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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 low-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 literature (1, 2).

This manual describes the usage of the APSY package in TOPSPIN, along with some background information about basic principles of projection spectroscopy, helping the user to adapt standard library pulse programs of TOPSPIN to APSY.

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

A license is required for APSY.

Commands, parameters and files

The APSY package uses partially new definitions in the parameter files, pulse programs and AU programs, which are presently introduced:

APSY Experiment names

1. The parameter file names start with the string *APSY*, followed by the dimensionality of the experiment, the dimensionality of the projections and the name of the experiment. As an example, the parameter file for 2D projections of a 4D HNCOCA experiment is *APSY_HNCOCA_42*. The parameter file can be loaded with the command 'rpar'. Please note that the dimensionality of the parameter file is identical to the dimensionality of the experiment, and not identical to the dimensionality of the projection
2. The file name of the pulse programs uses the same nomenclature, however with lower case letters and starting with *rd* rather than with *apsy*. As an example, the file name for the 4,2 HNCOCA experiment is *rd_hncoca_42*, and the file name for the 3,2 HNCA experiment is *rd_hnca_32*.

APSY Acquisition Parameters

Spectral widths *SW* and time domain data points *TD*: These spectral parameters are defined directly with *eda* in the common way. *Fig. 1* shows the top part of the acquisition parameter window for the 6,2 HNCOCANH experiment.

	F6	F5	F4	F3	F2	F1
▼ Experiment						
PULPROG	rd_hncocanh_62 ... E					
AQ_mod	DQD					
FnMODE		States	States	States	States	States
TD	2048	64	128	48	64	64
NS	16					
DS	4					
TD0	1					
▼ Width						
SW [ppm]	16.0182	35.5073	32.9916	11.1297	35.5073	3.3984
SWH [Hz]	8012.820	1800.000	4150.000	1400.000	1800.000	1700.000
IN_F [µs]		555.56	240.96	714.29	555.56	588.24
AQ [s]	0.1278452	0.0177778	0.0154217	0.0171429	0.0177778	0.0188235
FIDRES [Hz]	3.912510	28.125000	32.421875	29.166666	28.125000	26.562500
FW [Hz]	125000.00					

Figure 1: Acquisition parameters for the 6,2 HNCOCANH experiment. For the experiment parameters the FnMODE has to be States, the time domain data points TD can be set according to the required 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.

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.

The following table shows the additional parameters used for a 6D APSY-experiment. These parameters are explained in detail below.

Information		**Parameter**	**Set by**
Projection angles	α	cnst51	manageapsy
	β	cnst52	manageapsy
	γ	cnst53	manageapsy
	δ	cnst54	manageapsy
Acquisition and processing control		usera4	user
Experiment description		usera5	user

Table 1: Some additional acquisition parameters for APSY

Projection angles: N-2 projection angles describe the projection vectors for 2D projections of an N-dimensional experiment, as defined in the table below. These angles are stored in the parameters *cnst51*, *cnst52, …* by the AU program *manageapsy* according to the entries of the projection angle file.

Dimension	***N* = 6**	***N* = 5**	***N* = 4**	***N* = 3**
ω_1	$sin(\delta)$	$sin(\gamma)$	$sin(\beta)$	$sin(\alpha)$
ω_2	$sin(\gamma)cos(\delta)$	$sin(\beta)cos(\gamma)$	$sin(\alpha)cos(\beta)$	$cos(\alpha)$
ω_3	$sin(\beta)cos(\gamma)cos(\delta)$	$sin(\alpha)cos(\beta)cos(\gamma)$	$cos(\alpha)cos(\beta)$	-
ω_4	$sin(\alpha)cos(\beta)cos(\gamma)cos(\delta)$	$cos(\alpha)cos(\beta)cos(\gamma)$	-	-
ω_5	$cos(\alpha)cos(\beta)cos(\gamma)cos(\delta)$	-	-	-

Table 2: The Euler equations.

Acquisition and processing control: The AU program aqapsy is required to control the acquisition and the processing during an APSY experiment. The user definable parameter *usera4* is used to define this AU program.

Experiment description: The "experiment description" consists of the three elements ***dimensionality,*** ***experiment-code*** and ***quadrature-mode***. The experiment description is stored in the acquisition parameter *usera5*. The definition

has to be done by the user and the entry is evaluated by the AU program *manageapsy*.

- The ***dimensionality*** of the experiment: allowed values are 3D, 4D, 5D, 6D and 7D.
- 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*.
- Method for ***quadrature-mode***: allowed values are st and ea, for States-TPPI and echo-antiecho, respectively.

For example, for the 4D APSY-HNCOCA experiment with States-TPPI quadrature detection, the value for usera5 is **4D NOAH st**.

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 to define the offset. For that the experiment code of the string defined as acquisition parameter *usera5* is evaluated according to the following assignment:

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

Table 3: Assignment of experiment codes to offset parameters defined by constants.

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

15N: cnst29; CO: cnst21: Calpha: cnst22, amide proton: cnst18.

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.

APSY Processing Parameters

Size, phase correction and strip Fourier transformation: The processing parameters are defined directly with *edp* in the common way. *Fig. 2* shows the top part of the acquisition parameter.

	F6	F5	F4	F3	F2	F1
▼ Reference						
SI	2048	512	512	512	512	512
SF [MHz]	500.2300000	50.6938469	500.2300000	500.2300000	500.2300000	500.2300000
OFFSET [ppm]	12.70897	20.70018	4.63155	4.63155	4.63155	4.63155
SR [Hz]	0.00	0.00	0.00	0.00	0.00	0.00
▼ Window function						
WDW	QSINE	SINE	SINE	SINE	SINE	SINE
LB [Hz]	0.30	0.30	0.30	0.30	0.30	0.30
GB	0	0.1	0.1	0.1	0.1	0.1
SSB	2	2	2	2	2	2
TM1	0	0.1	0.1	0.1	0.1	0.1
TM2	0	0.9	0.9	0.9	0.9	0.9
▼ Phase correction						
PHC0 [degree]	14.439	0.000	0.000	0.000	0.000	0.000
PHC1 [degree]	-71.400	0.000	0.000	0.000	0.000	0.000
PH_mod	pk	pk	pk	pk	pk	pk
▼ Baseline correction						
ABSG	5	5	5	5	5	5
ABSF1 [ppm]	100.00000	1000.00000	1000.00000	1000.00000	1000.00000	1000.00000
ABSF2 [ppm]	4.69984	-1000.00000	-1000.00000	-1000.00000	-1000.00000	-1000.00000
BCFW [ppm]	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
COROFFS [Hz]	0.00	0.00	0.00	0.00	0.00	0.00
BC_mod	qpol	no	no	no	no	no
▼ Fourier transform						
TDeff	0	0	0	0	0	0
STSR	0	0	0	0	0	0
STSI	1024	0	0	0	0	0
ME_mod	no	LPfc	LPfr	LPfc	LPfc	LPfc

Figure 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 F1 dimension.