# A brief description of this implementation of APSY

**Initial setup**

The present implementation of APSY (1) consists of 2 parts:

- au-program `apsy`
- C-program `GAPRO`

Store the algorithm GAPRO at a location of your choice on the computer that operates the spectrometer. Store the au-program "apsy" in /opt/xwinnmr/exp/stan/nmr/au/src. Using a text editor, define the location of the GAPRO algorithm in line 16 of the au-program, the operating system in line 18 and the text editor in line 20. Start XWINNMR and compile the au-program.

**The au-program `apsy`**

The au-program `apsy` can be used with suitable pulse programs. Before starting `apsy`, pulse lengths, power levels, pulse shapes, gradients, sweep widths, etc. have to be defined, like in a normal experiment.

The following parameters are controlled (and overwritten) by `apsy`: ZGOPTNS and the constants defining the angles (CNST1, CNST2, ...). During runtime, `apsy` will create new datasets with increasing experimental numbers (expno). Therefore, it is required that the expnos following the data set where `apsy` is started do not exist. If they exist, `apsy` may result in unpredictable results and damage to the spectrometer may occur.

The main functionality of `apsy` is a full apsy run (Figure 1). During a full run, new experiments are being set up, measured, processed and analyzed (`GAPRO`), until convergence of the final result is achieved or a predefined number of angle sets was recorded. Alternatively, `apsy` can be used to create new data sets with desired angles but without measuring and analyzing data sets.

The file angles.dat is used to specify sets of angles. If this file does not exist, a template is created, which can be modified using a text editor.

The au-program `apsy` also creates the prosa macro apsy.pro for each data set for processing using the software PROSA (2).

Currently, a simple convergence criterion is implemented: Convergence is achieved, if the final number of peaks does not change in two consecutive convergence tests (Fig. 1).

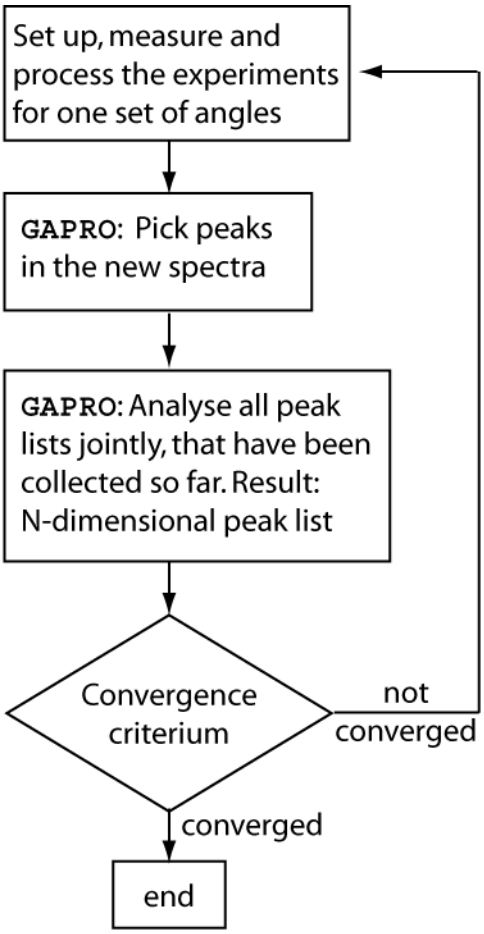


**Figure 1:** Scheme of the process executed by the au-macro `apsy`. `apsy` calls up the program `GAPRO` to peak pick spectra and to analyse the peak lists

**Requirements for pulse programs**

APSY-Pulse programs for XWINNMR must fulfill the following criteria: The first lines must consist of a header, which specifies parameters. Here an example from the 4D-HNCOCANH:

```
;Dimensionality: 4
;Experiment: NOAH
;Phaseoverlap: 0000
;trosy: 0
;DimProjections: 2
;Hfreq: BF1
;Cfreq: BF2
;Nfreq: BF3
;Carrier1: O3P
;Carrier2: CNST 23
;Carrier3: CNST 24
;Carrier4: O1P
;-----------------end header------------------
```

The order of the lines in this header shall not be changed. The header specifies the dimensionality of the experiment, an experiment identifier string, phase overlap, a potential trosy dimension, the dimensionality of the projections, further the basic frequencies for the nuclei, and the carrier frequencies. If one of the indirect dimensions has to be processed in Echo-Antiecho / TROSY mode, the value "trosy" must be the number of this dimension. Otherwise, trosy = 0. The experiment identifier describes the experiment with a 1-letter code for each dimension using the following definitions:

| 1-letter code | nucleus | description | rel. residue position |
|---|---|---|---|
| H | $^{1}H$ | amide proton | i |
| N | $^{15}N$ | amide nitrogen | i |
| A | $^{13}C$ | alpha carbon | i-1 |
| B | $^{13}C$ | beta carbon | i-1 |
| O | $^{13}C$ | carbonyl carbon | i-1 |
| M | $^{15}N$ | amide nitrogen | i-1 |
| J | $^{1}H$ | amide proton | i-1 |
| Z | $^{1}H$ | alpha proton | i-1 |
| Y | $^{1}H$ | alpha proton | i |
| z | $^{1}H$ | alpha proton | i & i-1 |
| a | $^{13}C$ | alpha carbon | i & i-1 |
| b | $^{13}C$ | beta carbon | i & i-1 |
| E | $^{13}C$ | alpha carbon | i |
| D | $^{13}C$ | beta carbon | i |
| I | $^{1}H$ | aliphatic proton | i-1 |
| K | $^{1}H$ | aliphatic proton | i |
| C | $^{13}C$ | aliphatic carbon | i-1 |
| G | $^{13}C$ | aliphatic carbon | i |

Examples: The 4D-APSY-HNCOCA experiment gets the indentifier NOAH, or AONH, depending on the order the evolution periods appear in the pulse program; the 3D-APSY-HNCA is identified by NaH or aNH; the 3D-APSY-HNCO has the identifier ONH or NOH and 5D-APSY-HACACONH has ZAONH.

The projection angles α β, … are defined as (1):

| dimension | $N = 5$ | $N = 4$ | $N = 3$ |
|---|---|---|---|
| $\omega_1$ | $\sin(\gamma)$ | $\sin(\beta)$ | $\sin(\alpha)$ |
| $\omega_2$ | $\sin(\beta)\cdot\cos(\gamma)$ | $\sin(\alpha)\cdot\cos(\beta)$ | $\cos(\alpha)$ |
| $\omega_3$ | $\sin(\alpha)\cdot\cos(\beta)\cdot\cos(\gamma)$ | $\cos(\alpha)\cdot\cos(\beta)$ | - |
| $\omega_4$ | $\cos(\alpha)\cdot\cos(\beta)\cdot\cos(\gamma)$ | - | - |

In an APSY pulse program, the constants CNST1, CNST2, CNST3, … are reserved for the angles α, β, γ, … For the sweep widths (in Hz), the constants CNST21, CNST22, CNST23, … are reserved for dimensions $\omega_1$, $\omega_2$, $\omega_3$, …
With the ZGOPTNS, the correct phases for acquisition are controlled. The dimensions are denoted by the last letters of the alphabet, the acquisition dimension is always z; e.g., in a 4D the four dimensions are w, x, y and z. with –DW, the w-dimension is shifted by 90°, with –DPw, the pseudo-2D acquisition is done on w. The ZGOPTNS are controlled by apsy and an interference is usually not necessary.

**Processing**
Processing is done by `apsy` in the course of a full run. Before processing, raw data is combined using the XWINNMR commands xf2, xf1, abs2 and abs1. Thus, the XWINNMR parameters for window functions, baseline corrections, zero filling, phase corrections, etc. have to be specified as desired. For the indirect dimensions, the phase corrections should be ph0 = 0° and ph1 = 0°.

**The C-program `GAPRO`**
`GAPRO` is the software routine, which performs the analysis of the peak lists (1). Additionally, it includes a peak picker for the projections. `GAPRO` has 4 main functions:

- pick peaks in projection spectra
- calculate coordinates of N-dimensional peaks
- calculate peaklists for the projections from an N-dimensional peak list
- simulate expected results from a known assignment (e.g. a BMRB file).

During a full run, `GAPRO` is executed automatically by `apsy`. It can alternatively be started by the user. This is typically done to repeat calculations or to analyze a set of already measured projections. If `GAPRO` is started without additional arguments, it enters the interactive mode and a dialog leads through the functionalities. If `GAPRO` is executed with additional arguments (e.g. `GAPRO` –c ...), it runs in the automatic mode.
The runtime parameters for `GAPRO` are specified in 3 different files: **parameter.gap**, **experiment.gap** and **spectra.gap**. If these files do not exist, templates will be generated.

- **parameter.gap**: The parameter for the geometric analysis: $S_{min1,2}$, $\Delta\nu_{min}$, $r_{min}$, S/N-ratio, width of waterline. These parameters can be changed for the analysis of data, for example, to improve the results or to test the influence of a given parameter on the result.
- **experiment.gap**: Parameters of the experiment. These are the carrier frequencies, the experiment identifier, basic frequencies, dimensionalities. These parameters are defined when the measurements are started; it is usually not necessary to change this file later.

- **spectra.gap**: This file contains a list of the projection spectra that are analyzed, together with the corresponding projection angles. Experiments with the same absolute values of the angles, but different signs are grouped in blocks. The number of experiments in the group (1, 2, 4, 8, …) is specified in a line with leading '#'. This can for example look like this:

```
# 1
../path/spec13 alpha=0.0 beta=90.0 gamma=0.0
# 1
../path/spec14 alpha=0.0 beta=0.0 gamma=90.0
# 2
../path/spec15 alpha=30.0 beta=0.0 gamma=0.0
../path/spec16 alpha=-30.0 beta=0.0 gamma=0.0
# 4
../path/spec17 alpha=60.0 beta=30.0 gamma=0.0
../path/spec18 alpha=-60.0 beta=30.0 gamma=0.0
../path/spec18 alpha=60.0 beta=-30.0 gamma=0.0
../path/spec18 alpha=-60.0 beta=-30.0 gamma=0.0
```

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The spectra need to be in XEASY (3) format. Alternatively, it is possible to use peak lists as input for GAPRO. The first step of GAPRO, peak picking, is then skipped. The peak lists must be in the XEASY format:

```
# Number of dimensions 2
#INAME 1 ?
#INAME 2 ?
       1      999.893      2748.579 1 U   4.122E+00  1.000E+00 e 0    0     0
       2      978.449      2442.960 1 U   4.403E+00  1.000E+00 e 0    0     0
       3      941.571      2836.361 1 U   4.128E+00  1.000E+00 e 0    0     0
       4      929.418      3356.701 1 U   4.415E+00  1.000E+00 e 0    0     0
       5      921.206      2662.443 1 U   4.093E+00  1.000E+00 e 0    0     0
       6      877.054      2346.930 1 U   4.218E+00  1.000E+00 e 0    0     0
       7      868.135      2554.440 1 U   4.069E+00  1.000E+00 e 0    0     0
       8      786.137      2294.889 1 U   4.397E+00  1.000E+00 e 0    0     0
       9      773.853      2920.435 1 U   4.319E+00  1.000E+00 e 0    0     0
       …
```

Calibration of spectra and peak lists: Spectra can not be calibrated using "ppm" values, as ppm is not defined for mixed evolution of different nuclei. The calibration must be in Hz, with 0 Hz being located at the carrier position in each dimension. All calculations within GAPRO are done in Hz. Only in at the very end of the calculation, the resulting *N*-dimensional peak list can be given out by GAPRO in ppm.

The current package is under continuous development and is currently available only as a beta-version for academic users. Please communicate questions, comments, bugs, feature requests or other suggestions to Sebastian Hiller.

**References**


(1) S. Hiller, F. Fiorito, K. Wüthrich & G. Wider, *Proc. Natl. Acad. Sci. USA* **102**, 10876–10881 (2005).

(2) P. Güntert, V. Dötsch, G. Wider & K. Wüthrich, *J. Biomol. NMR* **2**, 619–629 (1992).

(3) Bartels, C., Xia, T., Billeter, M., Güntert, P. & Wüthrich, K., *J. Biomol. NMR* **6**, 1–10 (1995).