APSY

Automated Projection Spectroscopy

User Manual

Version 003

Innovation with Integrity

NMR

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Contents

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1 Introduction

A*utomated* **P***rojection* **S***pectroscop***y** (APSY) gives access to N-dimensional NMR correlations (N ≥ 3) by means of recording and analyzing a series of two-dimensional projections. This procedure usually results in a drastic reduction of acquisition and analysis time for spectra of high dimensionality, compared to conventional schemes. The output of an APSY experiment is not a spectrum, but an N-dimensional peak list of high precision. Additionally, APSY offers the possibility to record projections iteratively with calculations of the peak list at each step until a convergence criterion has been reached. This feature minimizes the acquisition time and simultaneously ensures a high precision of the peak list.

More details about APSY can be found in the chapter *References [▷ 29]*.

This manual describes the usage of the APSY package in TOPSPIN, along with some background information about basic principles of projection spectroscopy.

The APSY package in TOPSPIN allows the convenient setup, the control, the execution and the reprocessing of APSY experiments with a graphical user interface.

Introduction

2 Commands, Parameters and Files

The APSY package relies on the recently introduced FnTYPE “projection spectroscopy”. APSY no longer requires dedicated pulse programs or parametersets. All suitable nD experiments (n ≥ 3) are now amendable for APSY. For the setup create a nD Dataset as would normally be acquired (e.g. FnTYPE traditional). The further handling of FnTYPE will then be done by the APSY program.

2.1 APSY Acquisition Parameters

Spectral widths SW and time domain data points TD: These spectral parameters are defined directly with eda in the common way. The figure below shows the top part of the acquisition parameter window for the 6,2 HNCOCANH experiment.

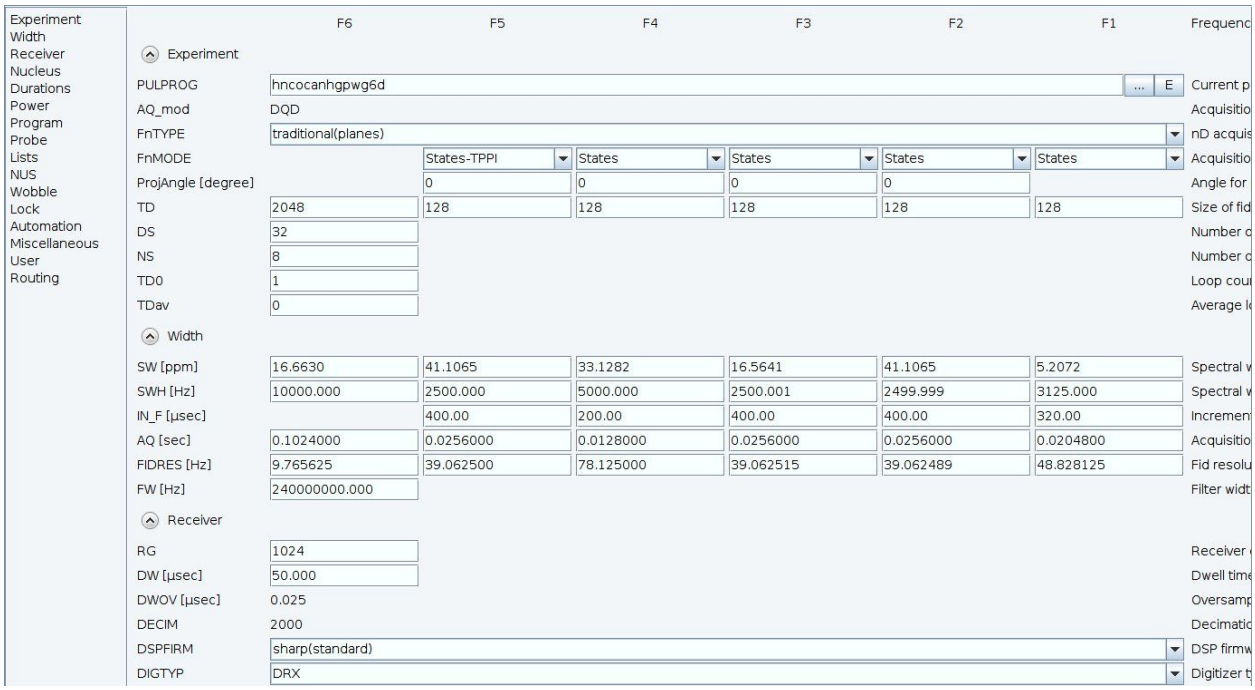


Figure 2.1: Acquisition parameters for the 6,2 HNCOCANH experiment. For the experiment parameters the FnMODE has to be States, except for the n-1 dimension (here F5) where the selection depends on the pulse program used, the time domain data points TD can be set according to the resolution required for the individual dimensions. For the HNCOCANH experiment, the nuclei H, N, CO, Calpha, and N are assigned to the F1, F2, F3, F4 and F5 dimension. The spectral windows have to be set accordingly.

Please note, that the entries for the nuclei in the indirect dimensions has to be defined, as well.

The APSY experiment will record 2-dimensional projections. The number of time domain data points used for projections at different projection angles will be calculated from the individual time domain data points from all indirect dimensions using the Euler equations. For a given nD experiment the projection will be onto the F(n-1) Fn plane.

In addition to the commonly used experiment parameters, such as spectral windows, offsets, pulse shapes and lengths, delays, etc., the recording of projections in APSY experiments requires some more parameters. They are defined during setup and handled by the APSY program.

Projection angles: N-2 projection angles describe the projection vectors for 2D projections of an N-dimensional experiment, as defined in the table below. They are defined during setup and stored in the parameter ProjAngle by the AU program manageapsy according to the entries of the projection angle file.

Dimension	N = 6	N = 5	N = 4	N = 3
ω1	*sin(δ)*	*sin(γ)*	*sin(β)*	*sin(α)*
ω2	*sin(γ)cos(δ)*	*sin(β)cos(γ)*	*sin(α)cos(β)*	*cos(α)*
ω3	*sin(β)cos(γ) cos(δ)*	*sin(α) cos(β)cos(γ)*	*cos(α)cos(β)*	-
ω4	*sin(α) cos(β)cos(γ) cos(δ)*	*cos(α) cos(β)cos(γ)*	-	-
ω5	*cos(α) cos(β)cos(γ) cos(δ)*	-	-	-

Table 2.1: The Euler equations

Experiment description (USERA5): The “experiment description” consists of the two elements **experiment-code** and **trosy option**. The experiment description is stored in the acquisition parameter **USERA5**. The definition has to be done by the user via the au program apsyuserA5 and the entry is evaluated by the AU program *manageapsy*.

- The **experiment-code** assigns a single letter to each dimension of the experiment, as defined in the appendix. The experiment code is also used to evaluate the chemical shift offset in the indirect dimensions. Details can be found below in the chapter Chemical shift offsets.
- The **trosy option** is required for trosy experiment in order to handle the phase correction properly for the different types of trosy experiments.

Chemical shift offsets: the calculation of the APSY peak list requires information about the chemical shift offsets of all indirect dimensions. If that information is incorrect, the APSY peak list will be wrong. Those chemical shifts offsets might differ from o1p, o2p as an example. Therefore constants like cnst18 are used in addition to define the offset. For that the experiment code of the string defined as acquisition parameter USERA5 is evaluated according to the assignment given in this tab.

As an example, for the 4,2 HNCOCA APSY experiment, which is defined by the experiment code AONH, the following parameters describe the chemical shift offsets:

Calpha: cnst22, CO: cnst21: 15N: o1p[F2](=3p), amide proton: O1p.

Please note the following: as the proton offsets in the 4,2 HNCOCA experiment is defined by O1P and no frequency jump is done during that experiment, the amide proton offset is identical to o1p, which corresponds to the frequency of the water proton.

2.2 APSY Processing Parameters

Size, phase correction and strip Fourier transformation: The processing parameters are defined directly with **edp** in the common way. The figure below shows the top part of the acquisition parameter.

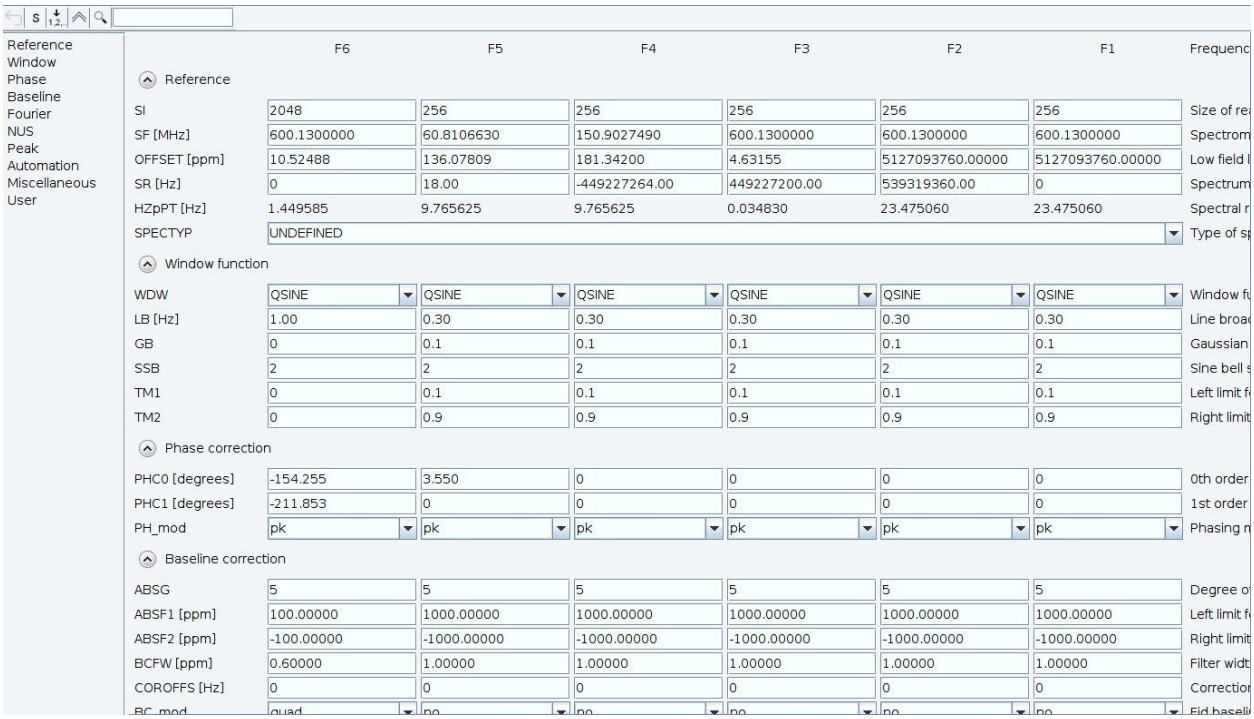


Figure 2.2: Processing parameter window of a 6-dimensional experiment.

As APSY will record 2-dimensional projection, the processing parameters of the n-dimensional parent data set will only partially be used. The processing parameters of the 2-dimensional projections will consist out of parameters of the acquisition and the original n-1 dimension.

Please note the following with respect to processing parameters:

- In case there are systematic additional phase corrections required in particular planes of a certain type of experiment (e.g. trosy, echo anti echo) manageapsy will automatically handle these.
- PH_mod has to be set to *pk* for the direct and indirect dimension.
- The values for the the phase correction constants PHC0 and PHC1 can be set before acquisition but also before re-processing.
- Strip Fourier transformation is allowed for the acquisition dimension, but not for the indirect dimensions.
- Processed data of an APSY experiment are 2-dimensional spectra. Therefore high values for the processing size SI are not an issue.

2.3 APSY Data Files

Data set organization in TopSpin: An APSY experiment is set up and started in a “parent data set” (filename/expno). The individual projections are acquired and stored in “child data sets”, which are created automatically (in filename_expno).

Example: The setup was done in a data set with the name APSY_HNCOCA and EXPNO 83. This is the parent data set. The child data sets will have the name APSY_HNCOCA_83 and consecutive EXPNO’s, starting with 1.

Dimensionality of parent and child data sets: The dimensionality of the parent data set corresponds to the dimensionality of the APSY experiment. The same holds for the child dataset. During the setup:

- Time domain data points TD are calculated from the individual entries of TD in the parent data set according to the Euler equations. In addition, the time domain data points are multiplied by a factor describing the number of projections and a factor given by the loop structure of the pulse program. Increments are also recalculated according to the Euler equations.
- The processing parameters correspond to those defined in the n-1 dimension of the parent data set.

Interleaved projections and file recombination: Depending on the projection angle, one acquired dataset results in one or more spectra. As an example in the 3,2 HNCO APSY experiment a projection angle of 0° or 90° gives one spectrum each, a projection angle of 30° results in two spectra corresponding to the projection angle +30° and -30°. As the +/- angle projections are recorded in an interleaved mode, the acquired SER file has to be split.

Assuming a 3,2 APSY experiment and that in EXPNO 3 and 4 the projection angle is +/- 30°. The acquisition of two interleaved projections will be done in EXPNO 1003 the linear combination of the two interleaved components will be stored in expnos 3 and 4, corresponding to projection angles +30° and -30°. This is done by the AU program manageapsy. While procno 1 still has the dimensionality of the nD experiment, Fourier Transforms will produce the 2D spectrum in procno 101.

In order to illustrate the data set handling the data browser of a 3,2 HNCO APSY experiment is shown in the figure below.

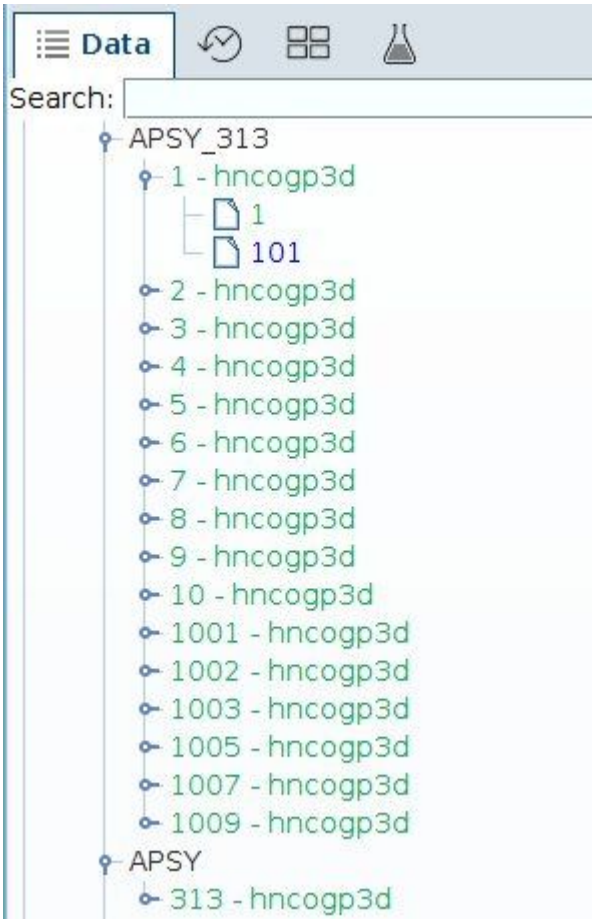


Figure 2.3: Expanded dataset browser of a 3,2 HNCO APSY experiment. The parent data set is APSY_HNCO with EXPNO 10. The NAME of the child data sets is APSY_HNCO_10. The experiment numbers EXPNO 1 to 8 have been created by APSY control panel after setting up the APSY series. In EXPNO 3, 5 and 7 the projection angle was 30°, 45° and 60°. This will result in two projection spectra, each, which are to be recorded in an interleaved mode. This is done in EXPNO 1003, 1005 and 1007.

The interleaved data are recombined in EXPNO 3 and 4 (source EXPNO 1003), in EXPNO 5 and 6 (source EXPNO 1005) and in EXPNO 7 and 8 (source EXPNO 1007). For the orthogonal planes (0° , 90°) the acquisition also happens in expnos 1001 and 1002. Data are then stored in expnos 1 and 2.

2.4 AU and Binary Programs

manageapsy: The AU program 'manageapsy' is the main program for control of the setup, the acquisition, the processing and the analysis of APSY experiments.

gapro: The binary file gapro is used for peak picking of the projection spectra and the final geometry analysis which will result in the final APSY peak list. In literature this procedure is described under the name GAPRO.

Files required for and created by GAPRO and APSY: the following files are stored in the parent data set and will be used and/or created by APSY and GAPRO, as shown in Table 4. The program PROSA is described in literature (3) and is not part of the APSY package.

file name	**description**	required by	created by
angles.dat	projection angles	manageapsy	manageapsy
procmodule	temporary protection file	manageapsy	manageapsy
experiment.gap	frequencies, type of experiment	gapro	manageapsy
parameter.gap	GAPRO processing parameters	gapro	manageapsy
spectra.gap	list of available projections	gapro	manageapsy
<xxx>.peaks	final peak list		gapro
APSY.log	log file of GAPRO		Gapro
APSY_setup.log	log file for setup		manageapsy
Analysis.dat	peaks and their support		Gapro
VsAPSY.txt	Version number		Manageapsy
sum_term_gag.txt	gapro communicator protocol		gapro

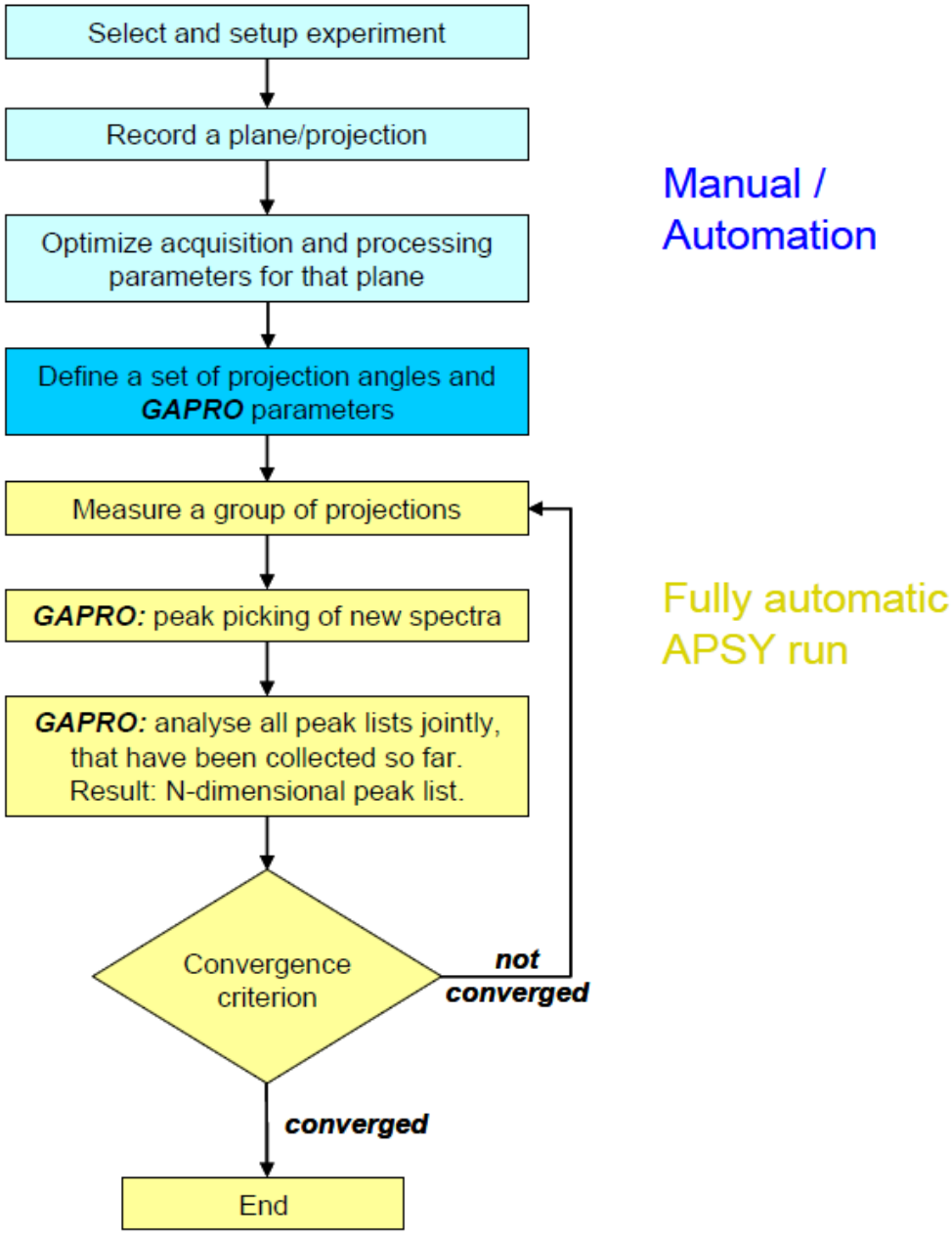
Table 2.2: Files required and created by GAPRO and APSY.

<xxx>.peaks is the experiment-code.

3 Getting Started

3.1 Overview

The flowchart in below shows the sequence of individual steps comprising the setup and execution of an APSY run.



Flowchart of an APSY experiment.

An APSY experiment has two main parts: In the first part (marked in Blue), the experiment is selected and setup. This is often done manually, but it can be done automatically using standard parameter sets, if desired.

In the second part (marked in Yellow), the APSY experiment runs in a fully automatic fashion.

3.2 Basic Setup and Processing of an APSY Experiment

3.2.1 The APSY Flow BAR and Command Panel

Setup, processing and acquisition of APSY experiments are supported by the APSY flow bar or a graphical command panel. The APSY flow bar can be started from the spectrometer menu of Topspin or by entering the command **apsy**. Alternatively the command panel, which was used in earlier Topspin versions, can be started with the command **bpan apsypan.** The APSY flow bar is shown in the figure below, the APSY command panel is shown in the following figure. The functionality of the flow bar and the command panel are identical. Details of the graphical user interface are described in the *Appendix [▸ 25]*.

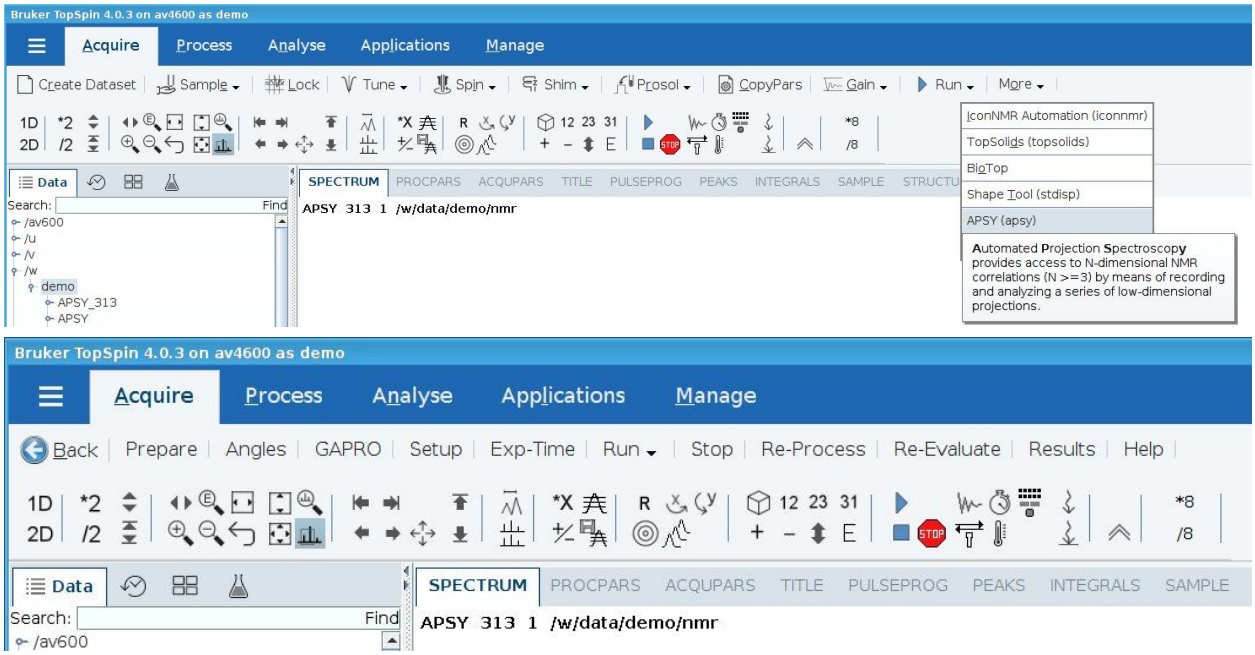


Figure 3.1: Starting APSY from the spectrometer menu (top) and the APSY flow bar (bottom).

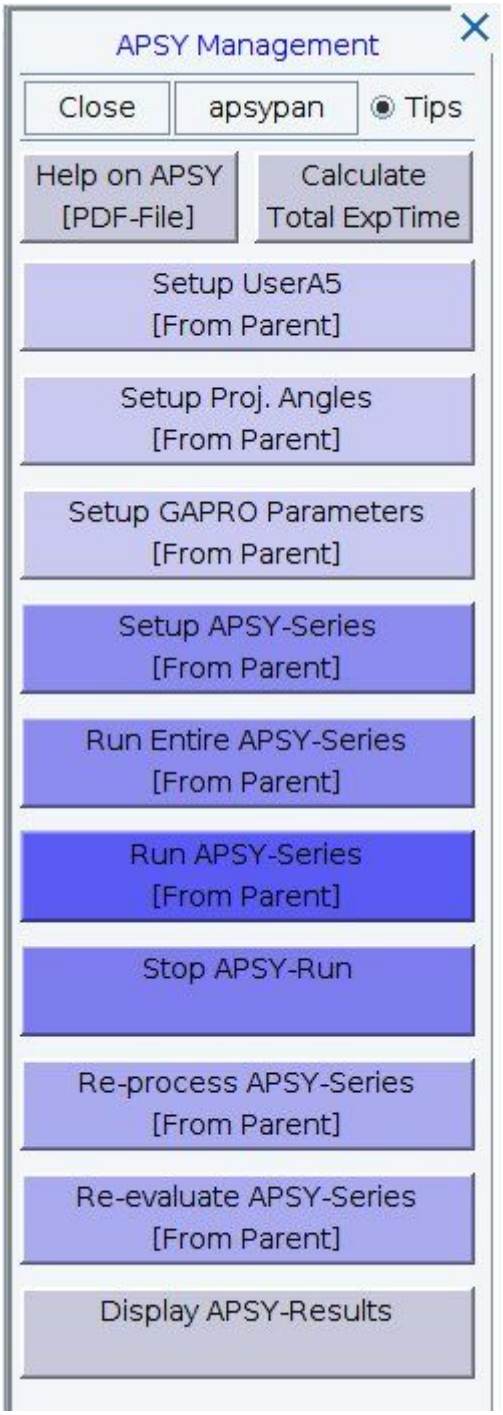


Figure 3.2: The APSY command panel.

3.2.2 Load Default Parameters and Basic Adjustments

Starting from a standard parameter file, the setup procedure is as follows. It is assumed that the probe is well tuned and matched, the sample is shimmed, the proton pulse, all frequency offsets have been adjusted and the spectral windows for indirect dimensions are known.

1. Load a parameterset for the desired experiment.
2. Load pulse parameters with **getprosol**. Do not forget to set the correct values of the proton pulse.

3. Define the desired number of scans.
4. Check the spectral widths and the number of increments in the individual indirect dimensions with **eda**.
5. Check the parameters for the offsets which are defined by different constants or offsets. The (To Do: tab 7?) table on page 7 given an overview of the constants used. Alternatively the comments of the pulse program can be checked. The offset parameters are used to define the centre frequencies for calculation of the APSY peak list, where it differs form O1p(Fn). Adjust the receiver gain.
6. Start the acquisition with **zg**. This will record a single projection in the parent data set, as defined by the parameter file.
7. Optimize the processing parameters. The processing parameters will be applied for all consecutive spectra and therefore have to be adjusted on the parent data set. There is nothing unusual, just ensure that the number of points SI2, SI1 and parameters for linear prediction are optimum and the phase is well adjusted in the F2 dimension. Two operations are not recommended for APSY experiments:
   - Strip FT is NOT allowed for the indirect (F1) dimension, since it will result in false values for chemical shifts in the indirect dimensions of the final peak list. Strip FT is allowed in the direct (F2) dimension.
   - Phase correction values in the indirect (F1) dimension different to (0, 0) for 0^{th} and 1^{st} order.

3.2.3 Define Projection Angles and Start the APSY Run

1. On the APSY panel select ‘Setup Proj. Angles’. Alternatively select Angles in the APSY flow bar. Default values for the projection angles will be loaded and might be changed. This step is optional. If it is skipped, the default angles are used for the APSY run.
2. On the APSY panel select ‘Setup GAPRO Parameters’. Alternatively select GAPRO in the APSY flow bar. Default values for the GAPRO parameters are loaded and might be changed. This step is optional. If it is skipped, the default parameters are used for the APSY run.
3. The APSY experiment can be started in two options:
   - Record all projections which are defined by the set of projection angles: On the APSY panel, select ‘Run Entire APSY-Series’, on the flow bar select “run”.
   - Run projections and calculate the result iteratively, stop, as soon as a convergence criterion has been fulfilled: On the APSY panel, select ‘Run APSY-Series’, on the flow bar select the arrow pointing down and select “Run APSY-Series”.

3.2.4 Inspecting the Result

The result of an APSY experiment is a peak list. This peak list is stored as text file in the data set directory of the parent data set under the name xxx.peaks, where xxx is the experiment code (see Appendix). The peak list together with additional information can be viewed by selecting ‘Display APSY-Results’ on the APSY panel. . Alternatively select Results in the APSY flowbar. 2D projections of the final APSY peak list are calculated for each individual projection and stored in the corresponding data set. This allows a fast verification of the quality of the result. It is advisable to look through all projections. If the peak list does not fit the spectral data, an error has occurred. An example of a projection with the projected result peak list is shown *Figure 3.3 [▷ 17]*.

3.2.5 Optimizing the Result

Once the projections have been recorded, the quality of the APSY peak list might be optimized by changing GAPRO processing parameters. For changing the GAPRO processing parameters, select ‘Setup GAPRO parameters’ on the APSY panel. . Alternatively select GAPRO in the APSY flowbar.

Three parameters have the main impact on the quality of the result:

1. ***S/Nratio***: this is the signal-to-noise threshold for the GAPRO-internal peak picking routine. Typical values are 3–10.
2. ***Threshold:*** This defines the threshold for the GAPRO-internal peak picking as fraction of the highest peak. Between S/Nratio and Threshold the larger of the two values is eventually used.
3. ***Smin1 and Smin2***: this parameter defines the minimum support required to create an N-dimensional correlation out of the peak lists from the projections. The minimal value possible is N-1 for 2D projections. Typical values with good artifact suppression are N+1 or N+2.

After changing GAPRO processing parameters, select ‘Re-evaluate APSY-Series’ on the APSY panel. Alternatively select Re-evaluate in the APSY flowbar. Peak picking will be done on all projections and the APSY peak list will be recalculated.

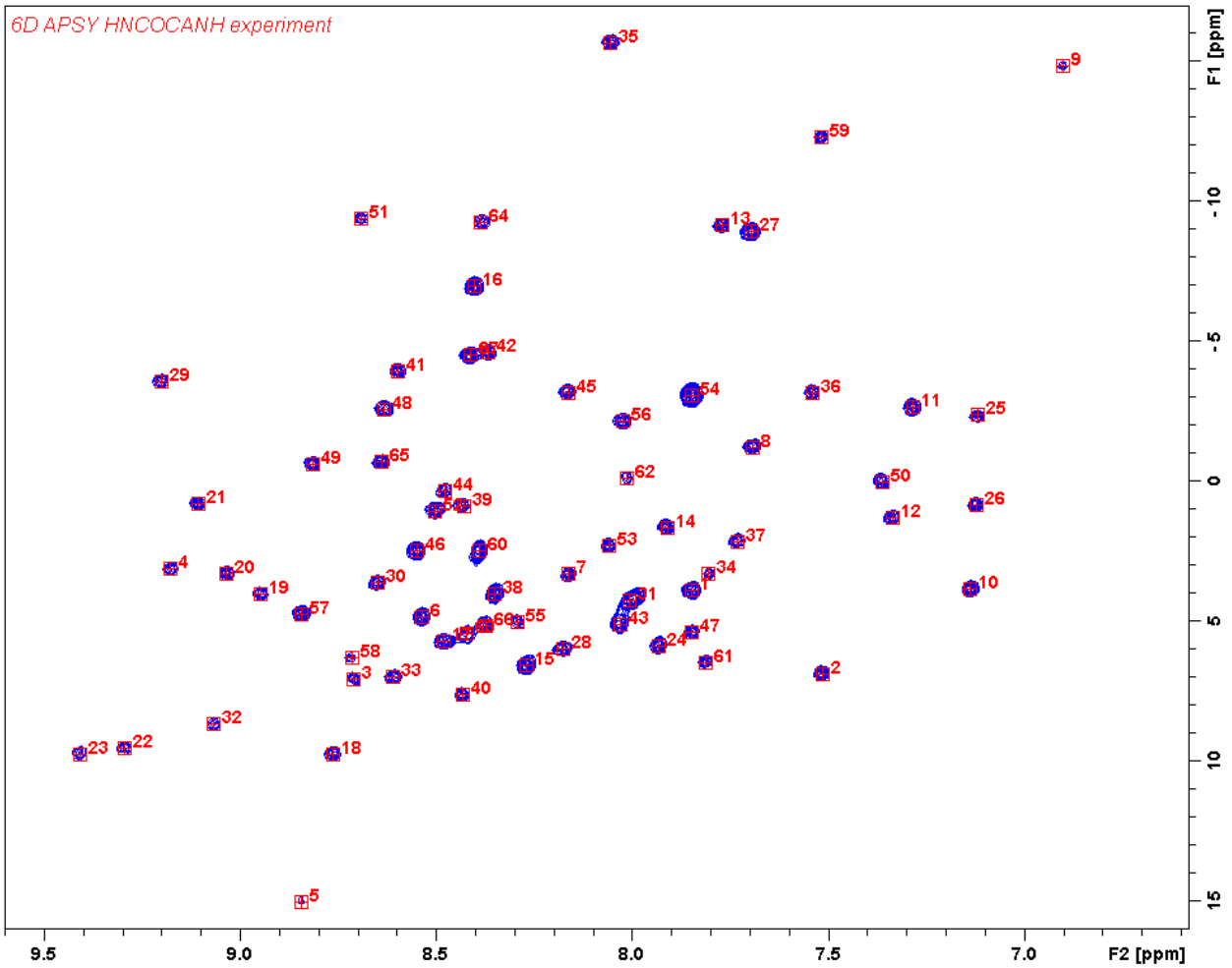
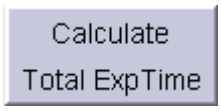


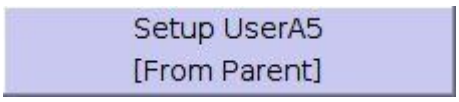
Figure 3.3: 2D projection of a 6D APSY-HNCOCANH experiment of ubiquitin at 500 MHz. The peak list shown is a projection of the resulting 6D APSY-peak list and allows inspecting the quality of the result.

4 Description of the APSY Command Panel

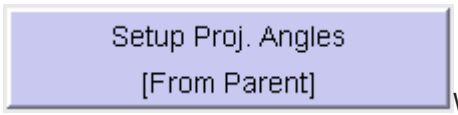
Opens this APSY manual



Calculates the experiment time for a single projection and for the whole set of projections defined in the angles file. If the APSY-run is executed with a convergence criterion, the total experiment time can be shorter than calculated with this option, because the run stops as soon as the convergence criterion is fulfilled.



Starts the AU program to setup the string for the acquisition parameter USERA5 (see *Experiment description [▶ 7]*). This comprises the definition of the experiment code and the trosy option.



With this button, the set of projection angles can be defined. Once this button has been pressed, a text editor will be opened. When the file has been changed, use File and Save to store the changes. This button should be used in a parent data set. The format of the projection table will automatically adapt to the dimensionality of the experiment. The figure below shows an example of a projection angle file for a 6-dimensional experiment.

The loaded projection angles are so-called matched projection angles, optimized for the spectra width of the indirect dimensions.

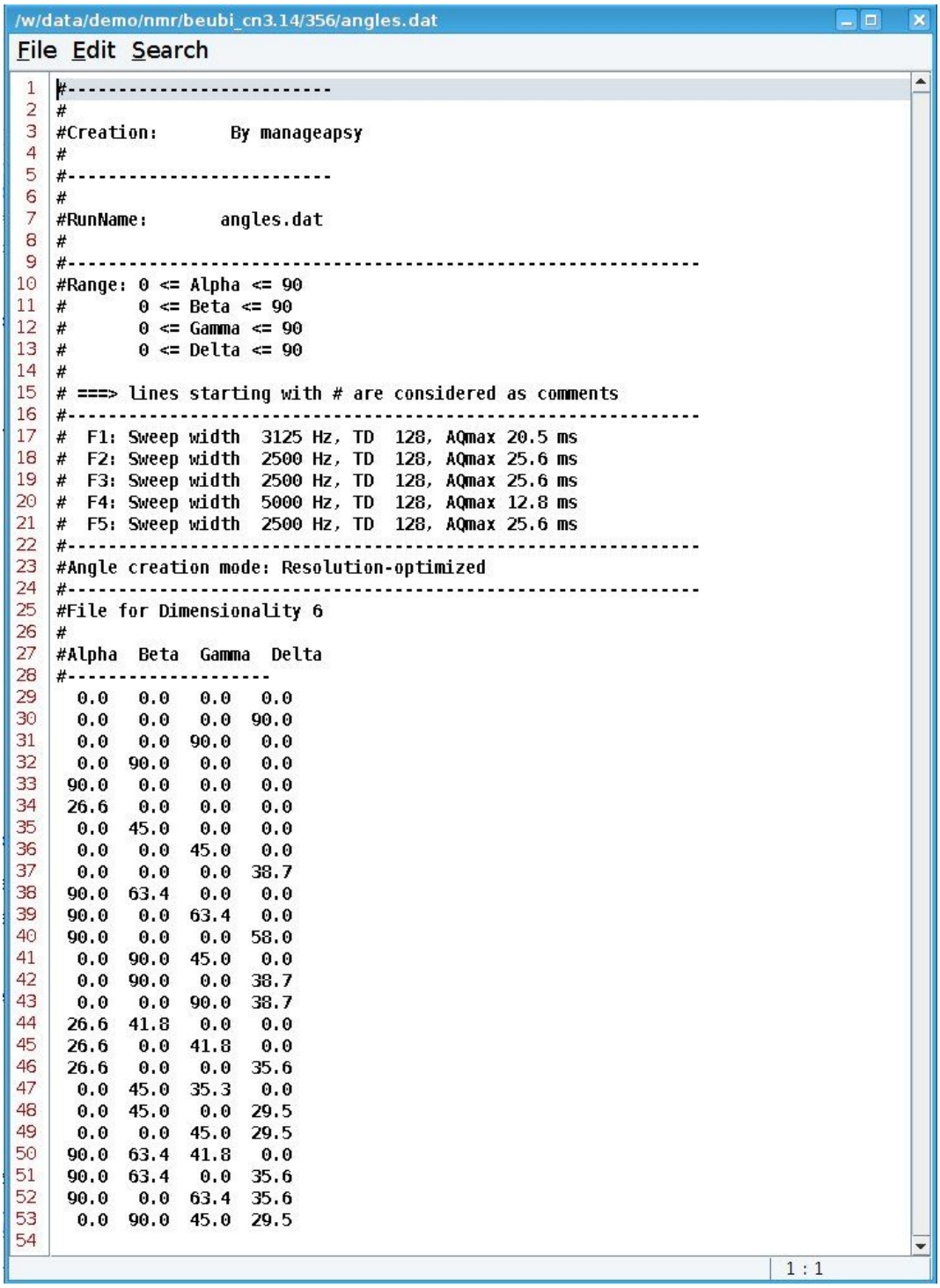
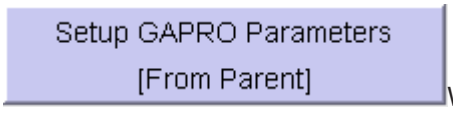


Figure 4.1: The text editor showing an angle file for a 6-dimensional experiment.



With this button, the parameters for peak picking and calculations of the final peak list by GAPRO can be edited. Once this button has been pressed, the text editor will be opened. When the file has been changed, use File and Save to store the changes. This button should be used in a parent data set. The figure below shows an example of a parameter file for a 4-dimensional experiment.

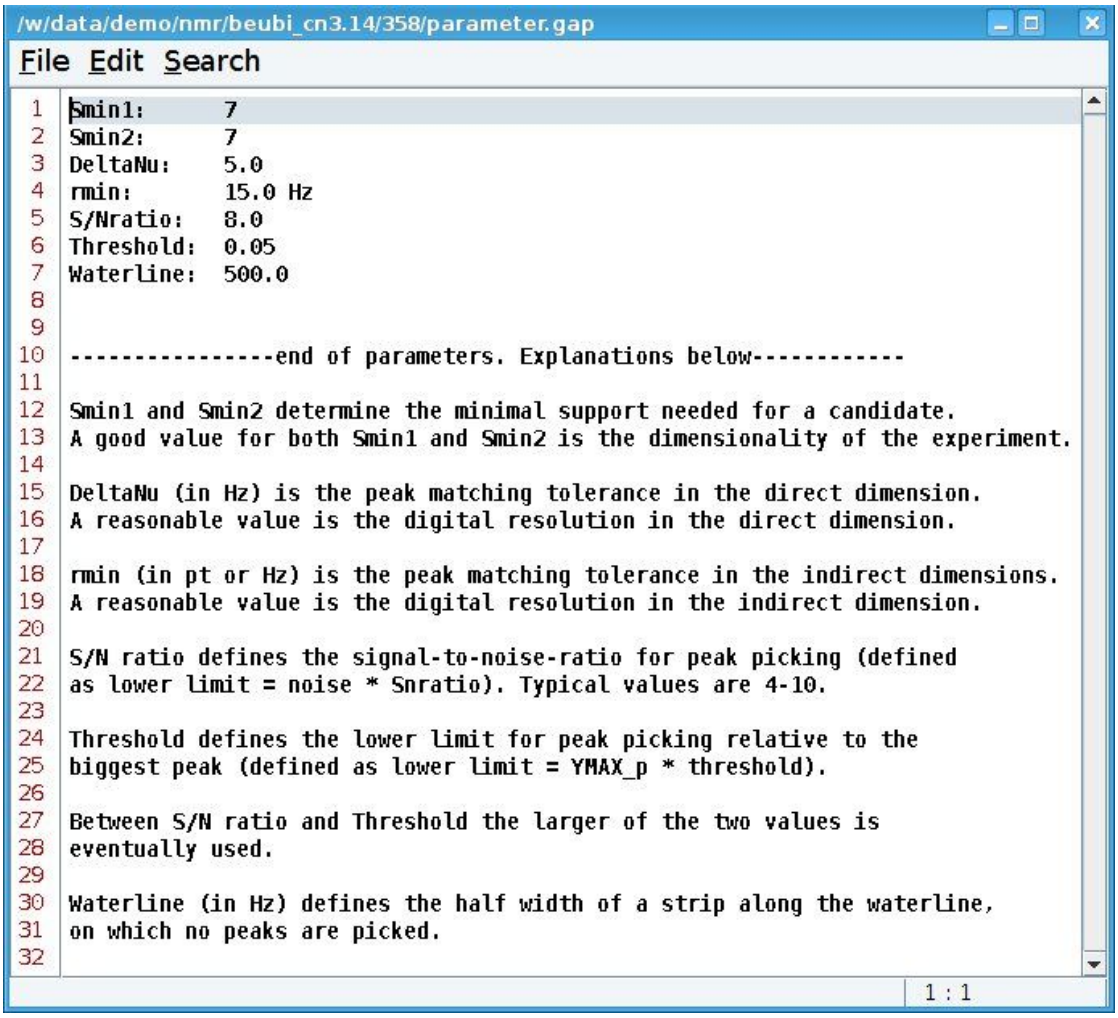


Figure 4.2: The text editor showing typical GAPRO processing parameters for a 4-dimensional experiment.

The processing parameters have the following function (see also (1)): (To Do: chapter?)

Parameters for peak picking:

S/Nratio: The signal-to-noise threshold for peak picking. Peaks with a lower value will not be picked. Typical values are 3–10.

Threshold: This defines the threshold for the peak picking as fraction of the largest peak [default 0.05]. Between S/Nratio and threshold the larger of the two values is eventually used. Please note that the computation time of GAPRO increases with the number of picked peaks.

Waterline: Defines the half-width of a strip centred on the carrier in the direct dimension (often the water line), which is excluded from peak picking. The unit is Hz.

Parameters for geometric analysis:

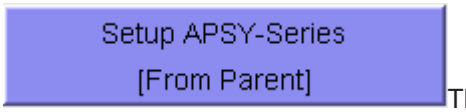
Smin1 and Smin2: The minimum support of a candidate point calculated from the intersection of subspaces. Typically the value is identical are larger than the dimensionality of the experiments, e.g. for a 4,2 HNCOCA, Smin1 and Smin2 are 4–6. When the value is lower than the dimensionality, the calculation of the peak list might stop with an error. Values between 0 and 1 are interpreted as fractions of the number of projections recorded.

DeltaNu: The tolerance for intersections in the direct dimension. A typical value is the digital resolution in Hz.

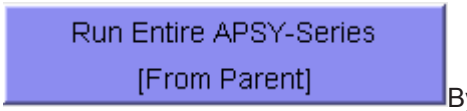
rmin: The tolerance for intersections in the indirect dimension. Values can be given either absolute in Hz or relative to the digital resolution in pt, e.g. 7.0 Hz or 0.6 pt.

To Do: neuer Screenshot für userA5

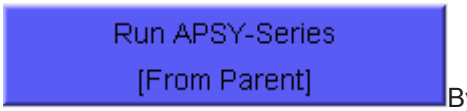
This tool can be used to correctly populate the userA5 shims. This comprises the definition of the experiment code and the trosy option.



The Button generates a series of child data sets according to the projection angles. The number of child data sets depends on the number of projection angles and the angles used. This option should be used in a parent data set. No Acquisition will be started. Data sets created with this button can subsequently be recorded without the APSY control panel [**apsyrun** or **fullapsyrun**].

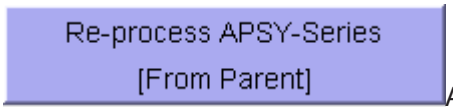


By selecting this button a complete APSY run including acquisition will start. The data sets for the individual projections (expno ≥ 1001) will be generated just prior to the start of the acquisition of a given projection. This function should be used in a parent data set. If this button is selected without prior definition of projection angles and GAPRO parameters, the default projection angles and GAPRO parameters are used. When starting APSY using this option, the convergence criterion is not used and projections for all angles will be recorded. [= **fullapsyrun**]

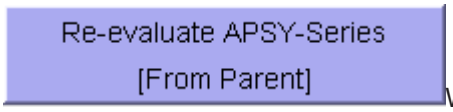


By selecting this button a complete APSY run including acquisition will start. The data sets for the individual projections (expno ≥ 1001) will be generated just prior to the start of the acquisition of a given projection. This function should be used in a parent data set. If this button is selected without prior definition of projection angles and GAPRO parameters, the default projection angles and GAPRO parameters are used. When starting APSY with this option, the acquisition stops once the convergence criterion has been fulfilled. The standard convergence criterion is fulfilled, if the number of peaks converges. Alternative convergence criteria can be implemented by the user in the au program manageapsy. [= **apsyrun**]

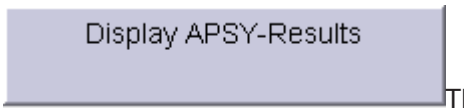
An active APSY-run can be stopped at any time using this button. After pushing this button, the acquisition of projections stops as soon as all data belong to the current projection angles have been recorded. The APSY peak list will be calculated using the available projections. This button can be used in parent as well as in child data sets.



After processing parameters have been changed in the parent, all spectra recorded for an APSY-run can be reprocessed. This includes Fourier transformation, baseline correction and recombination of 2D spectra. Please note that reprocessing has to be started on the parent data set.



With this button, the spectra of an APSY experiment can be reevaluated. Peak picking on all existing projections and recalculation of the APSY peak list according to the GAPRO parameters will be performed. Please note that the re-evaluation has to be started on the parent data set.



The display of the APSY results will display the following information:

- **<experiment>.peaks:** the final N-dimensional peak list, where <experiment> is the experiment code (see *Appendix [▶ 25]*).
- **angles.dat:** list of projection angles
- **parameter.gap:** GAPRO calculation parameters
- **experiment.gap:** experimental parameters used for calculation of the APSY peak list
- **spectra.gap:** list of all recorded projection spectra
- **APSY.log:** detailed log file of all GAPRO calculations.

This additional information can be used to judge the quality of the result and to optimize GAPRO processing parameters.

Manageapsy also fills the string for AUNM with manageapsy fullapsyrun. The argument can be changed to apsyrun. This allows to start the APSY run with **xaua**. Several APSY runs can be queued via the spooler by typing **qu xaua**.

5 Appendix

5.1 Description of the Manageapsy Options

Alternative to using the APSY panel, the AU-program manageapsy can be started with options and without the use of the graphical user interface, by typing manageapsy <option> in the command line. The following options are available:

help	Open APSY manual
setprojang	Open routine for definition of projection angles.
setpagean	Open routine for definition of GAPRO processing parameters.
expttime	Calculate maximum experiment time.
apsyprepare	Create data sets for all projection angles.
apsyrun	Start fully automated APSY run. Stop recording further projections when convergence criterion is fulfilled.
fullapsyrun	Start fully automated APSY run for all projections.
reprocess	Reprocess data.
neweval	Generate peak list and evaluate with GAPRO.
stopapsyrun	Stop an APSY run.
version	Show version of APSY.
removeprotect	Remove protection for manageapsy.

For example, entering **manageapsy apsyrun** will start a fully automated run of an APSY experiment using default values for projection angles and GAPRO processing parameters.

5.2 Definition of the Experiment Code

The experiment-code describes the experiment with a 1-letter code for each dimension using the following definition:

1-letter code	nucleus	description	rel. residue position	offset
J	^{1}H	amide proton	i-1	Cnst19
j	^{1}H	amide proton	i& i-1	Cnst18
H	^{1}H	amide proton	i	Cnst18
Z	^{1}H	alpha proton	i-1	Cnst18
z	^{1}H	alpha proton	i & i-1	Cnst18
Y	^{1}H	alpha proton	i	Cnst18
M	^{15}N	amide nitrogen	i-1	Cnst29
m	^{15}N	amide nitrogen	i & i-1	Cnst29
N	^{15}N	amide nitrogen	i	Cnst29
A	^{13}C	alpha carbon	i-1	Cnst22
a	^{13}C	alpha carbon	i & i-1	Cnst22
E	^{13}C	alpha carbon	i	Cnst22
O	^{13}C	carbonyl carbon	i-1	Cnst21
o	^{13}C	carbonyl carbon	i & i-1	Cnst21
U	^{13}C	carbonyl carbon	i	Cnst21
B	^{13}C	beta carbon	i-1	Cnst23
b	^{13}C	beta carbon	i & i-1	Cnst23
D	^{13}C	beta carbon	i	Cnst23
I	^{1}H	all aliphatic protons	i-1	Cnst24
K	^{1}H	all aliphatic protons	i	Cnst24
C	^{13}C	all aliphatic carbons	i-1	Cnst25
G	^{13}C	all aliphatic carbons	i	Cnst25

Table 5.1: The experiment code which is used for the description of an APSY experiment.

Examples:

- The 4D APSY-HNCOCA experiment
  H → … → Calpha (i-1, t1) → CO (i-1, t2) → N (i,t3) →H (i, acquisition, t4)
  This experiment gets the identifier AONH.

5.3 Frequently Asked Questions

The values of the chemical shifts in the final APSY peak list are wrong:

In the APSY command panel, select Display APSY Results and check the entries in the file 'experiment.gap'. The entries for Carries1, Carrier2 and so on have to correspond to the chemical shift offsets in the t1, t2, t3,.. dimension. The entries for those carries frequencies are taken from constants as shown in Table 3.

The content of the file 'experiment.gap' of a NOAH experiment is shown below. The carries Carrie1, Carrier2, Carrier3 and Carries4 correspond to 15N, CO, Calpha and proton offsets, respectively.

DimensionalityProjections	2
DimensionalitySpace:	4
Carrier1:	118.000000
Carrier2:	177.000000
Carrier3:	56.500000
Carrier4	4.700000
Hfreq:	500.230000
Nfreq:	50.687865
Cfreq:	125.782598
Experiment:	NOAH

Table 5.2: *** experiment.gap ***

Only positive peaks are picked in an experiment.

The peak picking routine of GAPRO picks positive peaks only. In order to pick the negative peaks, for all spectra change PHC0 by 180o and reprocess all spectra. Before the original APSY peaks list shall be stored under a different name.

GAPRO calculation times are very long.

The computation time basically depends on the number of peaks found in the projections. If the peak picking threshold is too low, the peak picker will also add noise peaks to the calculation. This will increase the calculation time. It therefore is recommended to start with a rather high peak picking threshold value (e.g. 20 - 30), do the calculation and subsequently reduce the peak picking threshold until a satisfactory result is obtained.

GAPRO calculation does not create a peak list.

1. Select Display APSY results on the APSY panel and check the result of the peak picking. Is the peak picking threshold correctly set, how many peaks are found? Below is an example from the peak picking protocol:

--*- Starting GAPRO in automatic mode -*-*-*
Automatic peak picking of topspin spectrum
Noise level: 808985.125000
Picking maxima above: 24269554.000000
65 Peaks found.
--*- Regular termination of GAPRO -*-*-*

2. Check the values for the GAPRO parameters. The entries for *Smin1* and *Smin2* shall be identical or higher than the dimensionality of the experiment. For a 6D experiment set *Smin1* and *Smin2* to 6 or higher.

Appendix

6 References

1. S. Hiller, F. Fiorito, K. Wüthrich & G. Wider, Proc. Natl. Acad. Sci. USA **102**, 10876–10881 (2005).
2. F. Fiorito, S. Hiller, G. Wider & K. Wüthrich, J. Biomol. NMR **35**, 27–37 (2006).
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References

7 Contact

Manufacturer

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

WEEE DE43181702

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