# 2009 NMR User Training Course I : New Software in HFNMRC
## Feb. 25, 2009

**Part I : Lectures**

**Place : B1B Meeting Room, IBMS, Academia Sinica, Taipei**

**10:00-10:50**

**Introduction to Topspin2.1 by Dr. Casper Wu, Rezwave Co.**

**11:00-11:50**

**Application using Topspin2.1: APSY and standard experiments in HFNMRC**
**by Dr. Chi-Fon Chang, HFNMRC, Academia Sinica**

**Part II : Hands-On (Getting start on Topspin2.1)**

**Place : B1A Conference Room, IBMS, Academia Sinica, Taipei**

**13:30-14:00**

**Overview for Practical Session by Dr. Chi-Fon Chang**
**Tips on using Topspin2.1**
**Set up HFNMRC standard experiments**
**Set up one APSY experiment**

**14:00-17:00**

**Group1 : AV600_CHEM, CHEMISTRY, B1, Academia Sinica (14:00-15:30)**
**Group2: AV600_CHEM, CHEMISTRY, B1, Academia Sinica (15:30-17:00)**
**Group3: AV600L, 1F , GRC, Academia Sinica**
**Group4: AVIII600, B1A, IBMS**
**Group5: AV600_IBMS, B1, IBMS**

# Application using Topspin2.1

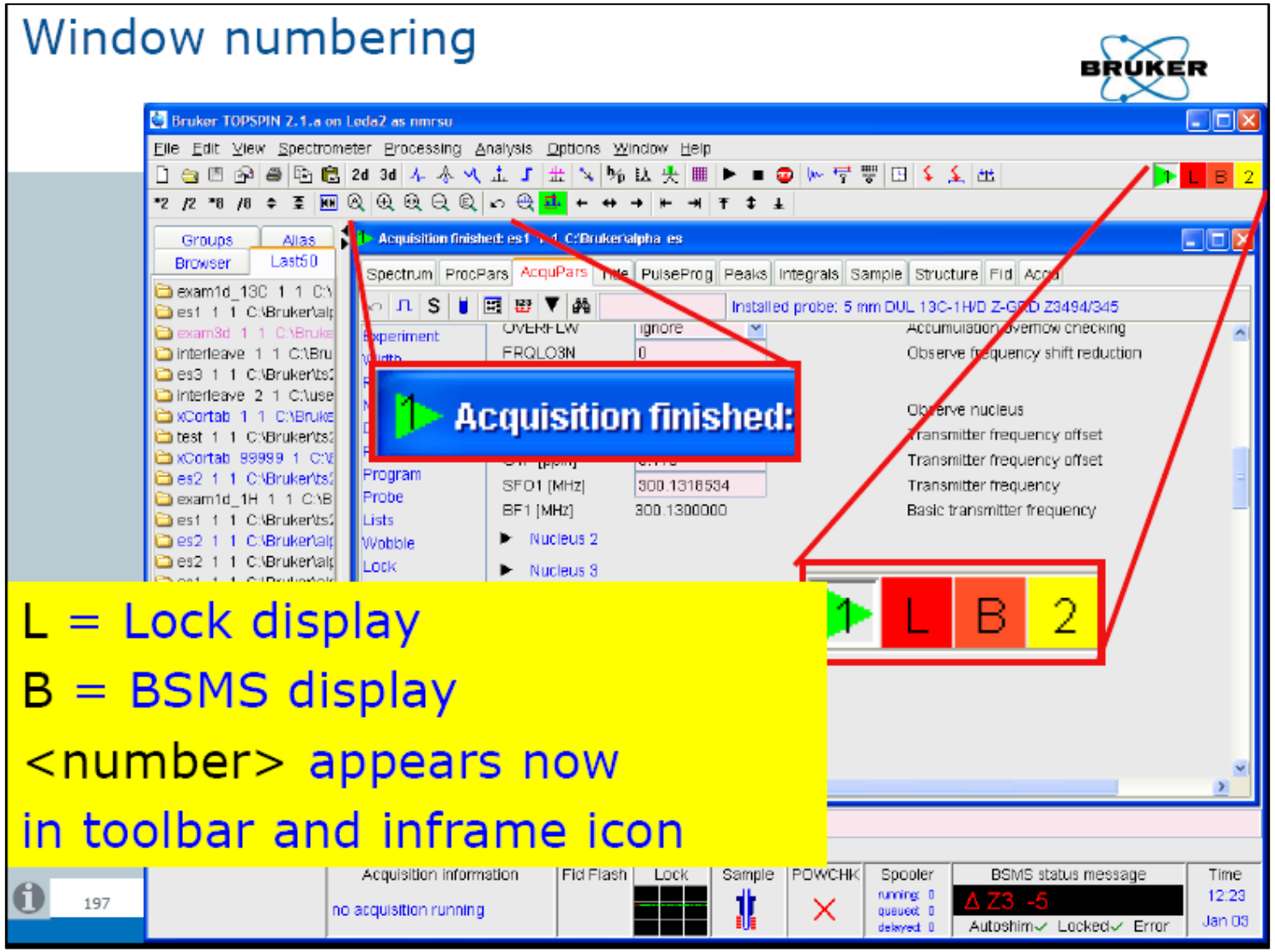
# APSY & Standard experiments in HFNMRC

Chi-Fon Chang, Ph.D.

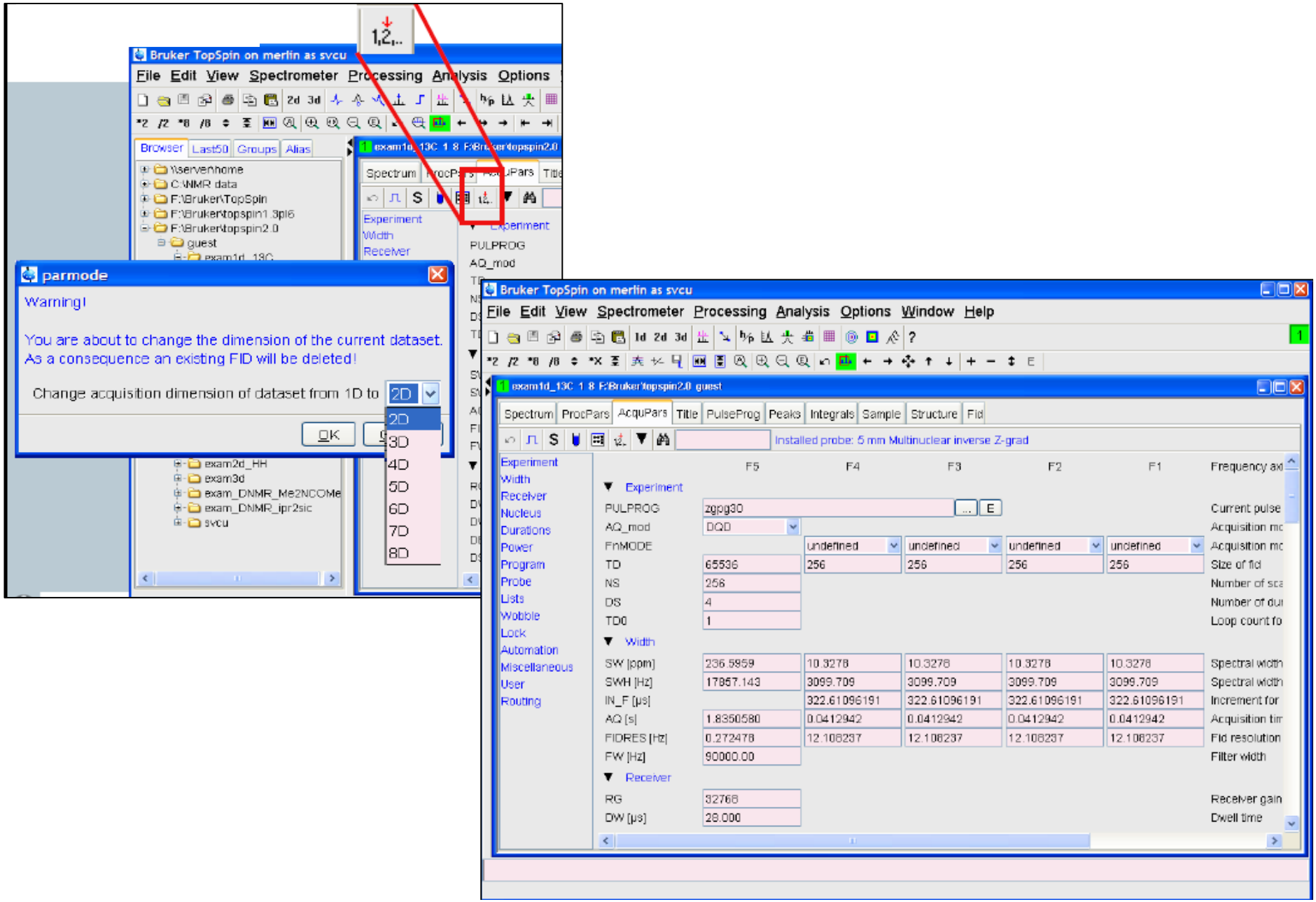
02.25.2009

# Nice/New functions in Topspin2.1
# (few examples)

**Example 1: multi windows**



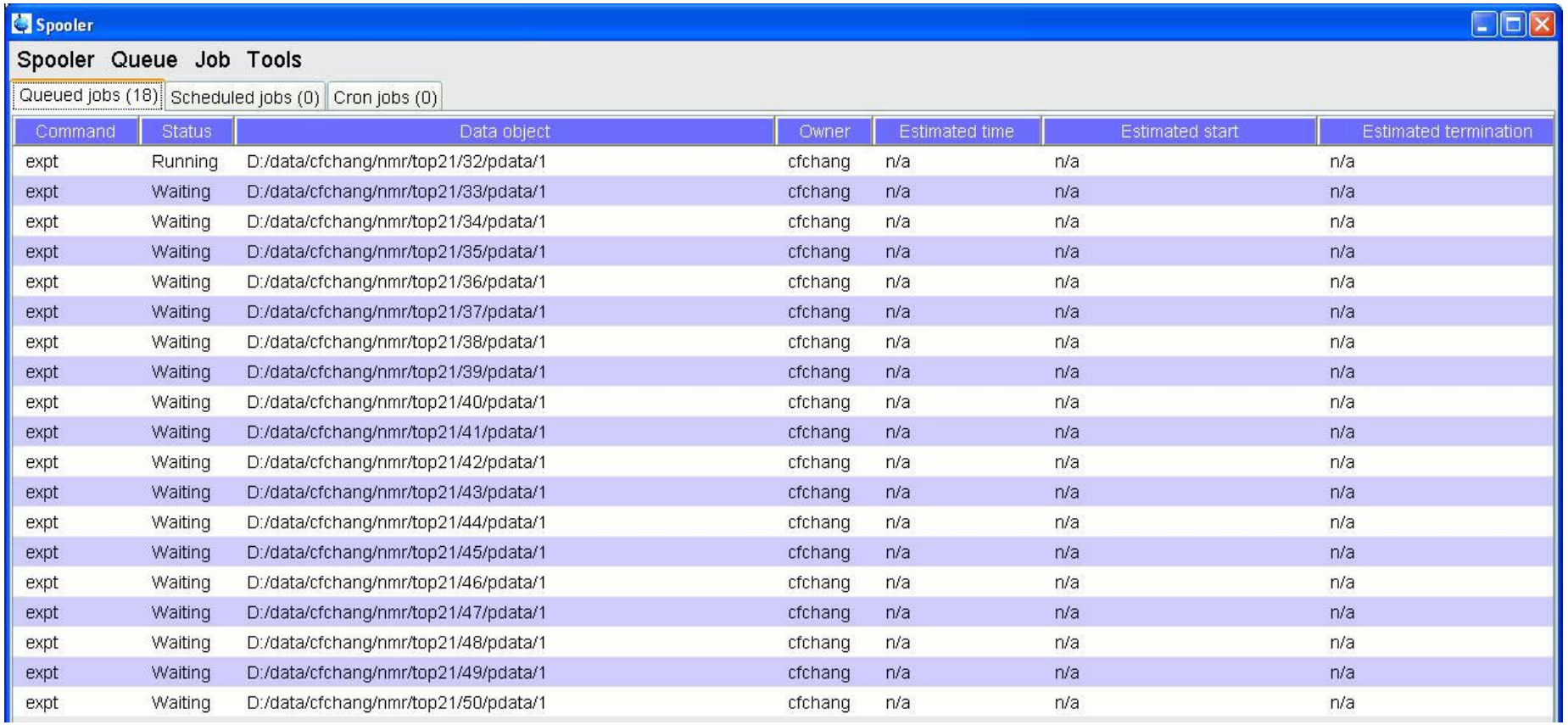
# Example 2: Acquisition and processing up to 8D



## Example 3: Topshim is faster than gradientshim

## Example 4: Spooler

(notice that not all commend will show on spooler, ex: multizg won't be there!!!!!)

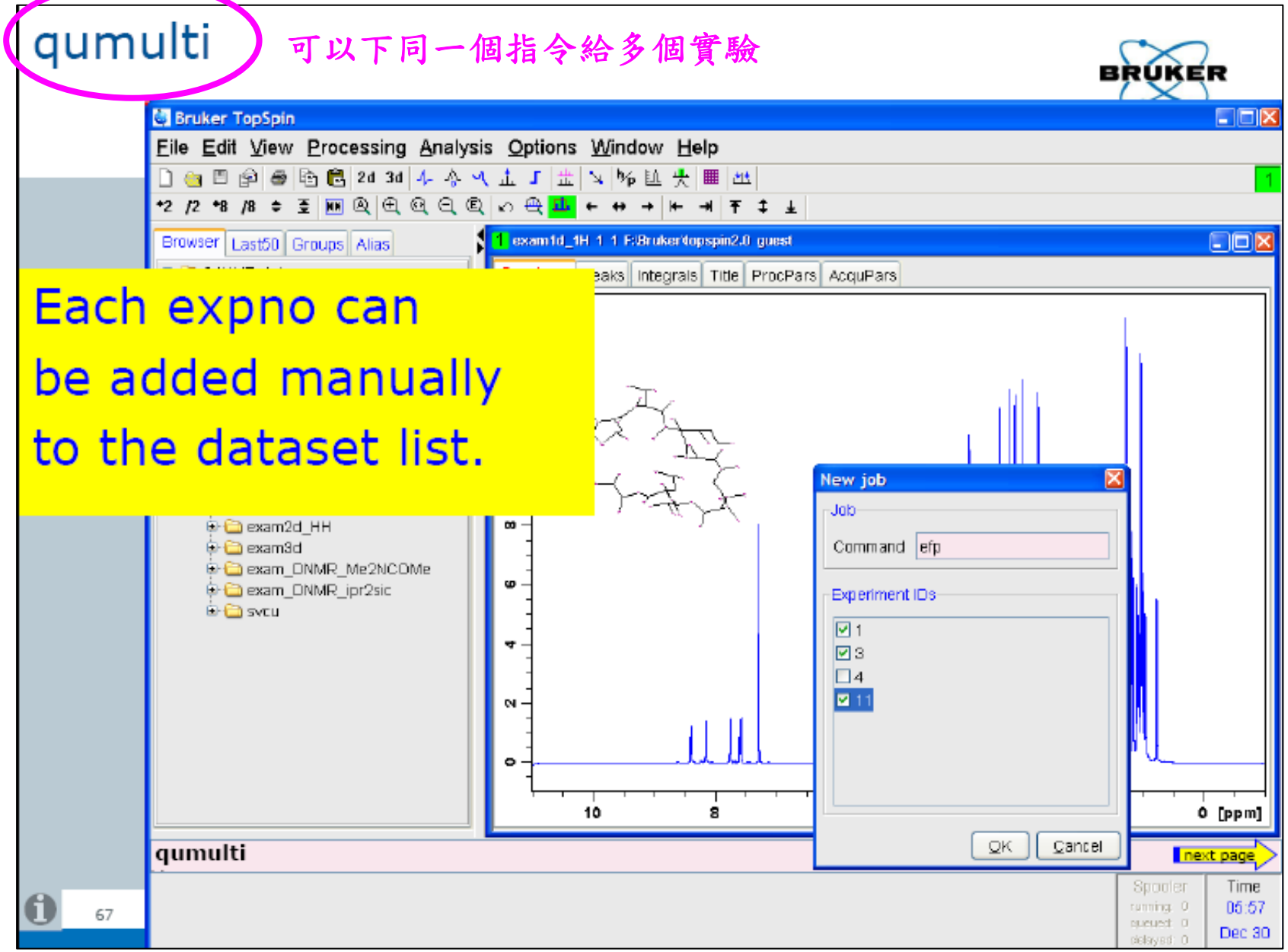



| Command | Status | Data object | Owner | Estimated time | Estimated start | Estimated termination |
|---|---|---|---|---|---|---|
| expt | Running | D:/data/cfchang/nmr/top21/32/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/33/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/34/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/35/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/36/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/37/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/38/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/39/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/40/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/41/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/42/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/43/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/44/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/45/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/46/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/47/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/48/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/49/pdata/1 | cfchang | n/a | n/a | n/a |
| expt | Waiting | D:/data/cfchang/nmr/top21/50/pdata/1 | cfchang | n/a | n/a | n/a |

**( click on Spooler → you can check or modify the running status )**

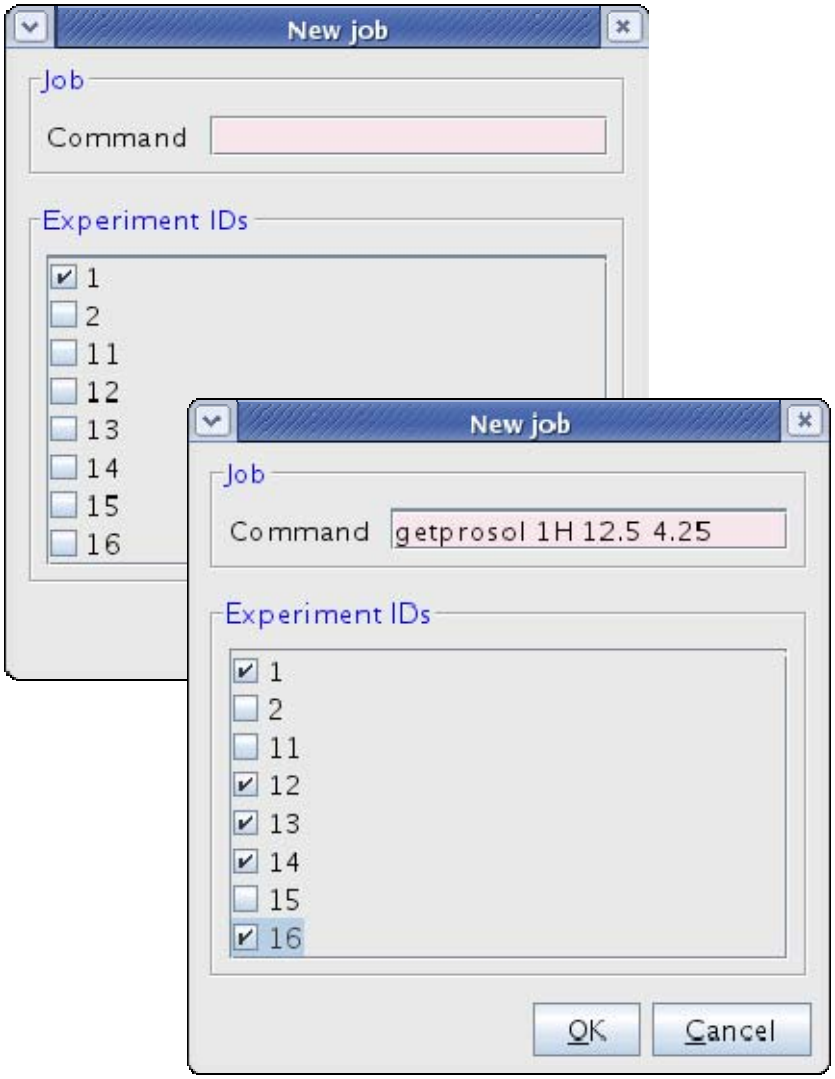
# qumulti

可以下同一個指令給多個實驗

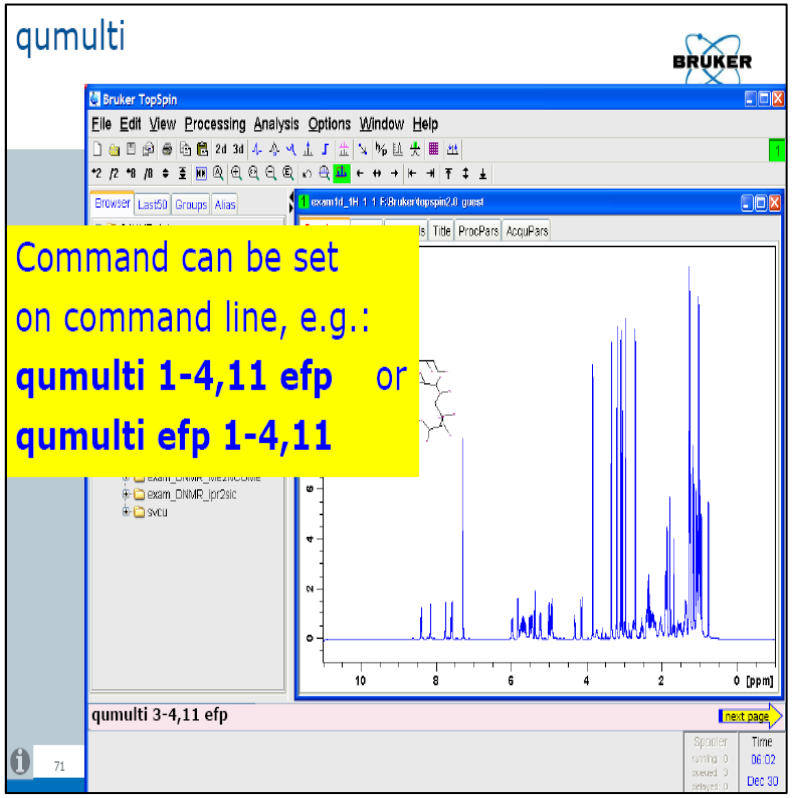



Each expno can be added manually to the dataset list.

## > qumulti




## > qumulti 1-4,11 efp

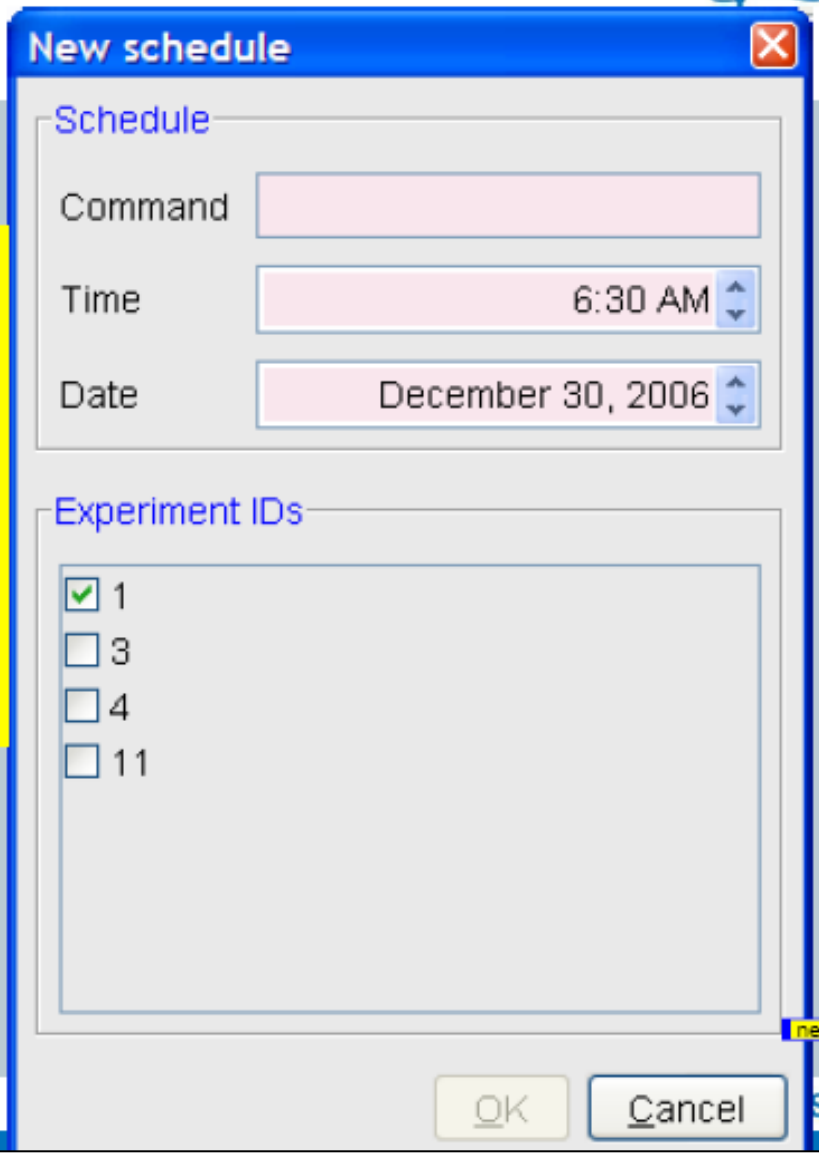



## > qu efp

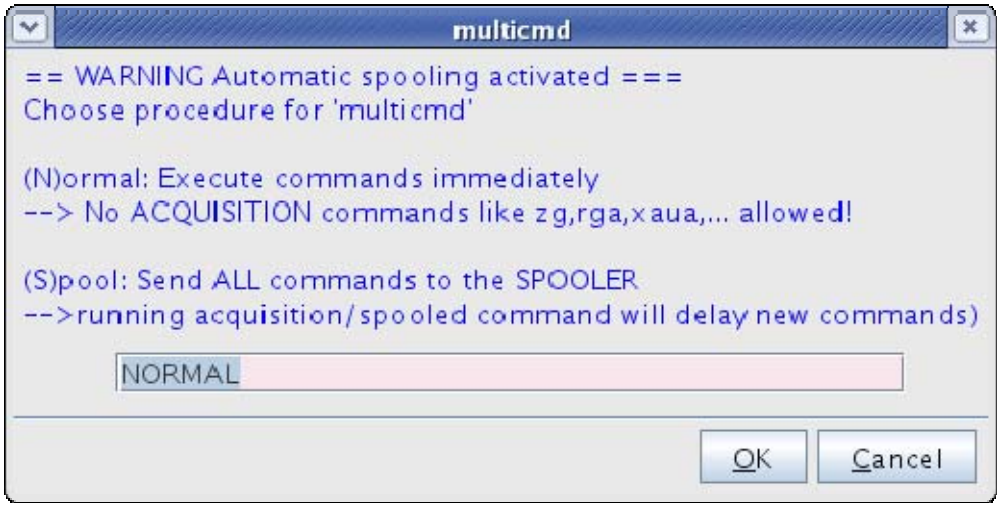
atmulti

可以下同一個指令給多個實驗;並指定開始的時間

Same functionalities are available for the list of delayed jobs with command: **atmulti**



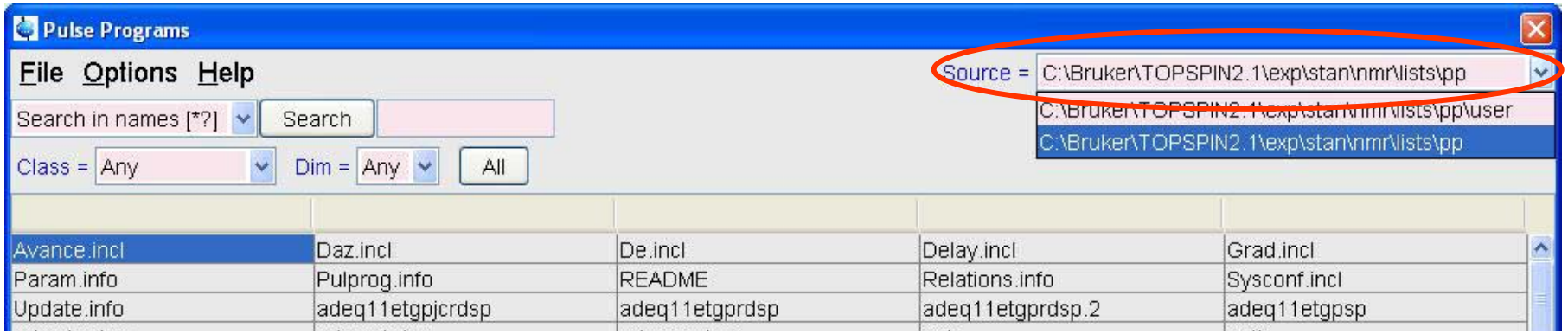
multicmd 可以下多個指令給多個實驗



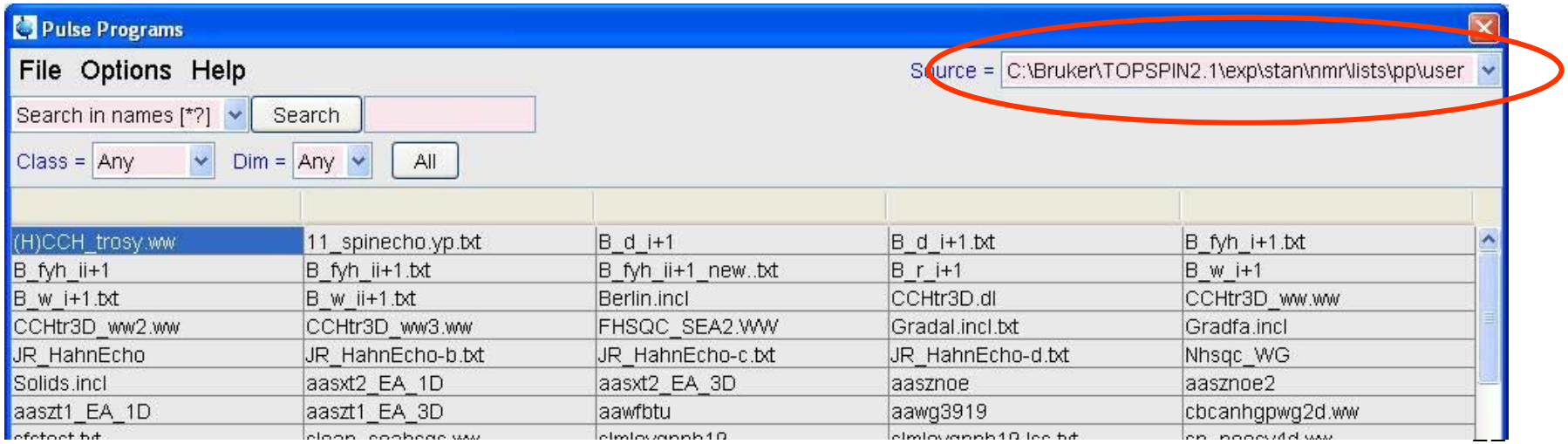


## Example 5: “Users” directory for pulseprogram and parameter set

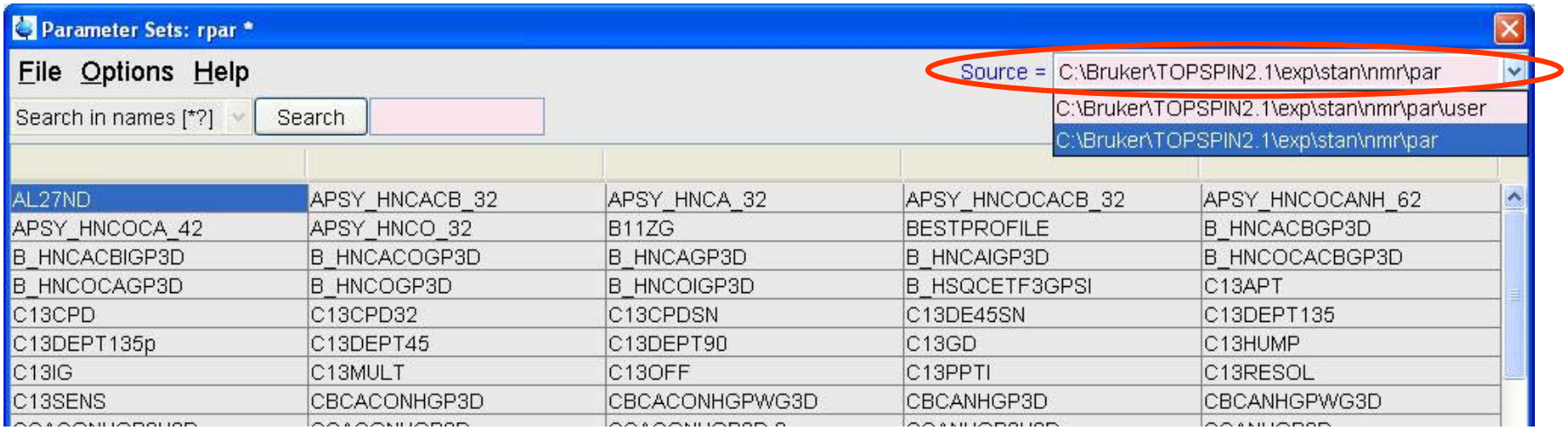
### Bruker default pulse program directory (xxx\Topspin2.1\exp\stan\nmr\list\pp )



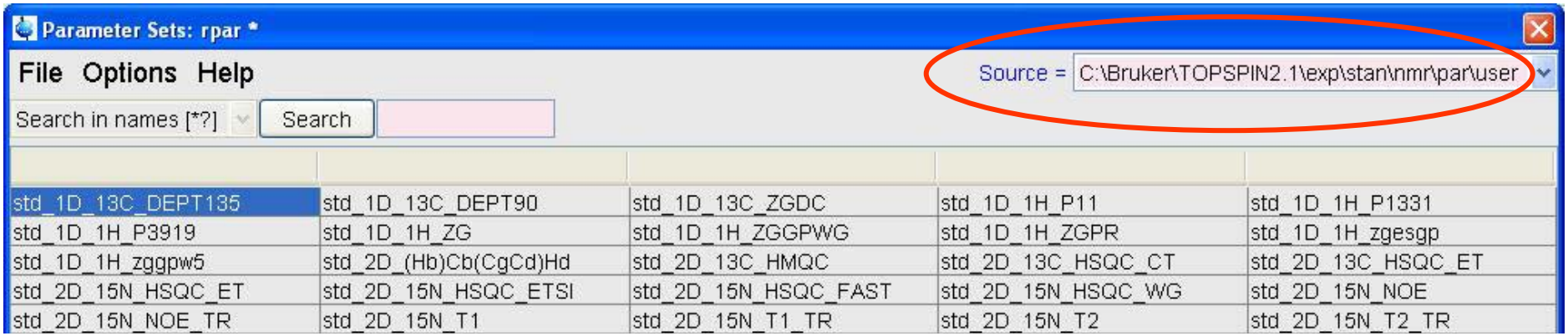

### User’s Pulse program directory
### (HFNMRC staff or users : xxx\Topspin2.1\exp\stan\nmr\list\pp\user )



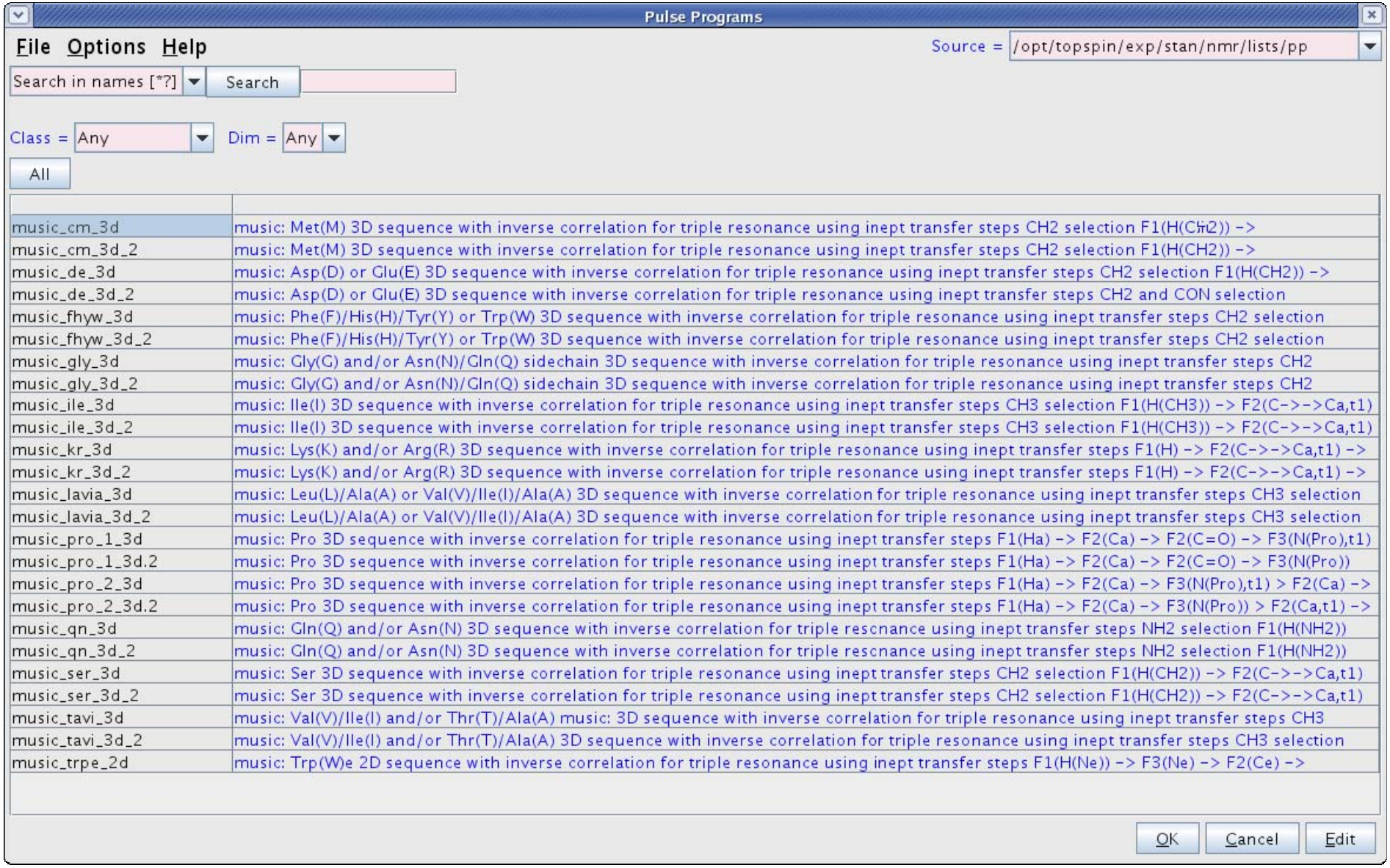
## Bruker default Parameter Set directory (xxx\TOPSPIN2.1\exp\stan\nmr\par )



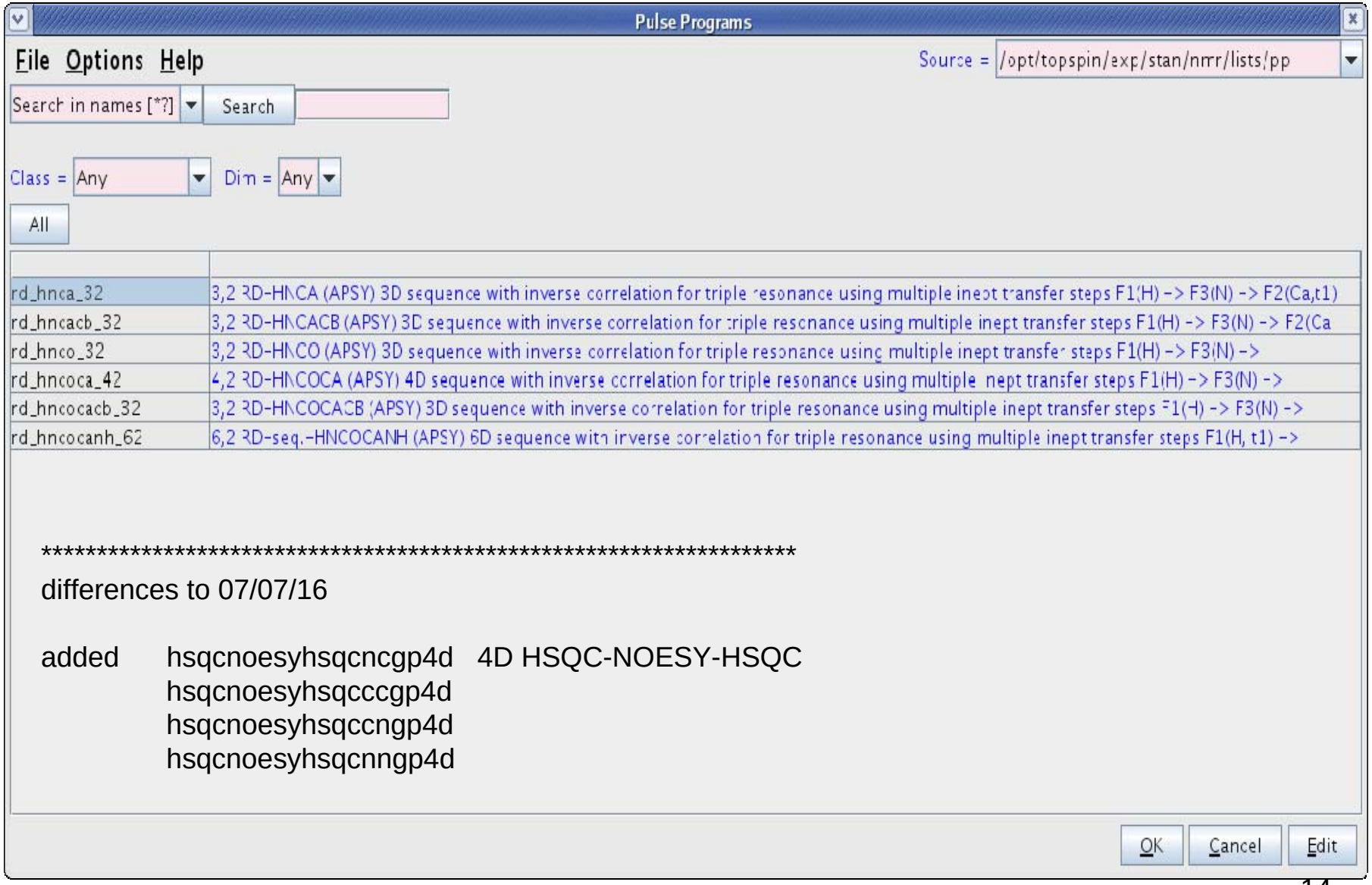

## HFNMRC standard Parameter Set directory (xxx\TOPSPIN2.1\ejxjp\stan\nmr\par\user )



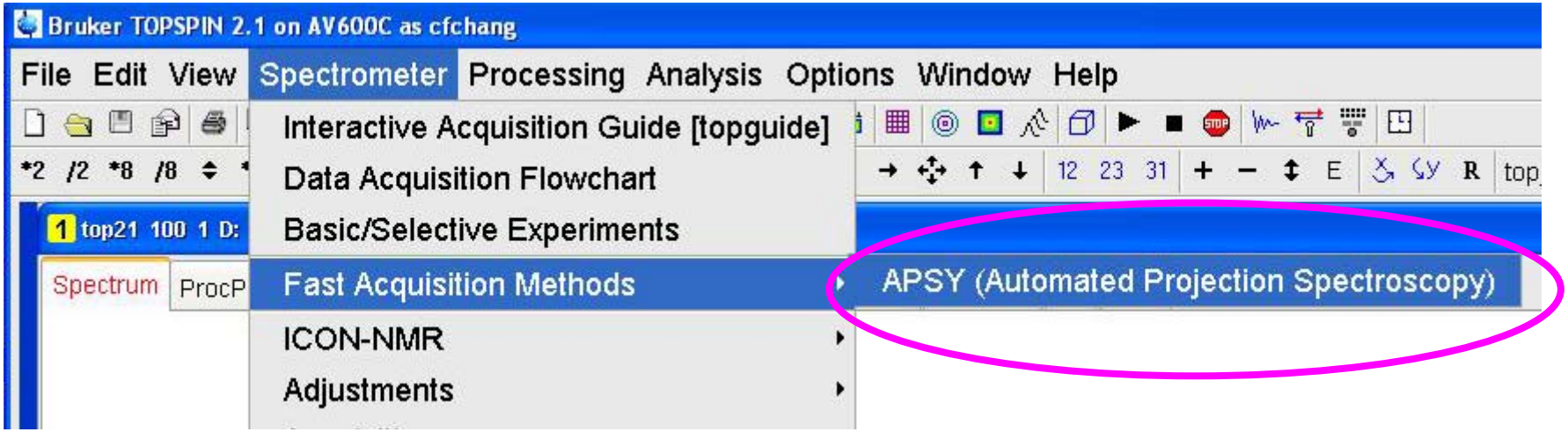
# Example 6: new pulseprogram (Music type)



# Example 6: new pulseprogram (for APSY & 4D experiments)



## Example 7: APSY




S. Hiller, F. Fiorito, K. Wüthrich and G. Wider, Proc. Nat. Acad. Sci. USA 102, 10876-10881 (2005).

**Automated Projection SpectroscopY (APSY).**

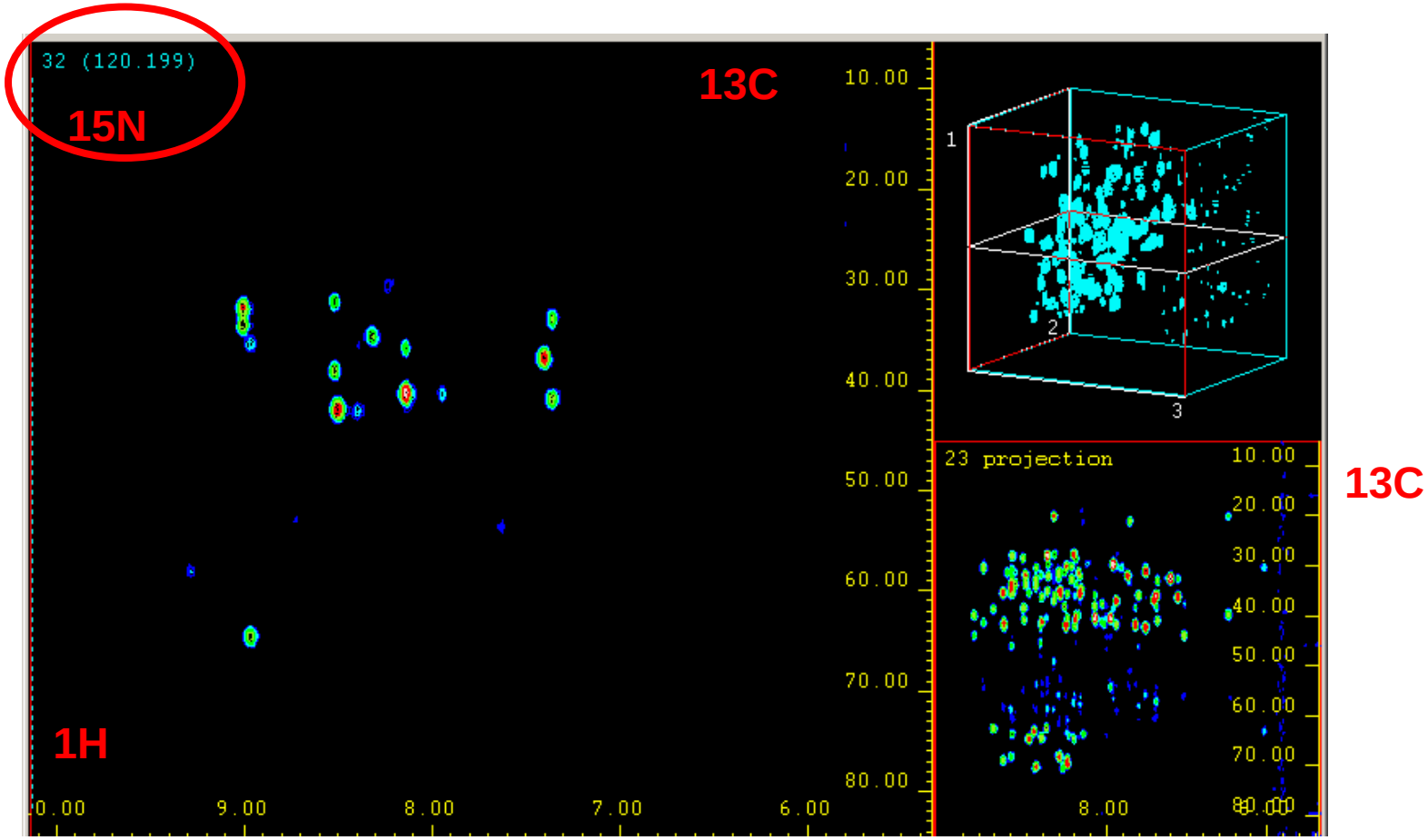
# Brief Introduction to APSY

## Automated Projection SpectroscopY (APSY)

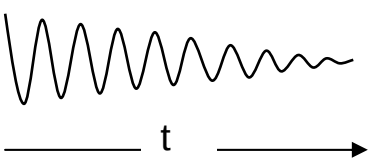
**Part I: Acquisition using Projection Spectroscopy**

### Background:

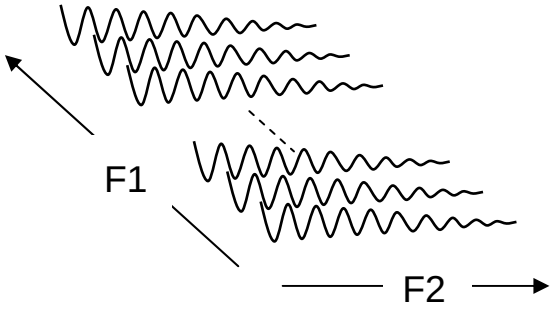
- Multidimensional NMR could help to solve the problem of overlapping



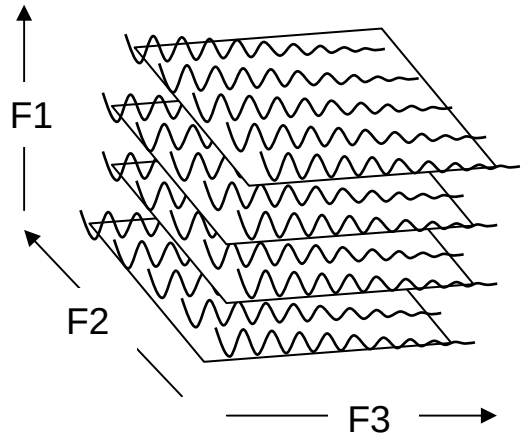
•However, multidimensional NMR is time consuming




一維實驗
1個FID, t=30秒



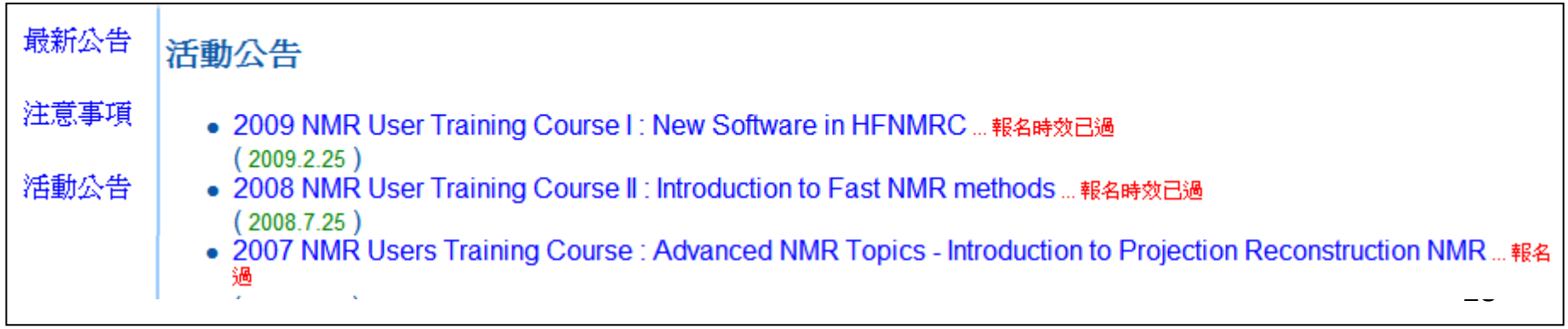

二維實驗
如F1=128, i.e. 128個FID
則需 64分鐘




三維實驗
如F1=128, F2=64
128*64個FID, 則需 68.2小時

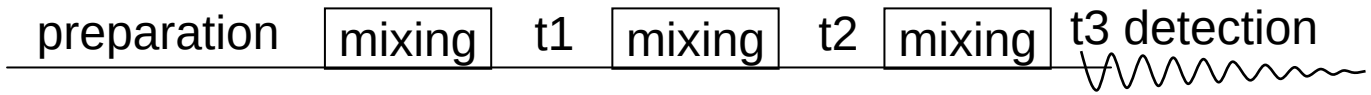
•How could we keep the resolution and also save the machine time?

→ Rapid Acquisition Methods !!

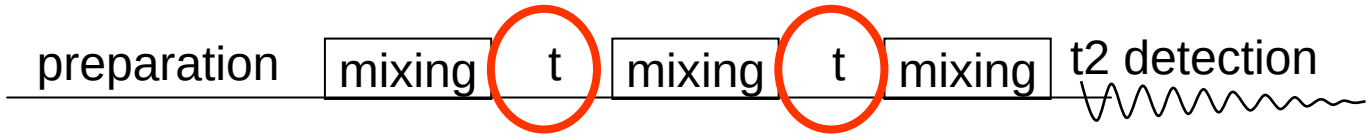


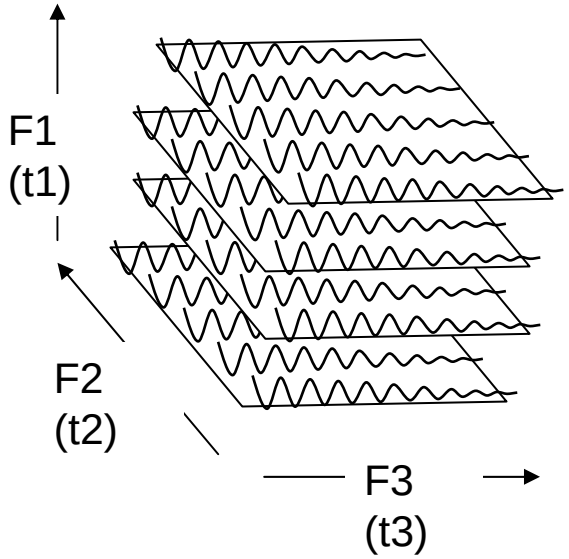
## Method: Projection Spectroscopy

傳統三維實驗

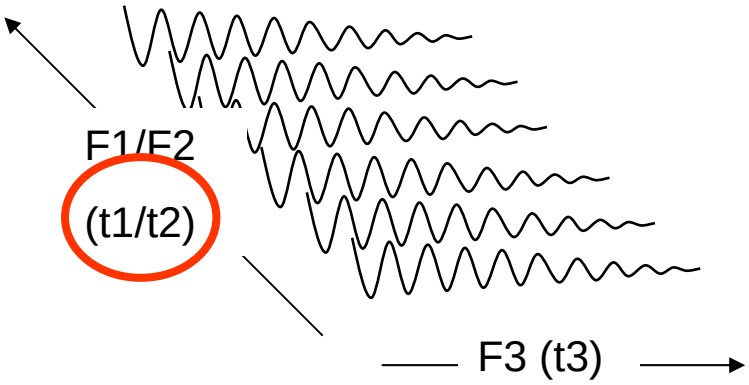



三維縮減成二維實驗




傳統三維實驗
t1及t2 各自獨立增加




維度縮減實驗
t1及 t2 若同時增加
則可共用同一維度

## Method: Projection Spectroscopy

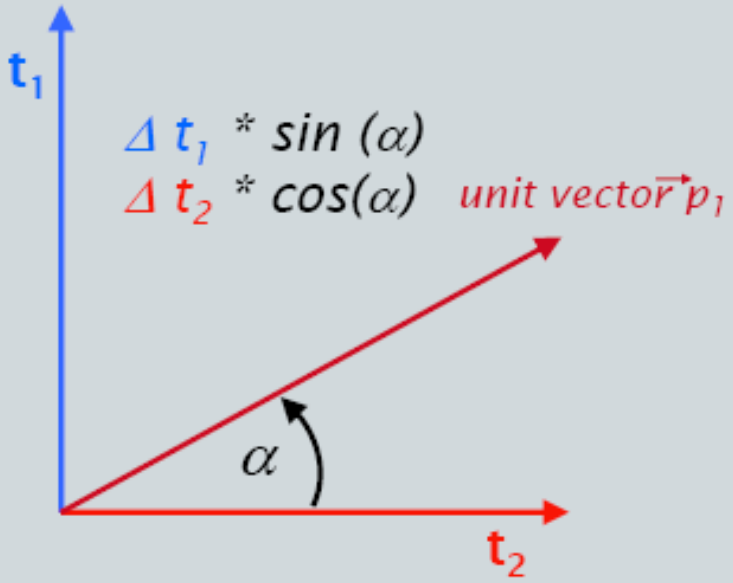
**t1=t*sin(α) & t2=t*cos(α)**

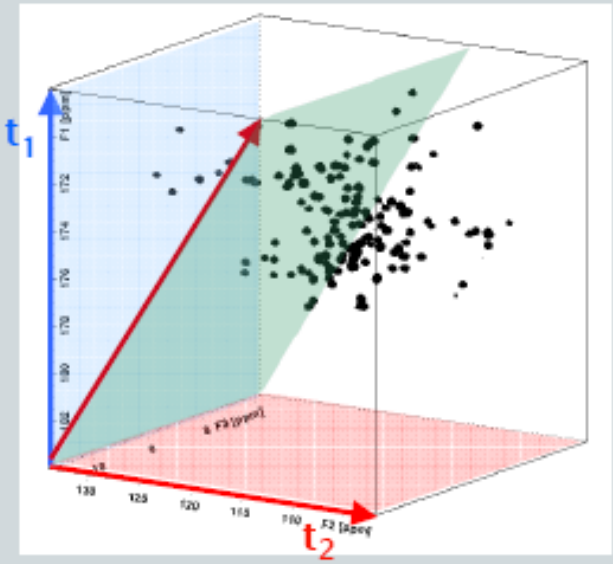
**Example: 3D HNCO experiment**

single evolution during $t_1$ only: 2D H,C plane ($\alpha = 90°$)

single evolution during $t_2$ only: 2D H,N plane ($\alpha = 0°$)

simultaneous evolution during $t_1$ and $t_2$: 2D H,NC plane ($\alpha = n°$)






(From Bruker APSY document)

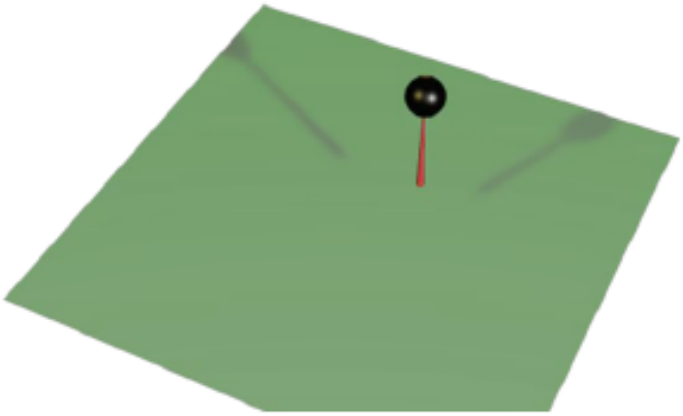
## Method: Projection Spectroscopy

Consequence of ***Reduction of dimensionality:***

Shift information of reduced dimensions is lost, but:

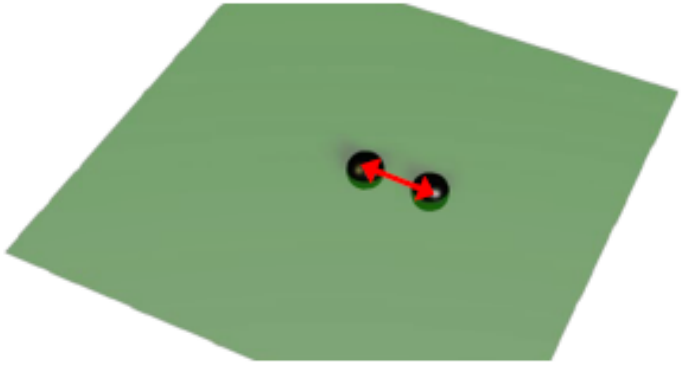
- Shift information is coded as a distance
- By additional splitting of single peaks

*2-dimensional, one selected peak*
*With information about shift in Z*



dimensionality:
full

*2-dimensional, one selected peak*
*Information about shift in Z coded in a distance*

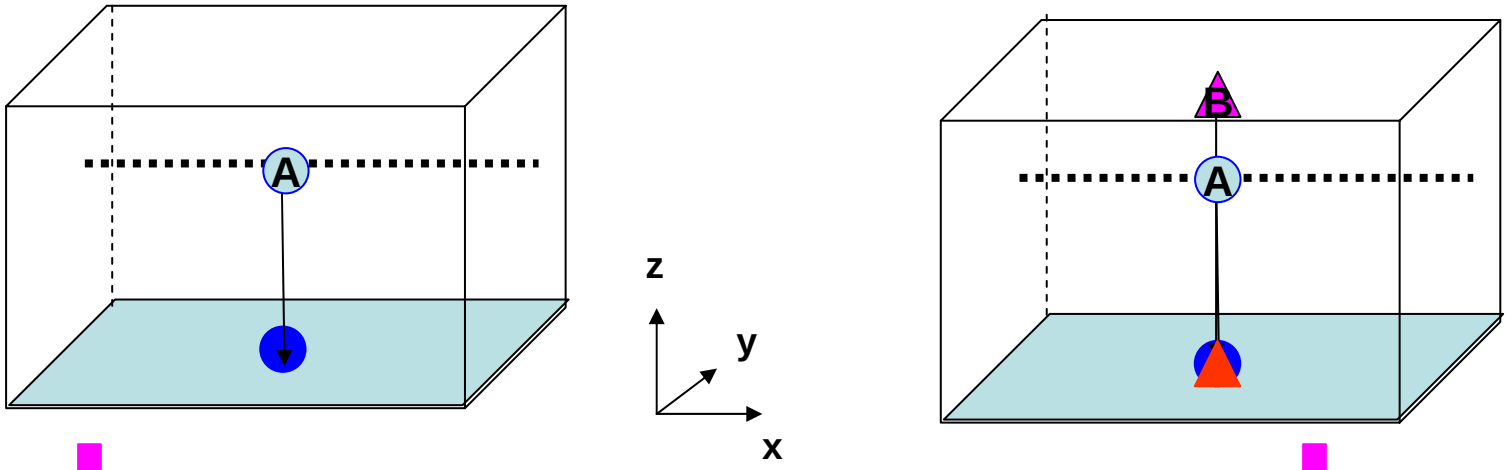


dimensionality:
reduced

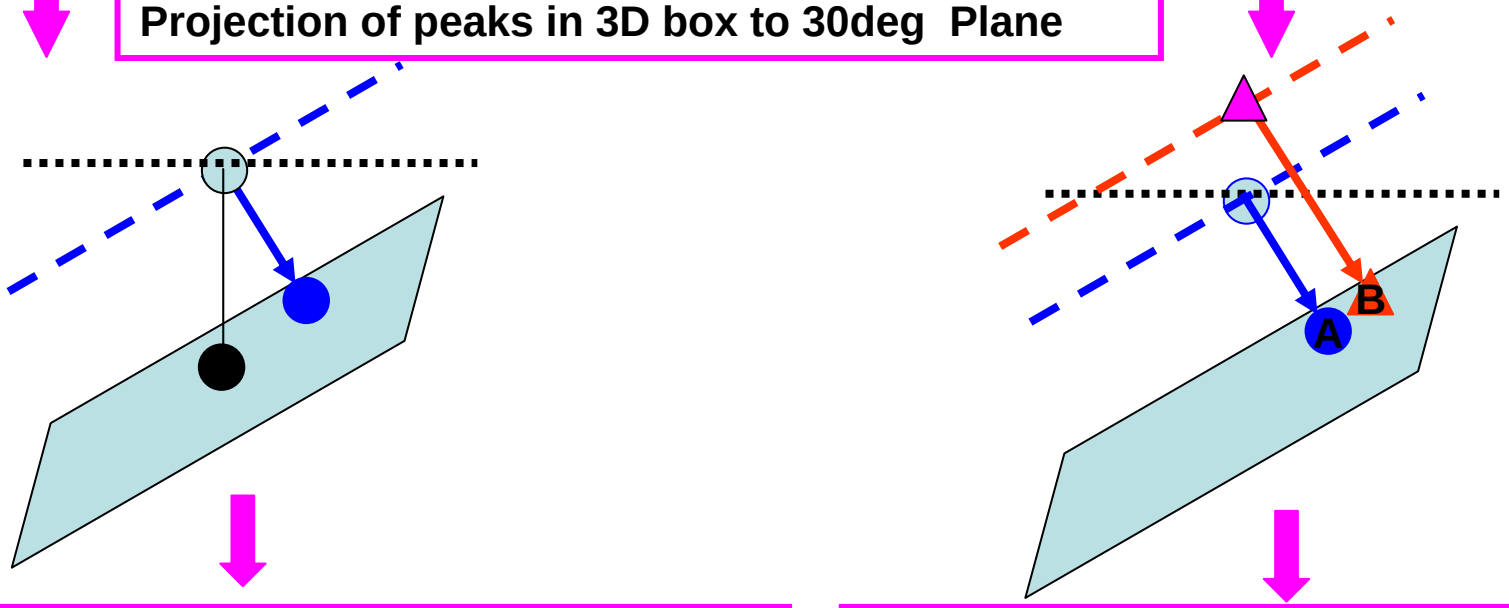


(From Bruker APSY document)

**Projection of peaks in 3D box to XY Plane**



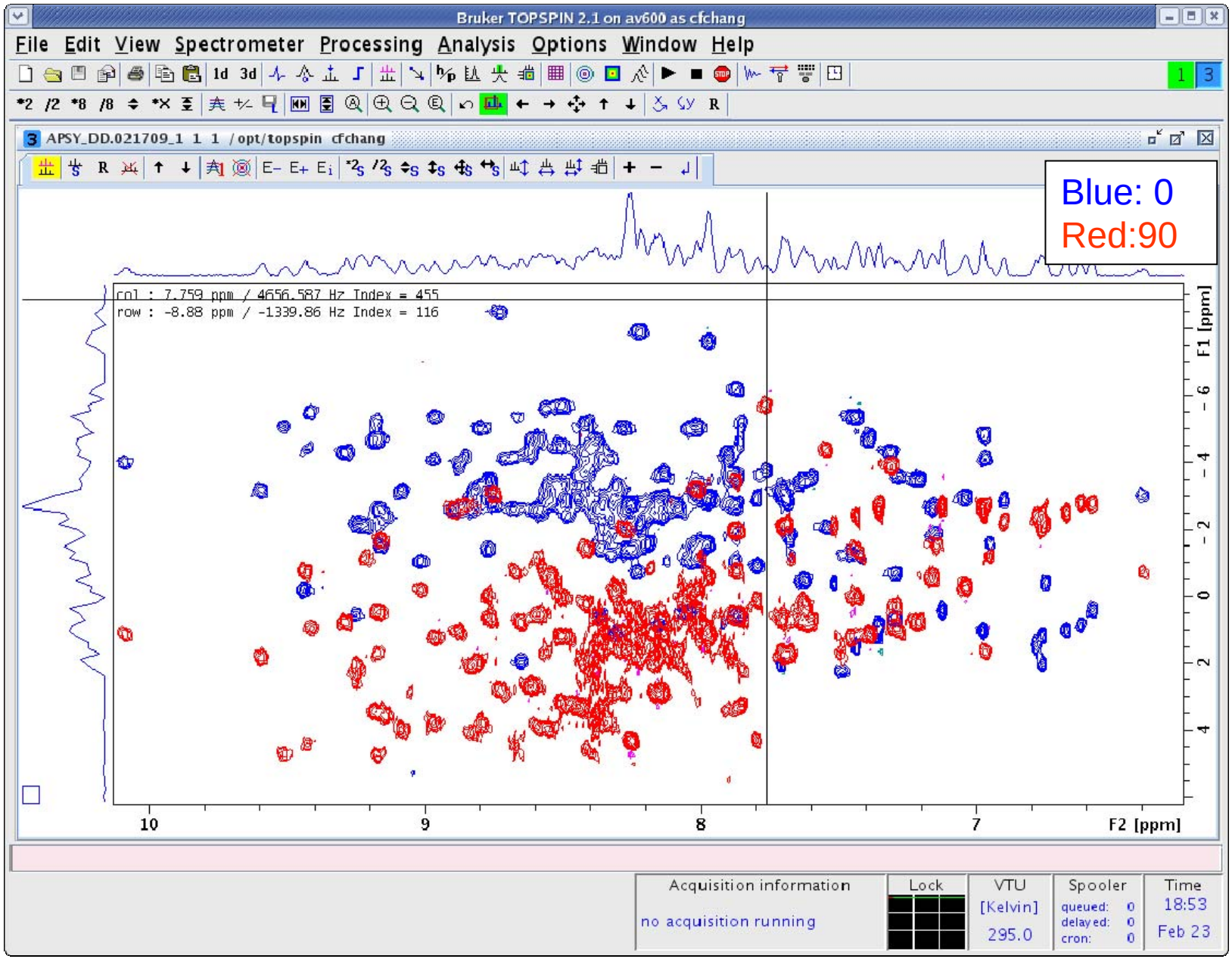

**Projection of peaks in 3D box to 30deg Plane**

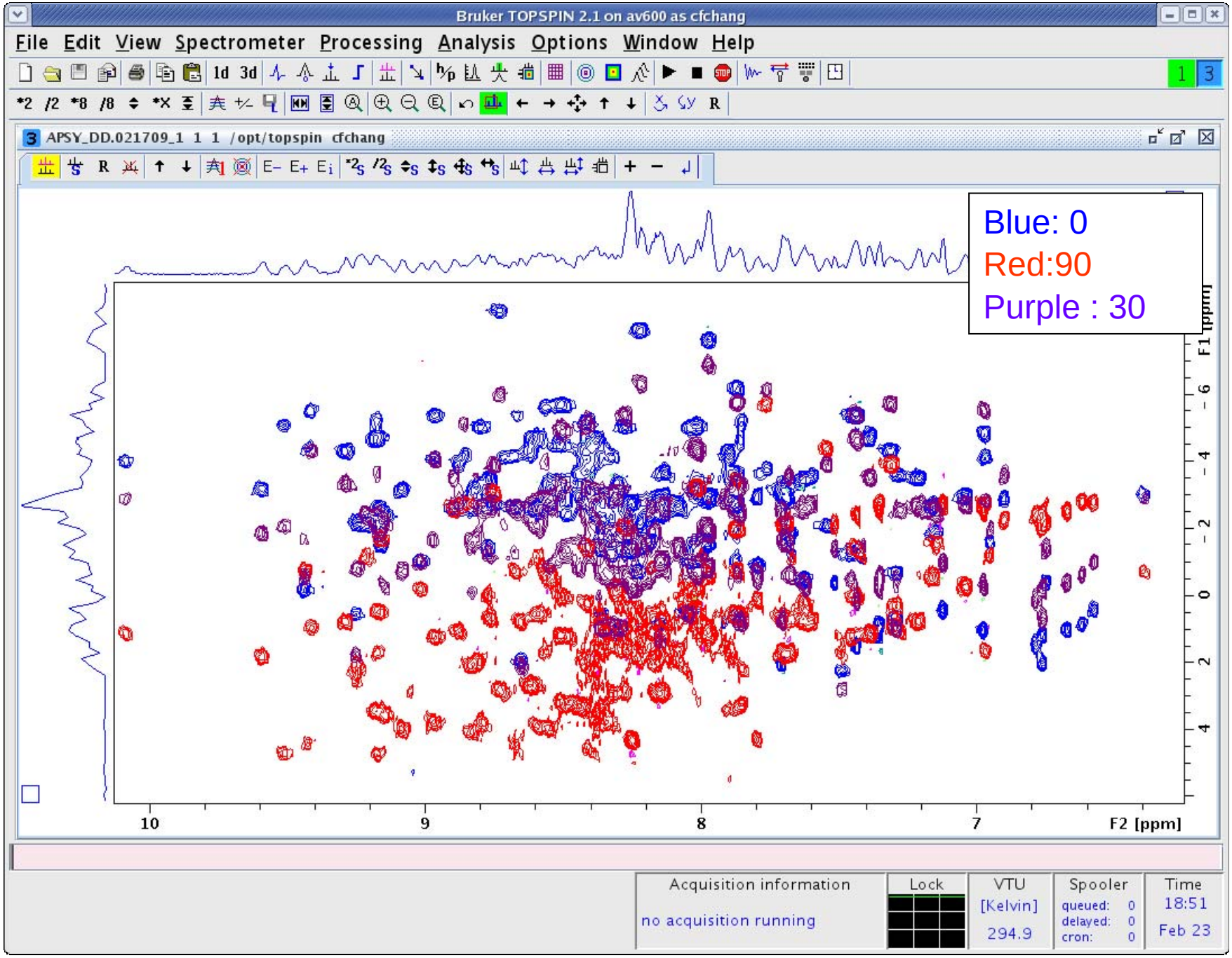



**Peak's information on Z-axis is coded in distance of peaks at different projection angles**

**Overlap in XY could be resolved by different projection angles**

# Method: Projection Spectroscopy

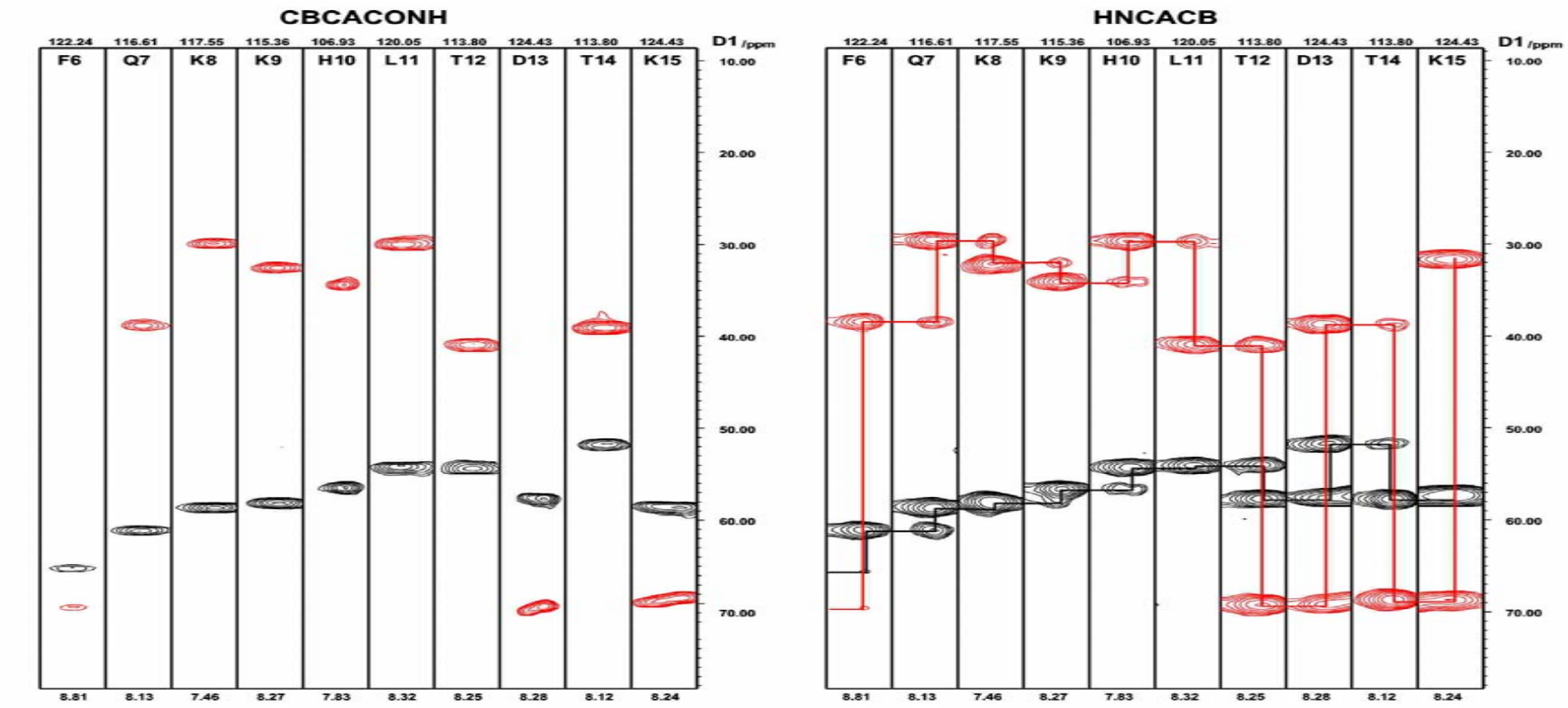



Bruker TOPSPIN 2.1 on av600 as cfchang
File Edit View Spectrometer Processing Analysis Options Window Help
APSY_DD.021709_1 1 1 /opt/topspin cfchang
Blue: 0
Red:90
Purple : 30
F1 [ppm]
F2 [ppm]
10
9
8
7
Acquisition information
no acquisition running
Lock
VTU
[Kelvin]
294.9
Spooler
queued: 0
delayed: 0
cron: 0
Time
18:51
Feb 23

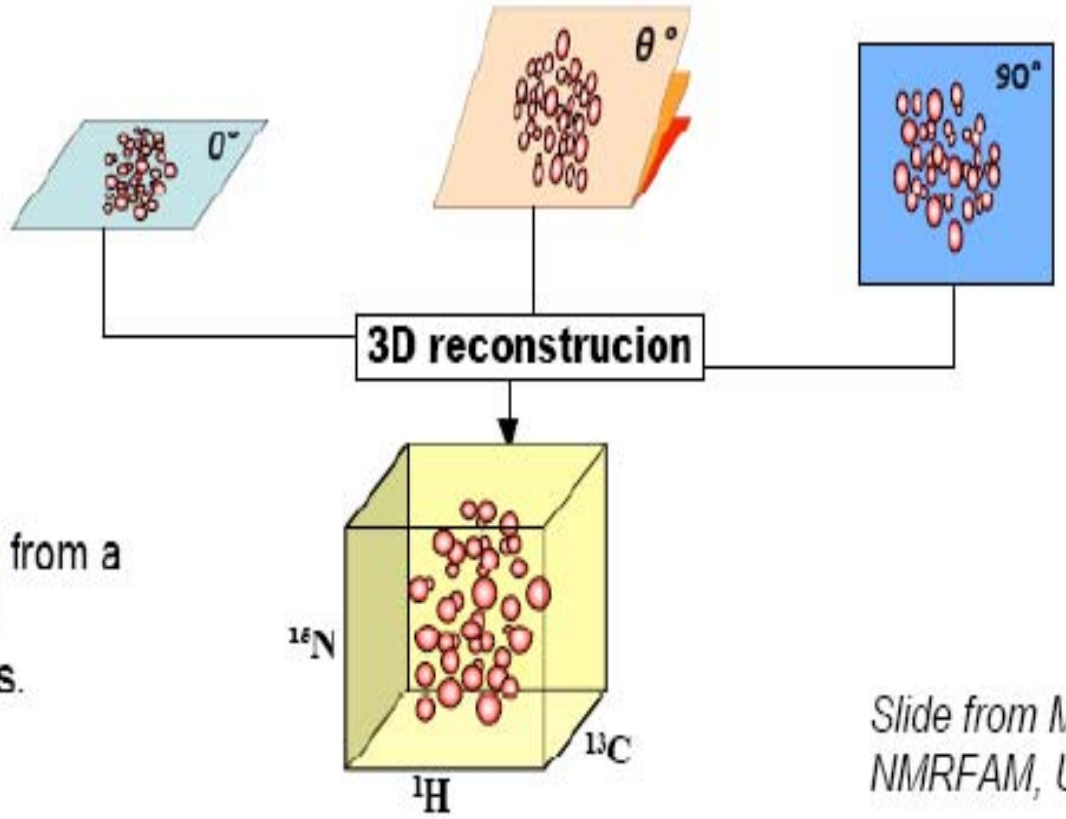
## Part II: Automatic data analysis

### Background:

- What we need is a peak lists with all chemical shift information
- Then, based on the peaks correlation between different spectra, chemical shift could be assigned



• Projection –Reconstruction Method




In principle, it is feasible to reconstruct a 3D spectrum from a number of 2D tilted planes collected at different angles.

*Slide from M. Tornelli*
*NMRFAM, UW-Madison*

• After reconstruction, go though traditional 3D data analysis process

## Method: Automatic peak analysis

**ATNOS : Peak picking program**

- Pick peaks for each projection spectrum

**GARPO: Geometric Analysis of Projections**

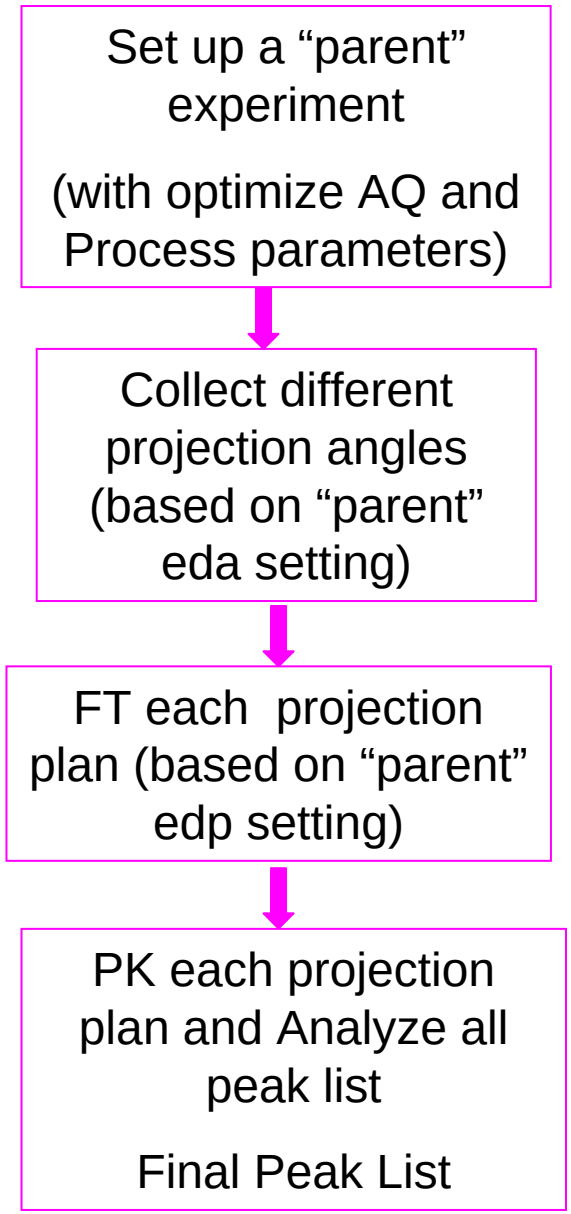
Proc. Nat. Acad. Sci. USA 102, 10876-10881 (2005).

- Analyze all peaks from different angles, and select real peaks

**Output: Final Peak List**

```
28 ********************************************************************************************************
29 *** NOH.peaks ***
30 ********************************************************************************************************
31 # Number of dimensions 3
32 #INAME 1 N
33 #INAME 2 O
34 #INAME 3 H
35        1     114.5913     168.7465       7.4410 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
36        2     118.4312     173.2002       7.7997 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
37        3     126.6306     169.4641       9.1843 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
38        4     116.7820     171.1912       8.5505 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
39        5     122.6248     171.1549       7.7035 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
40        6     118.0694     175.2307       8.3105 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
41        7     120.6934     170.2307       8.8864 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
42        8     110.6688     171.3524       8.7585 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
43        9     116.7303     166.4779       7.9777 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
44       10     111.4506     174.4963       6.5844 1 U   0.000E+00  0.000E+00 e 0      0      0      0      0
```

## Summary for APSY




- The same as setting up regular experiment

- Define the projection angles ( use default values)
- Could run all angles or let the program decide how many angles to collect

- Automatic FT (xfb)
- Change edp setting and re-process if necessary

- Automatic analyzing and show Result
- Change GARPO setting and re-analyze if necessary

# Running APSY in HFNMRC

## NMRs in HFNMRC

| System | AV500_ IBMS | AV600_ IBMS | AV600_ CHEM | AVIII600_ IBMS | AV800_ IBMS |
|---|---|---|---|---|---|
| Hardware | AVANCE Cryoprobe | AVANCE Cryoprobe | AVANCE | AVANCEIII Cryoprobe ATM | AVANCE Cryoprobe |
| Operation | Linux* | Linux | Windows | Linux | Linux* |
| Software | Topshin2.1* | Topshin2.1 | Topshin2.1 | Topshin2.1 | Topshin2.1* |
| APSY | YES * | YES | NO (demo) | YES | YES * |
| Process using Xwinnmr? | YES | YES | YES | NO | YES |
| BSMS Keybord | YES | YES | YES | NO | YES |

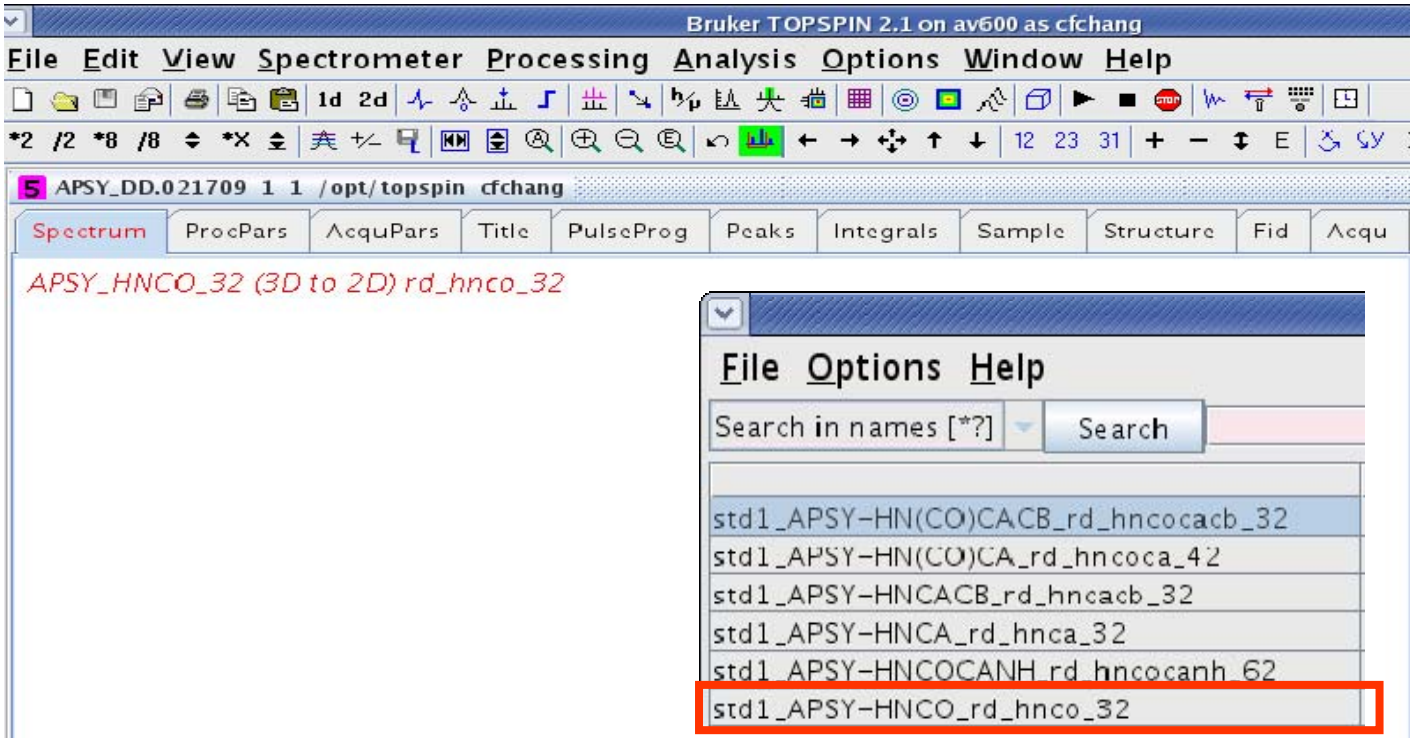
* Will be available in March, 2009

# How to run APSY in HFNMRC

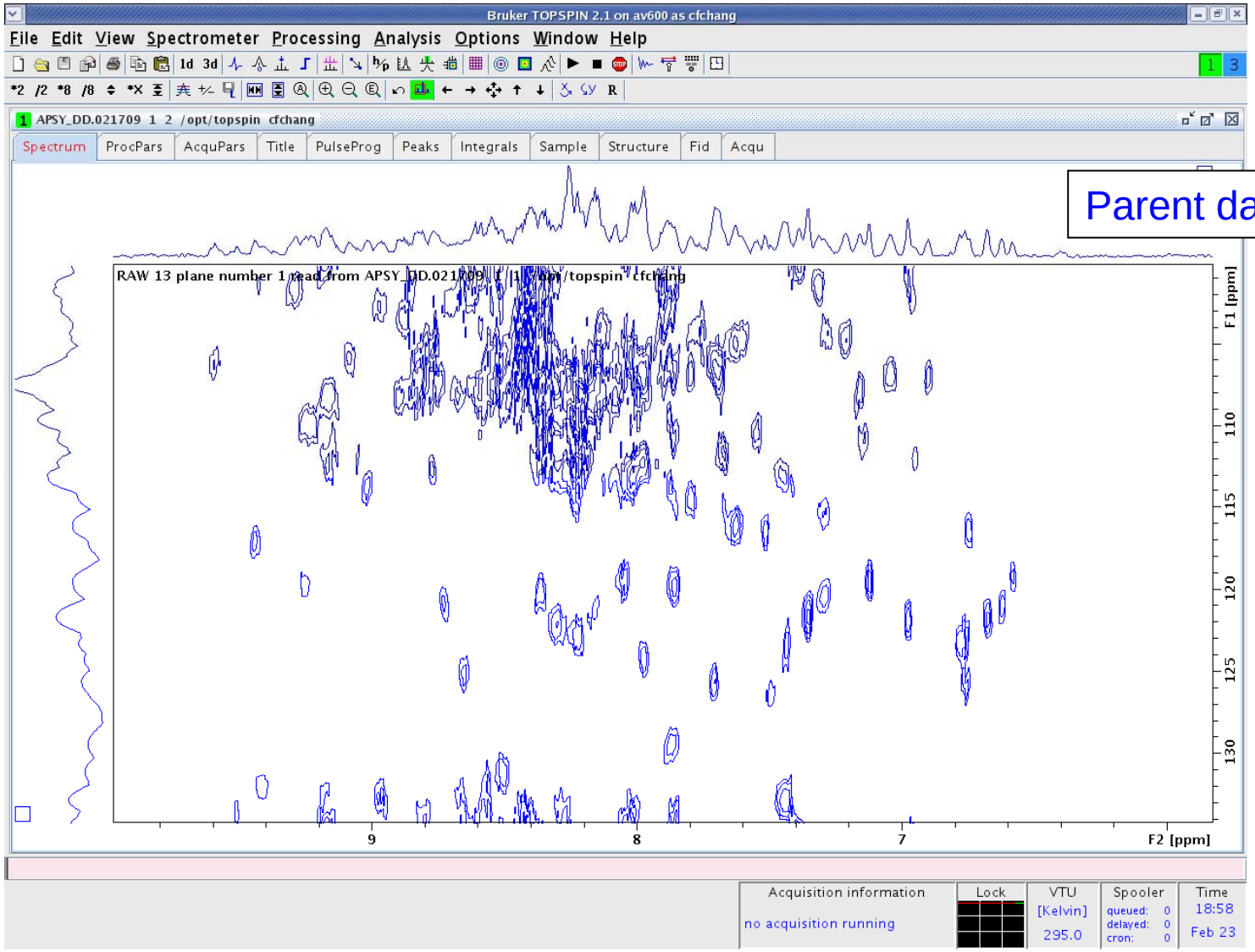
Set up a “parent” experiment

(with optimize AQ and Process parameters)

**STEP1 : rpar std1_APSY***



# STEP2 : set up eda and edp , then collect the “parent” spectrum




Parent data set

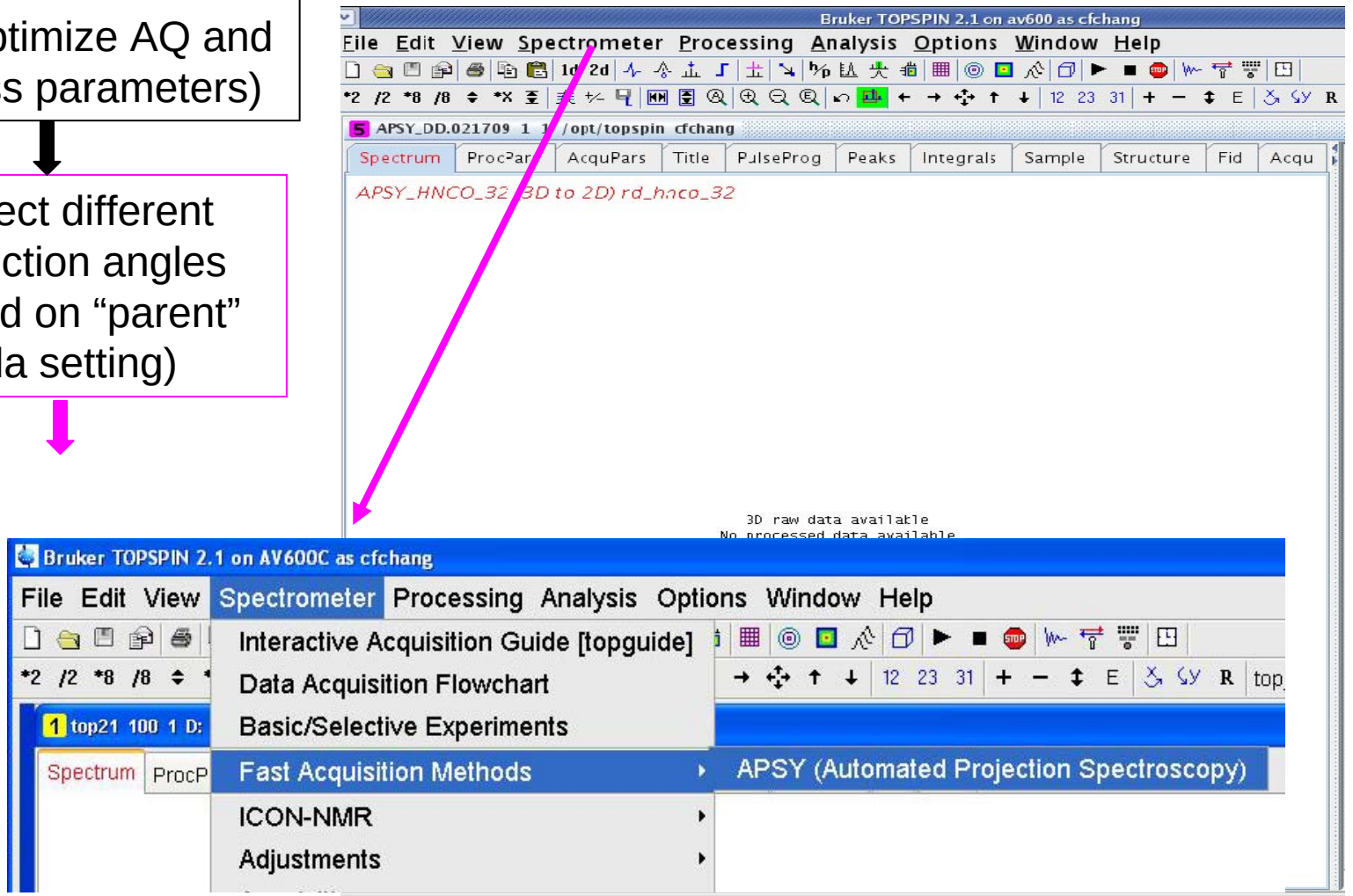
# How to run APSY in HFNMRC

Set up a “parent” experiment

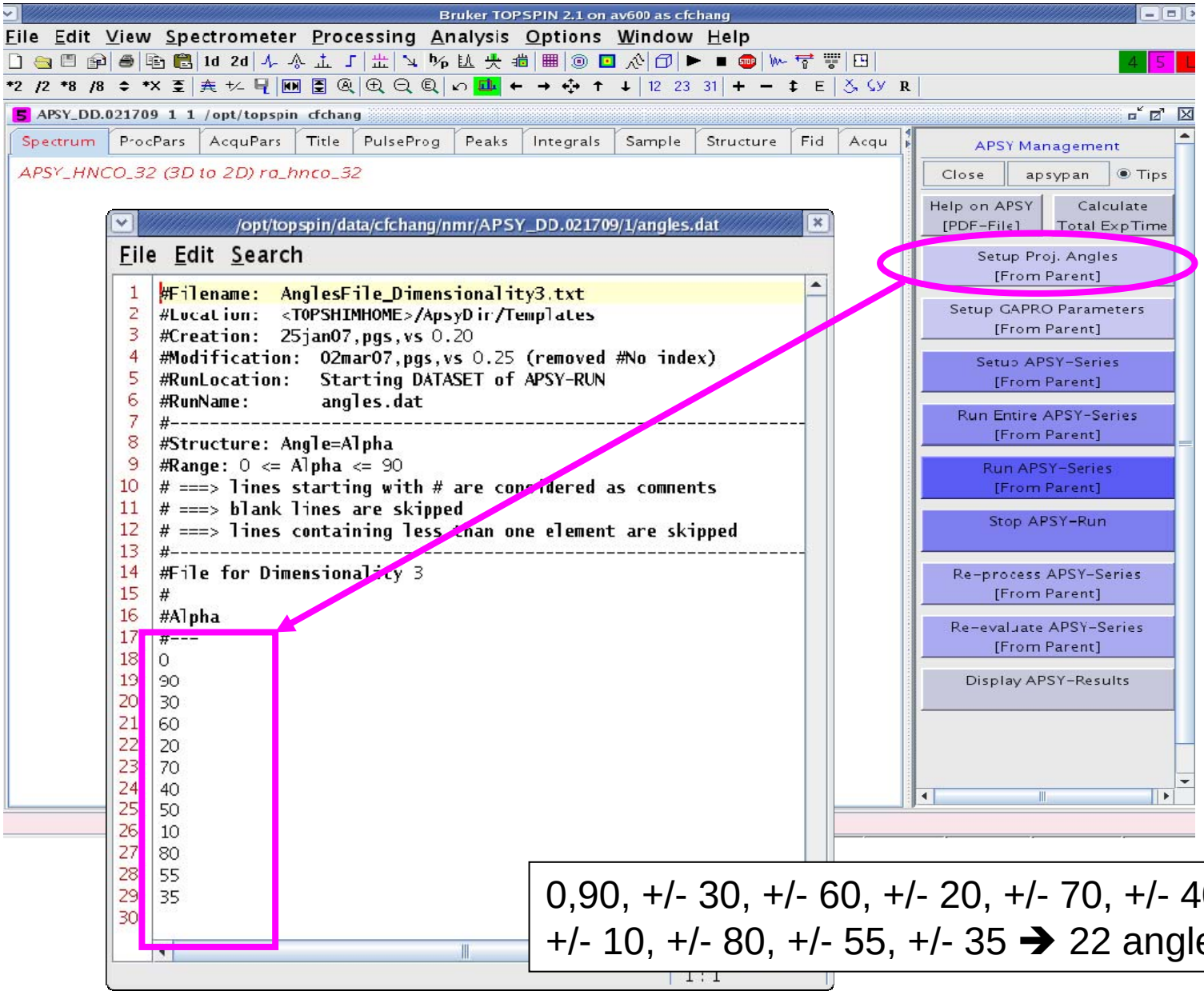
(with optimize AQ and Process parameters)

Collect different projection angles (based on “parent” eda setting)

**STEP3 : open APSY manual**

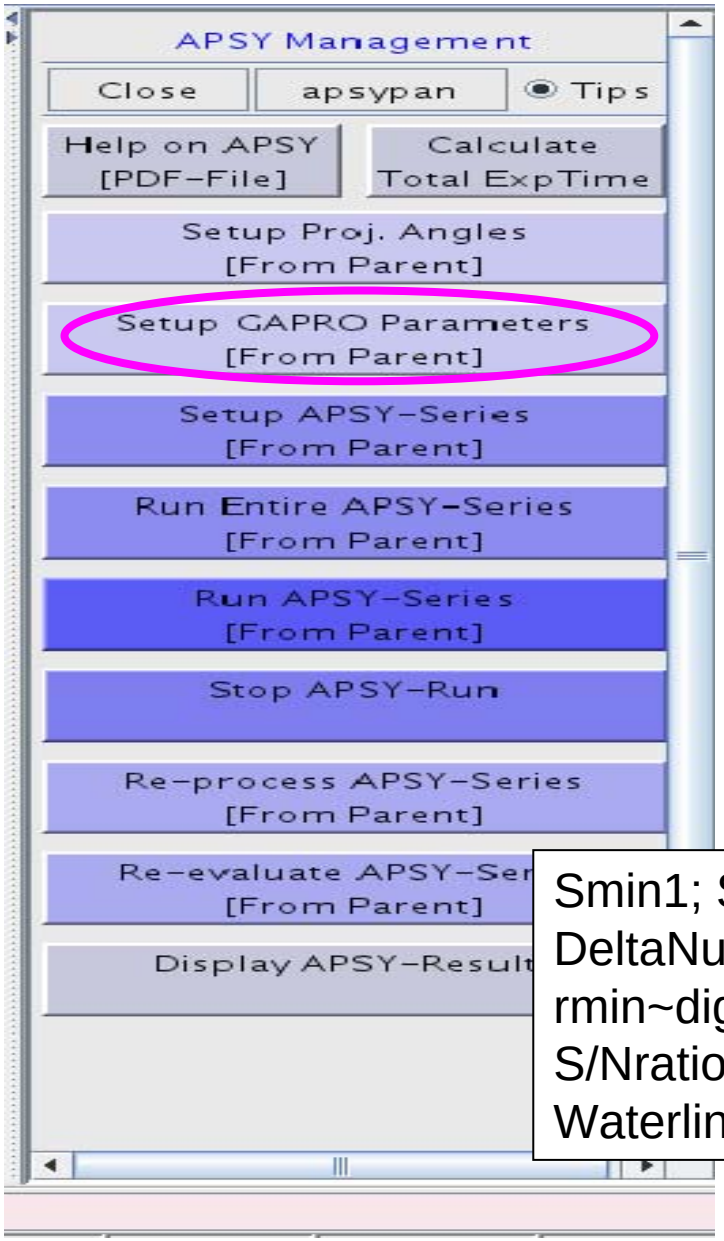


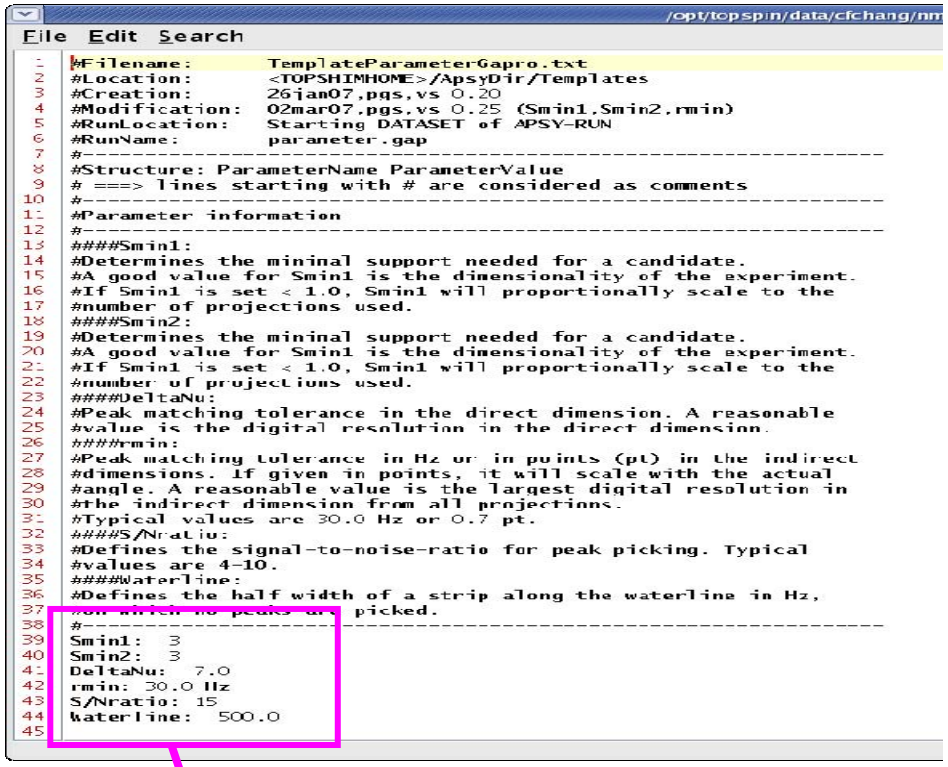
# STEP4 : Set up different projection angles




0,90, +/- 30, +/- 60, +/- 20, +/- 70, +/- 40, +/- 50, +/- 10, +/- 80, +/- 55, +/- 35 ➔ 22 angles

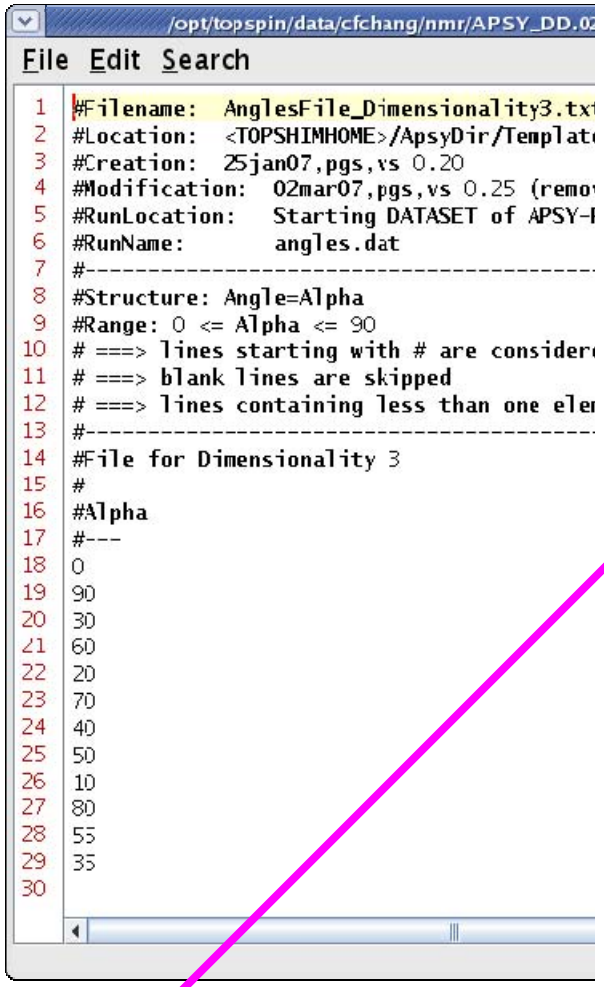
# STEP5 : Set up GARPRO parameters

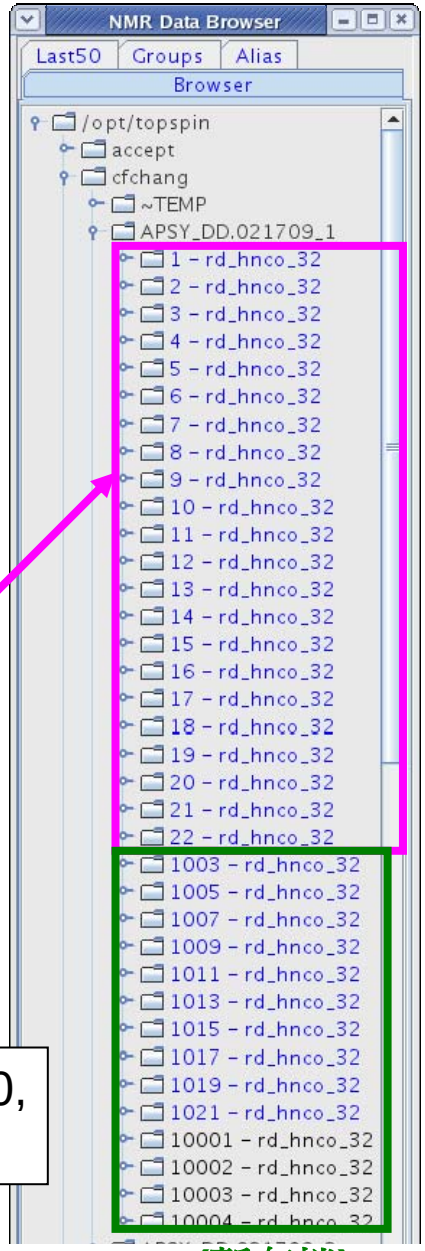



Smin1; Smin2 >= dimension (ex: 3)
DeltaNu ~ digital resolution in Hz in the direct dimension
rmin~digital resolution in Hz in the indirect dimension
S/Nration: typical values are 3-10
Waterline: halft-width water line in the direct dimension

# STEP6 : Set up different projection angles

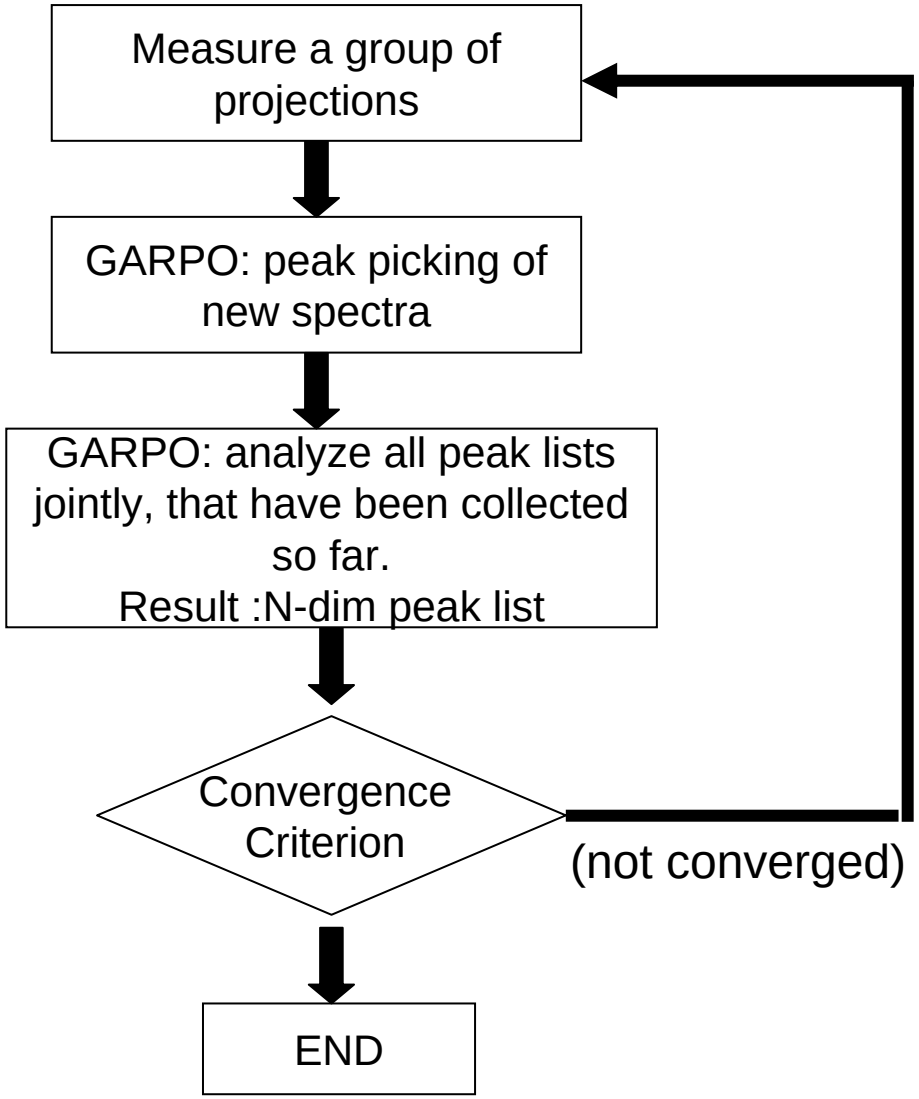



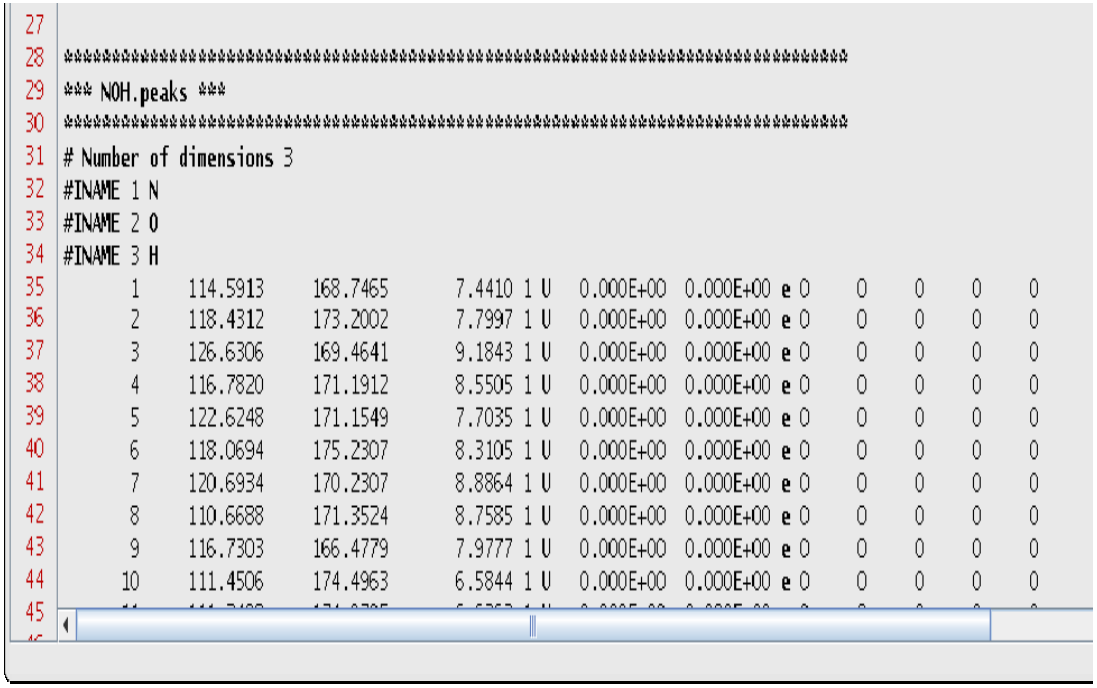

0,90, +/- 30, +/- 60, +/- 20, +/- 70, +/- 40, +/- 50, +/- 10, +/- 80, +/- 55, +/- 35 ➔ 22 angles

(暫存檔)

# STEP7 : Collect data for different projection angles

# STEP8 : Final Peak List Result

```
27
28 ********************************************************************************
29 *** NOH.peaks ***
30 ********************************************************************************
31 # Number of dimensions 3
32 #INAME 1 N
33 #INAME 2 O
34 #INAME 3 H
35       1    114.5913    168.7465      7.4410 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
36       2    118.4312    173.2002      7.7997 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
37       3    126.6306    169.4641      9.1843 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
38       4    116.7820    171.1912      8.5505 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
39       5    122.6248    171.1549      7.7035 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
40       6    118.0694    175.2307      8.3105 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
41       7    120.6934    170.2307      8.8864 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
42       8    110.6688    171.3524      8.7585 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
43       9    116.7303    166.4779      7.9777 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
44      10    111.4506    174.4963      6.5844 1 U   0.000E+00  0.000E+00 e 0    0    0    0    0
45
```

If change edp parameters

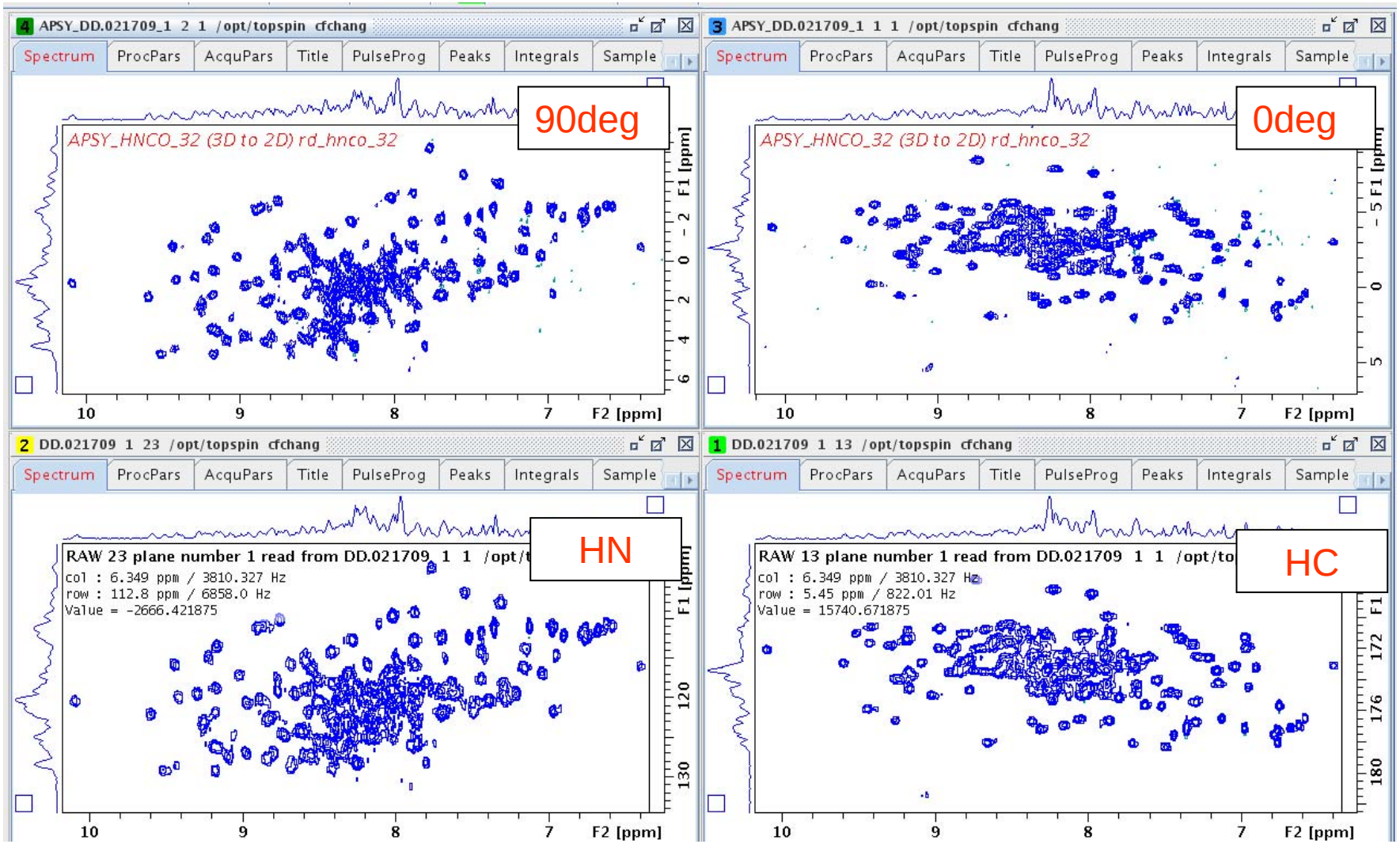
If change GARPO parameters

## Example

Sample : 145 assigned AA 13C/15N protein

System: AV600_IBMS ( regular TXI probe)

Experiment : rd_hnco_32 vs. 3D HNCO, NS=8

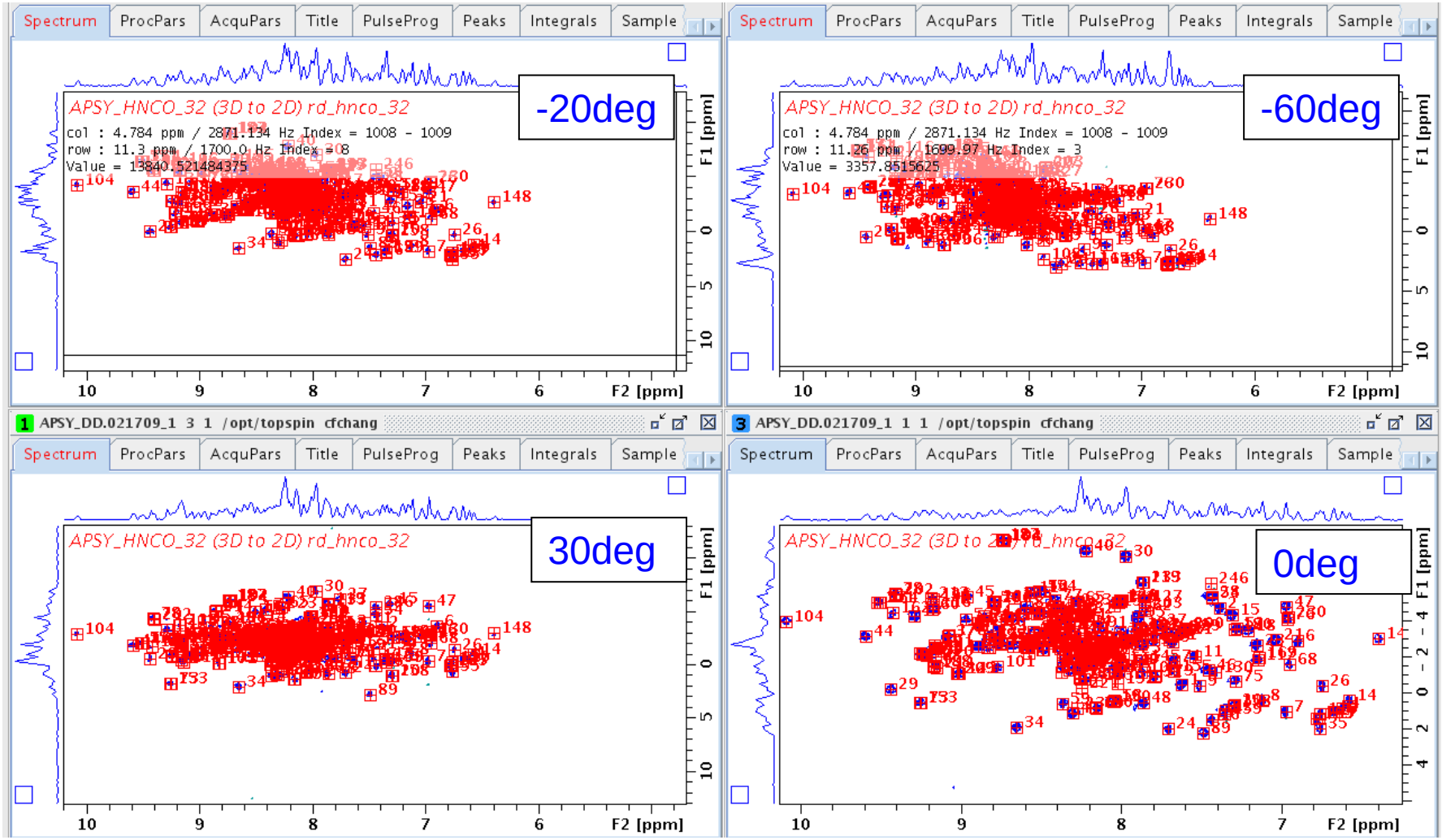


## Example

Sample : 145 assigned AA 13C/15N protein

System: AV600_IBMS ( regular TXI probe)

Experiment : rd_hnco_32, NS=8



## Example

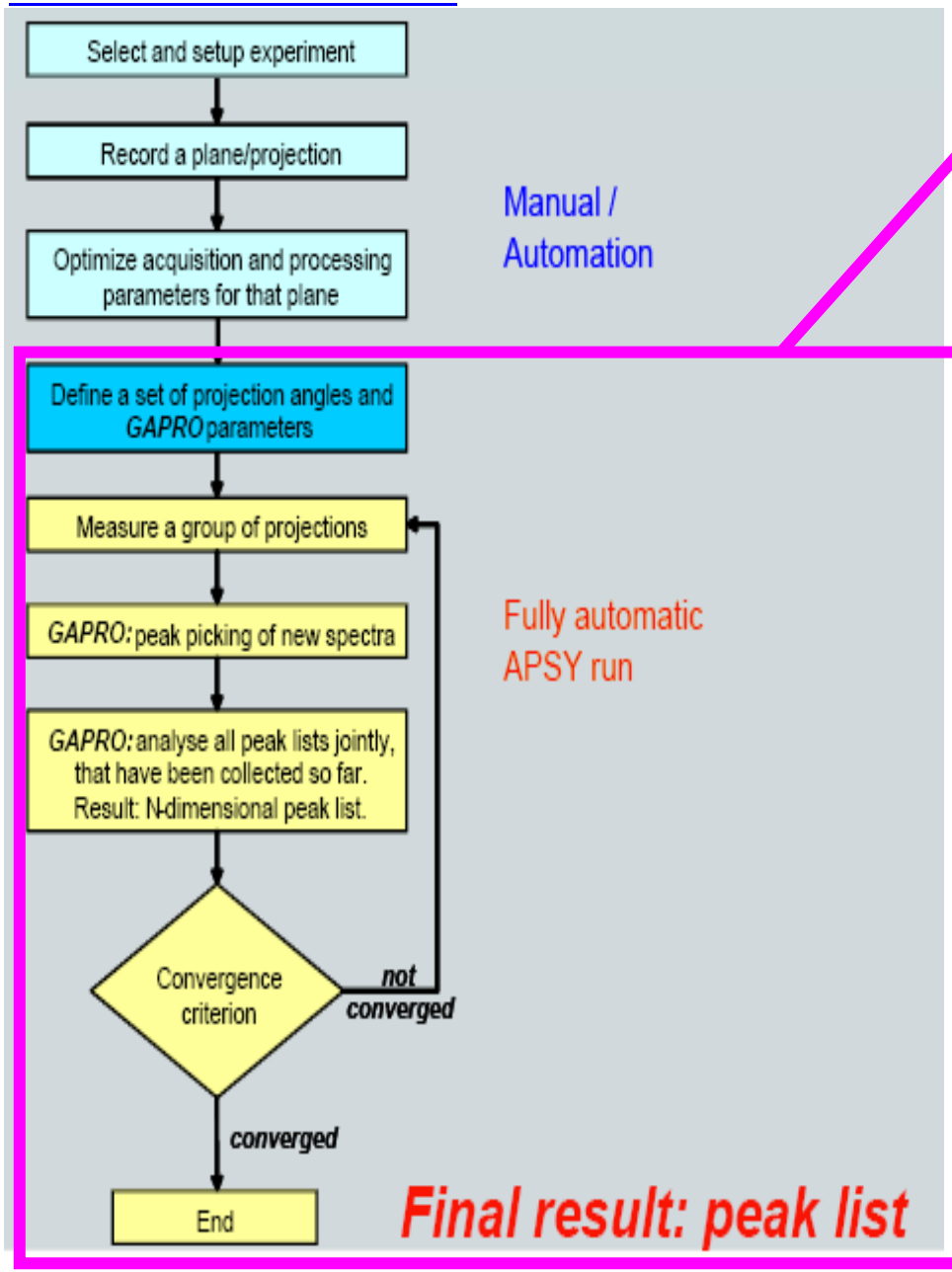
Sample : 145 assigned AA 13C/15N protein

System: AV600_IBMS ( regular TXI probe)

Experiment : HNCO, NS=8, 2K*64(CO)*32(NH)

| | 3D HNCO | APSY-HNCO |
|---|---|---|
| Expt. Time | ~ 6hours | ~9min/angles<br>~3.5 hours for 22 angles |
| Peaks | ~130/170 | |
| (1) S/N=10 | | ~130/242 |
| (2) S/N=15 | | ~125/170 |
| (3) S/N=20 | | ~120/170 |

# APSY: Flow Chart





(From Bruker APSY document)

# What's New in HFNMRC

# (Standard Parameter Set)

## Standard Parameter Sets in HFNMRC (std* )

### std*_nD_exptname_pp

(1) Bruker pulseprogram

**( pulseprogram from Bruker data base or with minor correction)**

**std0**_nD_expt_pp : for small molecules ( ~100% D-solvent)

**std1**_nD_expt_pp : for biomolecules ( ~10% D-solvent )

(2) Implemented/modify version

**( pulseprogram not in Bruker data base or with major modification)**

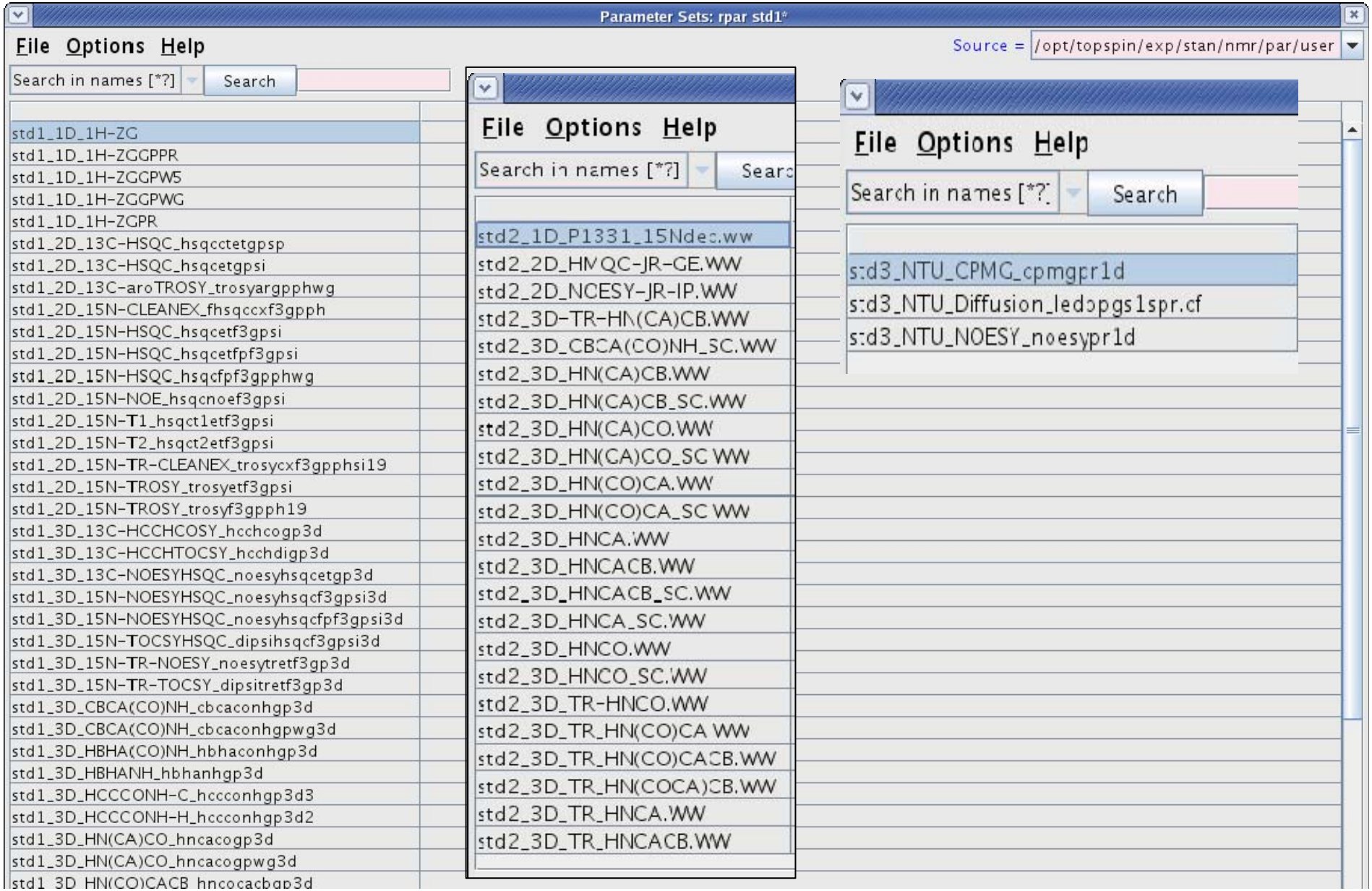
**std2**_nD_expt_pp

(3) Others

**( home-made experiments, or custom-requested experiments)**

**std3**_**

# Standard Parameter Sets in HFNMRC (std1* or std2* or std3* )



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