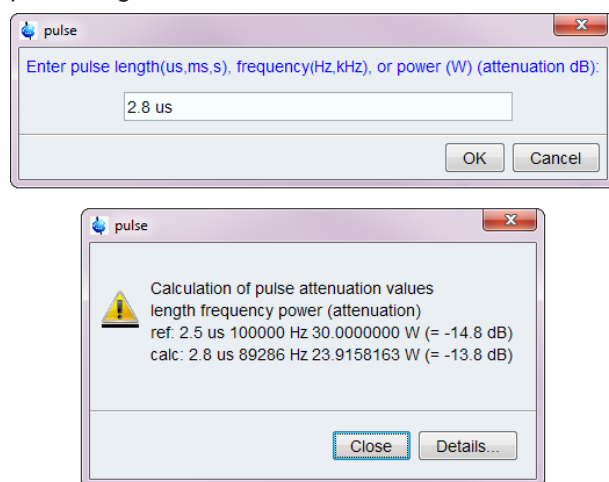
*Figure 6.19: The AU Program **pulse**.*

The AU program pulse. Example for using pulse to calculate the difference in dB when changing a pulse length from 2.5 µs (100 kHz) to 2.8 µs (90 kHz).

Another option is to calculate the new power level yourself:

$$pldB_2 = -20 \times \log_{10} \frac{v_2}{v_1} + pldB_1$$

pl2 is the power level corresponding to 90 kHz, while p1 and pl1 belong to the already known 100 kHz pulse. Solving Eq. 1 gives:

$$pldB_{90\ kHz} = -20 \times \log_{10} \frac{90\ kHz}{100\ kHz} - 14.77\ dB$$

$$pldB_{90\ kHz} = -13.9\ dB \approx 24.5\ W$$

$$\Delta pldB = -14.8\ dB - (-13.9\ dB) = 0.9 dB$$

Ignoring the rounding errors, we get the same result as when using calcpowlev or pulse.

Of course, Eq. 1 can also be solved for an unknown field strength v_2:

$$v_2 = 10^{-0.05 \times (pldB_2 - pldB_1)} \times v_1$$

By entering the known values into Eq. 2, we get:

$$\nu_2 = 10^{-0.05 \times (-13.9\,dB - (-14.8\,dB))} \times \nu_1$$

$$\nu_2 = 90\ kHz$$

Furthermore, there is a smartphone application called *Attenuator* (© Tim E. Burrow), which can be used for RF field strength calculations as well.

6.8 SR Value Calculation

In the chapter Shim Probe & Calibration, we calibrated the ^{13}C spectrum on adamantane by setting the left peak to 38.48 ppm* (referenced to TMS, *References [▷ 79]* Ref. #1. The resulting spectral reference value (SR) can be used to reference other ^{13}C spectra by simply copying and pasting.

Furthermore, the SR value can be recalculated for ^{1}H and ^{15}N spectra as well:

$$sr_H = (bf1_C + sr_C)\frac{bf1_H}{bf1_C} - bf1_H$$

$$sr_N = (bf1_C + sr_C)\frac{bf1_N}{bf1_C} - bf1_N$$

The basic transmitter frequency (bf1) for each nucleus has to be taken from *TopSpin* by typing **bf1** and should be entered in Hz. Bf1 can be found in the acquisition parameters as well.

Note that the ^{1}H and ^{13}C bf1 values are based on TMS as internal reference, while the ^{15}N bf1 is based on NH$_3$ (liquid) as external reference (*References [▷ 79]* - Ref. # 12).

* If ^{13}C spectra shall be referenced to DSS (DSS 0.5% in D$_2$O), the left adamantane peak should be set to 40.48 ppm, since DSS is shifted by -2 ppm toward neat TMS.

6.9 Glossary

HC CP: Cross polarization from ^{1}H to ^{13}C.

HN CP: Cross polarization from ^{1}H to ^{15}N.

HNCa DCP: Double cross polarization from ^{1}H to ^{15}N and in a second transfer to the aliphatic ^{13}C region.

HNCO DCP: Double cross polarization from ^{1}H to ^{15}N and in a second transfer to the carbonyl ^{13}C region.

MA: Magic Angle.

MAS: Magic Angle Spinning.

CP: Cross polarization.

DCP: Double Cross Polarization.

S/N: Signal to Noise.

KBr: Potassiumbromide; standard sample for Magic Angle adjustment.

Ada: Adamantane; standard sample for Shimming and ^{1}H/^{13}C pulse calibration.

Gly: Glycine in alpha-form; standard sample to measure S/N.

EDTE: Temperature control window.

MASDISP: Magic Angle Spinning control window of TopSpin.

VT: Variable Temperature.

BSMS: Control panel for shims and lock functions.

Prosol: Probe and solvent related parameter settings.

Q: Quality factor of a probe.

6.10   References

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