New for APSY

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APSY experiments and software

What is new:

☺ Finally, it is back ☺

1. Supported since Topspin 3.2pl2
2. Support for Topspin 3.0 & 3.1: on request, contact DMO
3. Matched projection angles for optimum resolution in projections
4. Easy setup using a flowbar interface
5. No license required

APSY experiments and software

Motivation for using APSY:

1. APSY is a method based on projection spectroscopy
2. 2D-projections are used to describe a nD space
3. Rapid acquisition method
4. Simple experiment setup and analysis

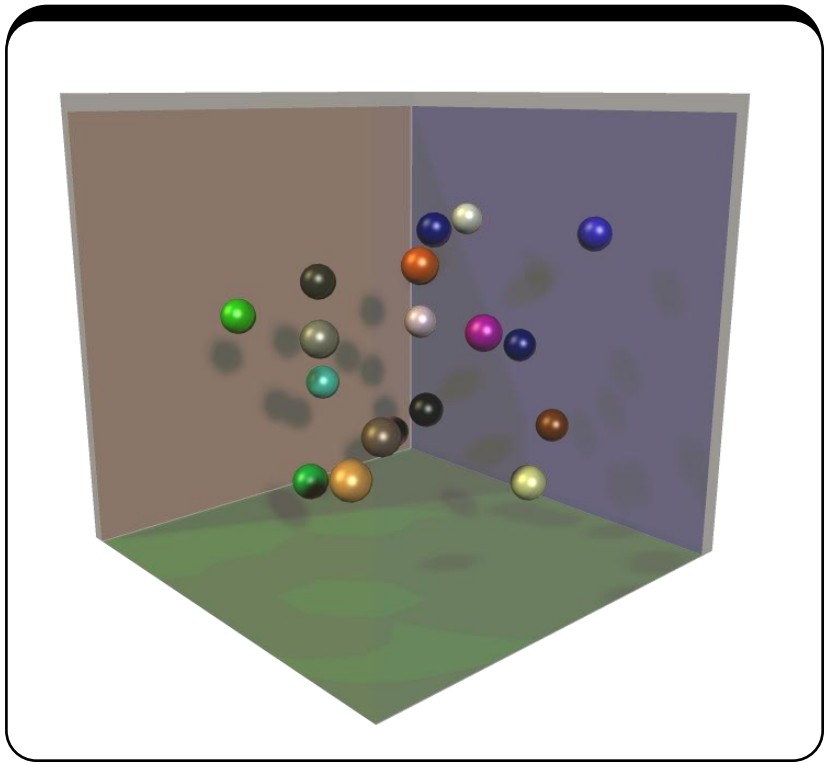
What do we get from APSY:

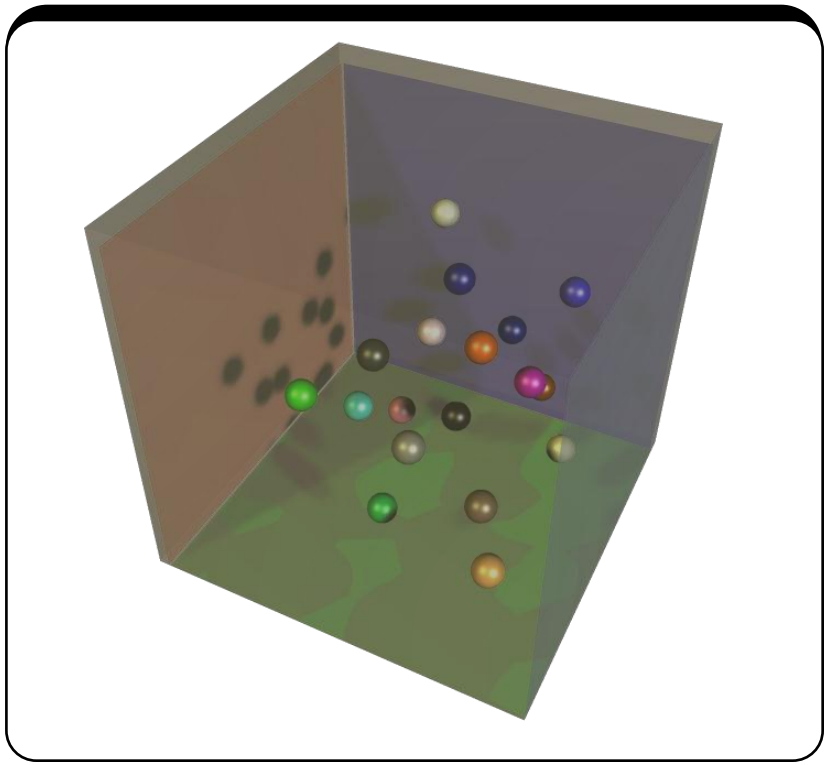
1. A nD peak list, not a spectrum
2. Peak list of high precision
3. Strength of APSY: high precision peak lists

Projection Spectroscopy

What is ‘projection spectroscopy’?

1. ***Projections*** use different viewpoints to evaluate content of a n-D space

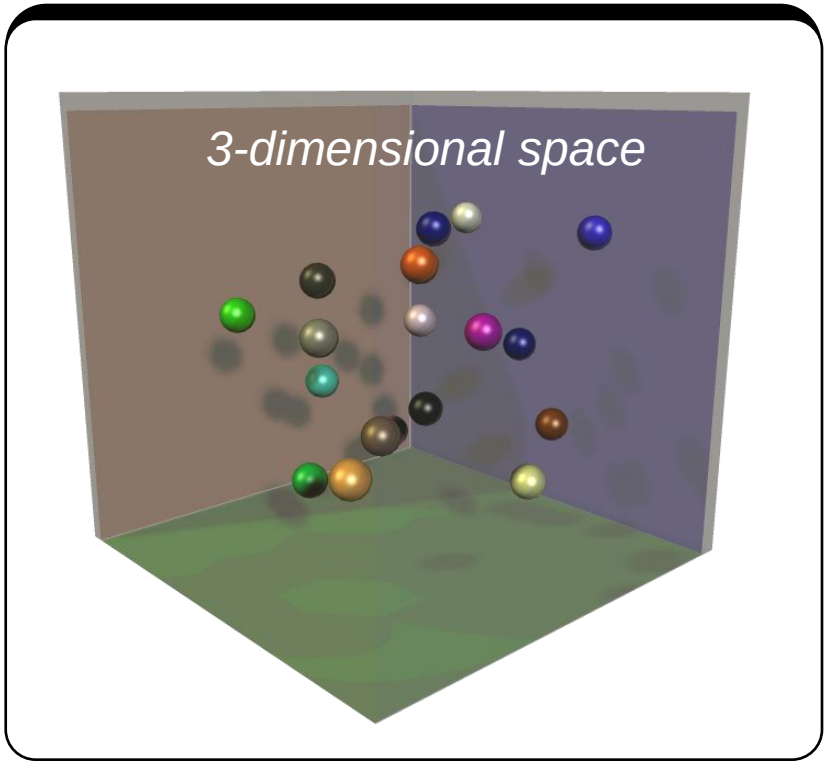


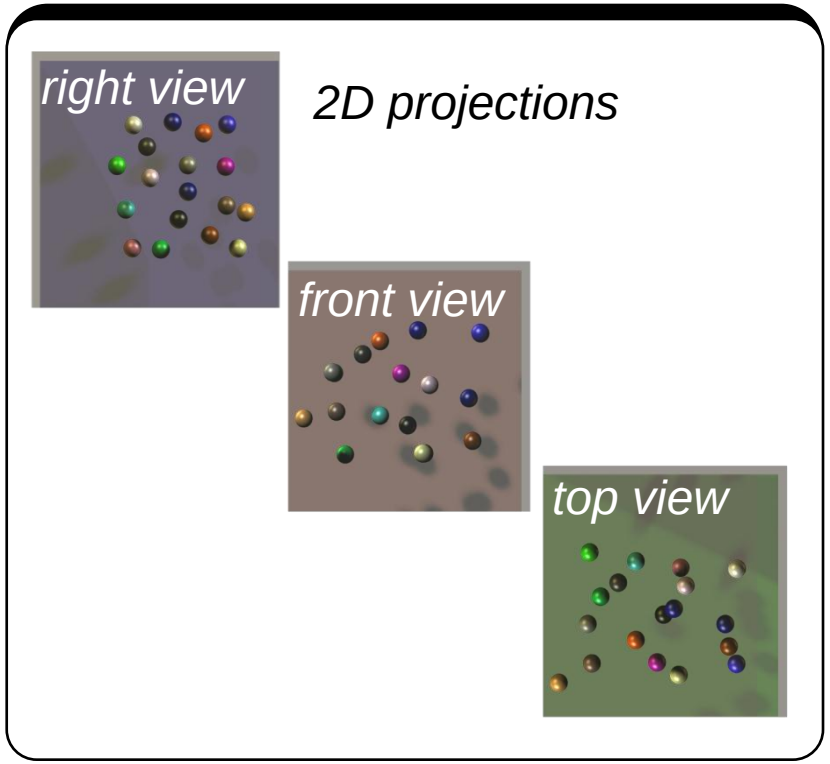


Projection Spectroscopy

What is ‘projection spectroscopy’?

1. ***Projections*** use different viewpoints to evaluate content of a n-D space
2. ***Reduction of dimensionality:*** example: 2D-projections for description of a n-D space.

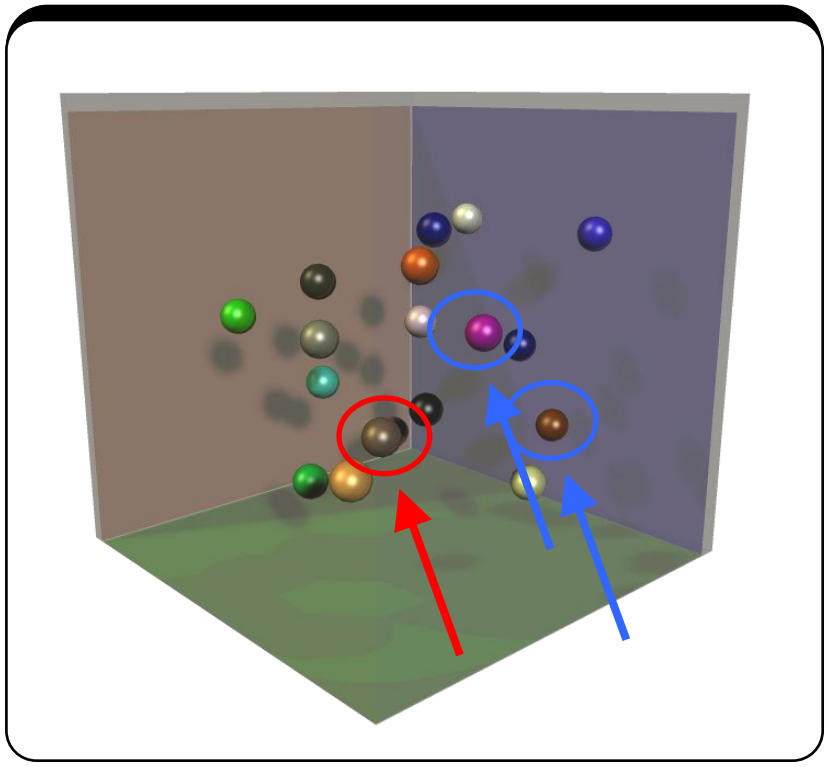


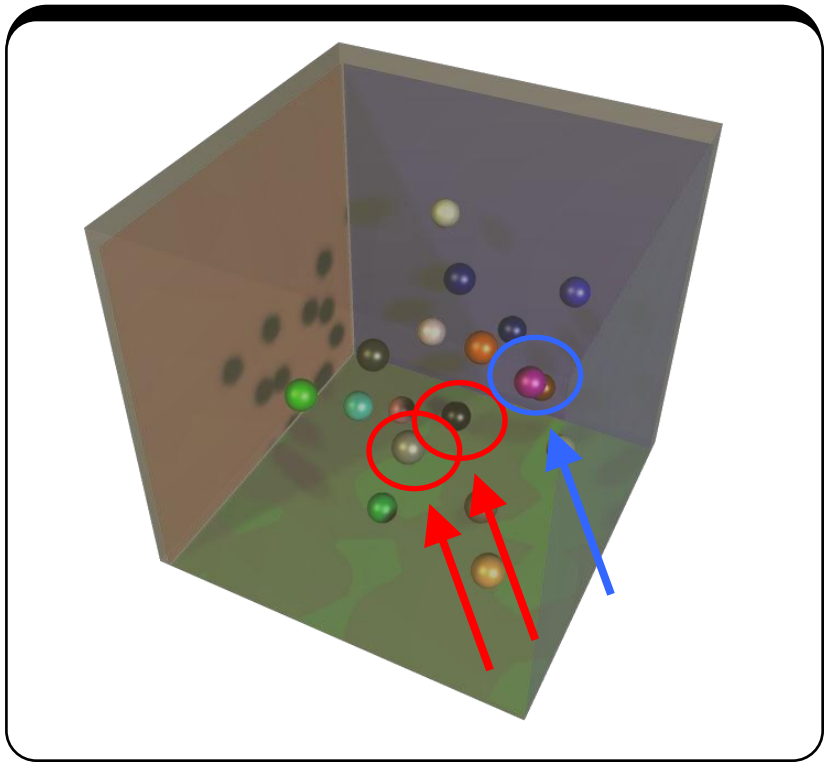


Projection Spectroscopy

What is ‘projection spectroscopy’?

Projection angles Multiple viewpoints are required

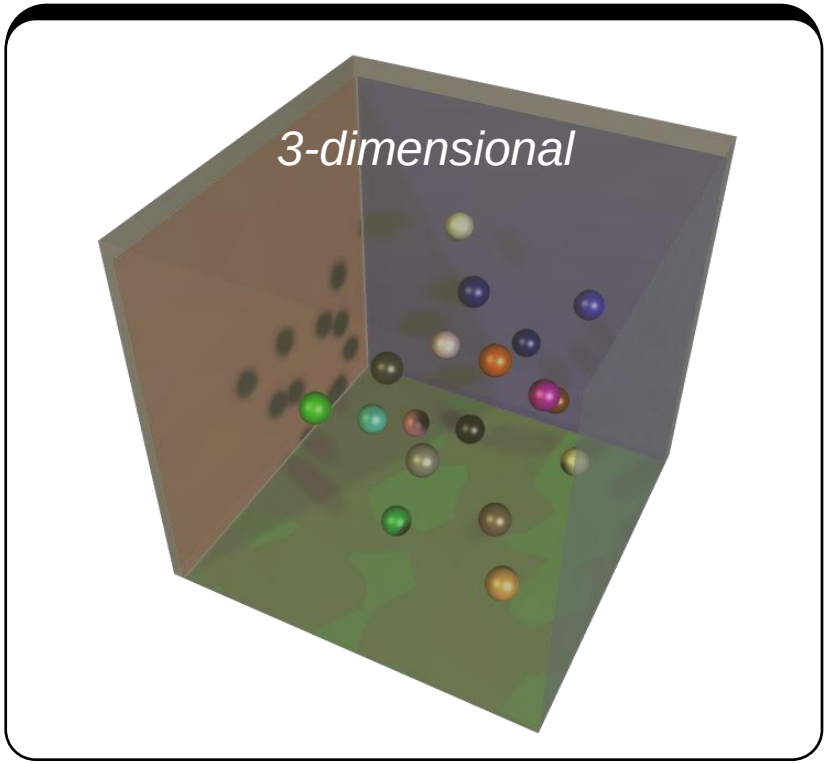


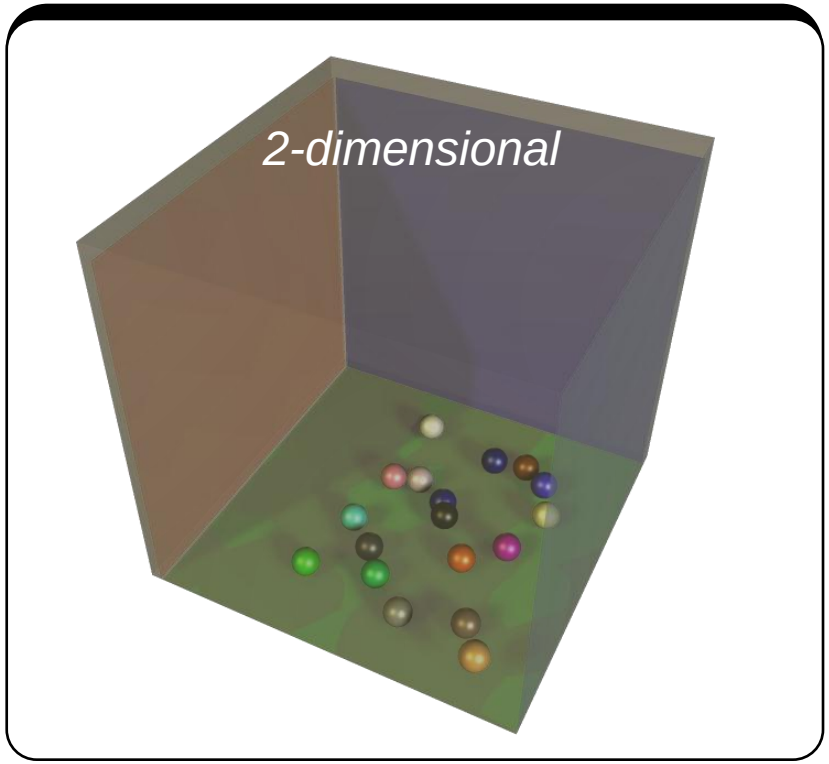


Projection Spectroscopy

Reduction of dimensionality:

Consequence: Information of additional dimensions is lost



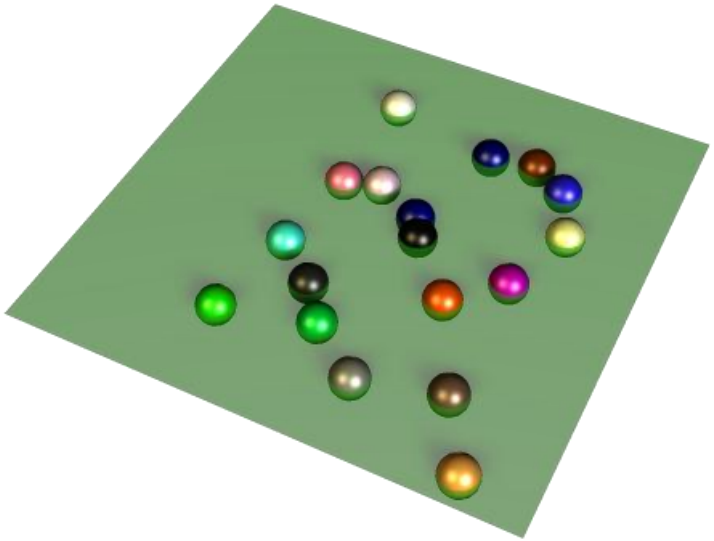


Projection Spectroscopy

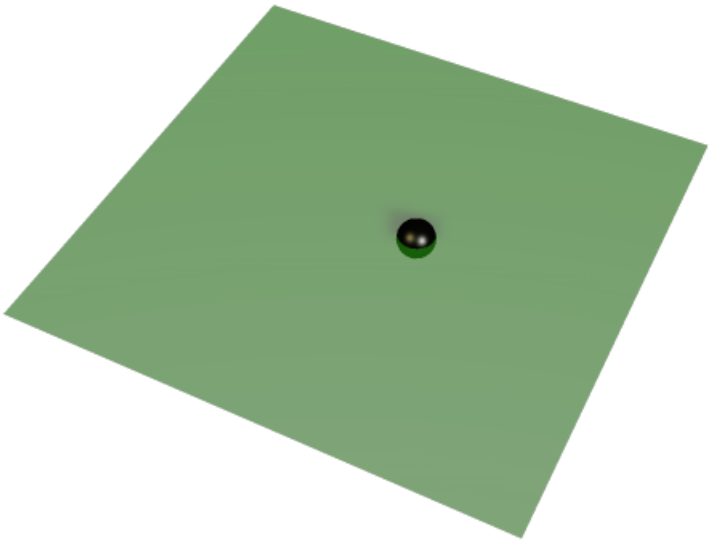
Reduction of dimensionality:

Consequence: Information of additional dimensions is lost

2-dimensional, all peaks



2-dimensional, one selected peak

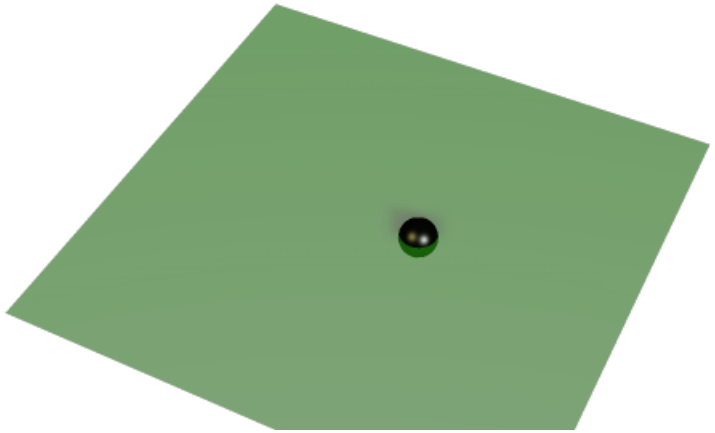


Projection Spectroscopy

Reduction of dimensionality:

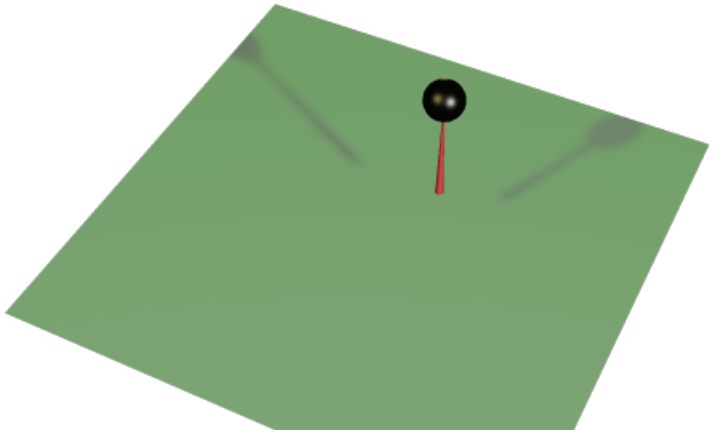
Consequence: Information of additional dimensions is lost

2-dimensional, one selected peak
No information about shift in Z



dimensionality:
reduced

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



dimensionality:
full

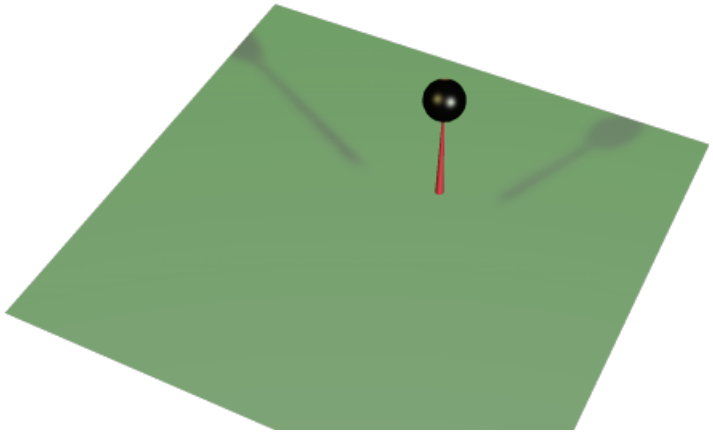
Projection Spectroscopy

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

Shift information of reduced dimensions is lost, but:

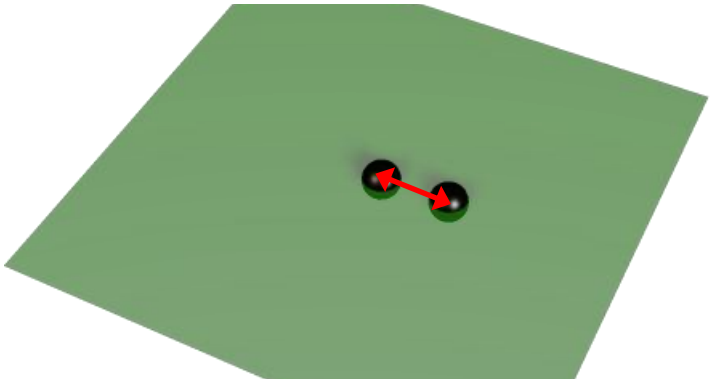
- Shift information is coded as a distance
- By additional splitting of single peaks

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

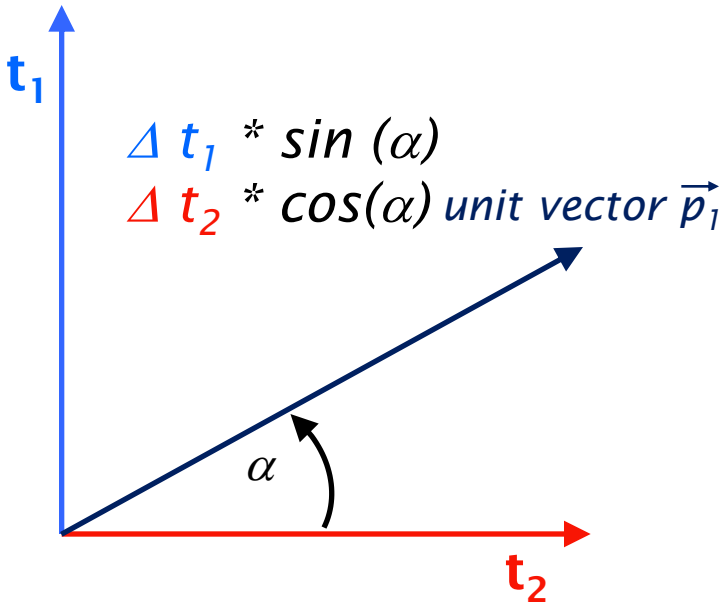
Recording of projection spectra

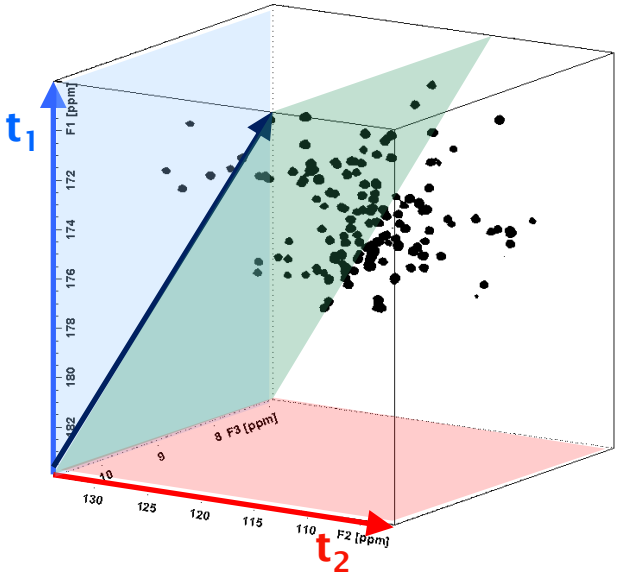
Example: **3D HNCO experiment**

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

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

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





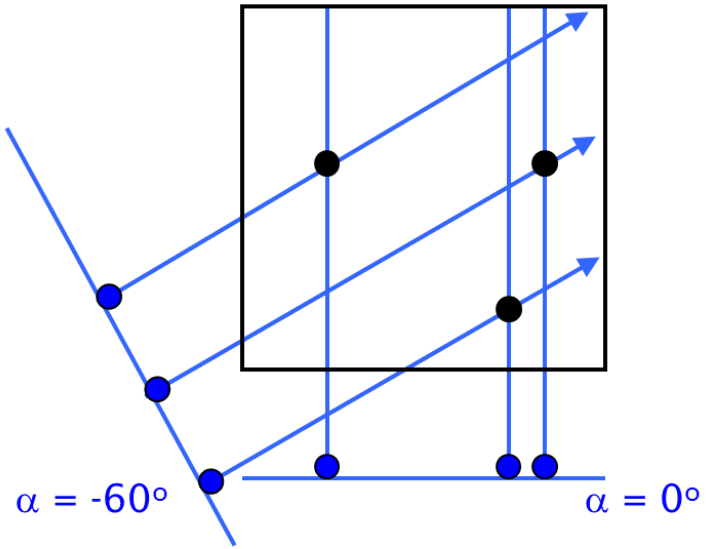
Projection Spectroscopy

What can be done with the projections?

1. Reconstruct a n-dimensional spectrum:
    - ***projection reconstruction***
2. Reconstruct a n-dimensional peak list:
    - ***APSY***

Principle of GAPRO

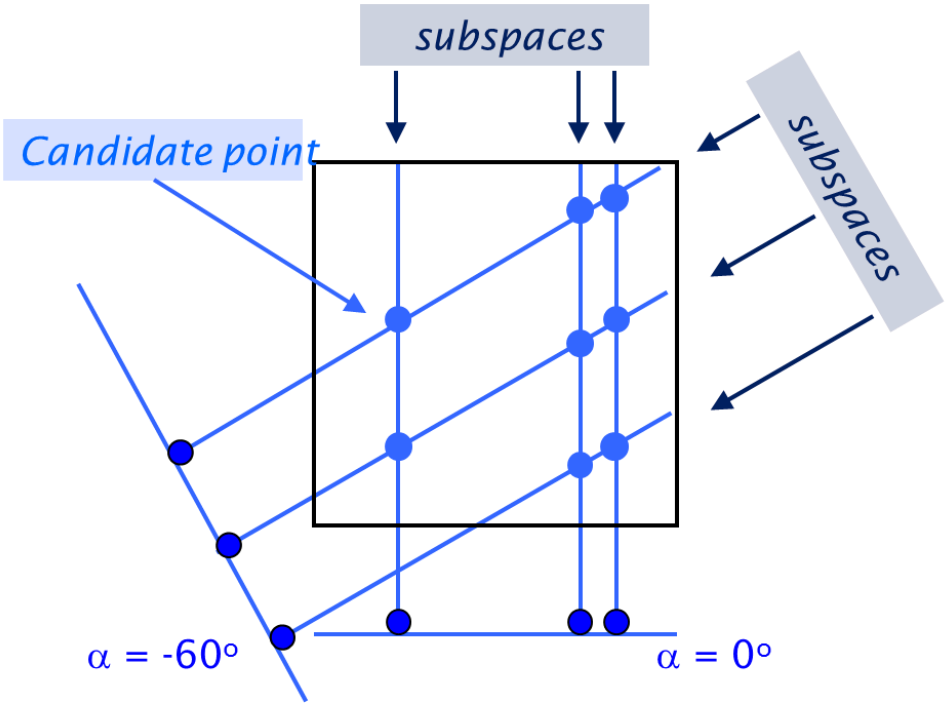
1. step: two projections are measured and selected arbitrarily:
e.g. 0° and -60°



Principle of GAPRO

1. step: two projections are selected arbitrarily:
e.g. 0° and -60°

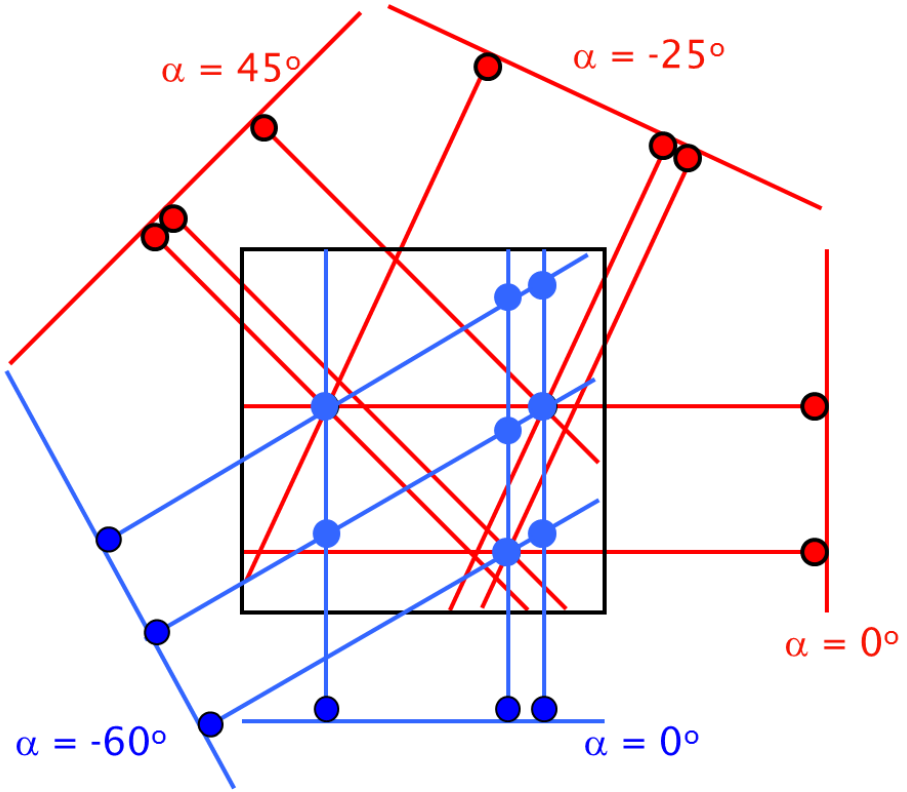
Intersection of subspaces creates candidate points



Principle of GAPRO

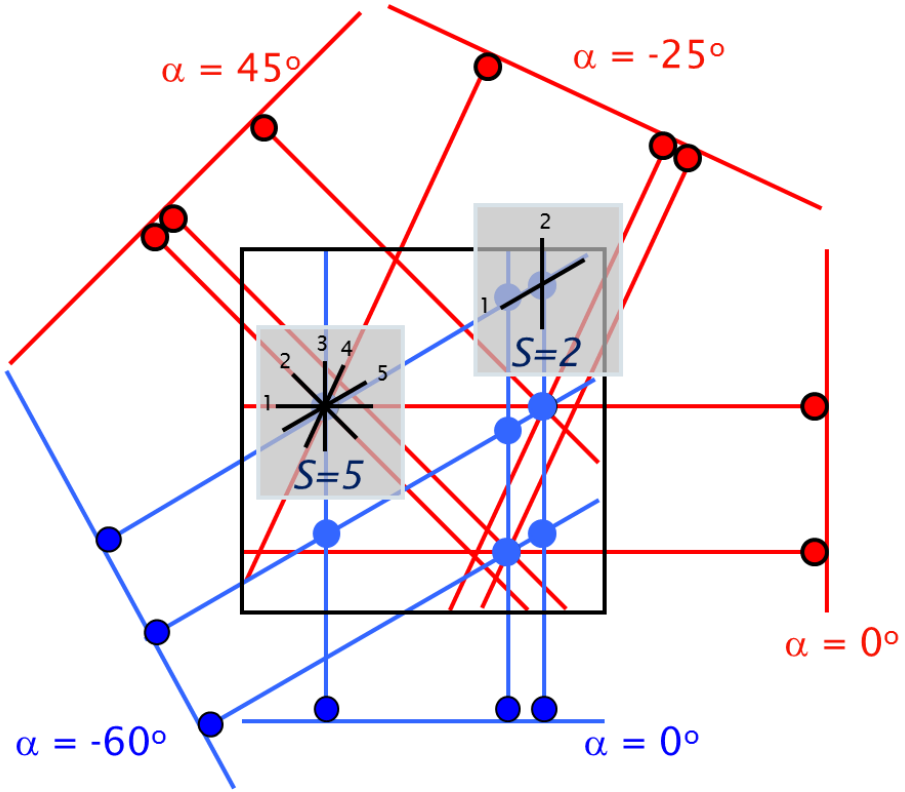
2. step: additonal projections included: e.g. 0°, -25° and 45°

Calculate additional intersections/subspaces



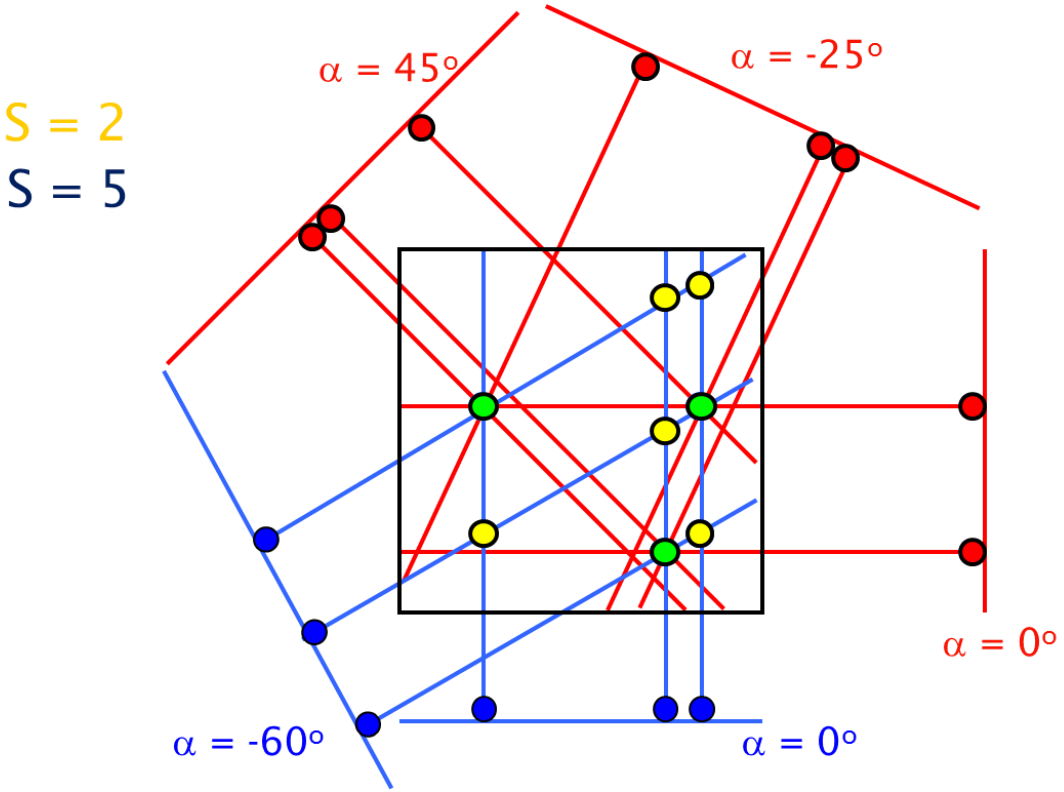
Principle of GAPRO

3. step: number of intersecting subspaces (*support S*) is calculated for each candidate point



Principle of GAPRO

3. step: *support S:* high values result in high ranking



APSY experiments and software

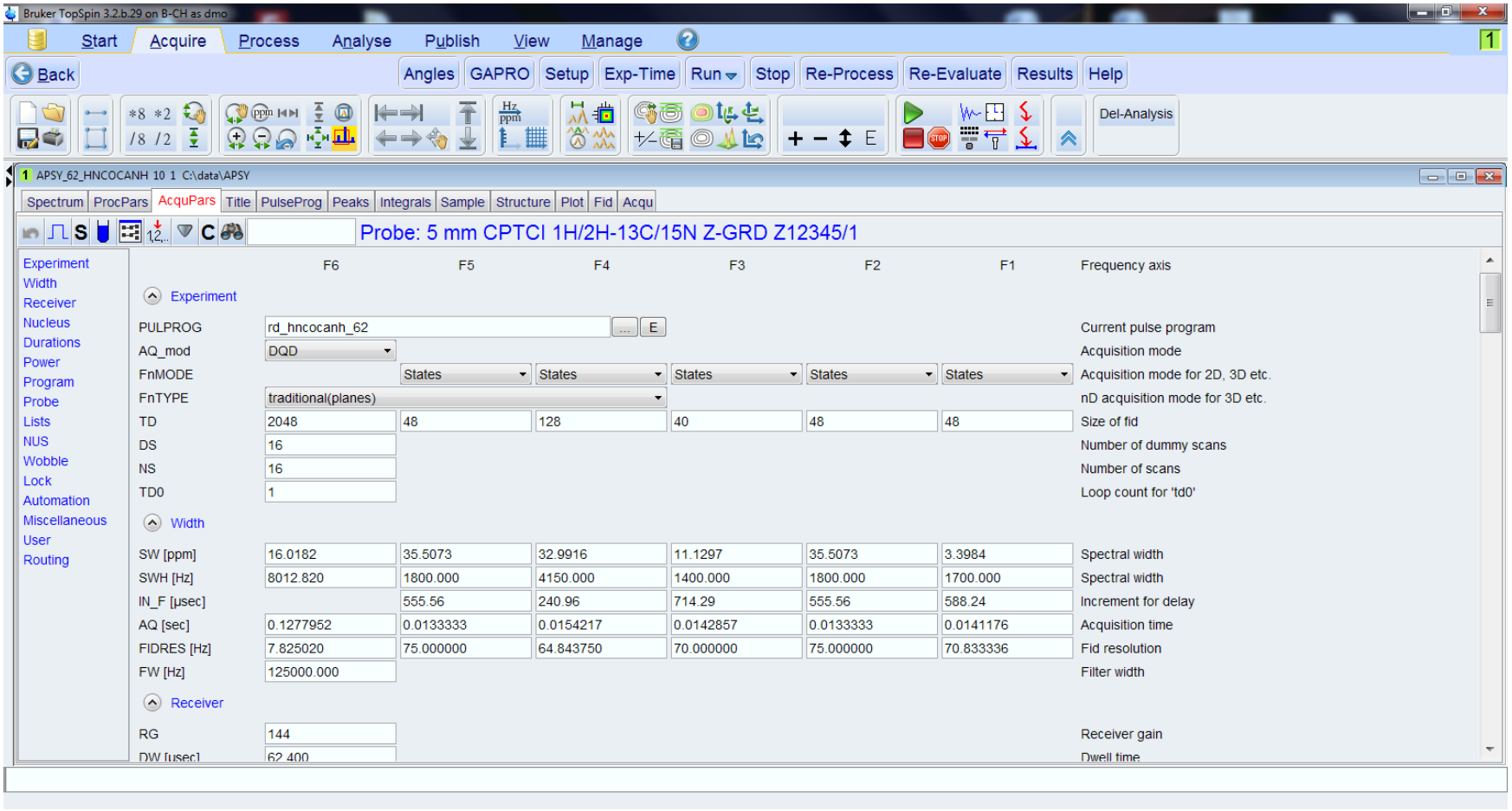
APSY as rapid acquisition method

	Conventional 48 and 128 increments for dimensions t_1 and t_2 32 increments for t_3 16 for t_4, t_5 and t_6	**APSY** 96 increments for all projection angles	**Time saving**
Dimensionality APSY	**exp. time [h]**	**#projections**	**exp. time [h]**	**factor conventional :**
3D	**2**	**20**	**0.5**	**4**
4D	**54**	**40**	**1.0**	**54**
5D	**864**	**60**	**1.5**	**576**
6D	**13824**	**80**	**2.0**	**6912**
7D	**221184**	**100**	**2.5**	**88474**

D1 + AQ = 1sec, number of scans = 1, sensitivity of different experiments not considered

APSY experiments and software

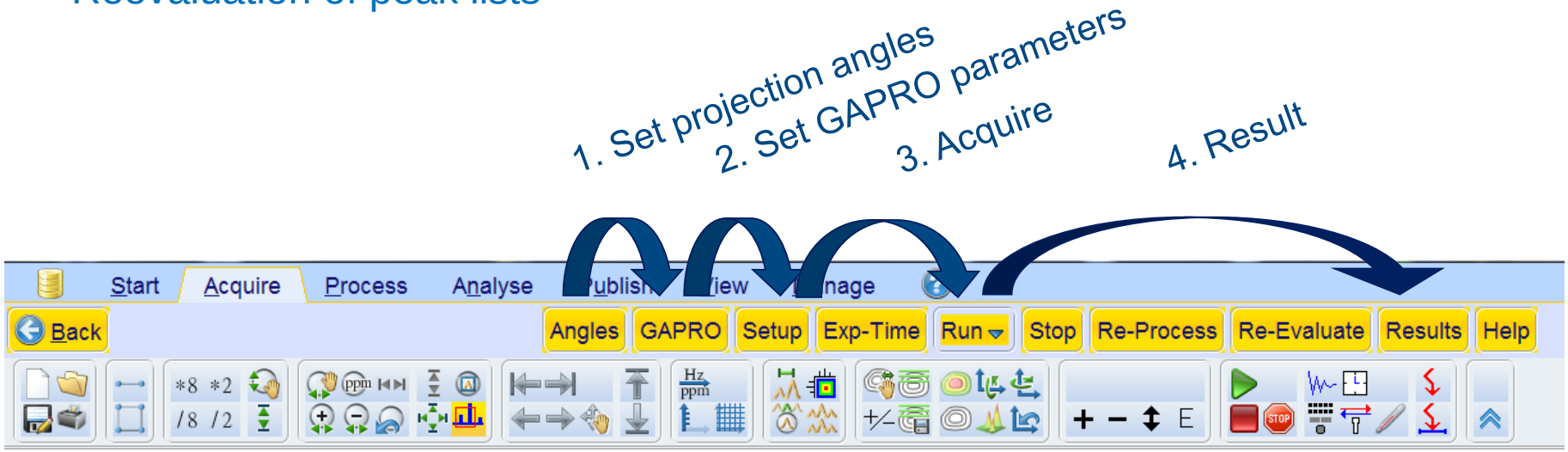
Acquisition parameters for APSY 6,2-HNCOCANH



APSY experiments and software

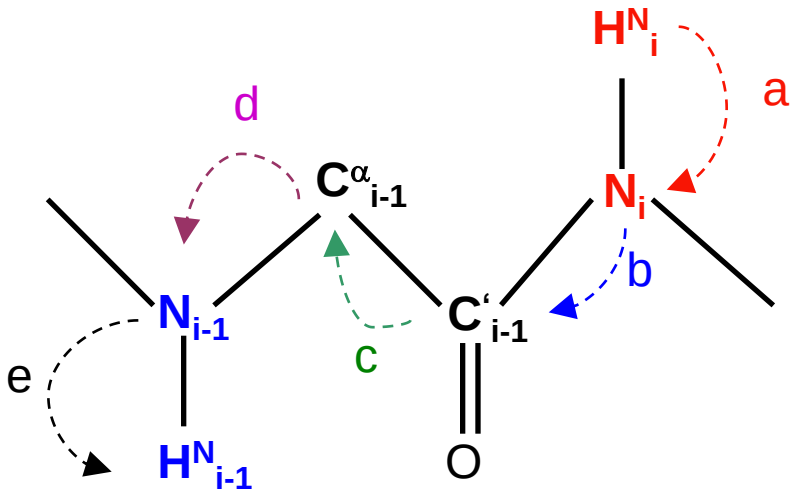
APSY flow bar

- Define projection angles
- Define GAPRO processing parameters
- Run APSY experiment
- Reprocessing of projections
- Reevaluation of peak lists



APSY experiments and software

6D sequential H_i-N_i-CO_{i-1}-CA_{i-1}-N_{i-1}-H_{i-1}



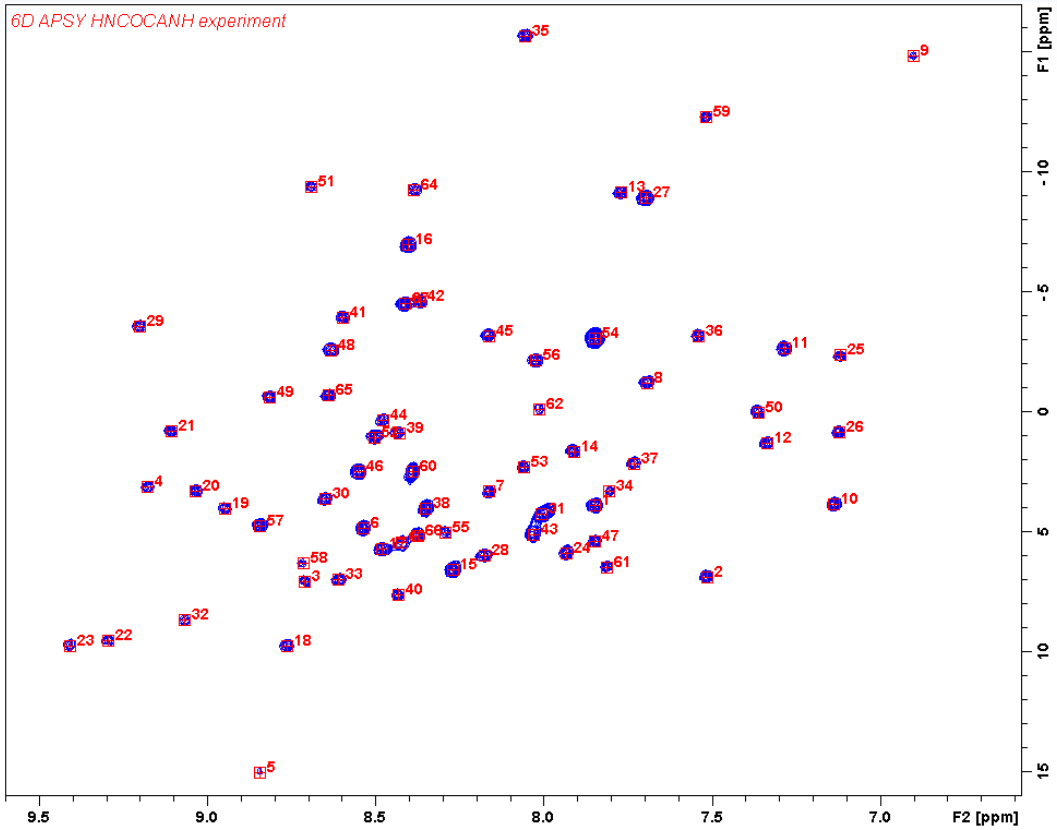
APSY: the peak list

•High Precision: peak lists of high precision from an optimum number of projections.

H_{i-1}	N_{i-1}	CO	CA	N_i	H_i	$\delta(N_{i-1}-N_i)$
ppm	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*Hz*
8.0613	102.4332	177.1224	52.3724	133.0131	8.8405	-4.635
8.8578	133.1058	174.3129	56.454	125.1379	8.7061	1.415
8.7125	125.1096	175.6223	58.7179	122.0751	8.9428	-1.24
8.9591	122.0999	174.9824	52.7983	124.366	8.7145	-4.17
8.716	124.4494	173.6779	54.8708	123.1657	8.378	---

Sequential assignment of [^{13}C,^{15}N]-ubiquitin using the peak list from a 6,2-APSY-HNCOCANH experiment.

APSY: the peak list

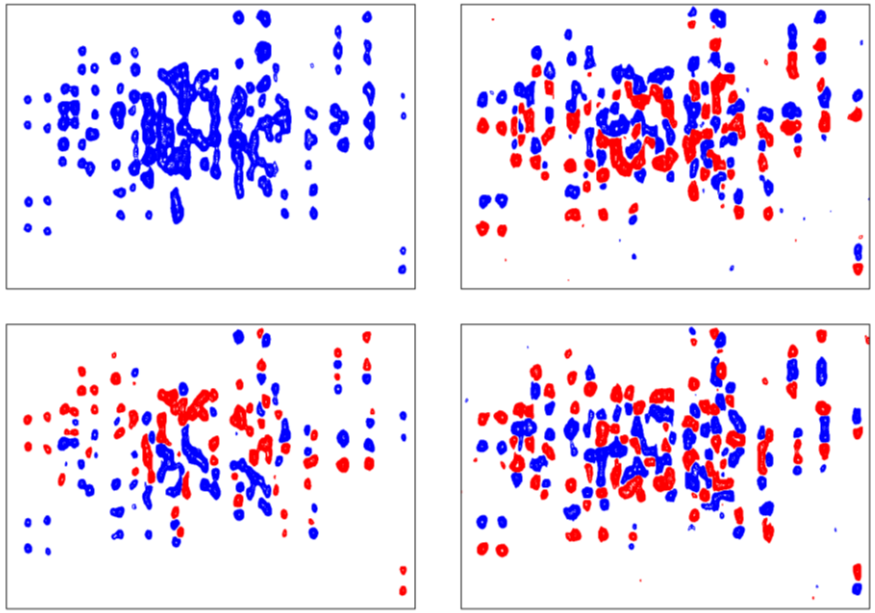


2D projection of a 6,2-APSY-HNCOCANH experiment. The displayed peak list was calculated from the 6D peak list and displayed on the projection

APSY challenges

Challenge / limitations:

- Signal loss for experiments of high dimensionality: relaxation
- Increased complexity of spectra due to simultaneous incrementation and multiple quadrature



4,2-HNCOCA
projection angles:
$\alpha, \beta \neq 0^{o}, 90^{o}$

APSY challenges

Challenge: APSY peak lists with high precision even if recorded on different:

- Spectrometers
- Date

Solution

- NMR-Thermometer!

Example

- 4D HNCOCA APSY
- Recorded @ 600 and 800MHz
- Compare peak lists
- Sample: 0.4mM ^{13}C, ^{15}N ubiquitin in 90% H_2O, 10% D_2O, NMR Thermometer compound: 10mM TSP-d $_{13}$

APSY and the NMR-Thermometer

Comparison of 4,2 HNCOCA APSY peak lists, 600 & 800MHz

0.4mM ^{13}C, ^{15}N ubiquitin in 90% H_2O, 10% D_2O, NMR-Thermometer compound: 10mM TSP-d_{13}

Δ[ppm] 800-600MHz				
	N	CA	Ca	H
	0.0034	-0.0112	0.0158	0
	-0.0058	-0.0024	0.0098	0.0014
	0.0038	-0.0047	0.0061	0.0002
	-0.0026	-0.0112	-0.0067	-0.001
	-0.0132	-0.0166	-0.003	0.0005
	-0.0032	0.0012	-0.0442	1E-04
	-0.0103	-0.0048	0.0165	-0.0005
	0.0019	-0.0116	0.0313	-0.0006
	-0.0153	-0.0051	0.0069	0.0005
	-0.0189	-0.0051	-0.0045	-0.0008
	-0.0033	-0.0102	0.0141	0.0001
	0.002	-0.0224	-0.0122	-0.0008
	0.0095	-0.0146	-0.0085	0.0006
	-0.0017	-0.0128	0.0328	-0.0004
	-0.0005	-0.0141	0.009	-0.0003
	-0.0092	-0.0078	0.0056	0.0007
	-0.0253	-0.0136	0.0315	0
STD/ppm:	***0.03086873***	***0.00776172***	***0.02262312***	***0.00065478***

Δ[Hz@800MHz] 800-600MHz				
	N	CA	Ca	H
	0.27574	-2.2512	3.1758	0
	-0.47038	-0.4824	1.9698	1.120182
	0.30818	-0.9447	1.2261	0.160026
	-0.21086	-2.2512	-1.3467	-0.80013
	-1.07052	-3.3366	-0.603	0.400065
	-0.25952	0.2412	-8.8842	0.080013
	-0.83533	-0.9648	3.3165	-0.400065
	0.15409	-2.3316	6.2913	-0.480078
	-1.24083	-1.0251	1.3869	0.400065
	-1.53279	-1.0251	-0.9045	-0.640104
	-0.26763	-2.0502	2.8341	0.080013
	0.1622	-4.5024	-2.4522	-0.640104
	0.77045	-2.9346	-1.7085	0.480078
	-0.13787	-2.5728	6.5928	-0.320052
	-0.04055	-2.8341	1.809	-0.240039
	-0.74612	-1.5678	1.1256	0.560091
	-2.05183	-2.7336	6.3315	0
STD/Hz:	***2.50345434***	***1.56010552***	***4.54724794***	***0.52391188***

Precision is in the order of the Hz/Pt resolution used for processing:

SI[F2] = 8K; SI[F1] = 1K; *Hz/Pt[F2]=0.78Hz; Hz/Pt[F1]=1.4 – 3.5Hz;*

APSY: outlook

GAPRO: peak picking, geometrical analysis, matched projection angles

1. Calculation of projection angles will be transferred to manageapsy

manageapsy: main program controlling setup, run, analysis & report

1. Calculation of matched projection angles: optimum resolution for indirect dimensions

Pulse programs:

1. Implementation of missing experiments

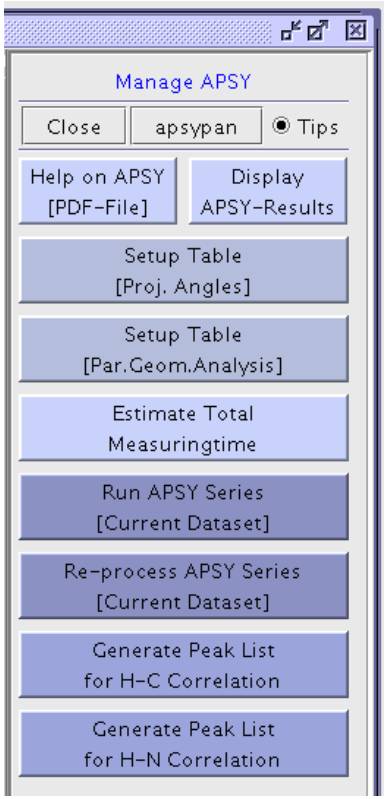
All: adaption to future Topspin versions

APSY experiments and software

Before I forget…How to start APSY:

“apsy”

“bpan apsypan”



Acknowledgement

- Gerhard Wider, Barbara Krähenbühl: ETH Zürich
- Sebastian Hiller: Biozentrum, University Basel

- Werner Mausshard, Wolfgang Bermel, Frank Schumann