TopSolids bio

- Assisted Biological Solid State NMR

User Manual

Version 002

Innovation with Integrity

NMR

This manual was written by

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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 the policy of Bruker 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. Support engineers are advised to regularly check with Bruker for updated information.

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

1.2 Symbols and Conventions

Safety instructions in this manual 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 which, if not avoided, will 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.

⚠ WARNING

WARNING indicates a hazardous situation, which, 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, which, if not avoided, may result in minor or moderate injury or severe material or property damage.

This is the consequence of not following the warning.

1. This is the safety condition.

▶ This is the safety instruction.

NOTICE

NOTICE indicates a property damage message.

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.

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 what you can enter"	Arial bold	Type or enter **fromjdx zg**
Button, Tab, Pane and Menu Names "All what 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	`REX` `PNO`

Table 1.1: Font and Format Conventions

About This Manual

2 Introduction

TopSolids^{bio} is an interactive flow bar that is especially designed to assist in the delicate setup of difficult experiments dedicated to solid state NMR in structural biology. It is readily accessible to users from a broad diversity of background and experience.

TopSolids^{bio} generates a project structure within *TopSpin* and organizes the available experiments automatically. It further offers a standard library of state of the art multinuclear multidimensional experiments aimed at protein backbone assignment and the measurement of distance restraints providing data for protein structure elucidation.

Based on specific spectrometer and probe configurations, a fully automated measurement of ^{1}H and ^{13}C RF hard pulses is rapidly realized. A guided magic-angle adjustment and automated shimming guarantee for best performance of the probe. *TopSolids*^{bio} guides the user successively through the optimization process, either on standard samples or directly on the biological sample of interest.

All optimized parameters and settings can be reviewed in a PDF report at any time to assist fast publication.

To use *TopSolids*^{bio} you need to have installed at least *TopSpin 3.2 patch level 6*.

This manual describes how to use the *TopSolids*^{bio} interface step by step to create your personal project, set up the general spectrometer environment for your experiments, and finally show you how to successfully run the desired correlation spectra on the biological sample of interest.

This manual is written primarily for Bruker AVANCE III instruments, but the experimental part will be identical, or similar, for AVANCE II instruments. If any special hardware (or software) knowledge is required, this will be indicated in the experimental section.

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.

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.

Introduction

3 Expected Expertise

3.1 General Requirements

Although *TopSolids*^{bio} is mainly addressed to users, who indeed have a sophisticated background in structural biology, but 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*^{TM} (acquisition, processing).
- Rotor (handling, sample changing, temperature, etc.).

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

  http://www.theresonance.com/2014/categories/material-science/nmr-tutorial-mas-rotor-filling
- 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*^{bio} 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^{bio} retrieves part 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*^{bio} 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*^{bio} workflow.

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 *Sol*vent 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 high resolution probes, therefore, usually separate PROSOL tables exist. For solid state NMR MAS 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.

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*^{bio} tool the command **getprosol** is executed automatically. The assignment of PROSOL parameters and pulse program parameters is provided by a so called pulse assignment file which needs to be referred to in the pulse program. For *TopSolids*^{bio} suitable assignment files are provided and referred to in the pulse programs.

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 exist or is created for a certain solvent.

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.

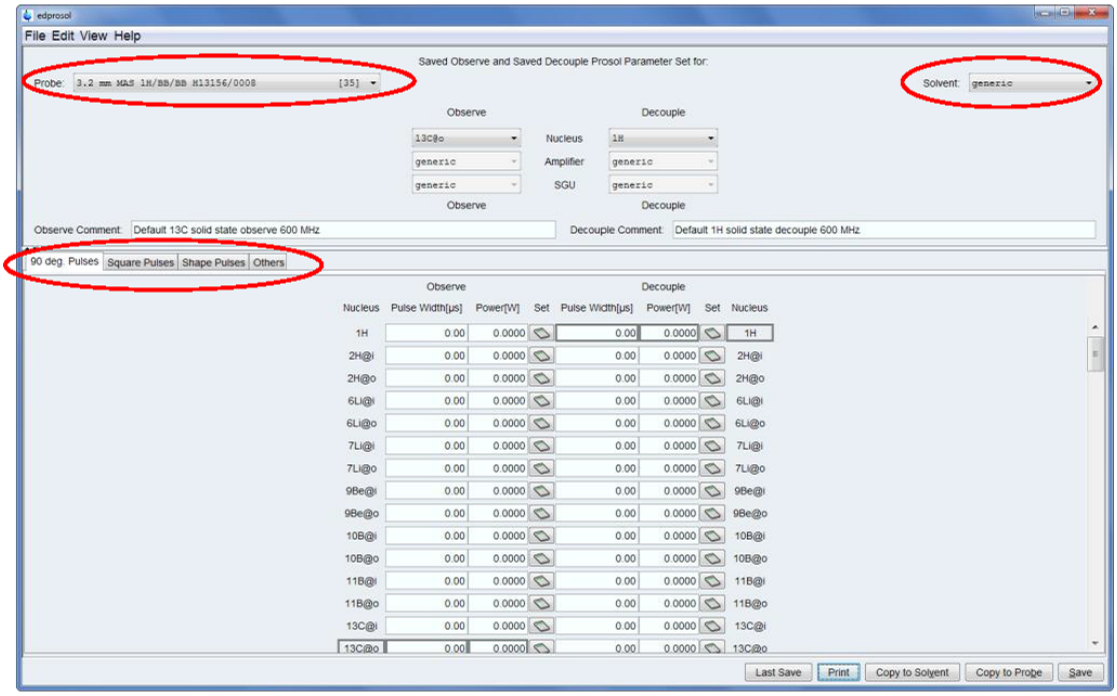

Figure 3.1: The edprosol Interface.

*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 tab **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:

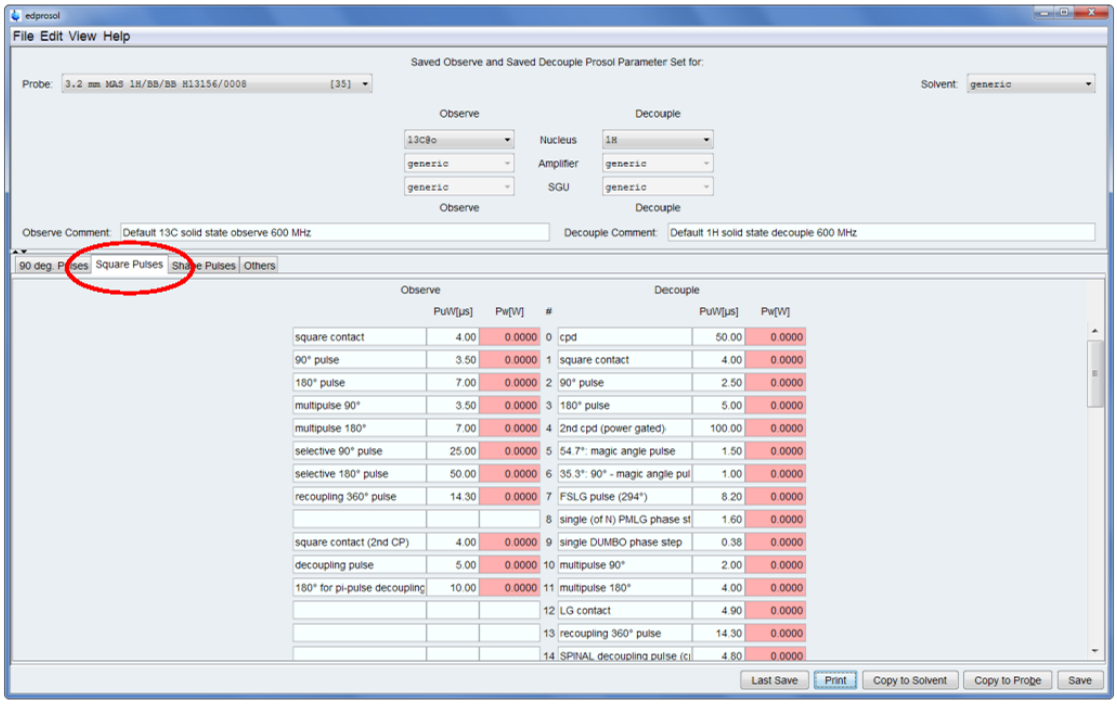

Figure 3.2: Square Pulse Tab in edprosol.

Square Pulse Tab in edprosol. No pulse calculation was performed, for this reason any power in Watts is zero and the entries are marked in red.

The tab **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.

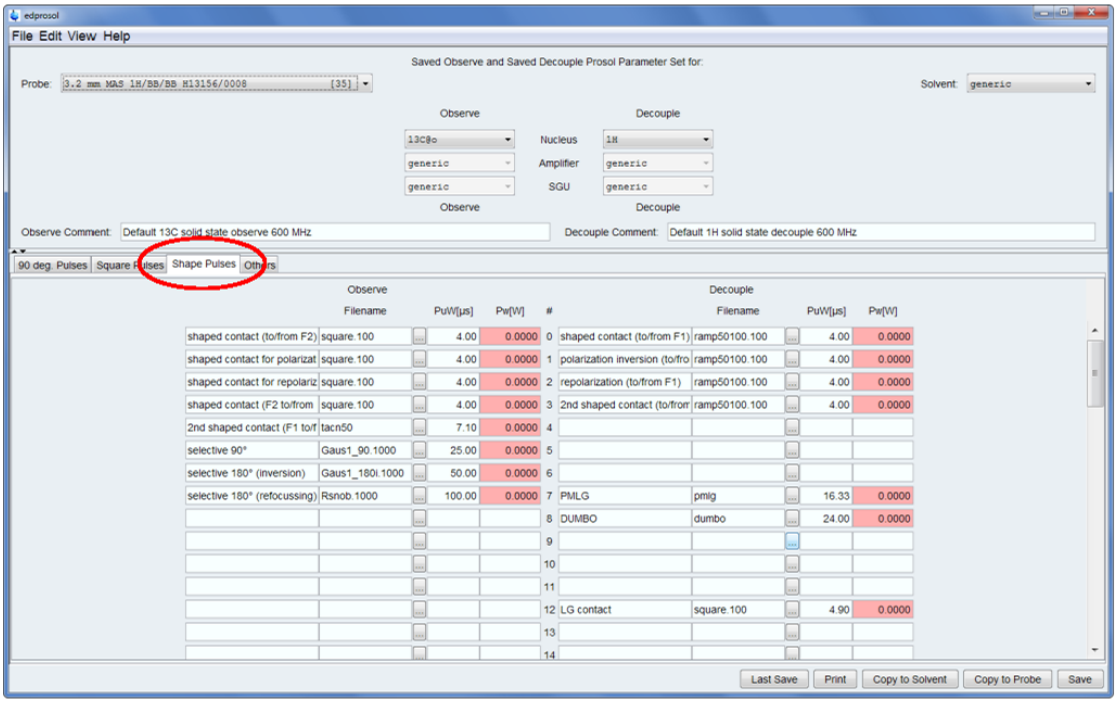

Figure 3.3: Shape Pulses Tab in edprosol.

Shape Pulses Tab in edprosol. No pulse calculation was performed, for this reason any power in Watts is zero and the entries are marked in red.

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. Usually, the 90° pulse and corresponding power level for a probe is known from previous experiments, e.g. from the acceptance tests during the probe's initial installation or from the latest experiments performed. These values are entered for the pulse width and power level as shown below:

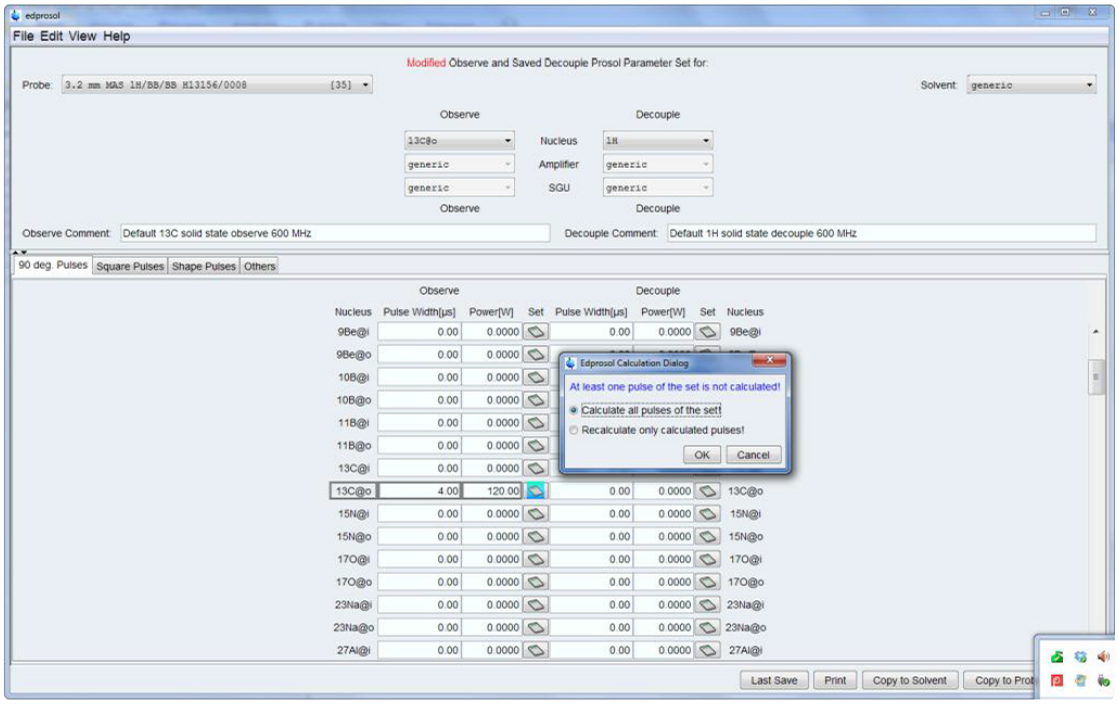

Figure 3.4: Filling in the PROSOL Table.

*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'** can be performed when clicking on the calculator icon (cyan).*

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 below.

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 below the parameter tables or by using the menu **File** > **Save**. If the user does not save, they will be prompted to do so before the next 90° pulse width and power level may be entered.

The discrimination of '**observe**' and '**decouple**' pulses is kept to keep the tables similar to that of high resolution NMR probes. Usually, there is no difference in the pulse widths and power levels for '**observe**' and '**decouple**' for solid state NMR probes. It is, therefore, recommended to use identical parameter values for both.

The kind of '**observe**' and '**decouple**' nuclei that can be chosen for a certain probe depends on the tuning ranges of the respective channel. For fixed frequency triple resonance probes, like standard bore H/C/N probes, only ^{1}H, ^{13}C (and ^{79}Br), and ^{15}N can be selected. For triple resonance probes with tunable X and Y channels like wide bore H/X/Y probes all nuclei within

the tuning range (typically ^{15}N to ^{31}P) are available. In this case the X and Y nuclei are labeled with **13C@o**, **13C@i**, **15N@o**, **15N@i**, etc. to indicate that the corresponding nuclei may be measured via different hardware channels (X or Y). The differences, however, typically are small and it is recommended to use identical tables for **@o** and **@i**.

3.2.3 PROSOL Parameters for TopSolids

TopSolids^{bio} 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 **Square Pulses** and **Shape Pulses**, respectively (the next two figures).

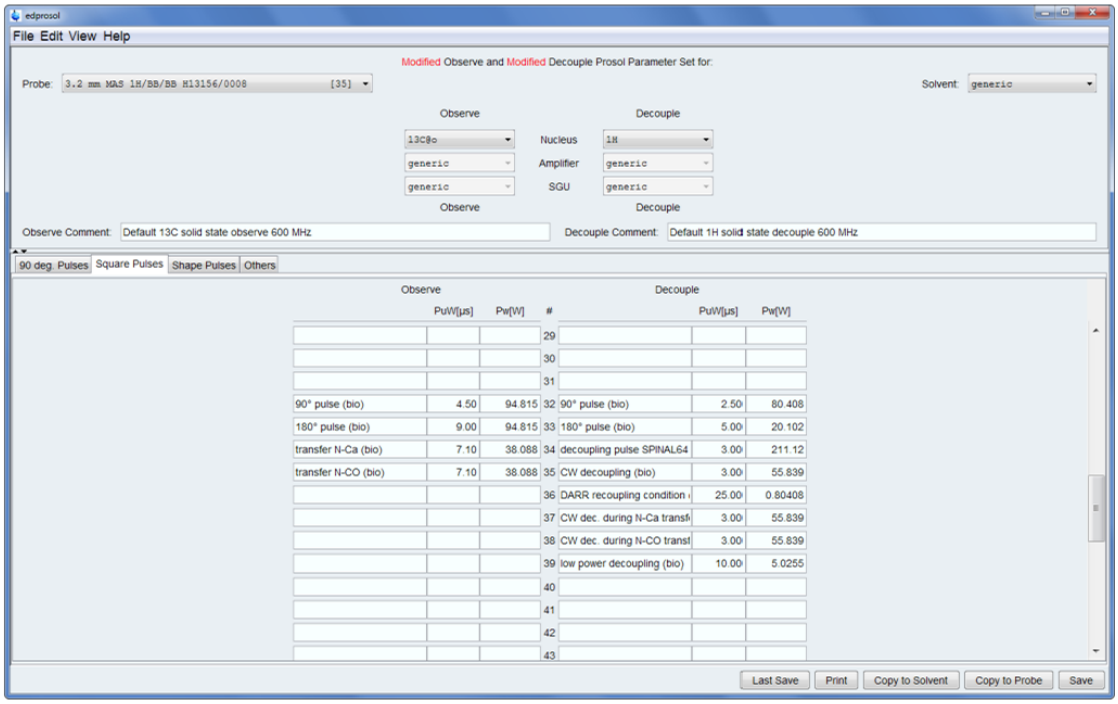

Figure 3.5: Dedicated Square Pulses for TopSolids^{bio} *Experiments.*

> *Dedicated **Square Pulses** for TopSolids*^{bio} *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. For this reason they are no longer shown in red (compare to the figures in The Command edprosol and the PROSOL Tables [▶ 12]).*

The following PROSOL tables should be updated, when entering the respective 90° pulse widths and power levels and doing a subsequent calculation:

- Fixed frequency triple resonance probes (e. g. SB H/C/N):
  - decouple: 1H, 13C, 15N, 79Br
  - observe: 1H, 13C, 15N, 79Br
- Tunable triple resonance probes (e. g. WB H/X/Y):
  - decouple: 1H, 13C@o, 13C@i, 15N@o, 15N@i, 79Br@o, 79Br@i
  - observe: 1H, 13C@o, 13C@i, 15N@o, 15N@i, 79Br@o, 79Br@i

As written above the tables for '**observe**' and '**decouple**' as well as the tables for **@o** and **@i** usually are identical.

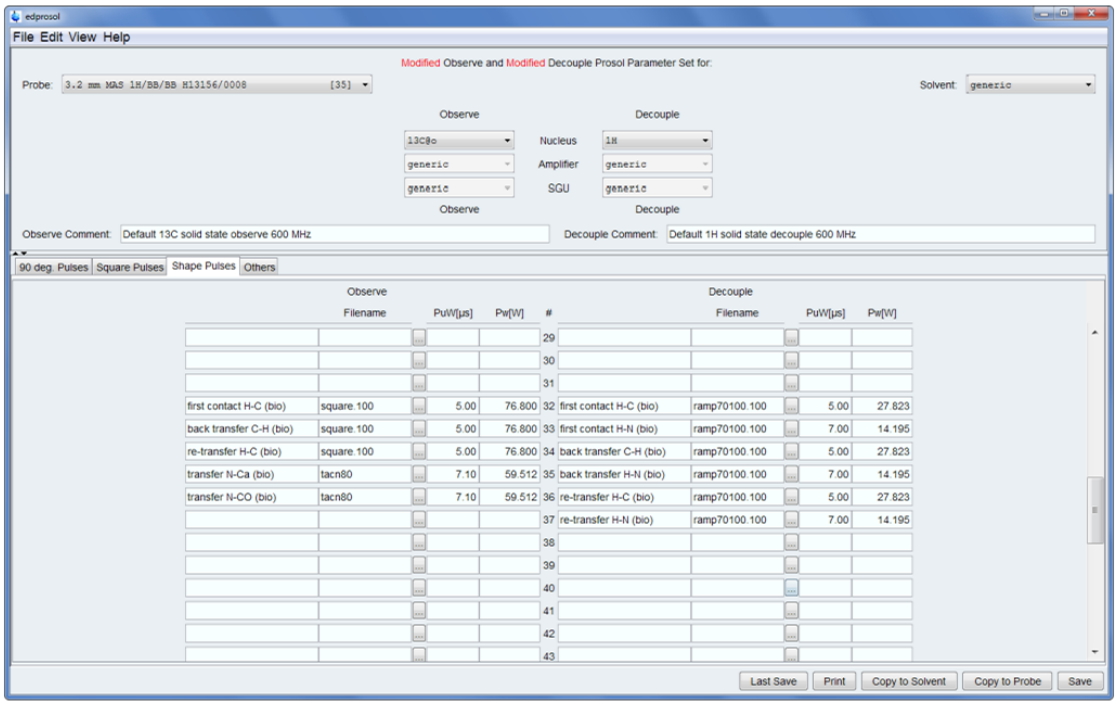

Figure 3.6: Dedicated Shape Pulses for TopSolids^{bio} Experiments.

> *Dedicated **Shape Pulses** for TopSolids^{bio} 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. For this reason they are no longer shown in red (compare to the figures in The Command edprosol and the PROSOL Tables [12]).*

3.2.4 Manipulating PROSOL Parameters

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

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. In this case 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 the figure” Filling in the PROSOL table”.
- For shape pulses the user may change the shape file. With respect to the shapes used by *TopSolids^{bio}* this is not recommended.
- For square pulses the user may change the flip angle. This usually makes no sense and is not recommended.

- The user may add parameters to the tables. These parameters, however, will not be used by *TopSolids*^{bio} and its pulse programs. Rather, the user needs to write an own pulse assign file and refer to this in the pulse program. This is an advanced feature and not covered in this manual!

3.2.5 Changing the Parameter Display in edprosol

The view menu of the PROSOL window allows changing the number of displayed values for each entry:

- Base view: description, pulse width, and power
- Full view: description, flip angle, pulse width, RF field, alignment (for shape pulses; the user may uncheck the boxes for flip angle, RF field, and alignment to reduce the number of displayed parameters).

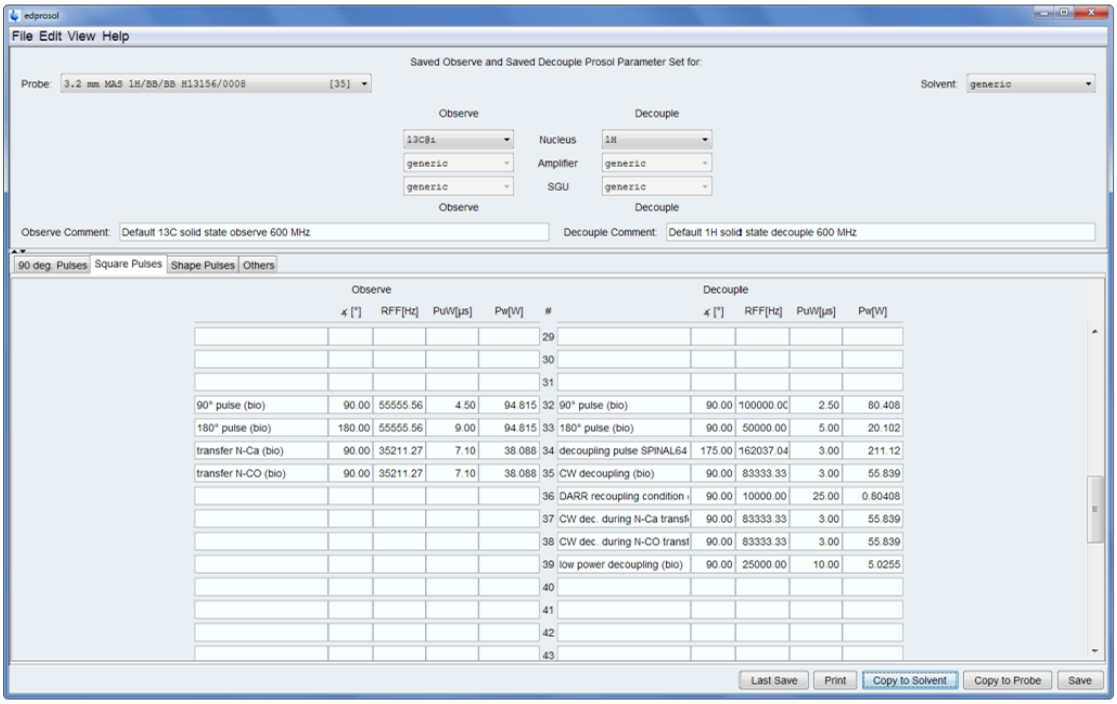

Figure 3.7: Full View of the Tab ***Square Pulses*** *for Parameters Related to TopSolids*^{bio}.

The RF field is not an independent parameter. It is derived from flip angle and pulse width. It may, however, be changed. This will change the pulse width and corresponding power level in turn. The flip angle cannot be changed by the user for shape pulses. It is read from the corresponding shape file entry instead. An extended view is shown for square pulses in the figure above.

The power level of a PROSOL parameter can either be shown in Watts or in dB (attenuation). The user may set this in the view menu.

3.2.6 Miscellaneous

3.2.6.1 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*^{bio} 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.

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*^{bio} there may be need to interact with *TopSpin*. You will find the most important acquisition functions summarized in the icon bar below:

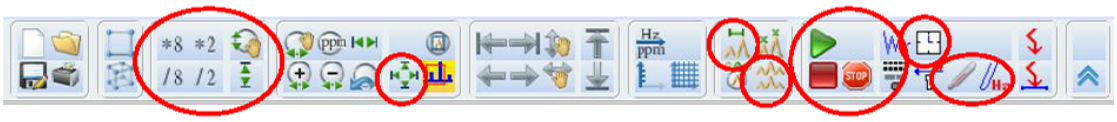

Figure 3.8: Icon Bar of the TopSpin ***Acquisition*** *Tab with TopSolids*^{bio} *Relevant Functions Indicated.*

The top symbols from left to right with **bold** indicating important functions during the use of *TopSolids*^{bio} 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*^{bio} 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

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*^{bio} in **bold**) summarized in a flow bar:

- **Process the spectrum**
- **Adjust the phase manually**
- **Calibrate the ppm axis**
- Pick Peaks
- Integrate the spectrum
- Advanced functions

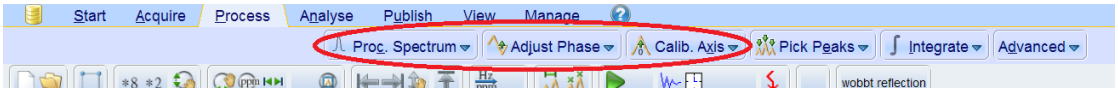

*Figure 3.9: Flowbar of the TopSpin **Process** Tab with TopSolids*^{bio} *Relevant Functions Indicated.*

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

3.3.2.1 Spectral Calibration

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

Before you do measurements:

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

At any time:

- Calibrate the spectrum of a standard sample and use the resulting **sr** value for all spectra.

Following the first approach, it is best to calibrate the field directly after shimming the probe when you are still in the '**gs**' mode (see chapter *BSMS Display [▷ 24]*):

1. If not already done, open the **BSMS** display (**bsmsdisp**) and choose the tab **Lock/Level** (see chapter *Shim Probe & Calibration [▷ 45]*).
2. Modify the '**field**' value carefully in units of 1 until the left (low field) peak of the adamantane spectrum is shifted to 38.48 ppm (*References [▷ 113]* Ref. # 1).
3. Stop the acquisition. Use for all experiments a **sr** value of zero.

Following the second approach you can calibrate the adamantane spectrum at any time (see chapter *BSMS Display [▷ 24]*):

1. Go to the adamantane reference spectrum in EXPNO 4 named '*Standard Setup/ probe shimming*'.
2. Zoom into the region of the left peak.
3. Go to the tab **Process** and click on **Calib. Axis**.
4. Do a left-click on the center of the peak and reference it to 38.48 ppm. After referencing click the arrow button to leave the menu (see figure below).
5. The resulting **sr** value can be used for referencing of ^{13}C dimensions and for recalculation of **sr** values for ^{1}H and ^{15}N dimensions (see the figure below and the chapter *SR Value Calculation [▷ 112]*).

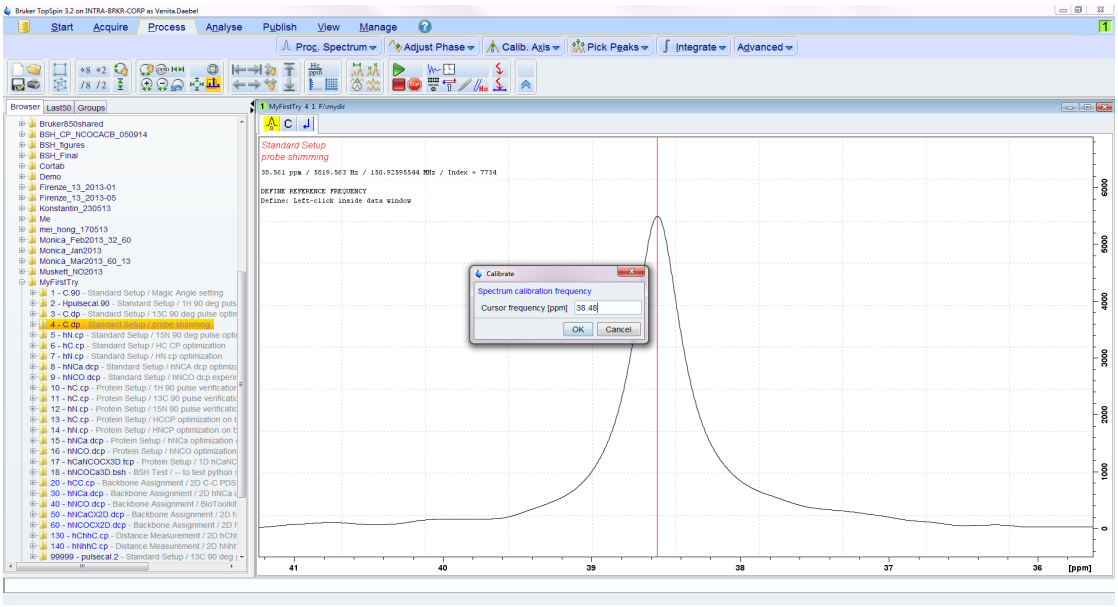

Figure 3.10: Calibration of the Left Peak of an Adamantane ^{13}C Spectrum to 38.48 ppm.

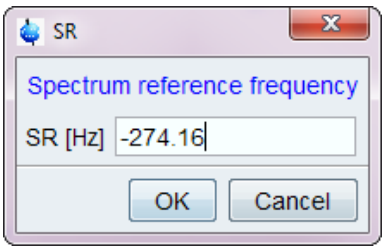

Figure 3.11: SR Value Used for Spectral Referencing.

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 [▸ 20]* or by typing '**bsmsdisp**' into the *TopSpin* command line.

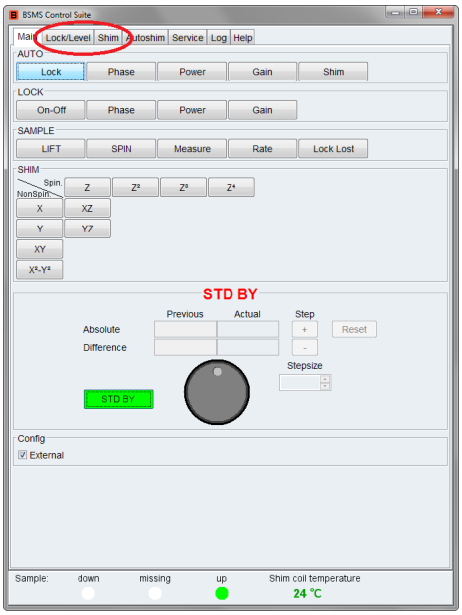

Figure 3.12: The BSMS Interface.

*Interface for the BSMS. The tabs **Shim** and **Lock/Level**, encircled in red, are especially important for TopSolids^{bio}.*

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 (see chapter *Shim Probe & Calibration [▸ 45]*).

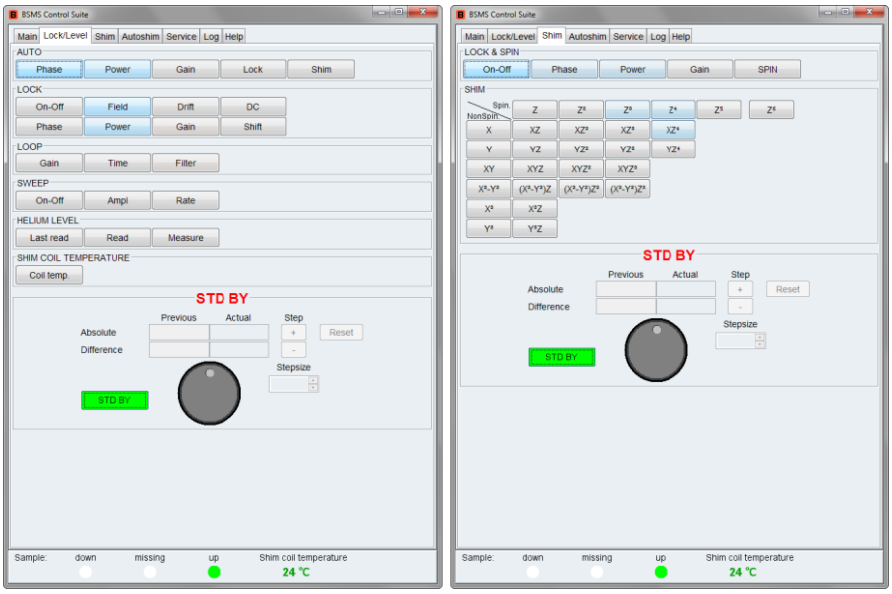

*Figure 3.13: BSMS Tabs **Lock/Level** (left) and **Shim** (right).*

3.3.4 Temperature Control - edte

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

Since *TopSolids*^{bio} is designed 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 to 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 '**high**' 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]*).

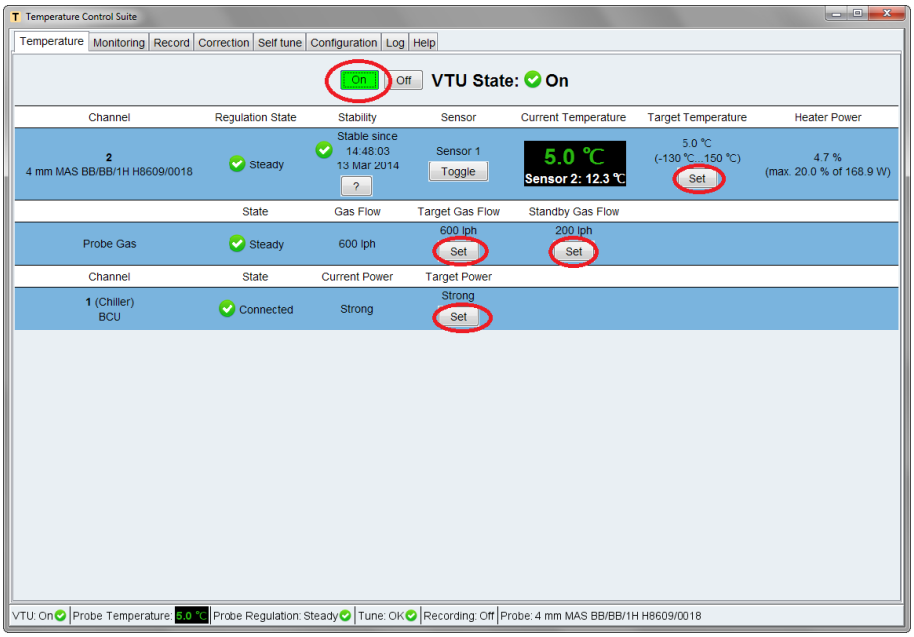

Figure 3.14: Temperature Control Suite (edte).

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 figure below shows the MAS display to control and change the MAS rate while using *TopSpin*. During the *TopSolids*^{bio} operation only the first overview 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*^{bio} 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 send to the MAS unit by pushing **ENTER** on the computer keyboard.

If the sample needs to be exchanged, first push the **HALT** 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 3.15: MAS Pneumatic Unit Control Display.

MAS 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 unit at the spectrometer.*

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 screws for each channel (first figure below) and those with labels (second figure below).

In the latter case, tuning screws are always thicker than those for matching. Furthermore, the tuning screw on the X channel is often not labeled, which can cause confusion with the screw for magic angle adjustment. Nevertheless, both screws can be distinguished quite easy: While the X channel tuning screw is thick and sticks out quite a lot, the MAS screw 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 **edhead**. A detailed description can be found in the **1D and 2D Step-by-Step-Basic** manual.

Maybe, you need to check the PROSOL parameters (**edprosol**). Further information can be found in the chapter *PROSOL for TopSolids [▶ 12]*.

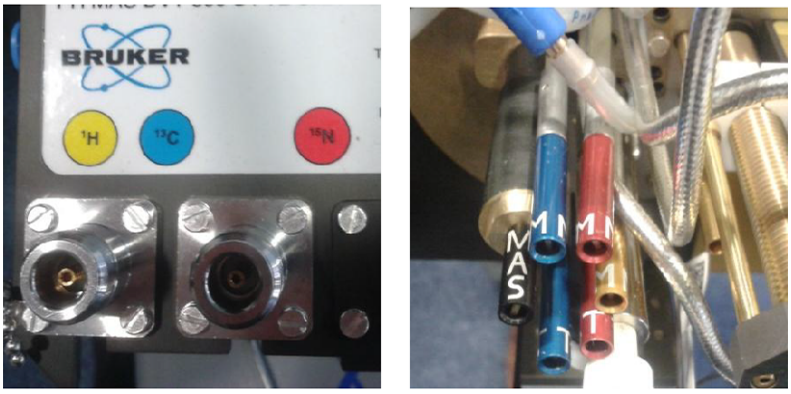

Figure 3.16: Probe with Color-Coded Screws for Each Channel.

Probe with color-coded screws 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) screws can be distinguished easily. Furthermore, the screw for adjusting the magic angle is seen in black (MAS).

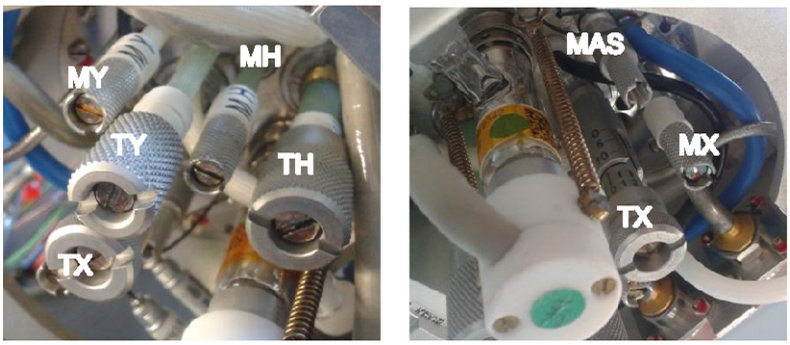

Figure 3.17: Probe with Labeled Screws.

Probe with labeled screws. As can be seen in the left figure, the tuning screws are labeled with T, matching screws 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 screw is visible. On the right figure, both tuning and matching screws for the X channel are depicted as well as the screw to adjust the magic angle (MAS). Usually TX is the only screw that is not labeled, which often causes confusion with the MAS screw. Note: The MAS screw is always shortest and thin, while the TX screw 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 20 %, 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 screw 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 correct rotation direction for probes produced before 2010 (figure below) is counterclockwise. The MA screw 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**).

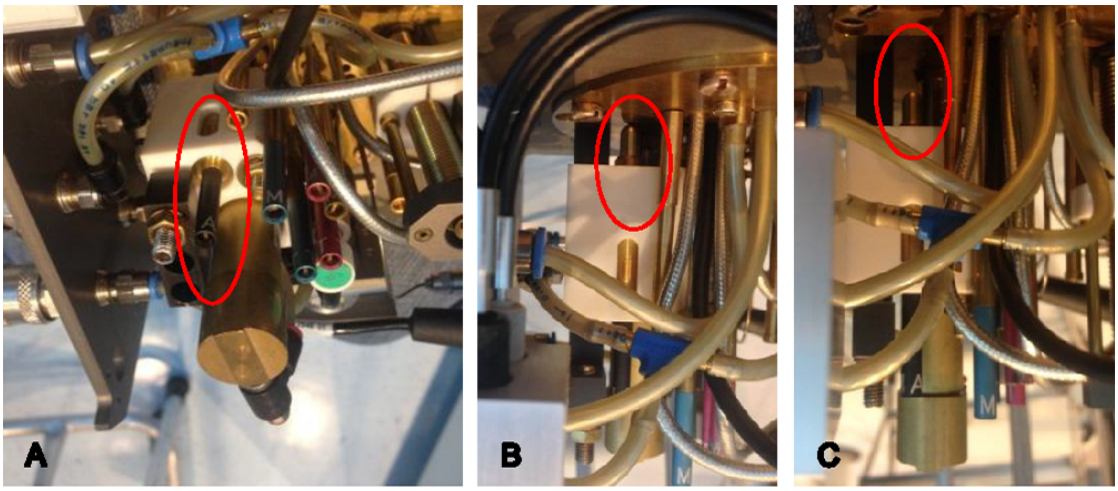

Figure 3.18: SB Probe Produced Before 2010.

SB probe produced before 2010. (A) Bottom view showing the black screw for MA adjustment (encircled in red). This screw 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 screw 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 screw 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 screw has been turned too far, turn it back slightly and toggle the stator by a sample exchange cycle before continuing.

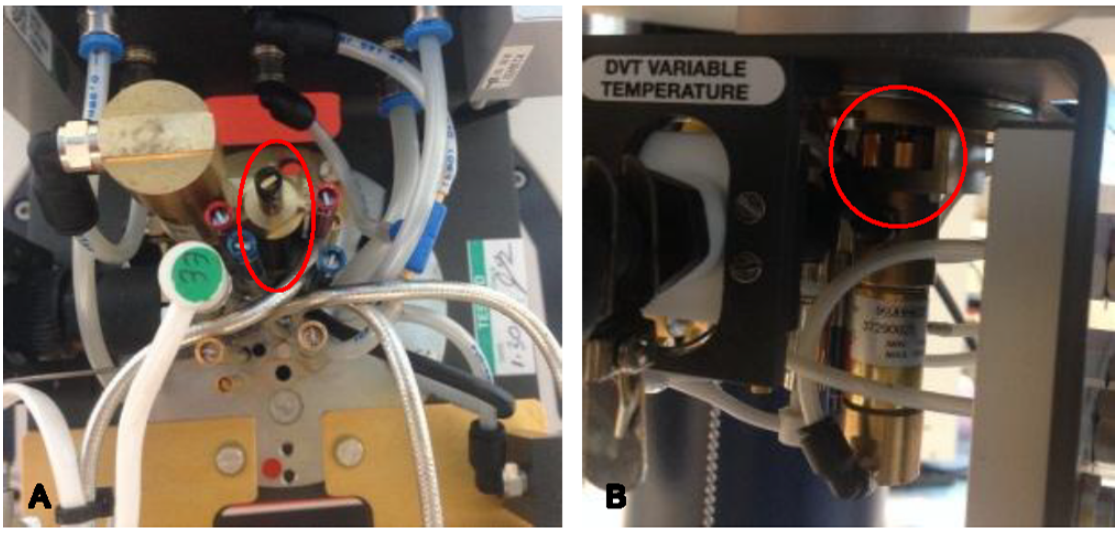

Figure 3.19: SB Probe Produced After 2010.

SB probe produced after 2010. (A) Bottom view showing the black screw for MA adjustment (encircled in red). This screw 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. This manual is built the same way the *TopSolids*^{bio} flow bar is constructed. Thus, in case of any question, read the relevant chapter. If there is further information available, there will be a link directing you to a detailed description of a topic.

4.1 General Information

To use *TopSolids*^{bio} you need to have installed at least *TopSpin 3.2 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*^{bio} button, saying '*Command not implemented*'.

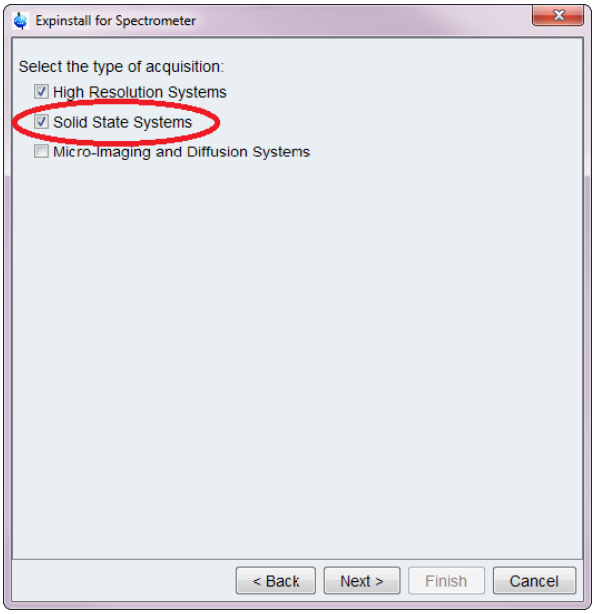

Figure 4.1: Activating Solid State Systems when Executing 'expinstall'.

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

TopSolids^{bio} is always checking if an existing EXPNO already contains acquired data. Therefore, if you e.g. repeat an optimization step, you will be asked, if you really want to continue and overwrite the existing data:

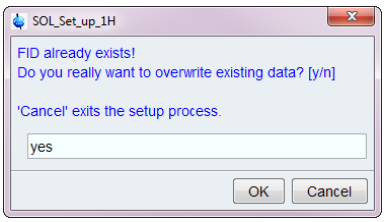

Figure 4.2: Warning Message used to prevent Overwriting of Acquired Data.

4.1.1 Where to Start TopSolids?

TopSolids^{bio} is integrated in the acquisition options of *TopSpin*. To open the flow bar:

- Go to the tab **Acquire**.
- Choose the tab **Options**.

  A list will pop up showing you i. a. ***TopSolids***^{bio}.

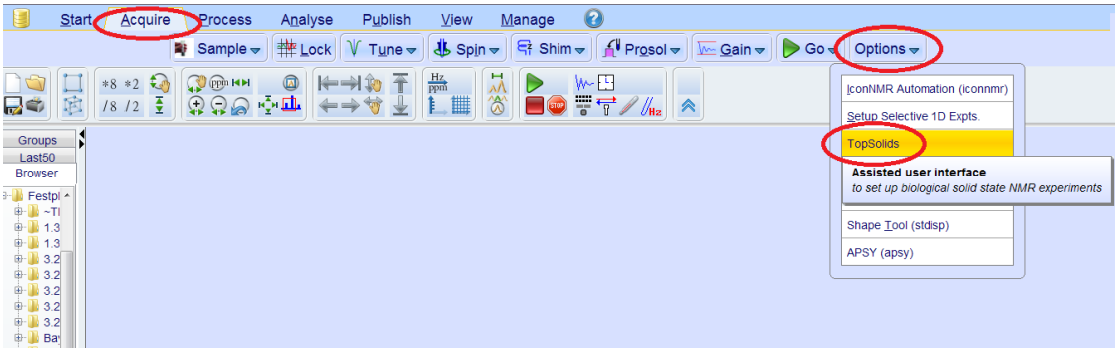

Figure 4.3: The TopSolids^{bio} *Menu Location.*

- Click on **TopSolids**^{bio} to open the assisted user interface.

By pushing the **Back** button, you can return to the **Acquire** menu at any time without really closing or even crashing *TopSolids*^{bio}. In the same way, you can go back to *TopSolids*^{bio} at any point.

Figure 4.4: The TopSolids^{bio} *Flow Bar and the* ***Back*** *Button.*

When you return to *TopSolids*^{bio} to continue an *already existing* optimization, make sure to be in the correct directory first! You do not have to take care about that, when you are just at the beginning of an optimization since *TopSolids*^{bio} includes the creation of a new directory (see chapter *Creating a Directory and Data Path [▷ 33]*).

4.1.2 Submenu Description

The *TopSolids*^{bio} interface comprises five tabs:

- I. *TopSolids bio – Create Directory [▷ 33]*
- II. *Standard Setup [▷ 38]*
- III. *Protein Setup [▷ 55]*
- IV. *Backbone Assignment [▷ 63]*
- V. *Distance Measurements [▷ 86]*

TopSolids^{bio} is designed as a flow bar. Thus, it is highly recommended to use it step by step. This is especially true for tabs I to III and their submenus, which comprise probe adjustment and optimization of parameters.

The optimization steps are built upon each other, making some of their execution mandatory. Nevertheless, a dialog window will inform you about any missing optimization and will ask you to execute it, before returning to the actual optimization step.

Tabs IV and V include the target multidimensional experiments, to first of all get backbone information about the protein of interest, followed by experiments that provide long range distance restraints. This part of the *TopSolids*^{bio} flow bar is under permanent progress. Soon, further experiments will be offered by *Bruker*.

4.2 Creating a Directory and Data Path

The first tab of the *TopSolids*^{bio} interface allows you to create a directory and a data path for all experiments that will be executed. We recommend using this feature to ensure a proper work flow.

In case you want to again measure a sample on the same setup as before (same spectrometer & probe), you can additionally copy an already existing setup into the new directory and directly continue with multidimensional (nD) experiments (or a short re-optimization on the sample of interest).

- Click on **TopSolids**^{bio} – **Create data path**.

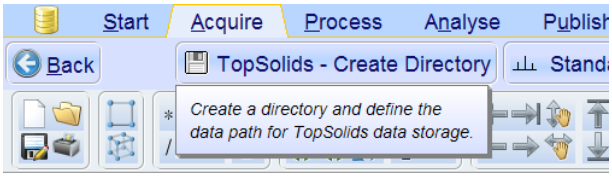

Figure 4.5: Creating a Directory and Data Path.

- A dialog window will pop up. Click **OK**.

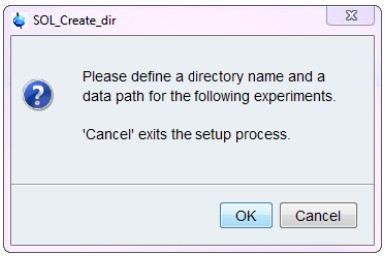

Figure 4.6: Defining the Directory and Data Path.

- Choose a directory name, e.g.: “`MyFirstTry`”.

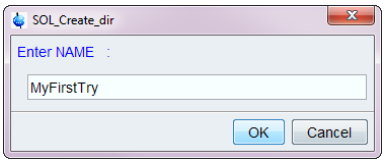

Figure 4.7: Selecting a Directory Name.

- As EXPNO and PROCNO choose “1”.

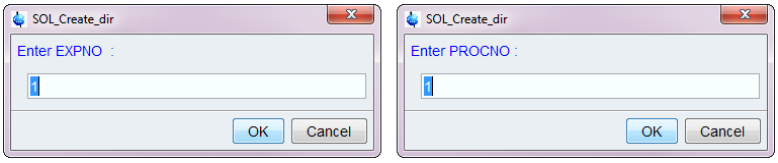

Figure 4.8: Selecting EXPNO and PROCNO.

- Define the data path, e.g.: “`C:/Bruker/data/Me`”.

  We recommend saving the data at your common local data folder.

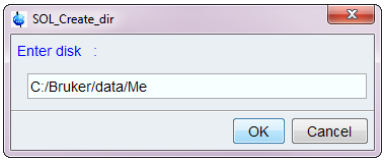

Figure 4.9: Selecting a Path for Data Storage.

- By choosing “**c**” you can continue the dialog. In case of any mistakes, repeat the steps 2 – 6 typing “**r**”.

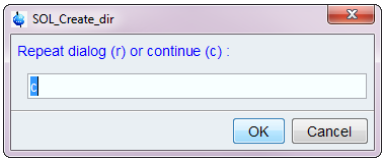

Figure 4.10: Confirming Whether to Repeat Dialog or Continue.

- Repeat the setup with entering “**r**” or continue using “**c**”.
- Your new directory has been created. In case you revisit an already measured sample using the same hardware (probe, preamplifier, filters, etc.), you can copy your previously recorded data to the new directory typing ‘y’ to directly continue with nD experiments. Else, enter ‘no’ and continue with step 10.

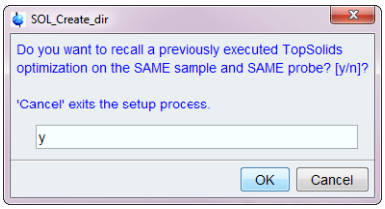

Figure 4.11: Confirming Whether to Recall a Previously Executed Setup.

- Enter the name of the directory that you want to copy and its data path. As in step 6, you will be asked to repeat (‘r’) or continue (‘c’) the process. E.g.: “`C:/Bruker/data/Me/PreviousExperiments`”

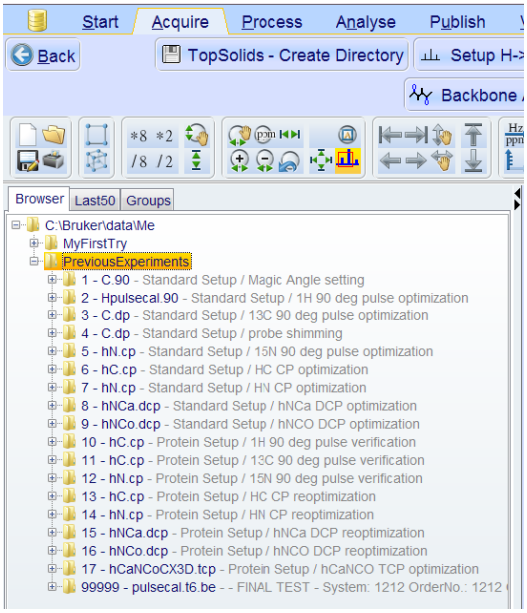

Figure 4.12: List of a Previously Executed TopSolids^{bio} Setup.

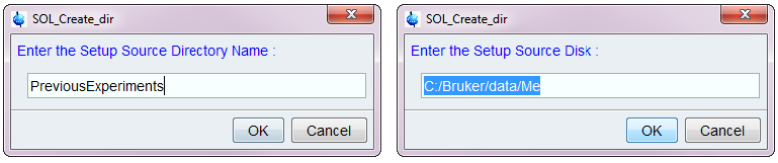

Figure 4.13: Entering the Name and Disk of the Source Directory.

- After the 17 Setup EXPNOs have been copied successfully, you can continue with the acquisition of nD experiments. Nevertheless, it is recommended to check, if an appropriate shim file is loaded and if the Magic Angle is adjusted well (see the chapters *Set Magic Angle Position [▶ 38]* and *Shim Probe & Calibration [▶ 45]*).

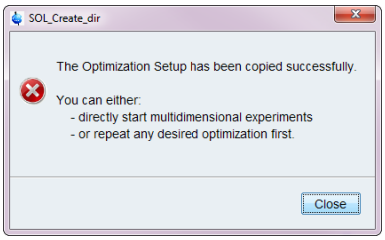

Figure 4.14: Confirmation of Successful Copying of Optimization Setup.

- Before continuing, there are some points worth mentioning to prevent any complication.
  - Do an '**edhead**' to tell the spectrometer which probe you are using. A more detailed explanation can be found in the chapter *Probes [▶ 27]*.
  - *TopSolids^{bio}* uses Prosol to get information about probe-specific parameters, such as 90° pulse powers for different nuclei as well as maximum decoupling values. In case you get an error corresponding to 'prosol', please read the chapter *PROSOL for TopSolids [▶ 12]*.

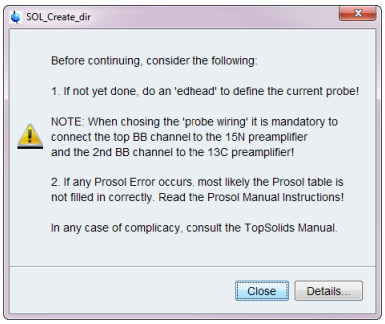

Figure 4.15: General Points to Consider Before Continuing.

- In the left column, the created directory will be displayed, either with only the first EXPNO as “C.90 – Magic Angle setting” or, in case you copied data, EXPNOs 1 to 17 will be shown. In this case, the title of each EXPNO comprises information about the source directory.

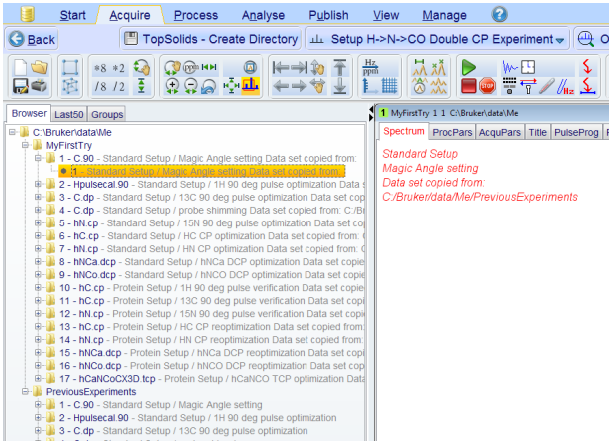

Figure 4.16: Browser Displaying the New Directory and its EXPNOs.

In case, data has been copied, information about the source directory is written in the title of each EXPNO.

- In case the data path you have chosen is not yet listed in your browser, do a right click within the browser window and choose “**Add New Data Dir**” to add a new data path (e.g. “`C:/Bruker/data/Me`”) to the list.

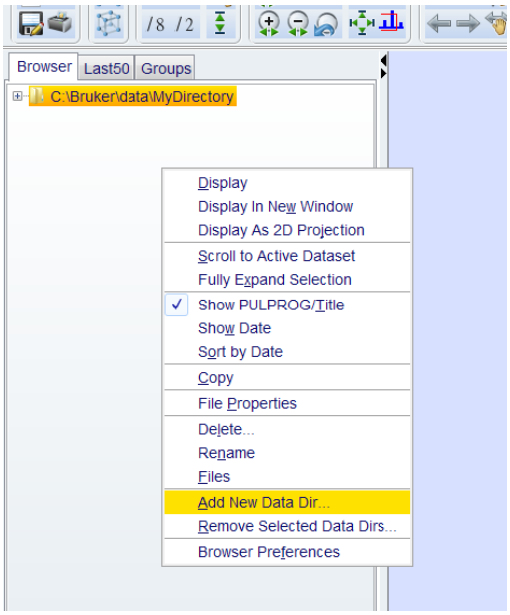

Figure 4.17: Selecting a New Data Directory.

- Browse for the Data Path Created in the previous step.

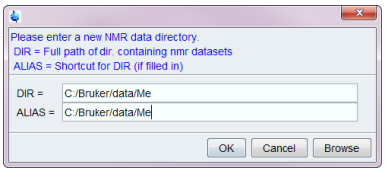

Figure 4.18: Browsing for the Data Path Created in the Previous Step.

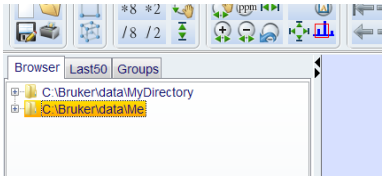

Figure 4.19: The Data Path Added to the Browser.

4.3 Setup of Experiments on Standard Samples

After the successful creation of a target directory, the probe requires to be adjusted and miscellaneous parameters need to be optimized to achieve best possible results. This can even be true, if you copied a previously recorded data set - depending on the elapsed time. Mostly it is sufficient to check the Magic Angle position and the shim file (see chapters *Set Magic Angle Position [▶ 38]* and *Shim Probe & Calibration [▶ 45]*).

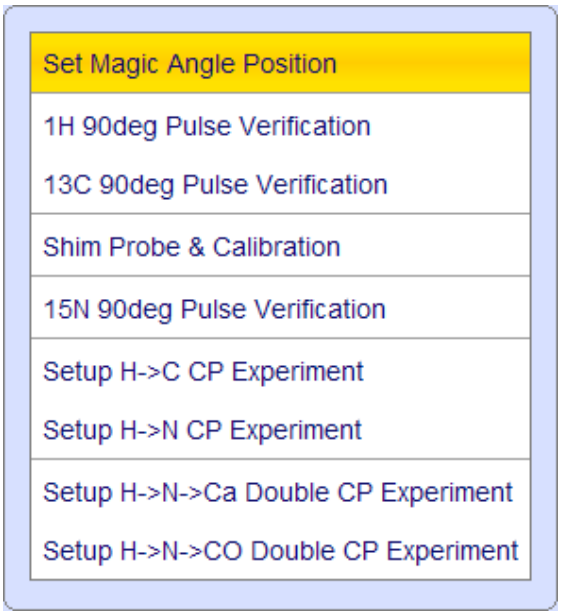

Figure 4.20: Standard Setup Dialog.

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 Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*) directly.

However, in most cases parameters should be optimized on standard samples first. This ensures best results in shortest time and avoids degradation of a sample by wrong power settings and overheating.

It is recommended to execute the Standard Setup consecutively.

TopSolids^{bio} 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 the chapter *Probes [▶ 27]*)!

4.3.1 Set Magic Angle Position

A new directory (e.g. “`MyFirstTry`”) was created and the first EXPNO is displayed as “`C.90 - Magic Angle setting`”. The calibration of the magic angle is of a great interest to a spectroscopist, since it ensures best cancelation of unwanted interactions.

- Go to the **Standard Setup** tab and click on **Set Magic Angle Position**.
- Insert a rotor filled with KBr (the common sample for adjusting the Magic Angle). Information about sample insertion can be found in the chapter *MAS [▶ 26]*.
- Spin the rotor at 5 kHz.

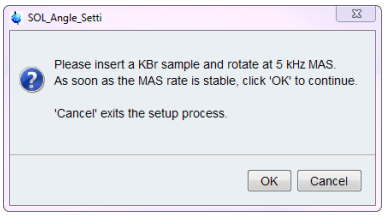

Figure 4.21: Magic Angle Adjustment Insertion Instructions.

- After the rotor is spinning stable, click **OK**.
- Usually, ^{79}Br is observed when adjusting the Magic Angle. If you are using a fixed H/C/N frequency probe, this option may not be possible though. Thus, in this case, *TopSolids*^{bio} will use the ^{13}C frequency, which is close to the one of ^{79}Br, and shift the spectrum correspondingly when you are entering '**y**', or else type '**n**'.

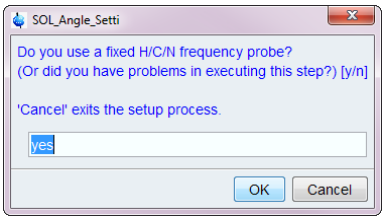

Figure 4.22: Frequency Selection for the Magic Angle Adjustment.

Frequency selection for the Magic Angle adjustment. If 'y' is entered, a shifted ^{13}C spectrum will be used. Else ^{79}Br is observed as typically.

If you had problems when previously executing this step, choose the 'yes' option as well.

- You will be asked for tuning and matching (= wobbling; see the chapter *Probes [▶ 27]*) the probe. By typing '**y**', *TopSpin* will start wobbling. By typing anything else, e.g. '**n**', it will not be started.

It is recommended to wobble the probe frequently, especially after a sample or temperature change. In case you miss information about wobbling, refer to the chapter *Probes [▶ 27]*.

- The wobbling curve of ^{79}Br or ^{13}C will appear (figure below). Tune and match the X channel.
- Once you are done, use the **Stop** button in the **Wobb** interface to quit. The setup will continue automatically.

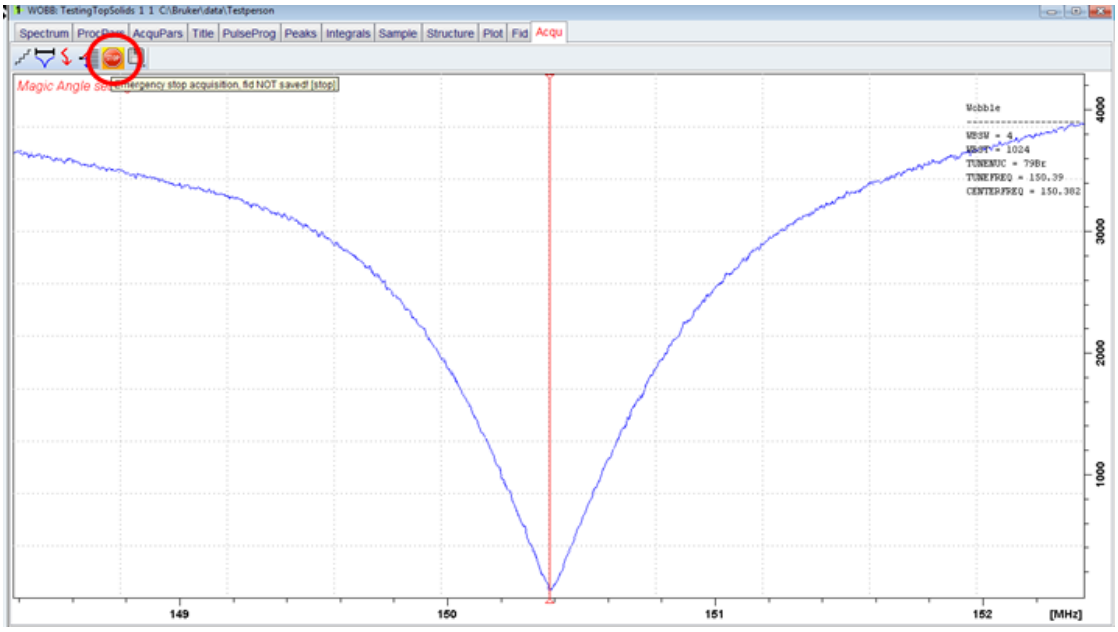

Figure 4.23: Wobb Menu Showing the Wobbling Curve for ^{79}Br.

*Wobb menu showing the wobbling curve for ^{79}Br. After successful tuning and matching, push the **STOP** button (encircled in red) to leave the menu. The setup will continue automatically.*

- A short spectrum will be recorded to reference the processing phase values, followed by another dialog window giving you instructions on how to adjust the Magic Angle. Besides, acquisition is turned on in the continuous '**gs**' mode to directly see changes when turning the MA screw. Maximize the visible spikes in the FID for best MA adjustment. If you need general instructions for the MA adjustment, please refer to the chapter *Magic Angle Adjustment [▶ 28]*. Continue by pushing the **STOP** button.

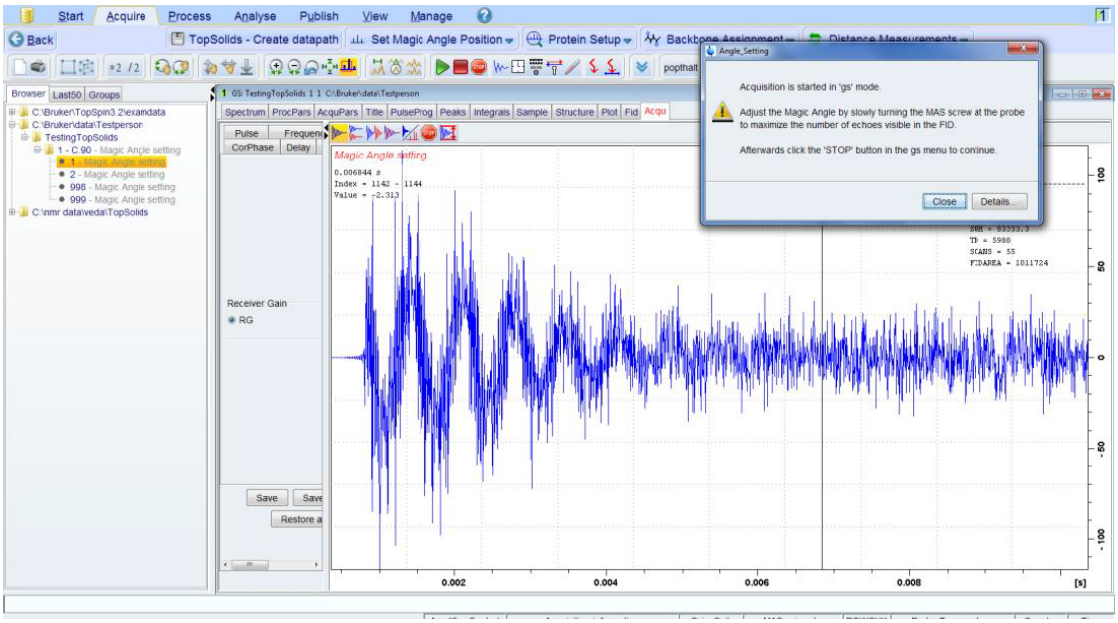

Figure 4.24: Acquisition Mode 'gs' Automatically Started for a Direct MA Adjustment.

*Acquisition mode 'gs' is started automatically for a direct MA adjustment. By pushing the **STOP** button in the acquisition window, the 'gs' mode will be stopped and a reference spectrum will be recorded.*

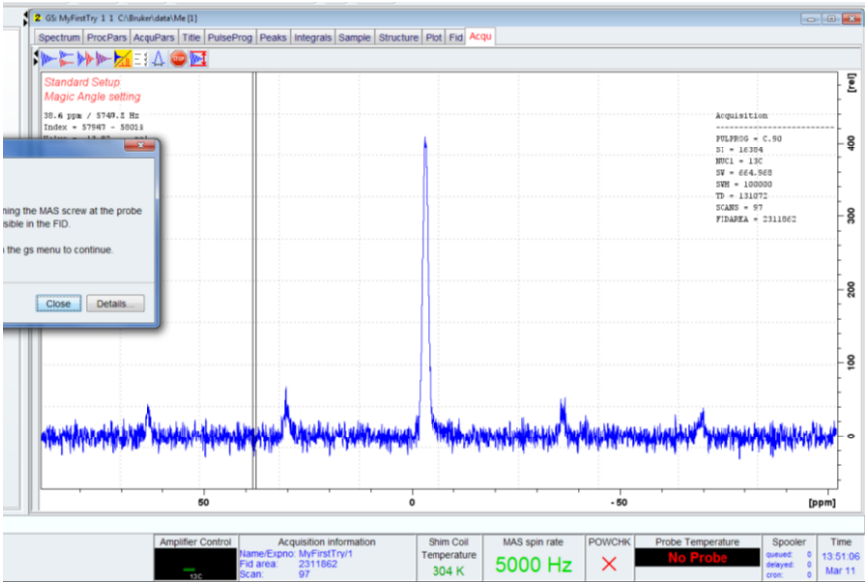

Figure 4.25: The Acquisition Displayed as a Spectrum.

*By clicking on the **Display Switch** button (marked in yellow) the acquisition is displayed as a spectrum. If you prefer this representation adjust the MA screw such that the spinning sideband peaks give maximum intensity.*

- A spectrum will be recorded. Compare the line-width of the center band with the line width of the 3rd or 5th spinning sideband (figure below) using the command '**peakw**'. If these differ less than 20 %, the magic angle setting is sufficient for ^{13}C CPMAS experiments.

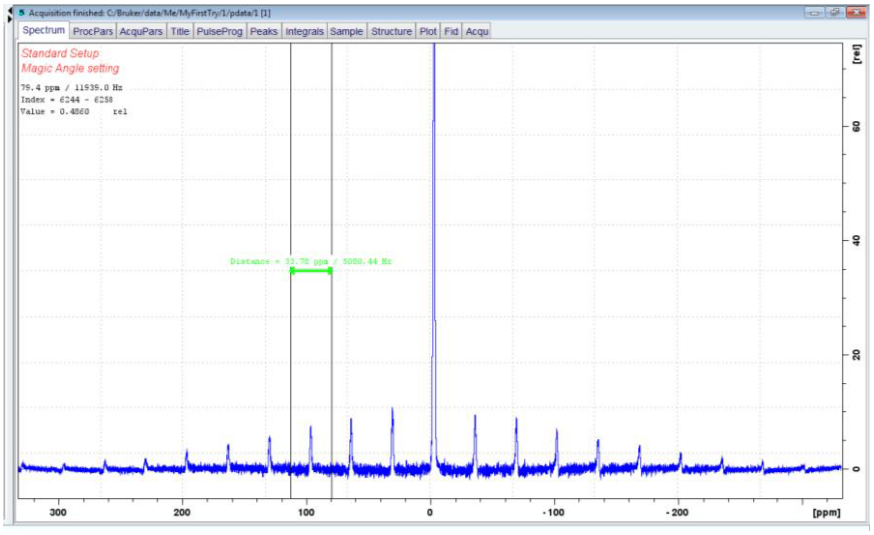

Figure 4.26: A Line Width Measurement of the 3rd Spinning Side Band.

Do a line width measurement of the central peak as well as the 3rd (or 5th) spinning side band (indicated by green line) by zooming into the respective region and typing 'peakw' into the command line. If the line widths differ less than 20 % the MA is sufficiently adjusted for ^{13}C CPMAS experiments.

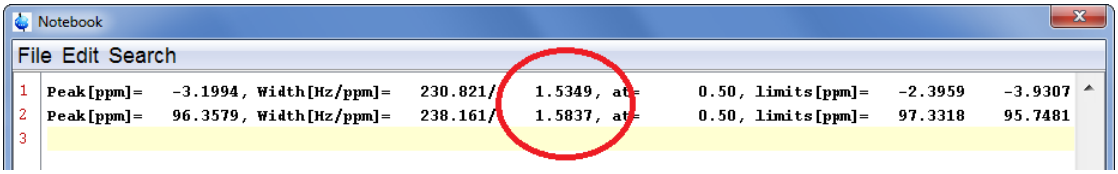

Figure 4.27: Dialog window that Opens when Executing 'peakw'.

Dialog window that opens when executing 'peakw'. The top line represents the line width measurement for the central peak, the second line for the third spinning side band. The encircled values represent these widths in ppm. Both differ less than 20 %, indicating a well-adjusted MA.

4.3.2 1H 90° Pulse Verification

- Go to the **Standard Setup** tab and click on **1H 90deg Pulse Verification**.
- Follow the dialog instructions and insert an adamantane standard sample at a MAS rate of 10 kHz. Click **OK**, once the spinning is stable.

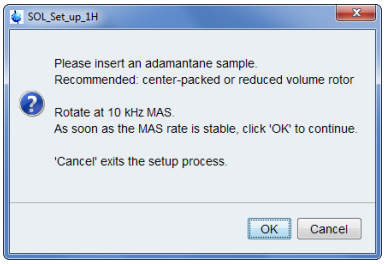

Figure 4.28: Setup for the 90° ^{1}H Hard Pulse.

- The appropriate EXPNO will be created.
- A program called `pulsecal` is started in the background, optimizing the 90° hard pulse on ^{1}H. This may take some seconds. Once it is done, a dialog window will pop up. Push **Close** to continue.

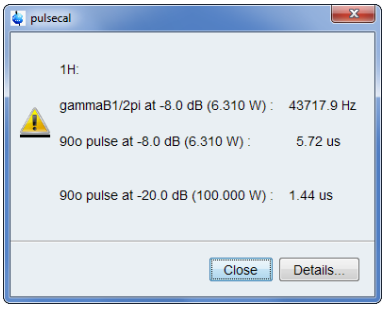

Figure 4.29: Pulsecal Information Window.

- A reference spectrum will be recorded and an information dialog will pop up to let you know the parameter values. Push **Close** to finish this setup.

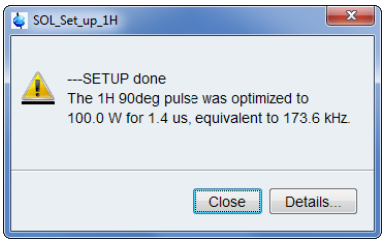

Figure 4.30: Setup Done Confirmation Dialog.

In case, any error messages concerning PROSOL occur, please refer to the chapter *PROSOL for TopSolids [▷ 12]*.

4.3.3 13C 90° Pulse Verification

- Go to the **Standard Setup** tab and click on **13C 90deg Pulse Verification**.
- If not already inserted and spinning, follow the dialog instructions and insert an adamantane standard sample at a MAS rate of 10 kHz. Click **OK**, once the spinning is stable.
- The appropriate EXPNO will be created.
- As for the ^{1}H 90° pulse optimization, `pulsecal` is started in the background, optimizing the 90° hard pulse on ^{13}C. This may take some seconds. Once it is done, a dialog window will pop up.

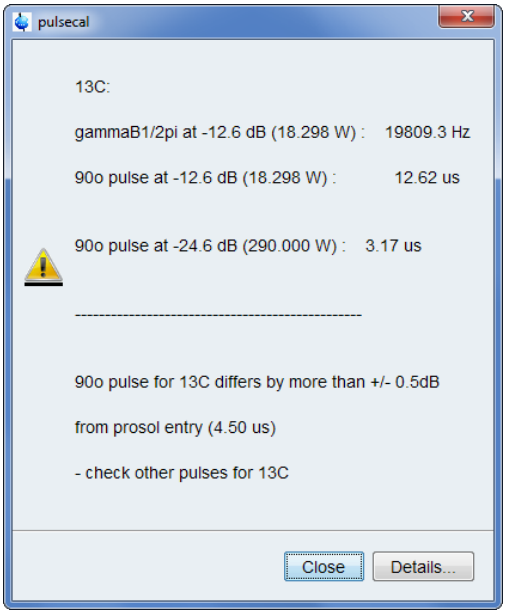

Figure 4.31: Pulsecal Information Window.

*Pulsecal information window. It is noted, that the found value differs from the given PROSOL value. This indicates inappropriate PROSOL values that most probably need to be refreshed. Nevertheless, the value can be used. Push **Close** to continue.*

- A reference spectrum will be recorded and an information dialog will pop up to let you know the parameter values. Push **Close** to finish this setup.

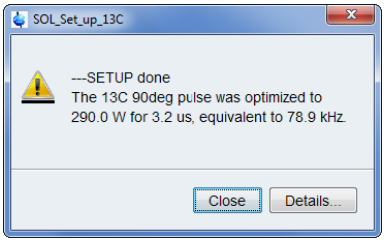

Figure 4.32: Setup Done Confirmation Dialog.

In case, any error messages concerning PROSOL occur, please refer to the chapter *PROSOL for TopSolids [▶ 12]*.

4.3.4 Shim Probe & Calibration

TopSolids^{bio} offers an automated shim procedure which is based on a Z shimming. Nevertheless, starting with reasonable values is always better than starting from scratch. Thus, if you have previously used the probe, load the latest shim file using '**rsh**'.

- Go to the **Standard Setup** tab and click on **Shim Probe & Calibration**.
- If not already inserted and spinning, follow the dialog instructions and insert an adamantane standard sample at a MAS rate of 10 kHz. Click **OK**, once the spinning is stable.

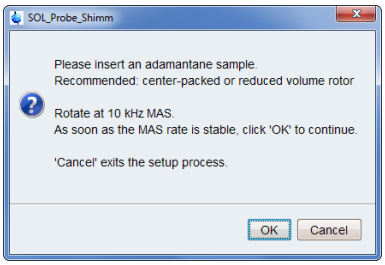

Figure 4.33: Standard Adamantane Sample Insertion Dialog.

- The appropriate EXPNO will be created. Wait until necessary parameters have been copied. This may take some seconds.
- As before, you will be asked for tuning and matching the probe. By typing '**y**', *TopSpin* will start wobbling (by typing anything else, e.g. '**n**', it will not be started.) Tune and match the ^{1}H channel. Once you are done, use the **Stop** button in the **Wobb** interface to quit. The setup will continue automatically.
- You can choose to use the automatic shim function:
  - If you already loaded a shim profile for your probe, you can select '**yes**'. *TopSolids*^{bio} will start to shim your probe by optimizing the line shapes. After the shim procedure has been finished, a dialog window will inform you.

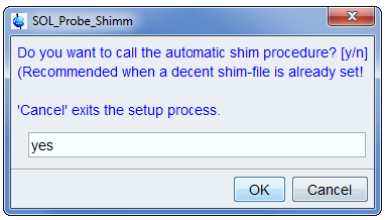

Figure 4.34: Confirmation Whether to Call the Automatic Shim Procedure.

  - If you are using a probe which was not shimmed before, we recommend to do a rough manual shimming first by typing '**no**' and repeating the whole setup again later (see *Performing a Rough Manual Shimming [▶ 46]*).

If not already been done, you can use this spectrum for **sr-** based calibration (see chapter *Spectral Calibration [▶ 22]*).

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 Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*) directly. Else continue with the Standard Setup.

4.3.4.1 Performing a Rough Manual Shimming

This section contains instructions when you have selected '**no**' when prompted if you want to call the automatic shim procedure in the section *Shim Probe & Calibration [▶ 45]*. When you wish to use the automatic shim function, but are using a probe which was not shimmed before, we recommend first doing a rough manual shimming as described below.

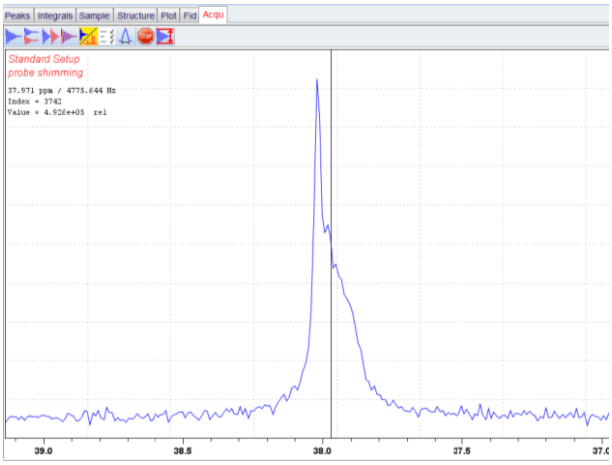

Figure 4.35: Spectral Display of an Acquisition in 'gs' mode on Adamantane.

Spectral display of an acquisition in 'gs' mode on adamantane. Shown is the left peak of the unshimmed spectrum. The line width is about 20 Hz.

- 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 chosen).

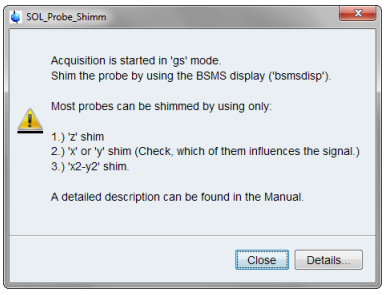

Figure 4.36: Instructions for Manual Shimming.

- Open the **BSMS display** by typing '**bsmsdisp**' and go to the tab **Shim**.

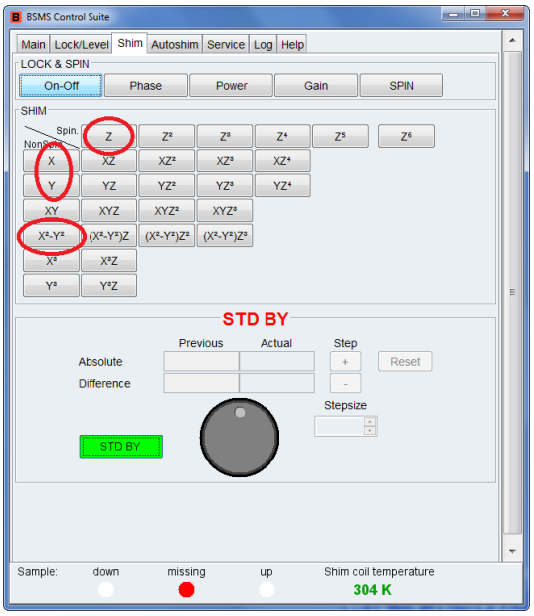

Figure 4.37: BSMS Display with the Shim Tab Activated.

BSMS display with the Shim tab activated. To turn off the Standby Mode push the green button. Important shims can be modified afterwards (encircled in red).

- 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 above).

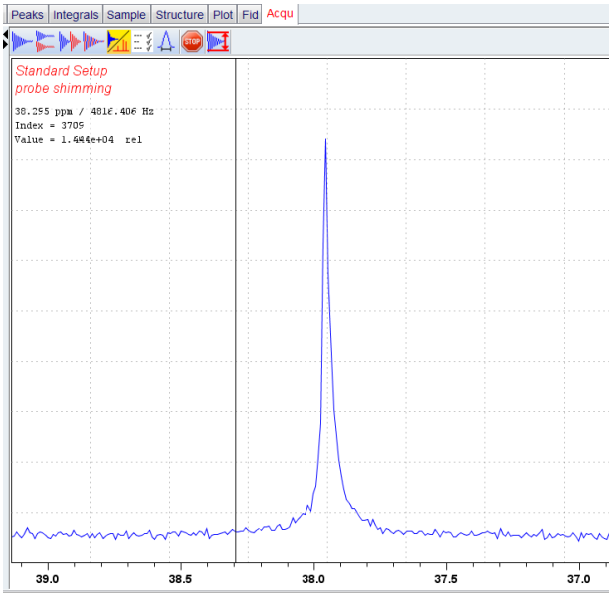

Figure 4.38: Peak already Shimmed on Z.

- Next select the **X** or **Y** shim. You need to change the **Stepsize** 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.

If wanted, you can calibrate the spectrum by changing the field value in the tab Lock/Level of the BSMS display, before stopping the acquisition. You can also calibrate the spectra later. For further reading refer to the chapter *Spectral Calibration [▷ 22]*.

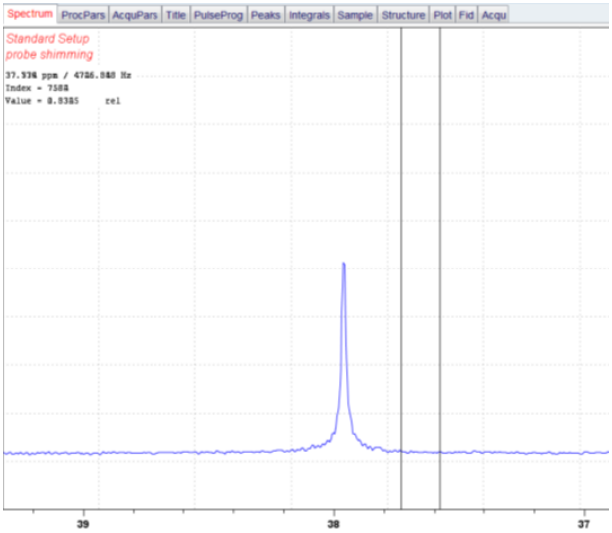

Figure 4.39: Left (low field) Adamantane Peak after Shimming.

- Once you are finished, you can save this shim for the next time by typing '**wshim**'. Name the shim file appropriately.

If not already been done, you can use this spectrum for **sr-** based calibration (see chapter *Spectral Calibration [▷ 22]*).

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 Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▷ 55]*) directly. Else continue with the Standard Setup.

4.3.5 15N 90° Pulse Verification

- Go to the **Standard Setup** tab and click on **15N 90deg Pulse Verification**.
- Follow the dialog instructions and insert a standard sample at 10 kHz of MAS rate. Click **OK**, once the spinning is stable.

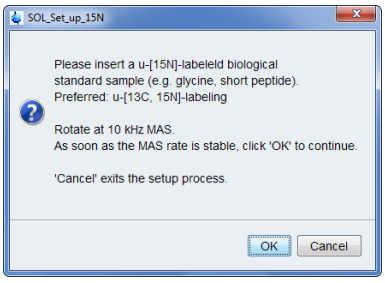

Figure 4.40: Standard Sample Insertion Dialog.

- In the next step you have to specify your standard sample to allow *TopSolids*^{bio} to set appropriate parameters. In case you are using a single amino acid, enter '**a**'. If you are using a short peptide or a standard protein (e.g. *SH3* or *GB1*) type '**p**'.

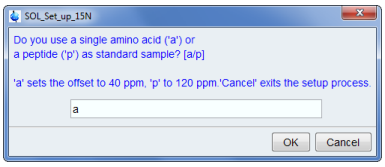

Figure 4.41: Specify the Standard Sample.

- Based on the PROSOL table as well as the EXPNOs 2 and 3, a window with parameter suggestions will pop up. You can use these or customized values. Also check for the correct MAS rate. Once you agree with the values, click **Accept**.

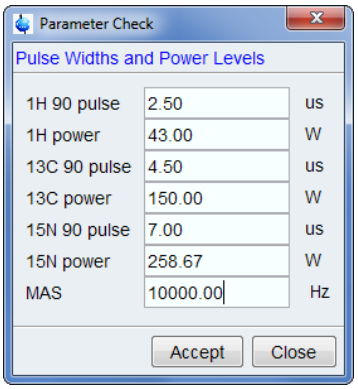

Figure 4.42: Parameter Check of Pulse Widths and Power Levels.

- The 90° pulse for ^{15}N will be optimized using an HN CP. Based on the parameter table with all 90° pulses *TopSolids*^{bio} will suggest a suitable Hartmann-Hahn condition. Nevertheless, you can use customized values as well.

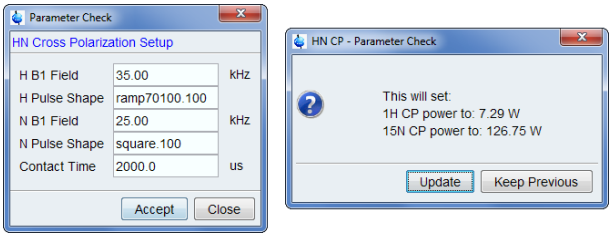

Figure 4.43: Parameter Check for the HN CP Condition and its Resulting Values.

- If you agree, push **Update**, else **Keep Previous**.
- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.

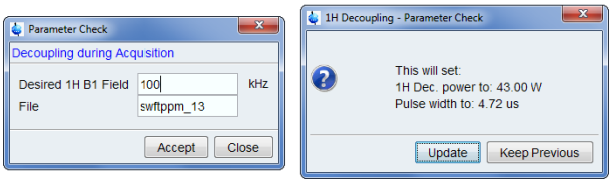

Figure 4.44: Setting of ^{1}H High Power Decoupling During Acquisition.

- If you agree, push **Update**, else **Keep Previous**.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- After wobbling, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (**poptau**) are recorded as can be seen in PROCNO 999. An information dialog will pop up to let you know the parameter values. Push **Close** to finish this setup.

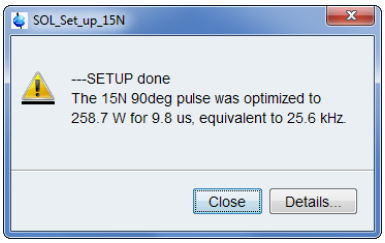

Figure 4.45: Setup Done Information Window.

- It is recommended to have a look at PROCNO 999 to control, if the automatically chosen value is appropriate.

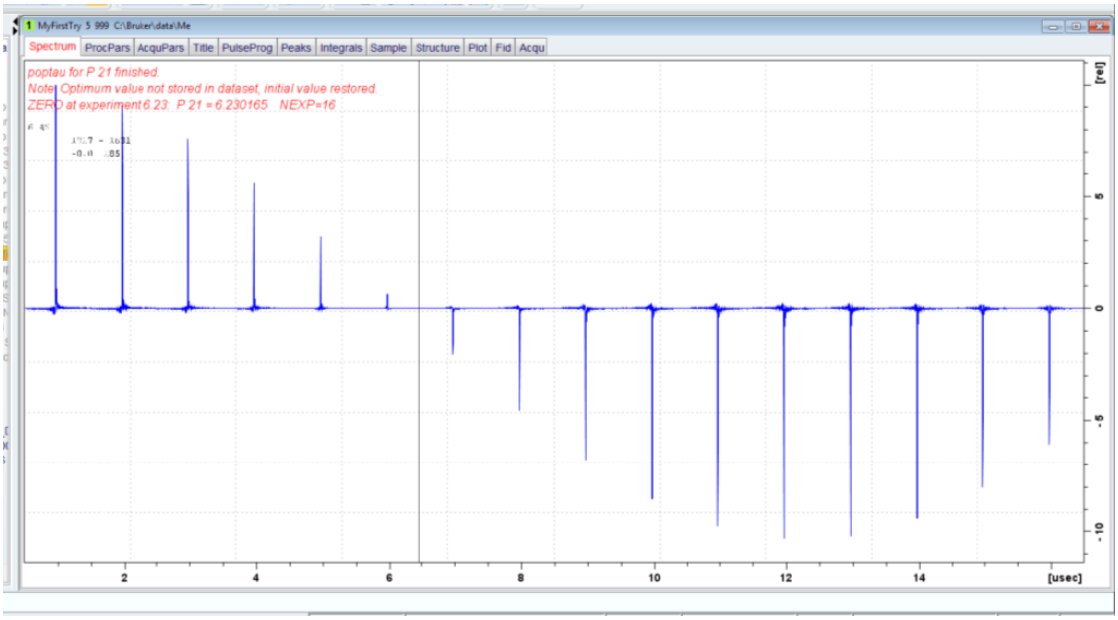

Figure 4.46: Spectrum of PROCNO 999 of EXPNO 5.

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.

4.3.6 Setup H-C CP Experiment

The general order of actions is the same as in the previous chapter.

- Go to the **Standard Setup** tab and click on **Setup H->C CP Experiment**.
- Follow the dialog instructions and insert a standard sample. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.

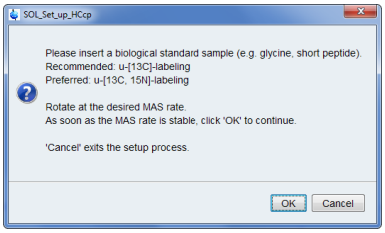

Figure 4.47: Standard Sample Insertion Dialog.

- You will have to specify your standard sample to allow *TopSolids^{bio}* to set appropriate parameters. In case you are using a single amino acid, enter '**a**'. If you are using a short peptide or a standard protein (e.g. *SH3* or *GB1*) type '**p**'.

- Based on the PROSOL table as well as the EXPNOs 2 and 3, a window with parameter suggestions will pop up. You can use these or customized values. Also check for the correct MAS rate. Once you agree with the values, click **Accept**.
- Based on the parameter table with all 90° pulses, *TopSolids*^{bio} will suggest a suitable Hartmann-Hahn condition. Nevertheless, you can use customized values as well.

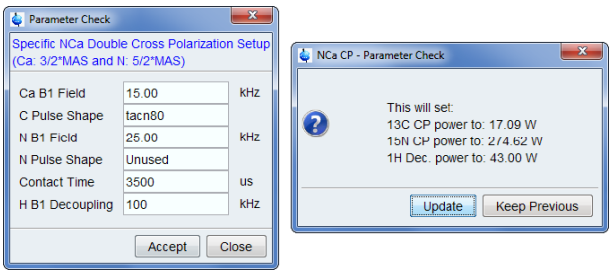

Figure 4.48: Parameter Check for the HC CP Condition and its Resulting Values.

- If you agree, push **Update**, else **Keep Previous**.
- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- Depending on the sample you are using, you will need more or less number of scans to get sufficient S/N for an optimization. Thus, choose if you want to use less scans by typing '**y**'.

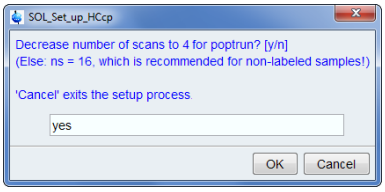

Figure 4.49: Setting Number of Scans for the Optimization Process.

- After wobbling, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (**poptau**) are recorded as can be seen in PROCNO 999. An information dialog will pop up to let you know the parameter values.

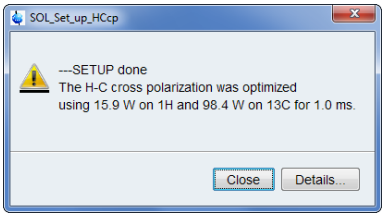

Figure 4.50: Setup Done Confirmation Dialog.

- Push **Close** to finish this setup.
- It is recommended to have a look at PROCNO 999 to control, if the automatically chosen value is appropriate.

4.3.7 Setup H-N CP Experiment

The general order of actions is the same as in the previous chapters *15N 90° Pulse Verification [▶ 48]* and *Setup H-C CP Experiment [▶ 50]*.

- Go to the **Standard Setup** tab and click on **Setup H->N CP Experiment**.
- If not already inserted and spinning, follow the dialog instructions and insert a standard sample. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.
- You will have to specify your standard sample to allow *TopSolids*^{bio} to set appropriate parameters. In case you are using a single amino acid, enter '**a**'. If you are using a short peptide or a standard protein (e.g. *SH3* or *GB1*) type '**p**'.
- Based on the previously optimized parameters, a window with parameter suggestions will pop up. You can use these or customized values. Also check for the correct MAS rate. Once you agree with the values, click **Accept**.
- Based on the parameter table with all 90° pulses, *TopSolids*^{bio} will suggest a suitable Hartmann-Hahn condition. Nevertheless, you can use customized values as well.
- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- Depending on the sample you are using, you will need more or less number of scans to get sufficient S/N for an optimization. Thus, choose if you want to use less scans by typing '**y**'.
- After wobbling, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (**poptau**) are recorded as can be seen in PROCNO 999. An information dialog will pop up to let you know the parameter values.

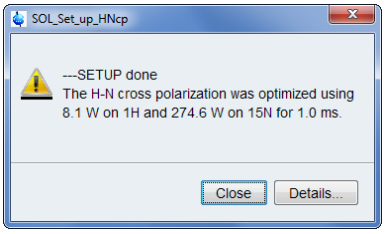

Figure 4.51: Setup Done Confirmation Dialog.

- Push **Close** to finish this setup.
- It is recommended to have a look at PROCNO 999 to control, if the automatically chosen value is appropriate.

4.3.8 Setup H-N-Ca Double CP Experiment

The general order of actions is the same as in the previous chapters *15N 90° Pulse Verification [▶ 48]* and *Setup H-C CP Experiment [▶ 50]*.

- Go to the **Standard Setup** tab and click on **Setup H->N->Ca Double CP Experiment**.
- If not already inserted and spinning, follow the dialog instructions and insert a standard sample. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.
- You will have to specify your standard sample to allow *TopSolids*^{bio} to set appropriate parameters. In case you are using a single amino acid, enter '**a**'. If you are using a short peptide or a standard protein (e.g. *SH3* or *GB1*) type '**p**'.

- Based on the previously optimized parameters, a window with parameter suggestions will pop up. You can use these or customized values. Also check for the correct MAS rate. Once you agree with the values, click **Accept**.
- Based on the parameter table with all 90° pulses, *TopSolids*^{bio} will suggest a suitable Hartmann-Hahn condition for the double CP. Nevertheless, you can use customized values as well.

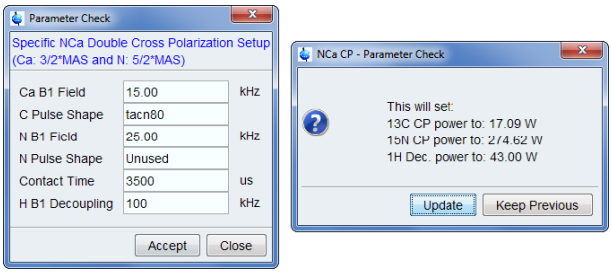

Figure 4.52: Parameter Check for the NCa DCP Condition and its Resulting Values.

> *Parameter check for the NCa DCP condition and its resulting values. Note: ^{1}H decoupling during the CP step can be chosen as well. If you agree, push Update, else Keep Previous.*

- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- Depending on the sample you are using, you will need more or less number of scans to get sufficient S/N for an optimization. Thus, choose if you want to use less scans by typing '**y**'.
- After wobbling, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (**poptau**) are recorded as can be seen in PROCNO 999. An information dialog will pop up to let you know the parameter values.

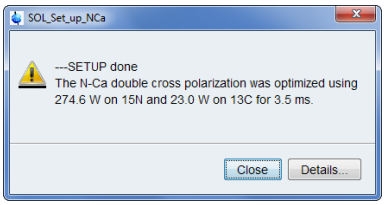

Figure 4.53: Setup Done Confirmation Dialog.

- Push **Close** to finish this setup.
- It is recommended to have a look at PROCNO 999 to control, if the automatically chosen value is appropriate.

4.3.9 Setup H-N-CO Double CP Experiment

The general order of actions is the same as in the previous chapters *15N 90° Pulse Verification [▶ 48]* and *Setup H-C CP Experiment [▶ 50]*.

- Go to the **Standard Setup** tab and click on **Setup H->N->CO Double CP Experiment**.
- Follow the dialog instructions and insert a standard sample. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.

Recording an NCO experiment requires at least one N to CO binding! Thus, a single amino acid, e.g. glycine, cannot be used!

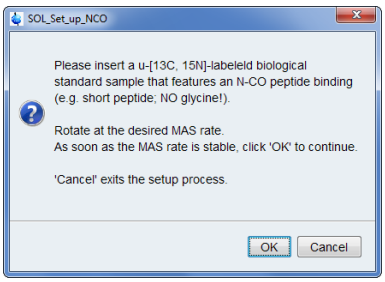

Figure 4.54: Standard Sample Insertion Dialog.

Insert a standard sample to optimize NCO DCP parameters. Note: Your sample has to feature at least one N-CO binding!

- Based on the previously optimized parameters, a window with parameter suggestions will pop up. You can use these or customized values. Also check for the correct MAS rate. Once you agree with the values, click **Accept**.
- Based on the parameter table with all 90° pulses, *TopSolids*^{bio} will suggest a suitable Hartmann-Hahn condition for the double CP. Nevertheless, you can use customized values as well.

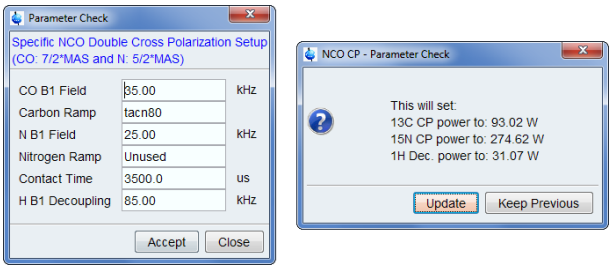

Figure 4.55: Parameter Check for the NCO DCP Condition and its Resulting Values.

Parameter check for the NCO DCP condition and its resulting values. Note: ^{1}H decoupling during the CP step can be chosen as well. If you agree, push Update, else Keep Previous.

- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.

- Depending on the sample you are using, you will need more or less number of scans to get sufficient S/N for an optimization. Thus, choose if you want to use less scans by typing '**y**'.
- After wobbling, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (**poptau**) are recorded as can be seen in PROCNO 999. An information dialog will pop up to let you know the parameter values.

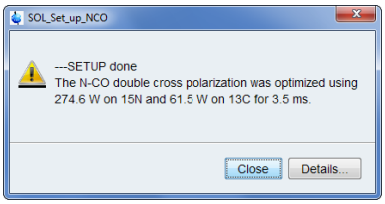

Figure 4.56: Setup Done Confirmation Dialog.

- Push **Close** to finish this setup.
- It is recommended to have a look at PROCNO 999 to control, if the automatically chosen value is appropriate.

4.4 Setup of Experiments on the Biological Sample of Interest

Since magic-angle spinning (MAS) 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 the chapter *Temperature Control - edte [▸ 25]*.

TopSolids^{bio} 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 the chapters *PROSOL for TopSolids [▸ 12]* and *Probes [▸ 27]*)!

Or else, the parameters that have been optimized in the Standard Setup (see chapter *Setup of Experiments on Standard Samples [▸ 38]*) are verified for your sample of interest.

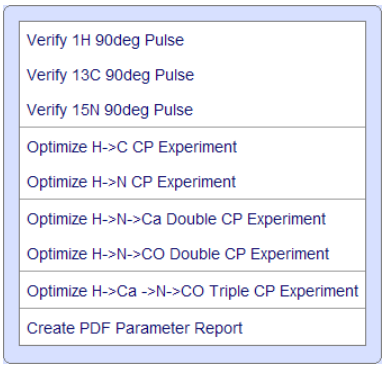

Figure 4.57: Protein Setup.

4.4.1 Verify 1H 90° Pulse

The 90° pulse on ^{1}H will be optimized in a HC CP experiment.

- Go to the **Protein Setup** tab and click on **Verify 1H 90deg Pulse**.
- Follow the dialog instructions and insert the biological sample of interest. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.

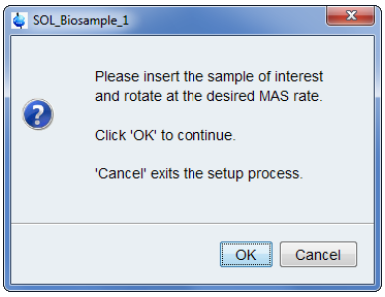

Figure 4.58: Inserting the Sample of Interest Dialog.

- Next, *TopSolids*^{bio} will collect data about necessary parameters. In case you performed the **Standard Setup** before (or you copied data before) *TopSolids*^{bio} will take data from there. Else, the parameters that are saved in the PROSOL table will be used. *TopSolids*^{bio} will inform you about the data source.

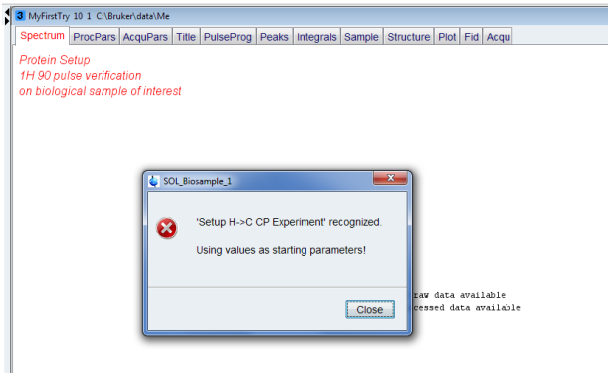

Figure 4.59: Setup Recognized.

> *There are data from the Standard Setup available that will be used instead of PROSOL parameters.*

- After having finished the parameter search, you will be asked for a parameter check.

Only parameters that are needed for the experiment will be shown. Here e.g., the ^{15}N parameters are not needed and will be displayed as zero.

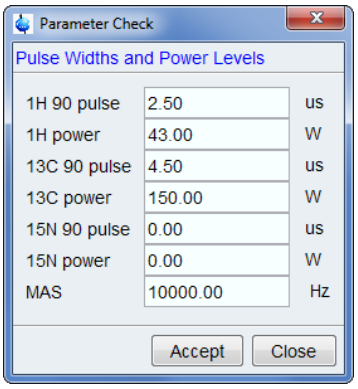

Figure 4.60: The Parameter Check.

> *The Parameter Check shows all necessary parameters that have been found in the Standard Setup or in the PROSOL table (though not necessarily recommended, all values can be customized and will be recalculated).*

- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.

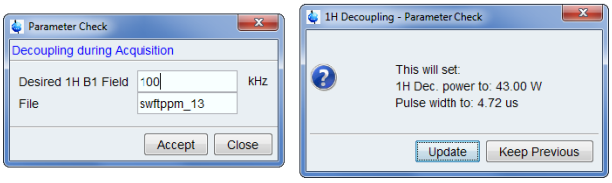

Figure 4.61: Customizing the ^{1}H High Power Decoupling During Acquisition and its RF Field Strength.

- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- You need to enter the number of scans you want the optimization to be recorded with. Depending on your sample, you will need more or less scans to get sufficient S/N. If you have no idea, 16 scans are usually good to start with. In later optimization steps you will have the possibility to in- or decrease the scans. Furthermore, if this optimization does not succeed, increase the number of scans and repeat this setup step.

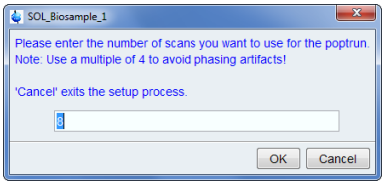

Figure 4.62: Entering the Number of Scans to Use for the poptrun.

- Afterwards, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (**poptau**) are recorded as can be seen in PROCNO 999. An information dialog will pop up to let you know the parameter values.

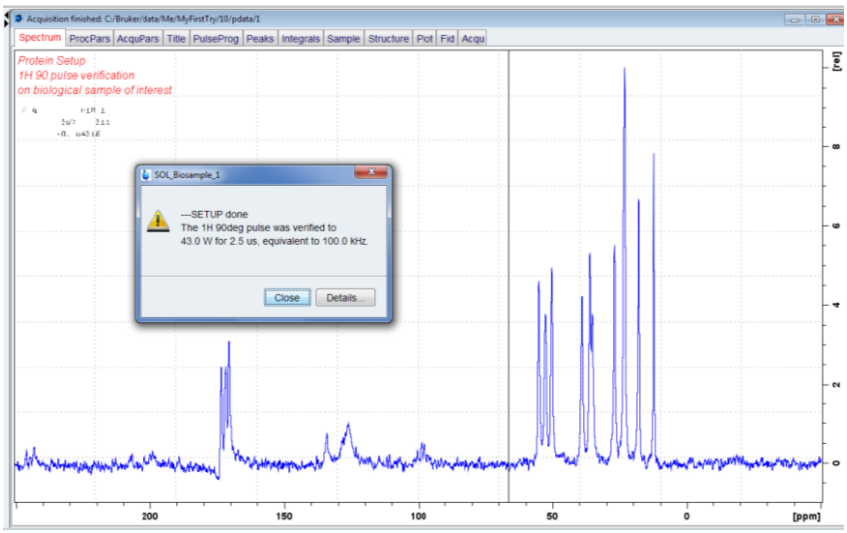

Figure 4.63: Dialog Window Showing Selected Parameter Values.

- It is recommended to have a look at PROCNO 999 to control, if the automatically chosen value is appropriate.

4.4.2 Verify 13C 90° Pulse

The general setup is the same as in the previous chapter *Verify 1H 90° Pulse [▶ 56]*.

- Go to the **Protein Setup** tab and click on **Verify 13C 90deg Pulse**.
- To run this setup the execution of the previous step **Verify 13C 90deg Pulse** is mandatory. If you did not execute it before, you will be asked to run it.

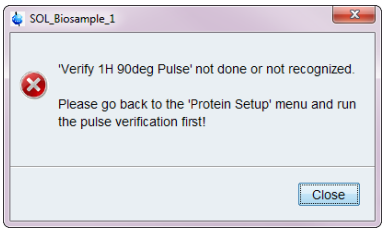

Figure 4.64: The Verification of the ^{1}H 90° pulse is mandatory to run this setup.

- If not already inserted and spinning, follow the dialog instructions and insert the biological sample of interest. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.
- Next, *TopSolids*^{bio} will collect data about necessary parameters. In case you performed the **Standard Setup** before (or you copied data before) *TopSolids*^{bio} will take data from there. Else, the parameters that are saved in the PROSOL table will be used. *TopSolids*^{bio} will inform you about the data source.
- After having finished the parameter search, you will be asked for a parameter check.
- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- You need to enter the number of scans you want the optimization to be recorded with. Depending on your sample, you will need more or less scans to get sufficient S/N. If this optimization does not succeed, increase the number of scans and repeat this setup step.

- Afterwards, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (`poptau`) are recorded as can be seen in PROCNO 999. An information dialog will pop up to let you know the parameter values.
- It is recommended to have a look at PROCNO 999 to control, if the automatically chosen value is appropriate.

4.4.3 Verify 15N 90° Pulse

The general setup is the same as in the chapter *Verify 1H 90° Pulse [▶ 56]*.

- Go to the **Protein Setup** tab and click on **Verify 15N 90deg Pulse**.
- To run this setup the execution of the step **Verify 13C 90deg Pulse** is mandatory. If you did not execute it before, you will be asked to run it.
- If not already inserted and spinning, follow the dialog instructions and insert the biological sample of interest. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.
- Next, *TopSolids*^{bio} will collect data about necessary parameters. In case you performed the **Standard Setup** before (or you copied data before) *TopSolids*^{bio} will take data from there. Else, the parameters that are saved in the PROSOL table will be used. *TopSolids*^{bio} will inform you about the data source.
- After having finished the parameter search, you will be asked for a parameter check.
- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- You need to enter the number of scans you want the optimization to be recorded with. Depending on your sample, you will need more or less scans to get sufficient S/N. If this optimization does not succeed, increase the number of scans and repeat this setup step.
- Afterwards, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (**poptau**) are recorded as can be seen in PROCNO 999. An information dialog will pop up to let you know the parameter values.
- It is recommended to have a look at PROCNOs 999 and 998 to control, if the automatically chosen value is appropriate.

4.4.4 Optimize H-C CP Experiment

The general setup is the same as in the chapters *Setup H-C CP Experiment [▶ 50]* and *Verify 1H 90° Pulse [▶ 56]*.

- Go to the **Protein Setup** tab and click on **Optimize H->C CP Experiment**.
- To run this setup the execution of the steps **Verify 1H 90deg Pulse** and **Verify 13C 90deg Pulse** are mandatory. If you did not execute them before, you will be asked to do so.
- If not already inserted and spinning, follow the dialog instructions and insert the biological sample of interest. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.
- Next, *TopSolids*^{bio} will collect data about necessary parameters. In case you performed the **Standard Setup** before (or you copied data before) *TopSolids*^{bio} will take data from there. Else, the parameters that are saved in the PROSOL table will be used. *TopSolids*^{bio} will inform you about the data source. Nevertheless, the 90° pulse parameters for ^{1}H and ^{13}C are taken from the optimized setups (see step 2).
- After having finished the parameter search, you will be asked for a parameter check.

- Based on the parameter table, *TopSolids*^{bio} will suggest a suitable Hartmann-Hahn condition for the CP step. Nevertheless, you can use customized values as well.
- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- You need to enter the number of scans you want the optimization to be recorded with. Depending on your sample, you will need more or less scans to get sufficient S/N. If this optimization does not succeed, increase the number of scans and repeat this setup step.
- Afterwards, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (`poptau`) are recorded as can be seen in PROCNOs 999 (CP power level on ^{1}H) and 998 (CP contact time). An information dialog will pop up to let you know the parameter values.
- It is recommended to have a look at PROCNOs 999 and 998 to control, if the automatically chosen value is appropriate.

4.4.5 Optimize H-N CP Experiment

The general setup is the same as in the chapters *Setup H-N CP Experiment [▶ 52]* and *Verify 1H 90° Pulse [▶ 56]*.

- Go to the **Protein Setup** tab and click on **Optimize H->N CP Experiment**.
- To run this setup the execution of the steps **Verify 1H 90deg Pulse** and **Verify 15N 90deg Pulse** are mandatory. If you did not execute them before, you will be asked to do so.
- If not already inserted and spinning, follow the dialog instructions and insert the biological sample of interest. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.
- Next, *TopSolids*^{bio} will collect data about necessary parameters. In case you performed the **Standard Setup** before (or you copied data before) *TopSolids*^{bio} will take data from there. Else, the parameters that are saved in the PROSOL table will be used. *TopSolids*^{bio} will inform you about the data source. Nevertheless, the 90° pulse parameters for ^{1}H and ^{15}N are taken from the optimized setups (see step 2).
- After having finished the parameter search, you will be asked for a parameter check.
- Based on the parameter table, *TopSolids*^{bio} will suggest a suitable Hartmann-Hahn condition for the CP step. Nevertheless, you can use customized values as well.
- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- You need to enter the number of scans you want the optimization to be recorded with. Depending on your sample, you will need more or less scans to get sufficient S/N. If this optimization does not succeed, increase the number of scans and repeat this setup step.
- Afterwards, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (`poptau`) are recorded as can be seen in PROCNOs 999 (CP power level on ^{1}H) and 998 (CP contact time). An information dialog will pop up to let you know the parameter values.
- It is recommended to have a look at PROCNOs 999 and 998 to control, if the automatically chosen value is appropriate.

4.4.6 Optimize H-N-Ca Double CP Experiment

The general setup is the same as in the chapters *Setup H-N-Ca Double CP Experiment [▶ 52]* and *Verify 1H 90° Pulse [▶ 56]*.

- Go to the **Protein Setup** tab and click on **Optimize H->N->Ca Double CP Experiment**.
- To run this setup the execution of the steps **Verify 13C 90deg Pulse** and **Optimize H->N CP Experiment** are mandatory. If you did not execute them before, you will be asked to do so.
- If not already inserted and spinning, follow the dialog instructions and insert the biological sample of interest. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.
- Next, *TopSolids*^{bio} will collect data about necessary parameters. In case you performed the **Standard Setup** before (or you copied data before) *TopSolids*^{bio} will take data from there. Else, the parameters that are saved in the PROSOL table will be used. *TopSolids*^{bio} will inform you about the data source. Nevertheless, the 90° pulse and HN CP parameters for ^{1}H and ^{15}N are taken from the optimized setups (see step 2).
- After having finished the parameter search, you will be asked for a parameter check.
- Based on the parameter table with all 90° pulses, *TopSolids*^{bio} will suggest a suitable Hartmann-Hahn condition for the double CP. You can use the suggested or a customized value.
- Furthermore, the field strength for ^{1}H decoupling during the double CP can be set. Typically, the field strength is the same as for the ^{1}H high power decoupling during acquisition. You can use the suggested or a customized value.
- Finally, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- You need to enter the number of scans you want the optimization to be recorded with. Depending on your sample, you will need more or less scans to get sufficient S/N. If this optimization does not succeed, increase the number of scans and repeat this setup step.
- Afterwards, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (`poptau`) are recorded as can be seen in PROCNOs 999 (DCP power level on ^{1}H) and 998 (DCP contact time). An information dialog will pop up to let you know the parameter values.
- It is recommended to have a look at PROCNOs 999 and 998 to control, if the automatically chosen value is appropriate.

4.4.7 Optimize H-N-CO Double CP Experiment

The general setup is the same as in the chapters *Setup H-N-CO Double CP Experiment [▶ 54]* and *Verify 1H 90° Pulse [▶ 56]*.

- Go to the **Protein Setup** tab and click on **Optimize H->N->CO Double CP Experiment**.
- To run this setup the execution of the steps **Verify 13C 90deg Pulse** and **Optimize H->N CP Experiment** are mandatory. If you did not execute them before, you will be asked to do so.
- If not already inserted and spinning, follow the dialog instructions and insert the biological sample of interest. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.

- Next, *TopSolids*^{bio} will collect data about necessary parameters. In case you performed the **Standard Setup** before (or you copied data before) *TopSolids*^{bio} will take data from there. Else, the parameters that are saved in the PROSOL table will be used. *TopSolids*^{bio} will inform you about the data source. Nevertheless, the 90° pulse and HN CP parameters for ^{1}H and ^{15}N are taken from the optimized setups (see step 2).
- After having finished the parameter search, you will be asked for a parameter check.
- Based on the parameter table with all 90° pulses, *TopSolids*^{bio} will suggest a suitable Hartmann-Hahn condition for the double CP. You can use the suggested or a customized value.
- Furthermore, the field strength for ^{1}H decoupling during the double CP can be set. Typically, the field strength is the same as for the ^{1}H high power decoupling during acquisition. You can use the suggested or a customized value.
- Finally, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- You need to enter the number of scans you want the optimization to be recorded with. Depending on your sample, you will need more or less scans to get sufficient S/N. If this optimization does not succeed, increase the number of scans and repeat this setup step.
- Afterwards, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (`poptau`) are recorded as can be seen in PROCNOs 999 (DCP power level on ^{1}H) and 998 (DCP contact time). An information dialog will pop up to let you know the parameter values.
- It is recommended to have a look at PROCNOs 999 and 998 to control, if the automatically chosen value is appropriate.

4.4.8 Optimize H-Ca-N-CO Triple CP Experiment

The general setup is the same as in the chapter *Verify 1H 90° Pulse [▶ 56]*.

- Go to the **Protein Setup** tab and click on **Optimize H->Ca->N->CO Triple CP Experiment**.
- To run this setup the execution of the steps **Optimize H->C CP Experiment**, **Optimize H->N->Ca Double CP Experiment** and **Optimize H->N->CO Double CP Experiment** are mandatory. If you did not execute them before, you will be asked to do so.
- If not already inserted and spinning, follow the dialog instructions and insert the biological sample of interest. Set the MAS to the rate you want to use in your multidimensional experiments. Click **OK**, once the spinning is stable.
- Next, *TopSolids*^{bio} will collect data about necessary parameters: the 90° pulses and all CP and DCP parameters for ^{1}H, ^{13}C and ^{15}N are taken from the optimized setups (see step 2).
- After having finished the parameter search, you will be asked for a parameter check.
- The field strength for ^{1}H decoupling during both double CP steps can be set consecutively. Typically, the field strengths are the same as for the ^{1}H high power decoupling during acquisition. You can use the suggested or customized values.
- Furthermore, the field strength for ^{1}H high power decoupling during acquisition needs to be set. Again, you can use the suggested value or a customized one.
- As in the previous experiments you will be asked for tuning and matching the probe, which is recommended after a rotor change.
- You need to enter the number of scans you want the optimization to be recorded with. Depending on your sample, you will need more or less scans to get sufficient S/N. If this optimization does not succeed, increase the number of scans and repeat this setup step.

- Afterwards, the optimization starts automatically. This may take several minutes, because parameter optimization scripts (`poptau`) are recorded as can be seen in PROCNOs 999 (NCa power level on ^{13}C) and 998 (NCO power level on ^{13}C). An information dialog will pop up to let you know the parameter values.
- It is recommended to have a look at PROCNOs 999 and 998 to control, if the automatically chosen value is appropriate.

4.4.9 Create PDF Parameter Report

Once the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▷ 55]*) succeeded, you can create a PDF that includes all relevant parameter information.

- Go to the **Protein Setup** tab and click on **Create PDF Parameter Report**.

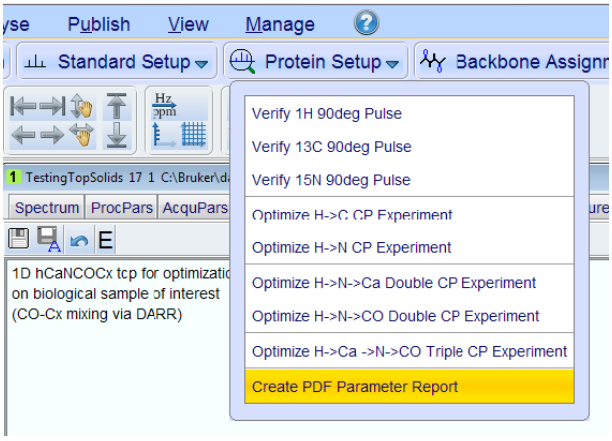

Figure 4.65: Protein Setup - Create Parameter Report.

- In case you did not execute every step of the Protein Setup, information boxes will pop up.

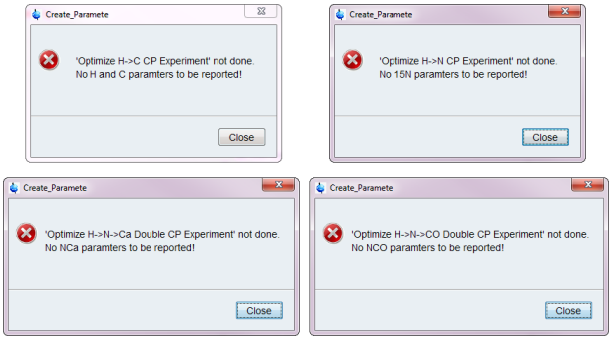

Figure 4.66: Information Messages about Missing Parameter Optimization Steps.

- Nevertheless, the PDF will be created, which can take some seconds.

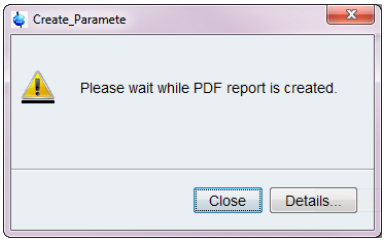

Figure 4.67: PDF Report Dialog.

- A dialog will tell you the data source of the PDF named “Param_report.pdf”.

  E.g.:

  – The PDF is saved in the directory that was created in the chapter *Creating a Directory and Data Path [▶ 33]*:

    “*C:/Bruker/data/Me/MyFirstTry/Param_report.pdf*”

    Data source of the PDF report named “Param_report.pdf” is given.

  – If you did not create a new directory, but revisited a former one, the PDF is saved in your chosen directory.

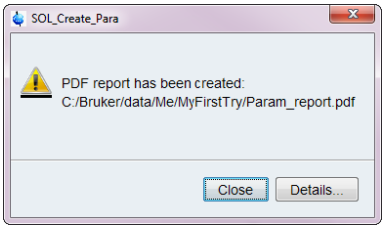

Figure 4.68: Confirmation that PDF Report has been Created.

The parameter report can be generated at any time. Note: A reapplication of the button ‘Create PDF Parameter Report’ is overwriting the existing PDF!

4.5 Backbone Assignment

You can choose between a diversity of experiments to gain information about the protein backbone of the sample of interest. There is no need for consecutive execution any longer. Rather, choose a dimensionality and select an experiment for the assignment process. Some general information about protein assignment is given in *Protein Assignment – A Brief Introduction [▶ 95]*.

Since magic-angle spinning (MAS) 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 the chapter *Temperature Control - edte [▶ 25]*.

A list of available 2D experiments is given in the figure below. The directions of a 2D experiment are indicated with the terms F2 (first direction - acquisition or direct direction) and F1 (second direction - indirect direction).

While DARR[1]/ PDSD[2] experiments provide homonuclear ^{13}C-^{13}C correlations, the other experiments include specific double CP (DCP) steps[3] from ^{1}H to ^{15}N to $^{13}Ca(^{13}Cx)$ or $^{13}CO(^{13}Cx)$, comprising sequential protein backbone correlations.

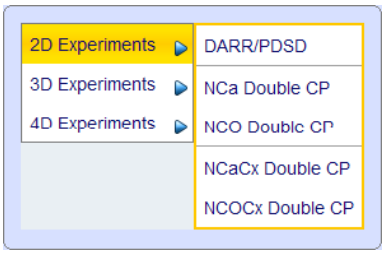

Figure 4.69: List of 2D Experiments used for Protein Backbone Assignment.

4.5.1 2D DARR/ PDSD

DARR and PDSD are recoupling techniques that use proton-driven spin diffusion to obtain homonuclear ^{13}C-^{13}C correlations in a time-dependent manner (see *References [▶ 113]* Ref. # 2 and 3).

- Go to the **Backbone Assignment** tab and choose **DARR/ PDSD** out of the list of **2D Experiments**.

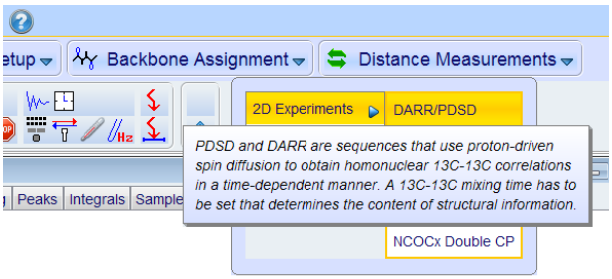

Figure 4.70: Selecting DARR/ PDSD as a 2D Experiment for the Protein Backbone Assignment.

- As in the Setup menus, you will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

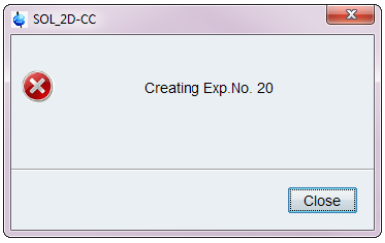

Figure 4.71: PDSD/ DARR Experiments Saved in EXPNOs 20 to 29.

Using *TopSolids*^{bio}, the EXPNOs 20 to 29 are kept for these PDSD/ DARR experiments only. If you want to record further PDSD/ DARR experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands ‘new’ or ‘edc’ (for further reading see TopSpin manual).

- A window will open listing the relevant 90° pulse lengths and power levels as well as the MAS rate (that have been optimized in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*)) that will be used for calculating parameters necessary for the experiment. Check these values carefully.

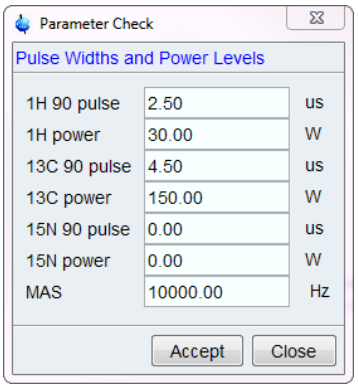

Figure 4.72: Parameter Check of Pulse Widths and Power Levels.

> *Parameter Check. Not only the 90° pulse lengths and power levels are important, but also the MAS rate. Based on these values experiment-specific parameters will be calculated. Note: Because for a PDSD or DARR experiment no ^{15}N is needed, it is listed as '0' here.*

- To avoid sample heating and arching you have to choose the ^{1}H decoupling program and field strength. If you are unsure what to use, the suggested values can be accepted as proper parameters.

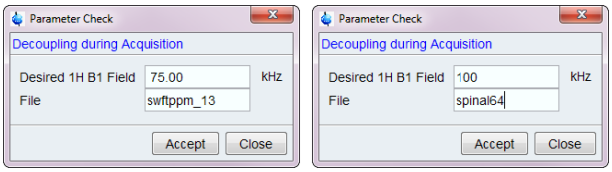

Figure 4.73: Suggested Decoupling Program and Field Strength (left). Customized (right).

- Depending on the entered field strength, the parameters will be recalculated (based on the previously optimized parameters in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▷ 55]*). If you agree with these values, click **Update**, else choose **Keep previous**.

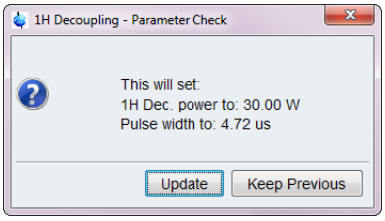

Figure 4.74: Decoupling Parameter Check.

- You can choose to acquire either a PDSD ('**y**') or a DARR ('**no**') as recoupling technique.

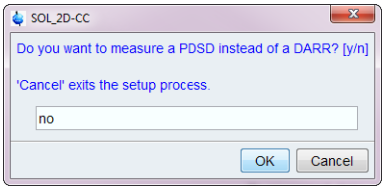

Figure 4.75: Choosing Either DARR (entering 'no') or PDSD ('y') as ^{13}C-^{13}C Recoupling Technique.

- Choose a ^{13}C-^{13}C mixing time. The length of the mixing time determines the content of structural information. Further information is given in the section *Mixing Times and Their Information Content [▶ 97]*.
  - 20 ms: If you choose “**y**”, 20 ms will be set, else see the next step. If you want to mix for 20 ms, choose ‘**yes**’, else enter ‘**n**’.
  - 150 ms: If you choose “**y**”, 150 ms will be set, else see the next step.
  - Enter a customized mixing time in ms, e.g. 80 ms.

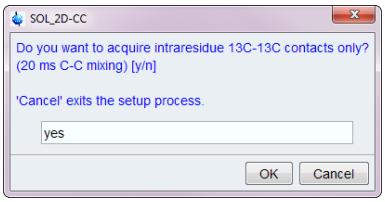

Figure 4.76: If you want to mix for 20 ms, choose ‘yes’, else enter ‘n’.

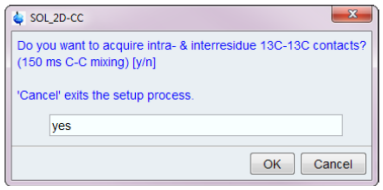

Figure 4.77: If you want to mix for 150 ms, choose ‘yes’, else enter ‘n’.

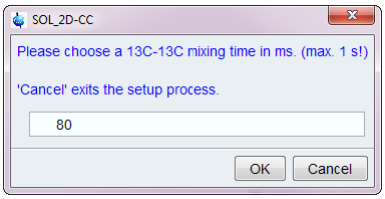

Figure 4.78: Entering a Customized Mixing Time in ms.

- To avoid unwanted side band backfolding artifacts, a rotor-synchronized spectral width can be set in the indirect carbon dimension. Depending on the needed spectral width it will be either a multiple or a divisor of the MAS frequency. Further information is provided in the chapters *Rotor Synchronized Spectral Widths [▶ 97]*, *Typical Carbon Spectral Widths [▶ 99]* and *Typical Nitrogen Spectral Widths [▶ 102]*.

Rotor-synchronization

- Enter the minimum spectral width that you need to cover all resonances in the spectrum (Check in the HC CP experiment recorded in the chapter *Setup H-C CP Experiment [▶ 50]*.). A rotor-synchronized spectral width will be calculated and set. Enter ‘**yes**’ if you want to use a rotor-synchronized spectral width, else enter ‘**n**’:

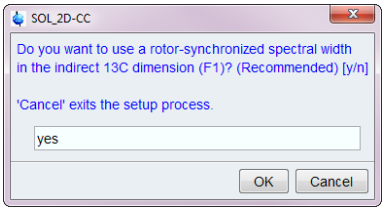

Figure 4.79: Entering ‘yes’ if you want to use a rotor-synchronized spectral width, else enter ‘n’.

Figure 4.80: Selecting the Desired Minimum Spectral Width.

Enter the minimum spectral width that you need to cover all resonances in a spectrum.
Note: The offset is set to 100 ppm, which defines the middle of your spectrum.

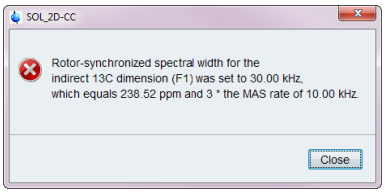

Figure 4.81: A rotor-synchronized spectral width is calculated and set.

No rotor-synchronization:

- A conventional spectral width of 200 ppm will be suggested, but a customized width in ppm can be entered in a further step as well.

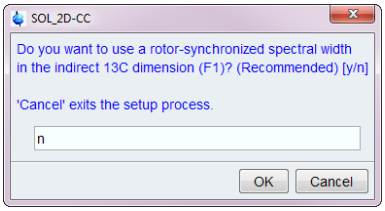

Figure 4.82: Enter 'n' if you do not want to use a rotor-synchronized spectral width.

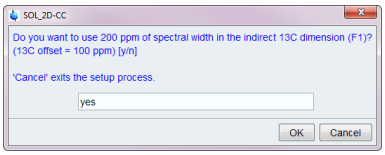

Figure 4.83: Spectral Width Dialog.

With the offset at 100 ppm, a spectral width of 200 ppm usually covers all ^{13}C resonances in a protein spectrum. Enter 'yes' and click OK for using 200 ppm. If you want to choose another width, enter 'n'.

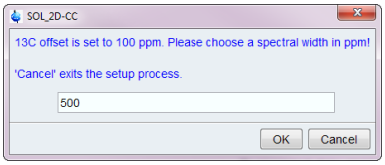

Figure 4.84: Entering a Spectral Width.

- Choose an acquisition time in ms in the indirect dimension.

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 *How to Choose an Appropriate Acquisition Time [▶ 105]*.

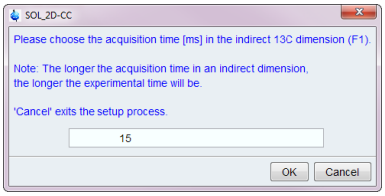

Figure 4.85: Selecting the Acquisition Time in ms for the Indirect Carbon Dimension.

- Enter the number of scans you want to acquire. Take care that the number of scans is a multiple of the phase cycle to avoid phase cycling artifacts.

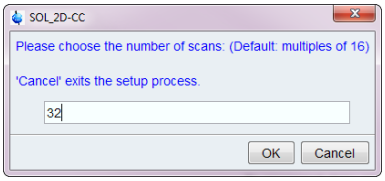

Figure 4.86: Selecting the Number of Scans as a Multiple of 16 to Avoid Phase Cycling Artifacts.

- The experimental time will be displayed.

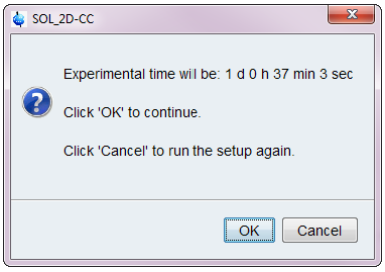

Figure 4.87: Experimental Time Displayed.

- If you agree with the duration of the experiment click **OK**, else click **Cancel** to repeat the last steps to make changes.
- You can tune and match the probe before the experiment will be started.
  - If you do not want to tune and match the probe, enter '**n**'. The experiment will be started consecutively.
  - If you want to wobble, enter '**y**'. The wobble interface will open. After tuning and matching will be finished, click **Stop**. The experiment will be started consecutively.

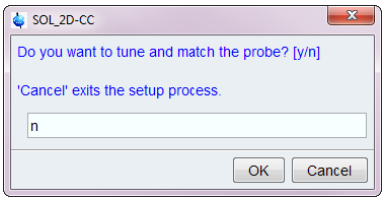

Figure 4.88: Confirming Whether to Tune and Match the Probe.

- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.5.2 2D NCa Double CP

The NCa experiment (see *References [▷ 113]* #4) is providing a specific heteronuclear cross polarization from ^{15}N to ^{13}C-alpha within the same amino acid residue, thus giving important intra residue information for assigning a protein.

The general order of actions is the same as in the chapter *2D DARR/ PDSD [▷ 65]*.

- Go to the **Backbone Assignment** tab and choose **NCa Double CP** out of the list of **2D Experiments**.

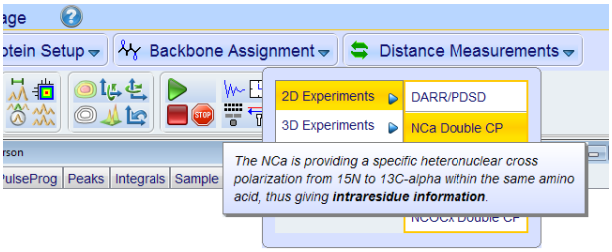

Figure 4.89: Selecting NCa Double CP as a 2D Experiment for the protein Backbone Assignment.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 30 to 39 are kept for these NCα double CP experiments only. If you want to record further NCα experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands **New** or **edc** (for further reading see TopSpin manual).

- A window will open listing the relevant 90° pulse lengths and power levels as well as the MAS rate (that have been optimized in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▷ 55]*)) that will be used for calculating parameters necessary for the experiment. Check these values carefully.

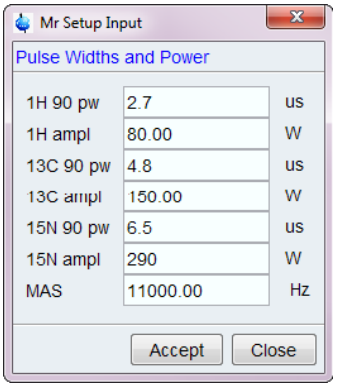

Figure 4.90: Parameter Check of Pulse Widths and Power Levels.

- As necessary, correct and **Accept**.
- During the specific NCα double CP, ^{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.

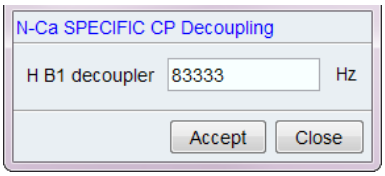

Figure 4.91: NCA Decoupling Input.

- Enter the field strength that you want to use for proton decoupling during the specific N-Ca CP in Hz.
- The power level is calculated. **Update** or **Keep previous**.

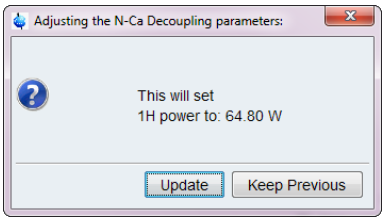

Figure 4.92: Adjusting the N-Ca Decoupling Parameters.

- To avoid sample heating and arching you have to choose the ^{1}H decoupling program and field strength that are used during the acquisition as well. If you are unsure what to use, the suggested values can be accepted as appropriate.
- Depending on the entered field strength, the parameters will be calculated (based on the previously optimized parameters in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*)). If you agree with these values, click **Update**, else choose **Keep previous**.
- To avoid unwanted side band backfolding artifacts, a rotor-synchronized spectral width can be set in the indirect nitrogen dimension. 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 chapters *Rotor Synchronized Spectral Widths [▶ 97]*, *Typical Carbon Spectral Widths [▶ 99]*, and *Typical Nitrogen Spectral Widths [▶ 102]*.
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all resonances in the spectrum (Check in the HN CP experiment recorded in the chapter *Optimize H-N CP Experiment [▶ 60]*.). A rotor-synchronized spectral width for the indirect nitrogen dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 46 ppm (backbone nitrogen) or a full spectral width of 240 ppm (backbone and side chain nitrogen) will be offered to choose (both with an offset at 120 ppm), but a customized width in ppm can be entered in a further step as well.
- Choose an acquisition time in ms in the indirect dimension.

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 *How to Choose an Appropriate Acquisition Time [▶ 105]*.

- Enter the number of scans you want to acquire. Take care that the number of scans are a multiple of the phase cycle to avoid phase cycling artifacts.
- The experimental time will be displayed. In case you want to make changes, click **Cancel** to repeat the previous steps.
- You can tune and match the probe before the experiment will be started.
  - If you do not want to wobble, enter '**n**'. The experiment will be started consecutively.
  - If you want to wobble, enter '**y**'. The wobble interface will open. After tuning and matching will be finished, click **Stop**. The experiment will be started consecutively.
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.5.3 2D NCO Double CP

The NCO (see *References [▶ 113]* #4) 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.

The general order of actions is the same as in the chapter *2D DARR/ PDSD [▶ 65]*.

- Go to the **Backbone Assignment** tab and select **NCO Double CP** from the list of **2D Experiments**.

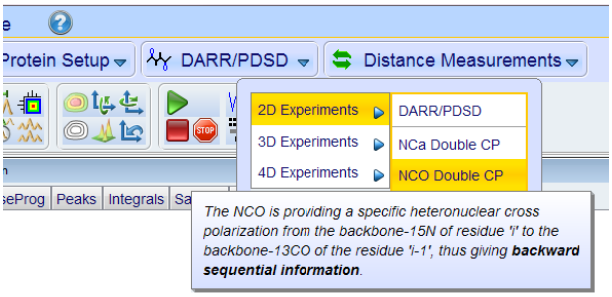

Figure 4.93: Selecting NCO Double CP as a 2D Experiment for the Protein Backbone Assignment.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 30 to 39 are kept for these NCa double CP experiments only. If you want to record further NCa experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands 'new' or 'edc' (for further reading see TopSpin manual).

- The order of actions is the same as for the NCa experiment, described in the chapter *2D NCa Double CP [▶ 70]*.
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.5.4 2D NCaCx Double CP

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 intra-residue information about the side chains of residues. The C-C transfer can be done either by PDSD or DARR (see chapter *2D DARR/ PDSD [▶ 65]*).

- Go to the **Backbone Assignment** tab and choose **NCaCx Double CP** out of the list of **2D Experiments**.

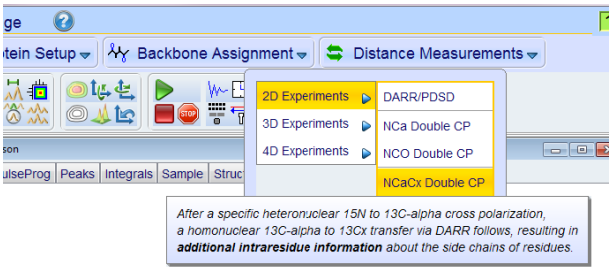

Figure 4.94: Selecting NCaCx Double CP as a 2D Experiment for the Protein Backbone Assignment.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 50 to 59 are kept for these 2D NCaCx double CP experiments only. If you want to record further 2D NCaCx experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands **new** or **edc** (for further reading see TopSpin manual).

- The order of actions is the same as for the NCa experiment (see chapter *2D NCa Double CP [▶ 70]*), including the setup steps of the DARR/ PDSD, described in (see chapter *2D DARR/ PDSD [▶ 65]*).

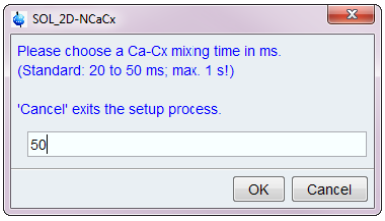

Figure 4.95: Entering a Customized Mixing Time for the ^{13}Cα-^{13}Cx Transfer.

- Enter a customized mixing time for the 13Cα-13Cx transfer (see *Mixing Times and Their Information Content [▶ 97]* for further reading).
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.5.5 2D NCOCx Double CP

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 (see chapter *2D DARR/ PDSD [▶ 65]*).

- Go to the **Backbone Assignment** tab and choose **NCOCx Double CP** out of the list of **2D Experiments**.

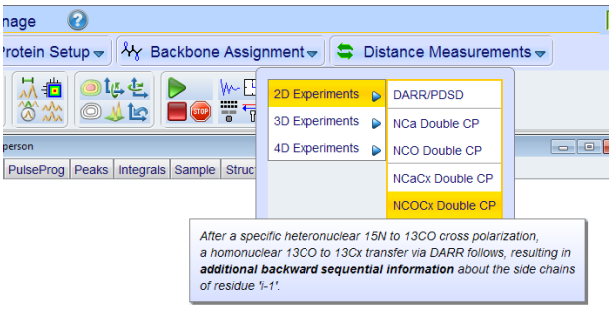

Figure 4.96: Selecting NCOCx Double CP as a 2D Experiment for the Protein Backbone Assignment.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 60 to 69 are kept for these 2D NCOCx double CP experiments only. If you want to record further 2D NCOCx experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands **new** or **edc** (for further reading see TopSpin manual).

- The order of actions is the same as for the NCO experiment (see chapter *2D NCO Double CP [▶ 72]*), including the setup steps of the DARR/ PDSD, described in (see chapter *2D DARR/ PDSD [▶ 65]*).
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.5.6 3D Experiments

A list of available 3D experiments is given in the figure below. The directions of 3D experiments are indicated with the terms F3 (first direction - acquisition or direct direction), F2 (second direction - indirect direction) and F1 (third direction - indirect direction).

All experiments include either specific double CP steps[3] 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.

With the introduction of a third dimension, the overlapping 2D signal can be further resolved. The third dimension should be handled as the indirect dimension of a 2D experiment in terms of spectral width and acquisition time (see chapter *How to Choose an Appropriate Acquisition Time [▶ 105]*).

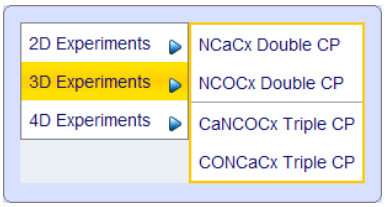

Figure 4.97: List of 3D Experiments used for Protein Backbone Assignment.

If you want to measure 3D experiments, make sure to have sufficient signal-to-noise, else the required measurement time to obtain appropriate 3D spectra will be excessively long. If you cannot measure a reasonable 2D in one day, we recommend not to measure 3D experiments on that sample.

4.5.6.1 3D NCaCx Double CP

As in the 2D- (see chapter *2D NCaCx Double CP [▶ 73]*) the 3D-NCaCx offers additional intra-residue information about the side chains of residues. 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. The C-C transfer can be done either using PDSD or DARR (see chapter *2D DARR/ PDSD [▶ 65]*).

- Go to the **Backbone Assignment** tab and choose **NCaCx Double CP** out of the list of **3D Experiments**.

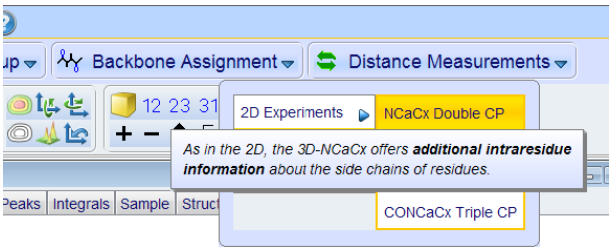

Figure 4.98: Selecting NCaCx Double CP as a 3D Experiment for the protein Backbone Assignment.

- Choose NCaCx Double CP as a 3D Experiment for the protein Backbone Assignment.
- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 70 to 79 are kept for these 3D NCaCx double CP experiments only. If you want to record further 3D NCaCx experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands **new** or **edc** (for further reading see TopSpin manual).

A window will open listing the relevant 90° pulse lengths and power levels as well as the MAS rate (that have been optimized in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*) that will be used for calculating parameters necessary for the experiment. Check these values carefully.

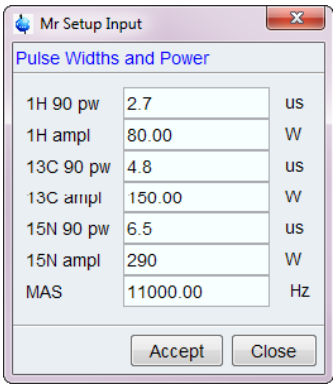

Figure 4.99: Parameter Checks of Pulse Widths and Power Levels.

- Correct the values in the figure above as necessary, and press **Accept**.
- During the specific NCa double CP, ^{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.

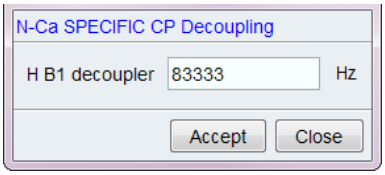

Figure 4.100: Suggested Field Strength Value.

- Enter the field strength that you want to use for proton decoupling during the specific N-Ca CP in Hz.
- The power level is calculated. **Update** or **Keep previous**.

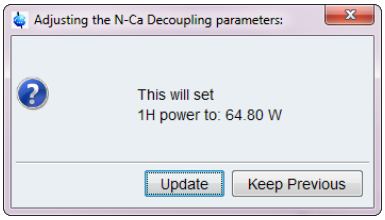

Figure 4.101: Calculated Decoupling Power.

- To avoid sample heating and arching you have to choose the ^{1}H decoupling program and field strength during the acquisition as well. If you are unsure what to use, the suggested values can be accepted as appropriate.
- Depending on the entered field strength, the parameters will be recalculated (based on the previously optimized parameters in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*)). If you agree with these values, click **Update**, else choose **Keep previous**.
- You can choose to acquire either a PDSD ('**y**') or a DARR ('**no**') as recoupling technique. The order of actions is the same as described in steps of the 2D DARR/ PDSD experiment (see chapter *2D DARR/ PDSD [▶ 65]*).

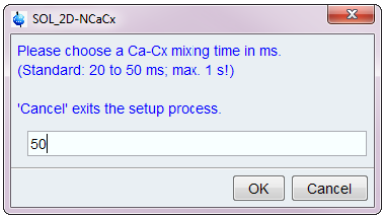

Figure 4.102: Selecting a Ca-Cx Mixing Time.

- Enter a customized mixing time.
- To avoid unwanted side band backfolding artifacts, a rotor-synchronized spectral width can be set in the indirect ^{15}N (F1) dimension. 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 chapters *Rotor Synchronized Spectral Widths [▶ 97]*, *Typical Carbon Spectral Widths [▶ 99]*, and *Typical Nitrogen Spectral Widths [▶ 102]*.
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all resonances in the spectrum (Check in the HN CP experiment recorded in the chapter *Optimize H-N CP Experiment [▶ 60]*). A rotor-synchronized spectral width for the indirect nitrogen dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 46 ppm (backbone nitrogen) or a full spectral width of 240 ppm (backbone and side chain nitrogen) will be offered to choose (both with an offset at 120 ppm), but a customized width in ppm can be entered in a further step as well.
- Choose an acquisition time in ms in the indirect F1 dimension (^{15}N).

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 chapter *Typical Nitrogen Spectral Widths [▶ 102]*.

- As for the F1 dimension, the spectral width for the indirect F2 dimension can be set rotor-synchronous as well (^{13}Cα).
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all Cα resonances in the spectrum (Check in the HC CP experiment recorded in the chapter *Optimize H-C CP Experiment [▶ 59]*). A rotor-synchronized spectral width for the indirect carbon dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 110 ppm will be offered to choose (with an offset at 55 ppm), but a customized width in ppm can be entered in a further step as well.

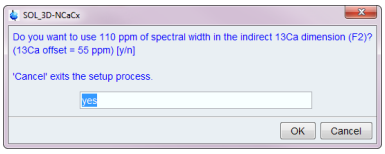

Figure 4.103: Conventional Spectral Width of 110 ppm for the Indirect ^{13}Cα Dimension F2.

- Choose an acquisition time in ms in the indirect F2 dimension (^{13}Cα).
- Enter the number of scans you want to acquire. Take care that the number of scans are a multiple of the phase cycle to avoid phase cycling artifacts.
- The experimental time will be displayed. In case you want to make changes, click **Cancel** to repeat the previous steps.
- You can tune and match the probe before the experiment will be started.
  - If you do not want to wobble, enter '**n**'. The experiment will be started consecutively.
  - If you want to wobble, enter '**y**'. The wobble interface will open. After tuning and matching will be finished, click **Stop**. The experiment will be started consecutively.
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.5.6.2 3D NCOCx Double CP

As in the 2D (see chapter *2D NCOCx Double CP [▶ 74]*) the 3D-NCOCx offers additional backward sequential information about the side chains of residue 'i-1'. 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. The C-C transfer can be done either by PDSD or DARR (see chapter *2D DARR/ PDSD [▶ 65]*).

- Go to the **Backbone Assignment** tab and choose **NCOCx Double CP** out of the list of **3D Experiments**.

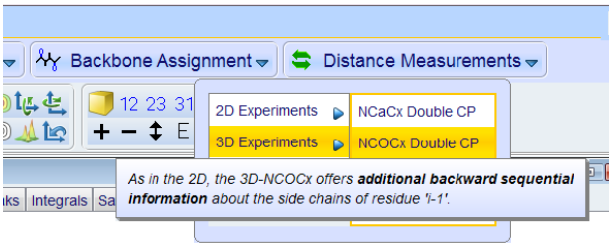

Figure 4.104: Selecting NCOCx Double CP as a 3D Experiment.

- Choose NCOCx Double CP as a 3D Experiment for the protein Backbone Assignment.
- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 80 to 89 are kept for these 3D NCOCx double CP experiments only. If you want to record further 3D NCOCx experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands ‘new’ or ‘edc’ (for further reading see TopSpin manual).

- The order of actions is the same as for the 3D NCaCx experiment (see chapter *3D NCaCx Double CP [▶ 75]*).
- As for the F1 dimension, the spectral width for the indirect F2 dimension (^{13}CO) can be set rotor-synchronous as well.
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all CO resonances in the spectrum (check in the HC CP experiment recorded in the chapter *Optimize H-C CP Experiment [▶ 59]*). A rotor-synchronized spectral width for the indirect carbon dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 30 ppm will be offered to choose (with an offset at 175 ppm), but a customized width in ppm can be entered in a further step as well.

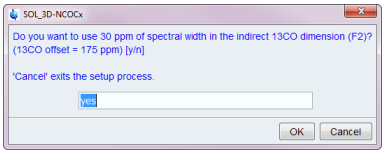

Figure 4.105: Conventional Spectral Width of 30 ppm for the Indirect ^{13}CO Dimension F2.

- Choose an acquisition time in ms in the indirect F2 dimension (^{13}CO).
- Enter the number of scans you want to acquire. Take care that the number of scans are a multiple of the phase cycle to avoid phase cycling artifacts.

- The experimental time will be displayed. In case you want to make changes, click **Cancel** to repeat the previous steps.
- You can tune and match the probe before the experiment will be started.
  - If you do not want to wobble, enter '**n**'. The experiment will be started consecutively.
  - If you want to wobble, enter '**y**'. The wobble interface will open. After tuning and matching will be finished, click **Stop**. The experiment will be started consecutively.
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.5.6.3 3D CaNCOCx Triple CP

Based on three heteronuclear cross polarization steps (^{1}H to ^{13}Ca to ^{15}N to ^{13}CO-Cx) this sequence provides backward sequential ('i' to 'i-1') information.

- Go to the **Backbone Assignment** tab and choose **CaNCOCx Triple CP** out of the list of **3D Experiments**.

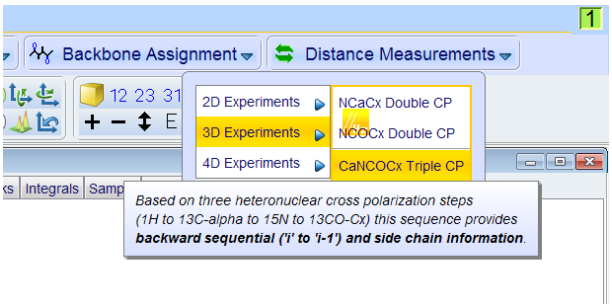

Figure 4.106: Selecting CaNCOCx Triple CP as a 3D Experiment.

- Choose CaNCOCx Triple CP as a 3D Experiment for the protein Backbone Assignment.
- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 90 to 99 are kept for these 3D CaNCOCx triple CP experiments only. If you want to record further 3D CaNCOCx experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands 'new' or 'edc' (for further reading see TopSpin manual).

A window will open listing the relevant 90° pulse lengths and power levels (that have been optimized in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▷ 55]*)) as well as the MAS rate that will be used for calculating parameters necessary for the experiment. Check these values carefully.

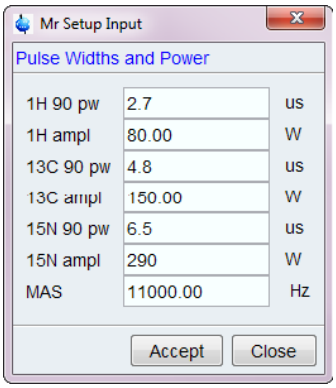

Figure 4.107: Parameter Check of Pulse Widths and Power Levels.

- Make any corrections necessary, and press **Accept**.
- During the Cα->N CP, ^{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.
- The power level is calculated. **Update** or **Keep previous**.
- Because there is another specific CP step, from N to CO, repeat steps 5 and 6 to set the 1H decoupling during this CP step as well (usually the same field strength is used).
- To avoid sample heating and arching you have to choose the ^{1}H decoupling program and field strength. If you are unsure what to use, the suggested values can be accepted as appropriate.
- Depending on the entered field strength, the parameters will be recalculated (based on the previously optimized parameters in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*)). If you agree with these values, click **Update**, else choose **Keep previous**.
- You can choose to acquire either a PDSD ('**y**') or a DARR ('**no**') as recoupling technique for the CO to Cx transfer, including a mixing time (see chapter *2D DARR/ PDSD [▶ 65]*).

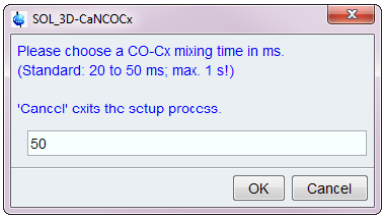

Figure 4.108: Selecting a CO-Cx Mixing Time.

- Enter a customized mixing time.
- To avoid unwanted side band backfolding artifacts, a rotor-synchronized spectral width can be set in the indirect F1 (^{13}Cα) dimension. 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 chapters *Rotor Synchronized Spectral Widths [▶ 97]*, *Typical Carbon Spectral Widths [▶ 99]*, and *Typical Nitrogen Spectral Widths [▶ 102]*.
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all Cα resonances in the spectrum (Check in the HC CP experiment recorded in the chapter *Optimize H-C CP Experiment [▶ 59]*). A rotor-synchronized spectral width for the indirect carbon dimension will be calculated and set.
  - No rotor-synchronization: A spectral width of 80 ppm will be offered to choose (with an offset at 55 ppm), but a customized width in ppm can be entered in a further step as well.

- Choose an acquisition time in ms in the indirect F1 dimension (^{13}Cα).

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 chapter *How to Choose an Appropriate Acquisition Time [▶ 105]*.

- As for the F1 dimension, the spectral width for the indirect F2 dimension can be set rotor-synchronous as well (^{15}N).
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all resonances in the spectrum (check in the HN CP experiment recorded in the chapter *Optimize H-N CP Experiment [▶ 60]*.). A rotor-synchronized spectral width for the indirect nitrogen dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 46 ppm (backbone nitrogen) or a full spectral width of 240 ppm (backbone and side chain nitrogen) will be offered to choose (both with an offset at 120 ppm), but a customized width in ppm can be entered in a further step as well.
- Choose an acquisition time in ms in the indirect F2 dimension (^{15}N).
- Enter the number of scans you want to acquire. Take care that the number of scans are a multiple of the phase cycle to avoid phase cycling artifacts.
- The experimental time will be displayed. In case you want to make changes, click **Cancel** to repeat the previous steps.
- You can tune and match the probe before the experiment will be started.
  - If you do not want to wobble, enter '**n**'. The experiment will be started consecutively.
  - If you want to wobble, enter '**y**'. The wobble interface will open. After tuning and matching will be finished, click **Stop**. The experiment will be started consecutively.
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.5.6.4 3D CONCaCx Triple CP

Based on three heteronuclear cross polarization steps (^{1}H to ^{13}CO to ^{15}N to ^{13}Ca-Cx) this sequence provides forward sequential ('i' to 'i+1') and side chain ('i+1') information.

- Go to the **Backbone Assignment** tab and choose **CONCaCx Triple CP** out of the list of **3D Experiments**.

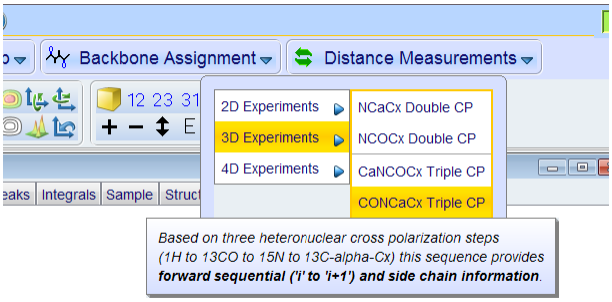

Figure 4.109: Selecting CONCaCx Triple CP as a 3D Experiment for the protein Backbone Assignment.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.

- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 100 to 109 are kept for these 3D CONCaCx triple CP experiments only. If you want to record further 3D CONCaCx experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands ‘new’ or ‘edc’ (for further reading see TopSpin manual).

The order of actions is the same as for the 3D CaNCOCx experiment (see chapter *3D CaNCOCx Triple CP [▶ 79]*), except that the sweep widths are changed corresponding to the nuclei:

- F1 dimension (^{13}CO):
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all CO resonances in the spectrum (check in the HC CP experiment recorded in the chapter *Optimize H-C CP Experiment [▶ 59]*). A rotor-synchronized spectral width for the indirect carbon dimension will be calculated and set.
  - No rotor-synchronization: A spectral width of 30 ppm will be offered to choose (with an offset at 175 ppm), but a customized width in ppm can be entered in a further step as well.
  - Choose an acquisition time in ms in the indirect F1 dimension (^{13}CO).
- F2 dimension (^{15}N):
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all resonances in the spectrum (check in the HN CP experiment recorded in the chapter *Optimize H-N CP Experiment [▶ 60]*). A rotor-synchronized spectral width for the indirect nitrogen dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 46 ppm (backbone nitrogen) or a full spectral width of 240 ppm (backbone and side chain nitrogen) will be offered to choose (both with an offset at 120 ppm), but a customized width in ppm can be entered in a further step as well.
  - Choose an acquisition time in ms in the indirect F2 dimension (^{15}N).

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 chapter *How to Choose an Appropriate Acquisition Time [▶ 105]*.

4.5.7 4D Experiments

A list of the two available 4D experiments is given in the figure below. The directions of 4D experiments are indicated with the terms F4 (first direction - acquisition or direct direction), F3 (second direction - indirect direction), F2 (third direction - indirect direction) and F1 (fourth direction - indirect direction).

Both experiments include specific triple CP steps[3] from ^{1}H to ^{13}C to ^{15}N and back to ^{13}C and a final ^{13}C-^{13}C mixing, comprising sequential protein backbone correlations. With the introduction of a fourth dimension, the overlapping 3D signal of the final ^{13}C-^{13}C mixing can be further resolved.

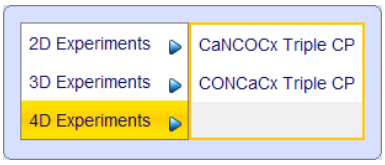

Figure 4.110: List of 4D Experiments used for Protein Backbone Assignment.

The third and fourth dimensions should be handled as the indirect dimension of a 2D experiment in terms of spectral widths and acquisition times (see chapters *Rotor Synchronized Spectral Widths [▷ 97]*, *Typical Carbon Spectral Widths [▷ 99]*, *Typical Nitrogen Spectral Widths [▷ 102]* and *How to Choose an Appropriate Acquisition Time [▷ 105]*).

If you want to measure 4D experiments, make sure to have sufficient signal-to-noise, else the required measurement time to obtain decent 4D spectra will be excessively long. If you cannot measure a reasonable 2D in one day, we do not recommend measuring 4D experiments on that sample.

4.5.7.1 4D CaNCOCx Triple CP

As in the 3D (see chapter *3D CaNCOCx Triple CP [▷ 79]*), this sequence provides backward sequential ('i' to 'i-1') and side chain information.

- Go to the **Backbone Assignment** tab and choose **CaNCOCx Triple CP** out of the list of **4D Experiments**.

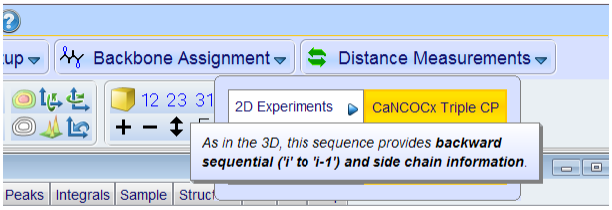

Figure 4.111: Selecting CaNCOCx Triple CP as a 4D Experiment for the protein Backbone Assignment.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 110 to 119 are kept for these 4D CaNCOCx triple CP experiments only. If you want to record further 4D CaNCOCx experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands ‘new’ or ‘edc’ (for further reading see TopSpin manual).

The order of actions is exactly the same as for the 3D CaNCOCx experiment (see chapter *3D CaNCOCx Triple CP [▷ 79]*), except that another indirect dimension is introduced (F3 for ^{13}CO). The sweep widths are changed corresponding to the nuclei:

- F1 dimension (^{13}Cα):

- Rotor-synchronization: Enter the minimum spectral width that you need to cover all Cα resonances in the spectrum (check in the HC CP experiment recorded in the chapter *Optimize H-C CP Experiment [▶ 59]*). A rotor-synchronized spectral width for the indirect carbon dimension will be calculated and set.
- No rotor-synchronization: A spectral width of 80 ppm will be offered to choose (with an offset at 55 ppm), but a customized width in ppm can be entered in a further step as well.
- Choose an acquisition time in ms in the indirect F1 dimension (^{13}Cα).

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 chapter *How to Choose an Appropriate Acquisition Time [▶ 105]*.

- F2 dimension (^{15}N):
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all resonances in the spectrum (check in the HN CP experiment recorded in the chapter *Optimize H-N CP Experiment [▶ 60]*.). A rotor-synchronized spectral width for the indirect nitrogen dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 46 ppm (backbone nitrogen) or a full spectral width of 240 ppm (backbone and side chain nitrogen) will be offered to choose (both with an offset at 120 ppm), but a customized width in ppm can be entered in a further step as well.
  - Choose an acquisition time in ms in the indirect F2 dimension (^{15}N).
- F3 dimension (^{13}CO):
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all CO resonances in the spectrum (check in the HC CP experiment recorded in the chapter *Optimize H-C CP Experiment [▶ 59]*). A rotor-synchronized spectral width for the indirect carbon dimension will be calculated and set.
  - No rotor-synchronization: A spectral width of 30 ppm will be offered to choose (with an offset at 175 ppm), but a customized width in ppm can be entered in a further step as well.
  - Choose an acquisition time in ms in the indirect F1 dimension (^{13}CO).
  - The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.5.7.2 4D CONCaCx Triple CP

As in the 3D (see chapter *3D CONCaCx Triple CP [▶ 81]*), this sequence provides forward sequential ('i' to 'i+1') and side chain information.

- Go to the **Backbone Assignment** tab and choose **CONCaCx Triple CP** out of the list of **4D Experiments**.

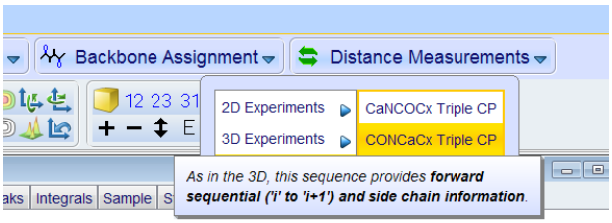

Figure 4.112: Selecting CONCaCx Triple CP as a 4D Experiment for the protein Backbone Assignment.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 120 to 129 are kept for these 4D CONCaCx triple CP experiments only. If you want to record further 4D CONCaCx experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands '**new**' or '**edc**' (for further reading see TopSpin manual).

The order of actions is the same as for the 3D CONCaCx experiment (see chapter *3D CONCaCx Triple CP [▶ 81]*), except that another indirect dimension (^{13}Cα) is introduced (F3). The sweep widths are changed corresponding to the nuclei:

- F1 dimension (^{13}CO):
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all CO resonances in the spectrum (check in the HC CP experiment recorded in the chapter *Optimize H-C CP Experiment [▶ 59]*). A rotor-synchronized spectral width for the indirect carbon dimension will be calculated and set.
  - No rotor-synchronization: A spectral width of 30 ppm will be offered to choose (with an offset at 175 ppm), but a customized width in ppm can be entered in a further step as well.
  - Choose an acquisition time in ms in the indirect F1 dimension (^{13}CO).

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 chapter *How to Choose an Appropriate Acquisition Time [▶ 105]*.

- F2 dimension (^{15}N):
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all resonances in the spectrum (check in the HN CP experiment recorded in the chapter *Optimize H-N CP Experiment [▶ 60]*). A rotor-synchronized spectral width for the indirect nitrogen dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 46 ppm (backbone nitrogen) or a full spectral width of 240 ppm (backbone and side chain nitrogen) will be offered to choose (both with an offset at 120 ppm), but a customized width in ppm can be entered in a further step as well.
  - Choose an acquisition time in ms in the indirect F2 dimension (^{15}N).
- F3 dimension (^{13}Cα):

– Rotor-synchronization: Enter the minimum spectral width that you need to cover all Cα resonances in the spectrum (check in the HC CP experiment recorded in the chapter *Optimize H-C CP Experiment [▷ 59]*). A rotor-synchronized spectral width for the indirect carbon dimension will be calculated and set.
– No rotor-synchronization: A spectral width of 80 ppm will be offered to choose (with an offset at 55 ppm), but a customized width in ppm can be entered in a further step as well.
– Choose an acquisition time in ms in the indirect F1 dimension (^{13}Cα).
– The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.6 Distance Measurements

A list of available experiments is given in the figure below. Select an experiment to gain long-range distance restraints that are essential for structure elucidation.

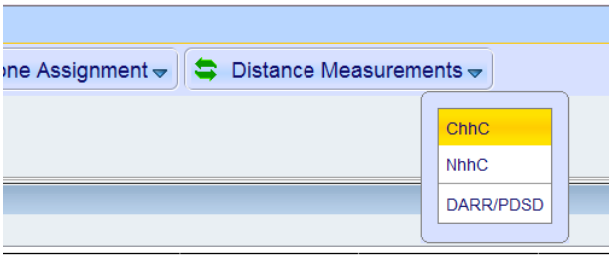

Figure 4.113: List of Experiments used to get Protein Distance Information.

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 the chapter *Temperature Control - edte [▷ 25]*.

4.6.1 2D ChhC

The ChhC (see *References [▷ 113]* #5) 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 chosen short.

- Go to the **Distance Measurements** tab and choose **ChhC**.

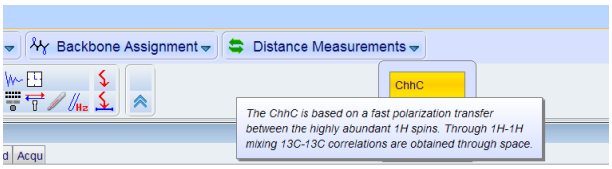

Figure 4.114: Selecting ChhC from the List of Experiments used for Distance Measurements.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 130 to 139 are kept for these ChhC experiments only. If you want to record further ChhC experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands **new** or **edc** (for further reading see TopSpin manual).

- A window displaying the found parameters will open. Please check the important values for ^{13}C, ^{1}H and the MAS rate.
- To avoid sample heating and arching you have to choose a decoupling field strength and decoupling program. If you are unsure what to use, the suggested values can be accepted as proper parameters.
- Depending on the entered field strength, the parameters will be recalculated (based on the previously optimized parameters in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*)). If you agree with these values, click **OK**, else choose **Keep previous**.
- Choose a ^{1}H-^{1}H mixing time. Do not use more than 400 µs in a uniformly labeled sample to avoid unspecific magnetization transfer and with that signal loss.

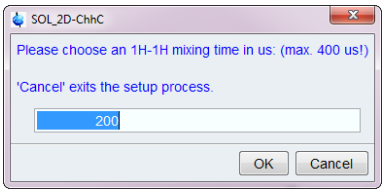

Figure 4.115: Selecting a 1H-1H Mixing.

- Enter a contact time in µs for the first short CP step (from ^{13}C to ^{1}H). Do not use more than 300 µs to ensure magnetization transfer only from directly bound protons and carbons.

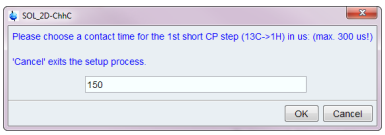

Figure 4.116: Selecting a Contact Time for the 1st Short CP Step.

- Enter a contact time in µs for the second short CP step (from ^{1}H back to ^{13}C). Do not use more than 300 µs to ensure magnetization transfer only from directly bound protons and carbons. Usually the same value is used as for the first short CP.

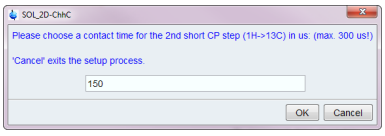

Figure 4.117: Selecting a Contact Time for the 2nd Short CP Step.

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 55 ppm to choose a smaller SW.

- To avoid unwanted side band backfolding artifacts, a rotor-synchronized spectral width can be set in the indirect ^{13}C (F1) dimension. 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 chapters *Rotor Synchronized Spectral Widths [▶ 97]*, *Typical Carbon Spectral Widths [▶ 99]*, and *Typical Nitrogen Spectral Widths [▶ 102]*.
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all resonances in the spectrum (check in the HC CP experiment recorded in the chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*). A rotor-synchronized spectral width for the indirect nitrogen dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 120 ppm will be offered to choose (with an offset at 55 ppm), but a customized width in ppm can be entered in a further step as well.
- Choose an acquisition time in ms in the indirect F1 dimension (^{13}C).

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 chapter *How to Choose an Appropriate Acquisition Time [▶ 105]*.

- Enter the number of scans you want to acquire. Take care that the number of scans are a multiple of the phase cycle to avoid phase cycling artifacts.
- The experimental time will be displayed. In case you want to make changes, click **Cancel** to repeat the previous steps.
- You can tune and match the probe before the experiment will be started.
  - If you do not want to wobble, enter “**n**” or “**no**”.
  - If you want to wobble, enter “**y**” or “**yes**”. The wobble interface will open. After tuning and matching will be finished, click **Stop**.
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.6.2 2D NhhC

The NhhC (see *References [▶ 113]* #5) 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.

- Go to the **Distance Measurements** tab and choose **NhhC**.

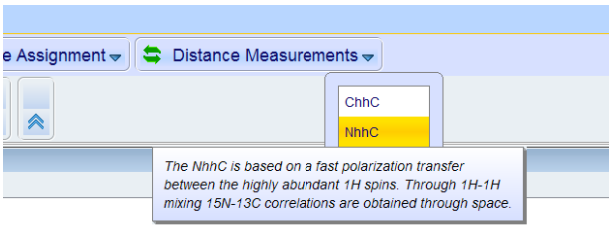

Figure 4.118: Selecting NhhC from the List of Experiments used for Distance Measurements.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 140 to 149 are kept for these NhhC experiments only. If you want to record further NhhC experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands **new** or **edc** (for further reading see TopSpin manual).

- A window displaying the found parameters will open. Please check the important values for ^{13}C, ^{1}H, ^{15}N and the MAS rate.
- To avoid sample heating and arching you have to choose a decoupling field strength and decoupling program. If you are unsure what to use, the suggested values can be accepted as proper parameters.
- Depending on the entered field strength, the parameters will be recalculated (based on the previously optimized parameters in the Protein Setup (see chapter *Setup of Experiments on the Biological Sample of Interest [▶ 55]*)). If you agree with these values, click **OK**, else choose **Keep previous**.
- Choose a ^{1}H-^{1}H mixing time. Do not use more than 500 µs in a uniformly labeled sample to avoid unspecific magnetization transfer and with that signal loss.
- Enter a contact time in µs for the first short CP step (from ^{15}N to ^{1}H). Do not use more than 300 µs to ensure magnetization transfer only from directly bound protons and nitrogen.

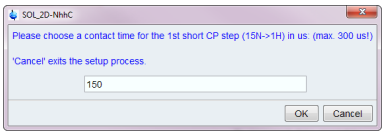

Figure 4.119: Selecting a Contact Time for the 1st Short CP Step.

- Enter a contact time in µs for the second short CP step (from ^{1}H to ^{13}C). Do not use more than 300 µs to ensure magnetization transfer only from directly bound protons and carbons.

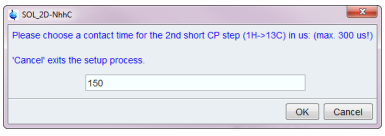

Figure 4.120: Selecting a Contact Time for the 2nd Short CP Step.

- To avoid unwanted side band backfolding artifacts, a rotor-synchronized spectral width can be set in the indirect ^{15}N (F1) dimension. 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 chapters *Rotor Synchronized Spectral Widths [▶ 97]*, *Typical Carbon Spectral Widths [▶ 99]*, and *Typical Nitrogen Spectral Widths [▶ 102]*.
  - Rotor-synchronization: Enter the minimum spectral width that you need to cover all resonances in the spectrum (check in the HN CP experiment recorded in the chapter *Optimize H-N CP Experiment [▶ 60]*). A rotor-synchronized spectral width for the indirect nitrogen dimension will be calculated and set.
  - No rotor-synchronization: A conventional spectral width of 46 ppm will be offered to choose (with an offset at 120 ppm), but a customized width in ppm can be entered in a further step as well.
- Choose an acquisition time in ms in the indirect F1 dimension (^{15}N).

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 *How to Choose an Appropriate Acquisition Time [▶ 105]*.

- Enter the number of scans you want to acquire. Take care that the number of scans are a multiple of the phase cycle to avoid phase cycling artifacts.
- The experimental time will be displayed. In case you want to make changes, click **Cancel** to repeat the previous steps.
- You can tune and match the probe before the experiment will be started.
  - If you do not want to wobble, enter “**n**” or “**no**”.
  - If you want to wobble, enter “**y**” or “**yes**”. The wobble interface will open. After tuning and matching will be finished, click **Stop**.
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.6.3 2D DARR/ PDSD

The DARR/ PDSD recoupling technique can not only be used for protein backbone assignment (see chapter *2D DARR/ PDSD [▶ 65]*), 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 (see *References [▶ 113]* Ref. # 6-10).

- Go to the **Distance Measurements** tab and choose **DARR/ PDSD**.

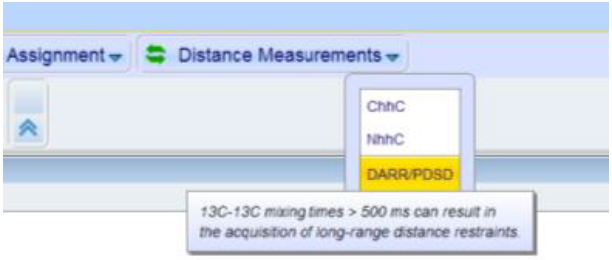

Figure 4.121: Selecting DARR/PDSD from the Distance Measurements List.

- You will be asked to insert the sample of interest and to spin it at the desired MAS rate. If your sample is already inserted, continue.
- The appropriate EXPNO will be created.

Using *TopSolids*^{bio}, the EXPNOs 150 to 159 are kept for these PDSD/ DARR experiments only. If you want to record further PDSD/ DARR experiments, you have to copy the dataset into another EXPNO (recommended: EXPNO > 999 to avoid complications!) using the commands new or edc (for further reading see TopSpin manual).

- The order of actions is the same as in the chapter *2D DARR/ PDSD [▶ 65]*. Choose a longer acquisition time, but take care to mix shorter than 1 sec to avoid sample heating. This is especially important when choosing DARR, where there is ^{1}H-decoupling during the ^{13}C-^{13}C mixing.

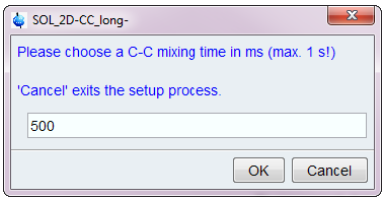

Figure 4.122: Selecting a C-C Mixing Time.

- Select a long ^{13}C-^{13}C mixing time to acquire long range restraints. If you choose DARR, take care not to mix longer than 1 second since ^{1}H decoupling is turned on during the whole mixing period.
- The experiment will be started directly or, in case acquisition is already running, put into the spooler.

4.7 Processing

Detailed information about data processing in *TopSpin* is given in the manual *Proc. Commands & Parameters* (see chapter *General Requirements [▶ 11]*).

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).

Error: Entering the MAS Rate Incorrectly:

In case, you enter the MAS rate in kHz (left figure), a dialog will pop up asking you to repeat the entry again in Hz (right figure).

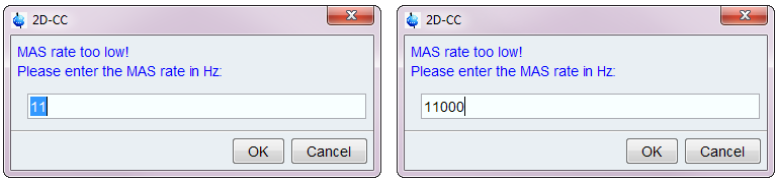

Figure 5.1: Error: MAS Rate Too Low.

Error: Entering PROSOL incorrectly

If PROSOL is not filled incorrectly, the program **pulsecal** that is used in the chapters *1H 90° Pulse Verification [▶ 43]* and *13C 90° Pulse Verification [▶ 44]* 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 the chapter *Filling in the PROSOL Table [▶ 16]* in order to check the PROSOL table.

Ensure B_0 Field is well calibrated

In addition for using the *TopSolids*^{bio} package it is important to have a well calibrated B_0 field. To recalibrate the B_0 field and how to check whether the B_0 field is set correctly, please refer to the shim section of the *TopSolids*^{bio} manual. A quick check is to measure the adamantane sample and see if the left (low field peak) is at 38.56 ppm chemical shift (relative to TMS).

See also

General Information [▶ 31]

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?

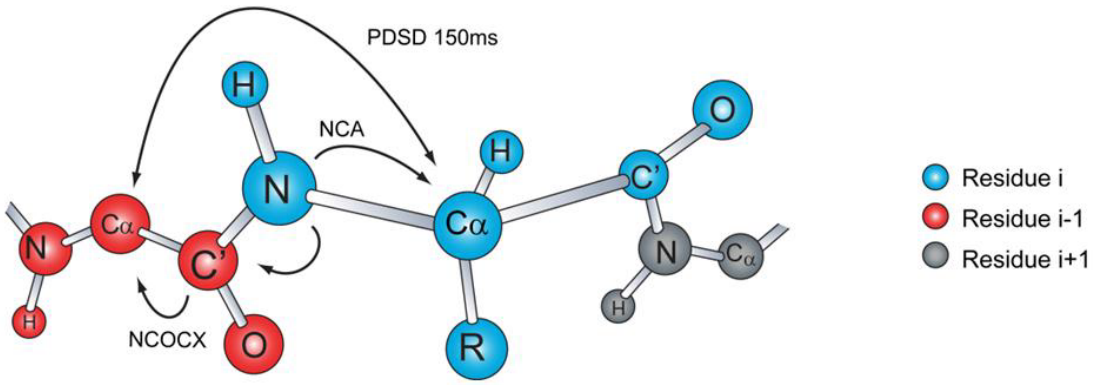

Figure 6.1: Schematic Representation of a Protein Backbone and the Correlations the Different Experiments Reveal.

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 Cα and Cβ shifts to random coil chemical shifts (*References [▶ 113]* Ref. # 11), you get information about the secondary structure of these residues.

Experiments: e.g. PDSD/ DARR with short mixing times (20 ms), NCα

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:

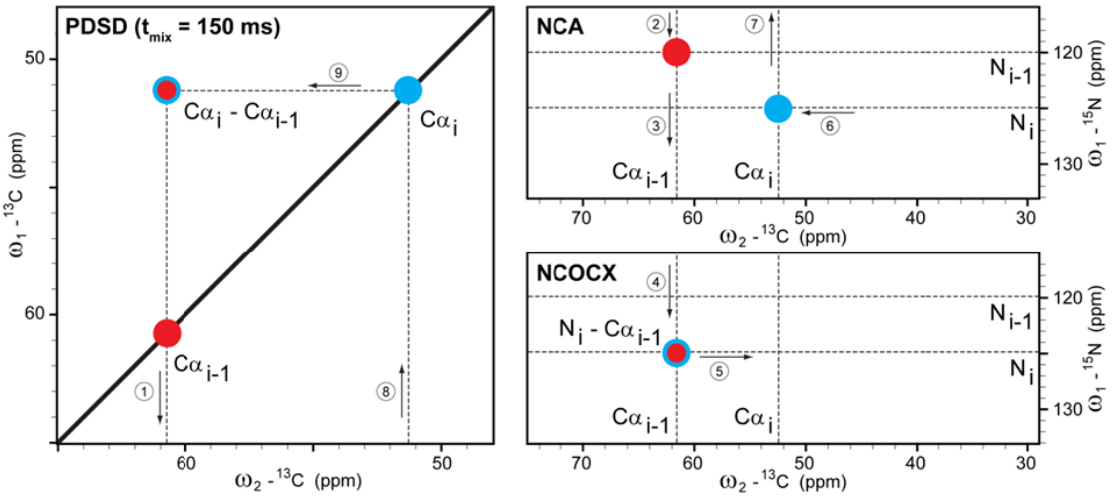

Figure 6.2: Schematic Representation of a Sequential Assignment.

Schematic representation of a sequential assignment. After having obtained chemical shifts of carbons within one amino acid in a short PDSD (t_{mix} ≈ 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} ≈ 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 Cα 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 [▷ 113]* 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 (ν_r), SSBs are separated from the isotropic signal by integer multiples of the MAS frequency:

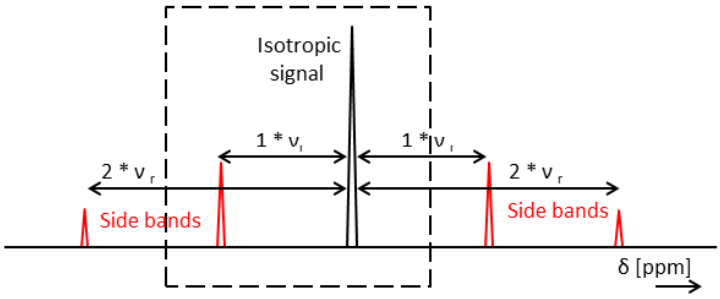

Figure 6.3: Schematic Representation of an Isotropic Peak (black) and its Corresponding Side Bands.

*Schematic representation of an isotropic peak (black) and its corresponding side bands (red), which occur at integer multiples of the MAS frequency (n * ν_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.

The preset acquisition mode for nD experiments in *TopSolids*^{bio} 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*^{bio} 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.

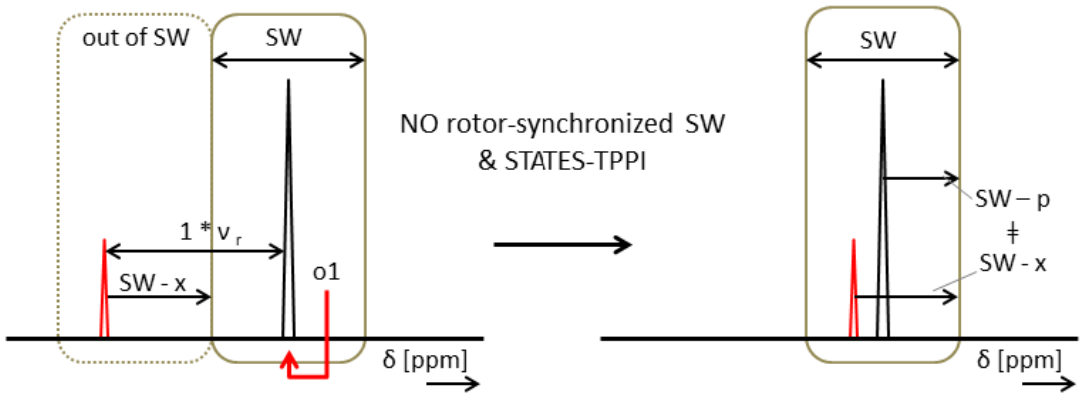

Figure 6.4: Non-synchronized SW.

*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!

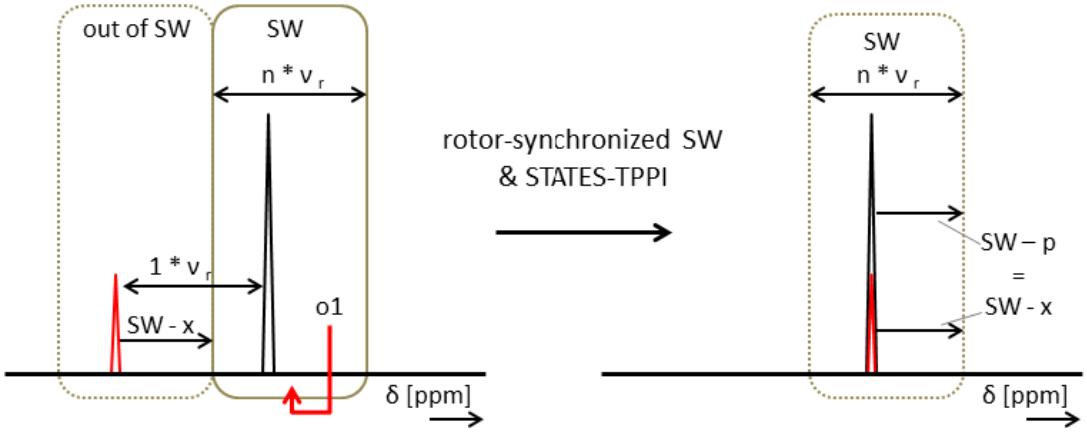

Figure 6.5: Rotor Synchronization.

*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*".

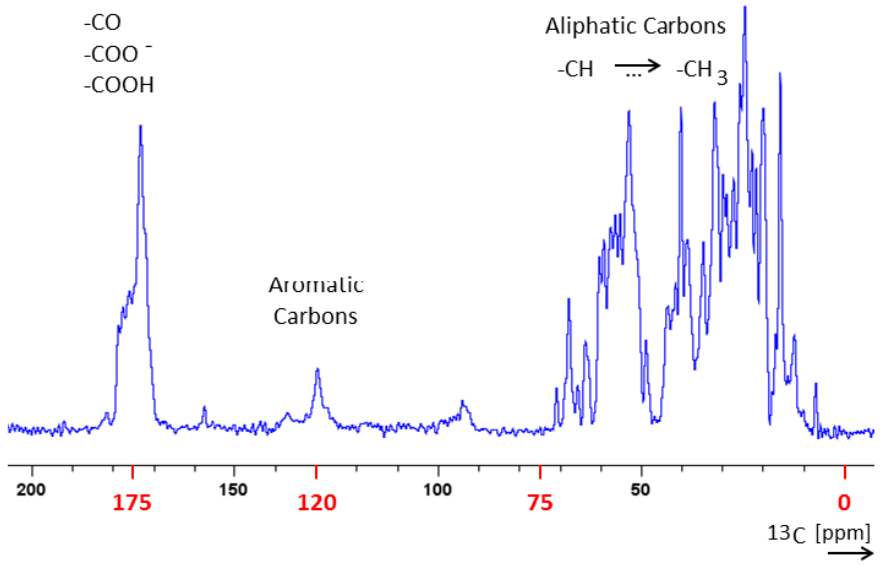

Figure 6.6: Typical Peak Pattern for a ^{13}C-detected HC CP Experiment on a Biological Sample.

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:

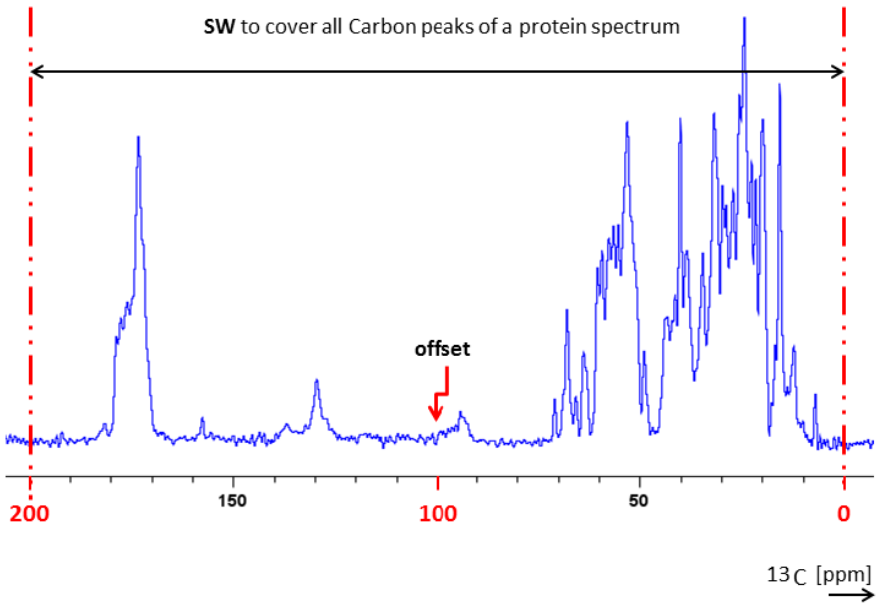

Figure 6.7: Typical Peak Pattern for a ^{13}C-detected HC CP Experiment on a Biological Sample.

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.

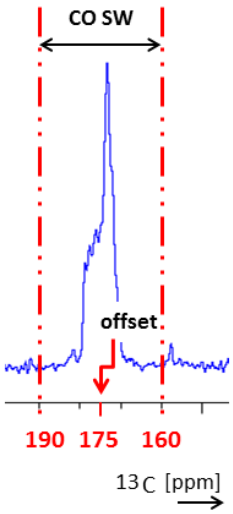

Figure 6.8: Typical Peak Pattern for a ^{13}C-detected Specific NCO Double CP Experiment on a Biological Sample.

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 $^{13}C\alpha$ (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 $^{13}C\alpha$ 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:

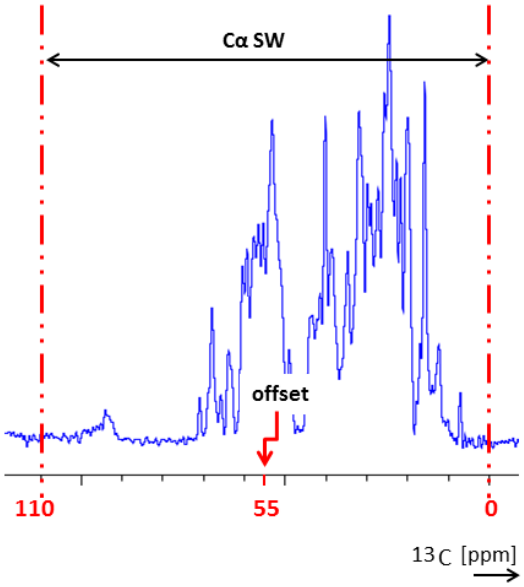

Figure 6.9: Typical Peak Pattern for a ^{13}C-Detected Specific NCα Double CP Experiment on a Biological Sample.

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 (see chapter *Optimize H-N CP Experiment [▷ 60]*), 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*".

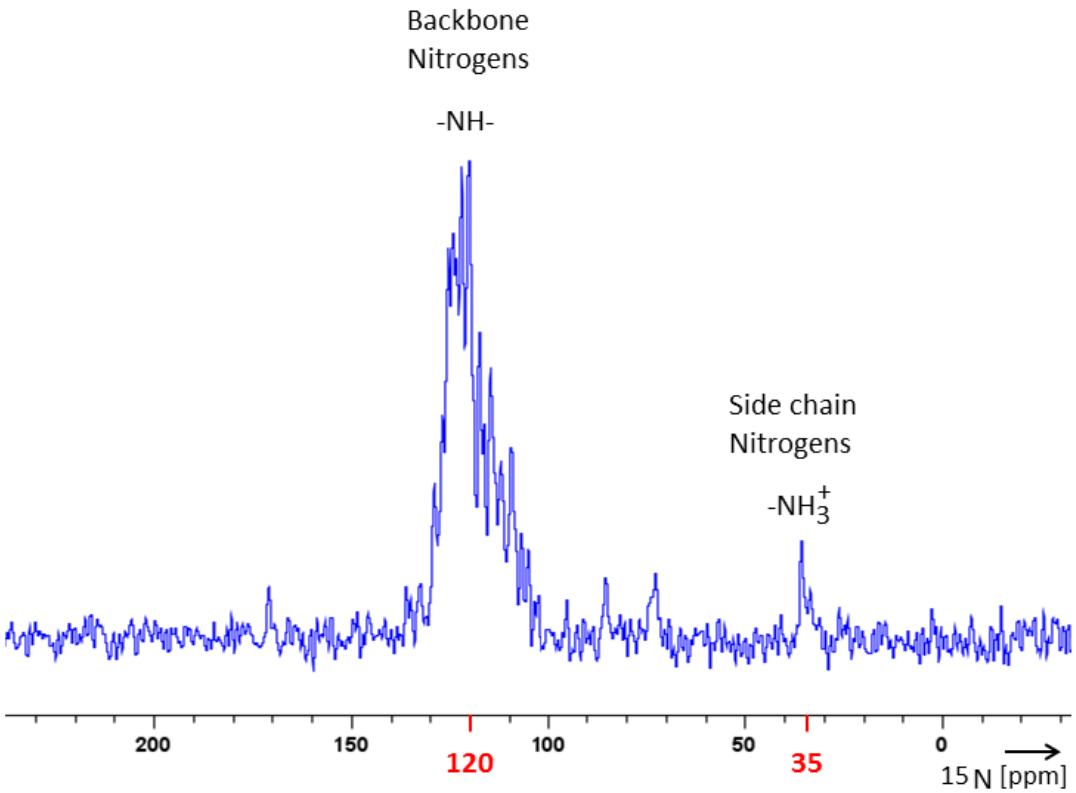

Figure 6.10: Typical Peak Pattern for a ^{15}N-detected HN CP Experiment on a Biological Sample.

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.

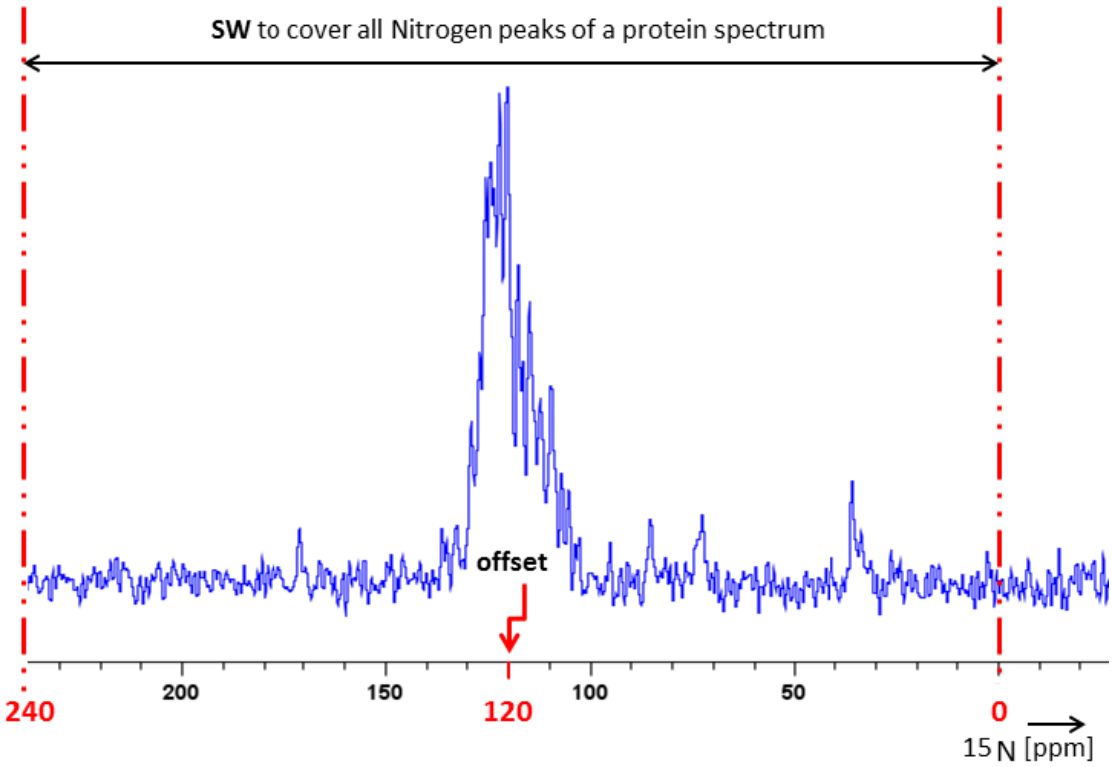

Figure 6.11: Typical Spectral Width for a ^{15}N-detected HN CP Experiment on a Biological Sample.

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.

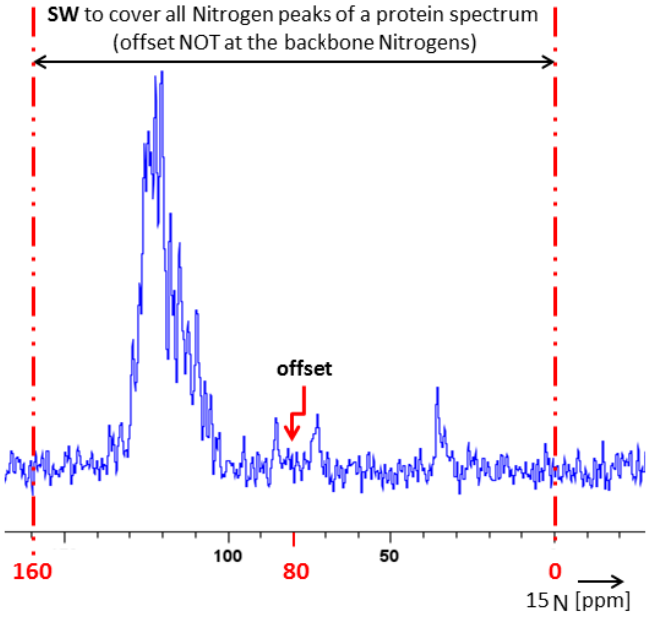

Figure 6.12: Reduced Spectral Width for a ^{15}N-detected HN CP Experiment on a Biological Sample.

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.

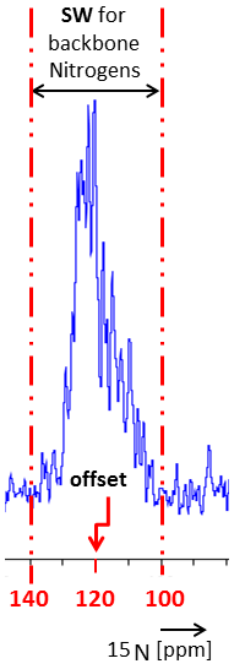

Figure 6.13: Small Spectral Width for a ^{15}N-detected HN CP Experiment on a Biological Sample.

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).

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.

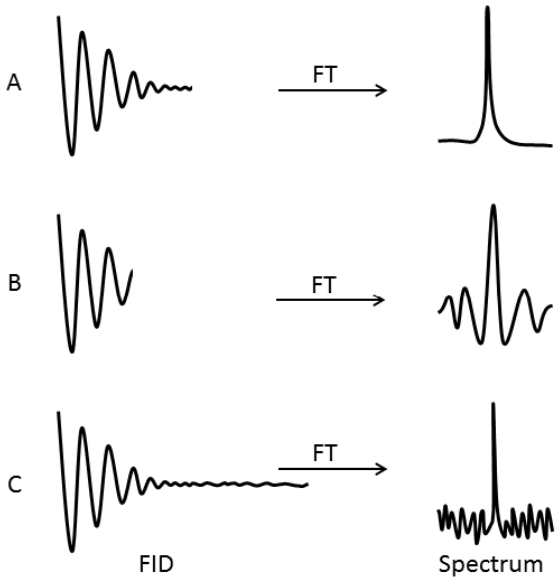

Figure 6.14: Different Acquisition Times Lead to Different Spectra.

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.

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 *Setup of Experiments on the Biological Sample of Interest [▶ 55]*).

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

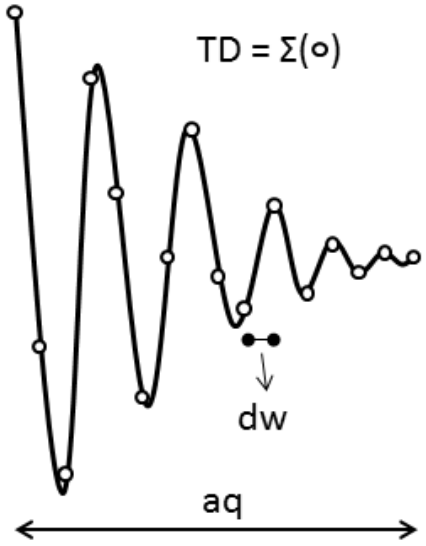

Figure 6.15: Schematic Illustration of Important Acquisition Parameters.

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

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.

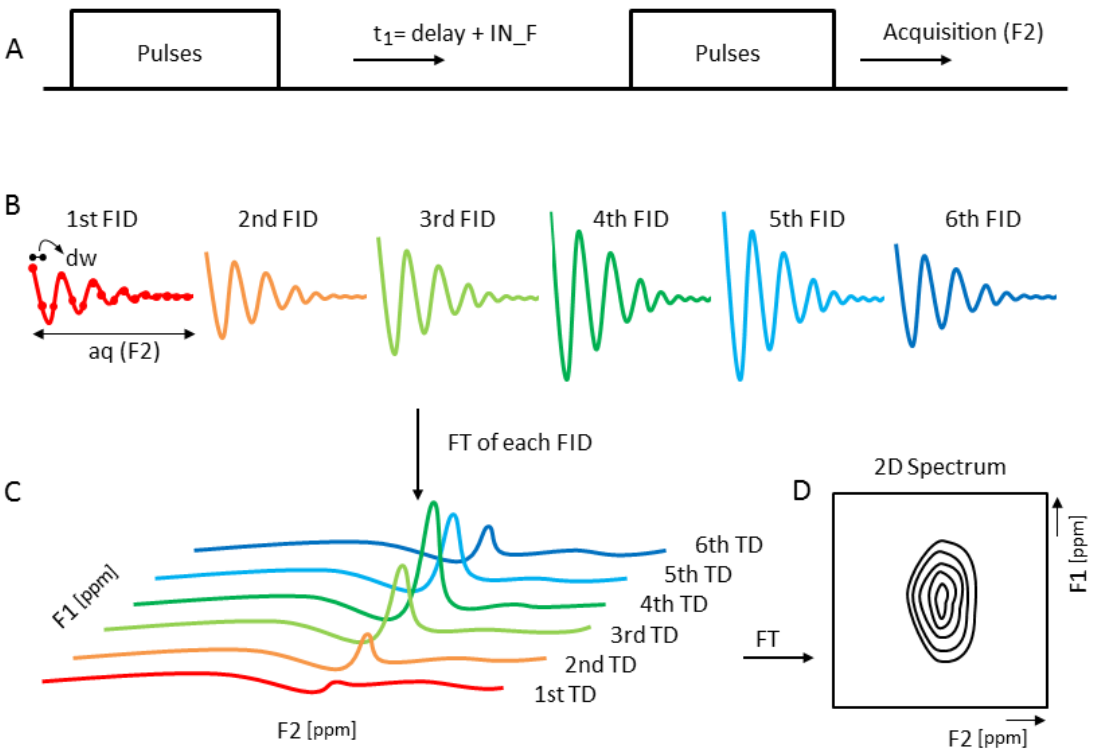

Figure 6.16: Schematic Illustration of Important Acquisition Parameters in Multidimensional (here: 2D) Experiments.

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): $aq_{F1} = ½TD_{F1} / SWH_{F1}$

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

- Equation (8): $aq_{F1} = ½TD_{F1} * IN\_F$

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:

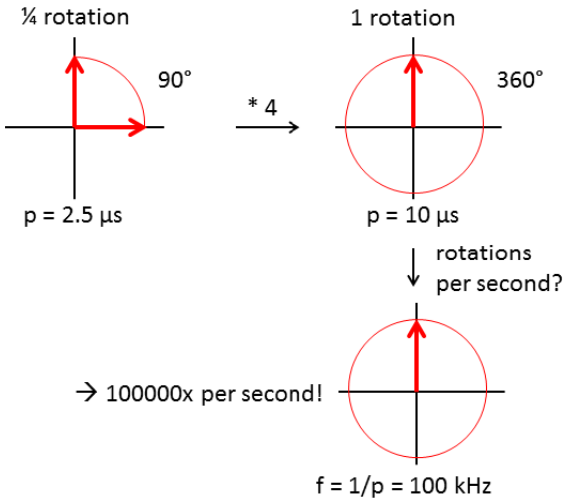

Figure 6.17: Converting the Length of a 90° Pulse p_{90} to its Frequency.

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:

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

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

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:

$$\nu = 90\ kHz = 90000\ Hz$$

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

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

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:

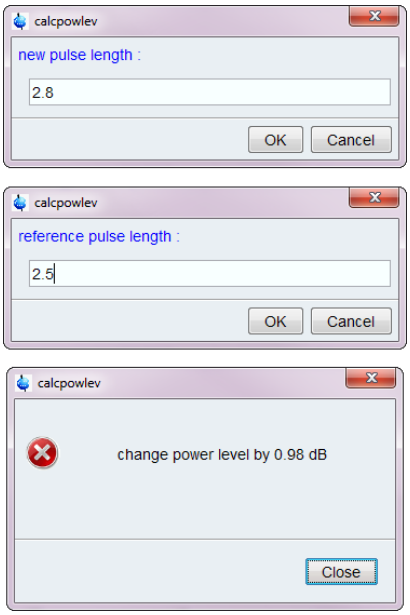

Figure 6.18: The AU Program calcpowlev.

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).

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.

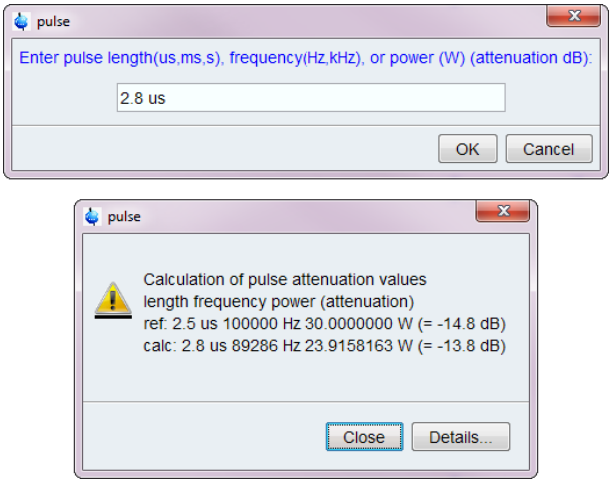

*Figure 6.19: The AU Program **pulse**.*

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:

$$pldB_2 = -20 \times \log_{10} \frac{\nu_2}{\nu_1} + pldB_1$$

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 ν_2:

$$\nu_2 = 10^{-0.05 \times (pldB_2 - pldB_1)} \times \nu_1$$

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 [▶ 45]*, we calibrated the ^{13}C spectrum on adamantane by setting the left peak to 38.48 ppm* (referenced to TMS, *References [▶ 113]* 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 [▶ 113]* - Ref. # 12).

* If ^{13}C spectra shall be referenced to DSS (DSS 0.5% in D_2O), 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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Appendix

7 Contact

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