Fast Methods

Detlef Moskau

May 2, 2014

Content

Covariance

Non-uniform Sampling

APSY / Projection Spectroscopy: brief introduction

Sofast experiments

Best triple resonance experiments

Accelerate NMR experiments

Reduced number of Increments in nD experiments:

- Non linear sampling
- Reduced dimensionality — APSY
- Projection Reconstruction
- Spectrum folding
- Covariance

Slice selection:

- Single scan

Enhanced Repetition rate:

- Rapid Pulsing — Sofast-HMQC, BEST triple resonance experiments

Pseudo-2D Representation of multiple selective 1D-experiments:

- Hadamard

Combine several experiments in one experiment:

- COCONOESY
- Multiple Receiver

Accelerate NMR experiments

Reduced number of Increments in nD experiments:

- Non uniform sampling NUS
- APSY
- Covariance

Enhanced Repetition rate:

- Rapid Pulsing

Sofast-HMQC, BEST triple resonance experiments

Covariance

Processing Method

Covariance

Principle

After FT in direct dimension:

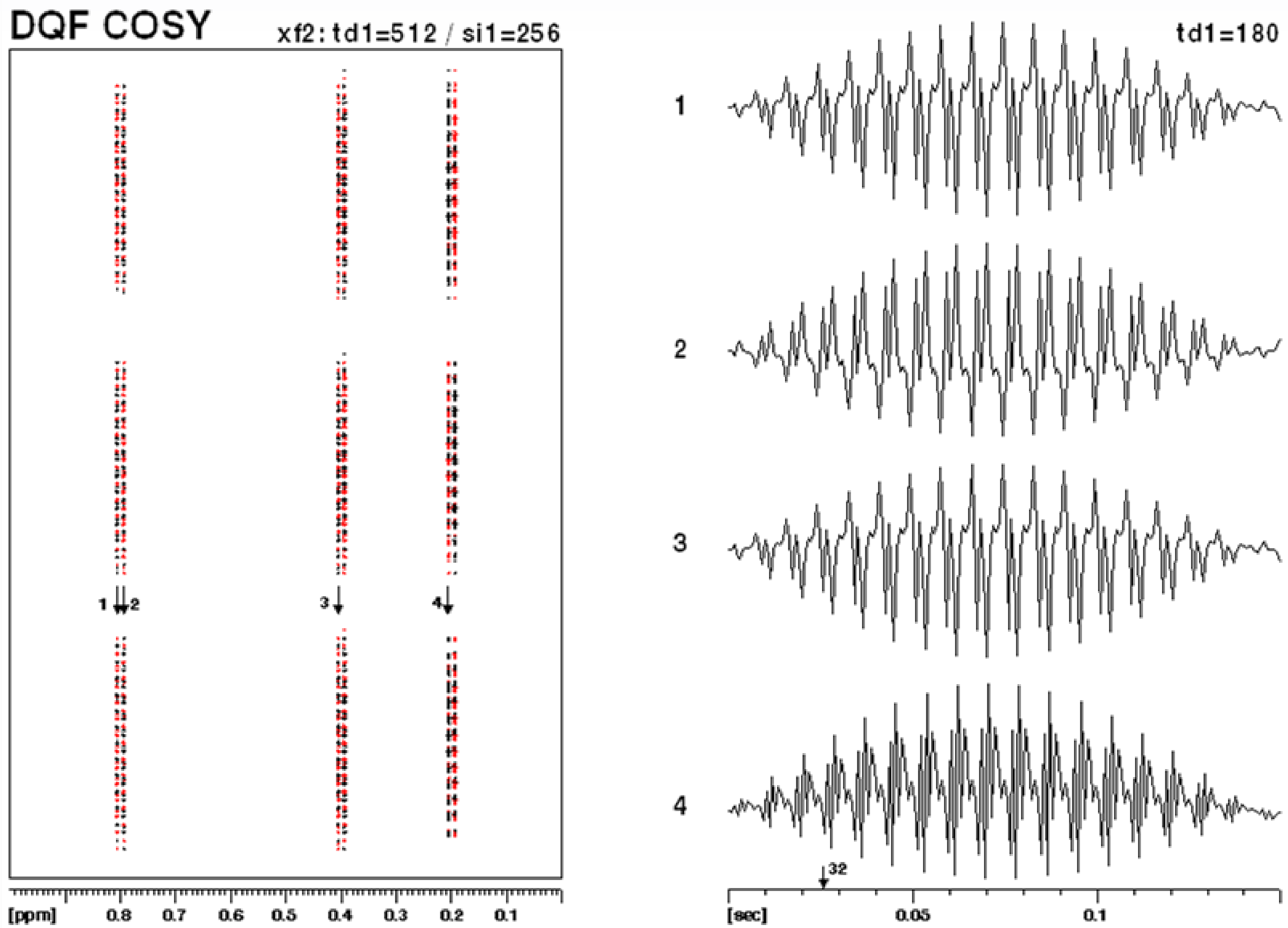
Compare all columns (indirect dimension)

For columns which are similar:
transfer info of frequency in direct dimension to the indirect dimension

No further FT applied

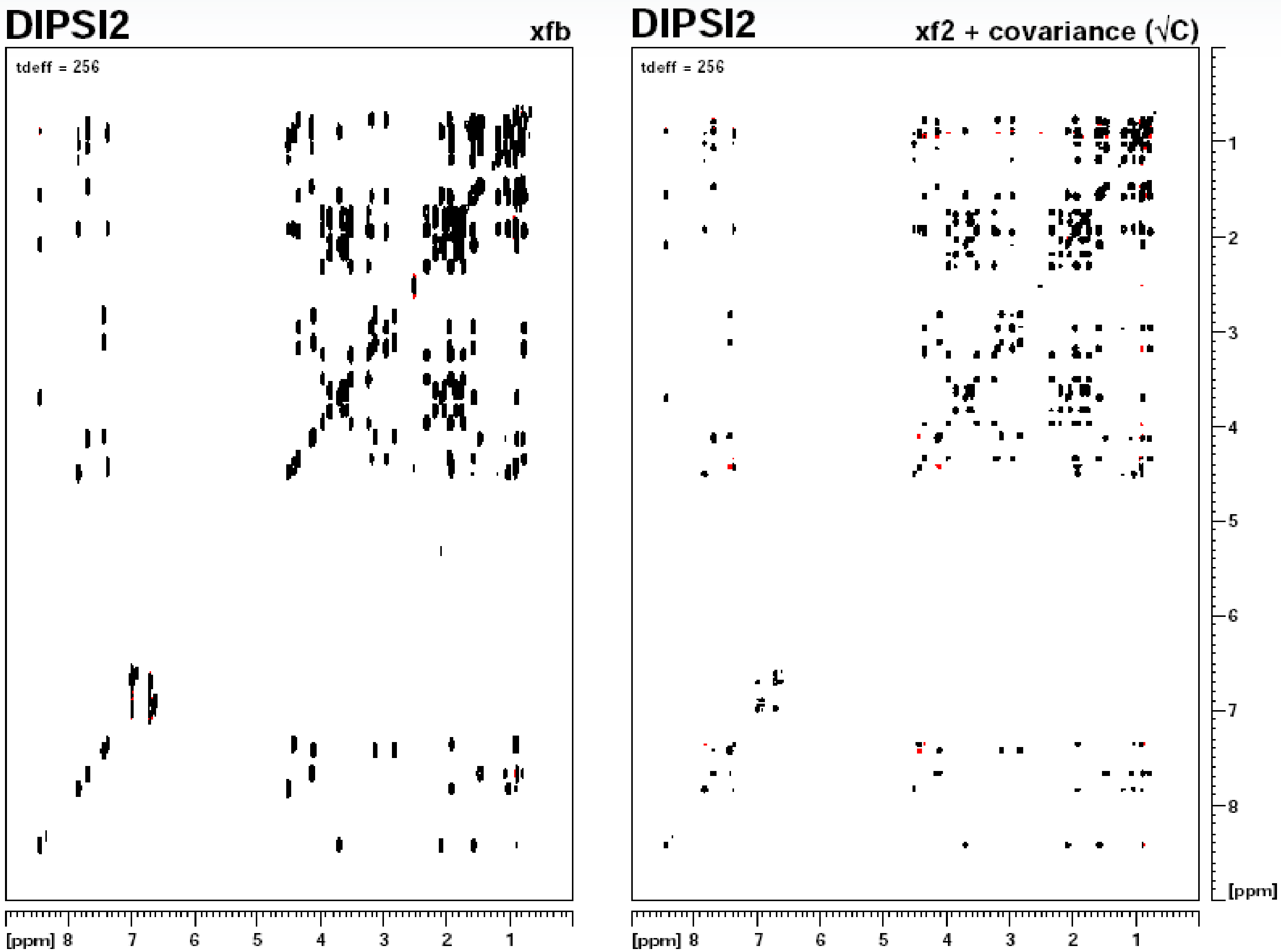
Advantance: enhanced resolution in indirect dimension

Covariance



DQF COSY
xf2: td1=512 / si1=256
1
2
3
4
[ppm]
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
td1=180
32
[sec]
0.05
0.1

Covariance


DIPSI2
xfb
tdeff = 256
[ppm] 8 7 6 5 4 3 2 1
DIPSI2
xf2 + covariance (√C)
tdeff = 256
[ppm] 8 7 6 5 4 3 2 1
1 2 3 4 5 6 7 8 [ppm]

NUS

Non Uniform Sampling

non uniform sampling

MultiDimensionl Decomposition (MDD)

MDD-NMR	Orekhov et al.

Maximum Entropy (MaxEnt)

Rowland Toolkit	Hoch et al.
Forward Maximum Entropy	Wagner et al.
Azara (CCPN)	Laue et al.

Multidimensionl Fourier Transformation (MFT)

MFT	Kozminski et al.

TopSpin 3.1 – **News**

Non Uniform Sampling

- NUS-Paket now shows seamless integration with GUI

- … and is available on all platforms
  (first version was only for Linux)

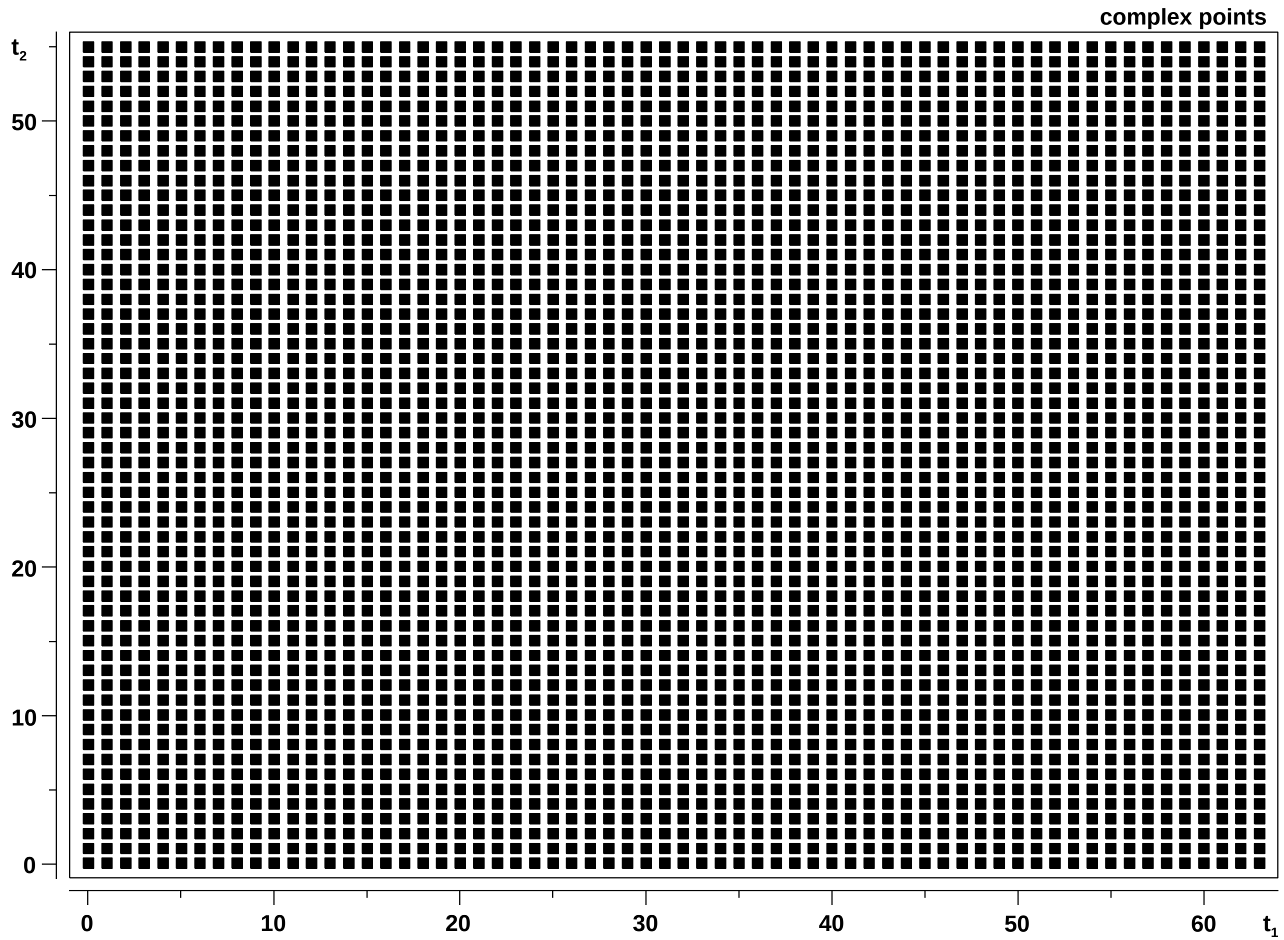
- New **Compressed Sensing**
  gives significant speed-up

V. Orekhov
K. Kazimierczuk

W. Mausshardt
W. Bermel

non uniform sampling

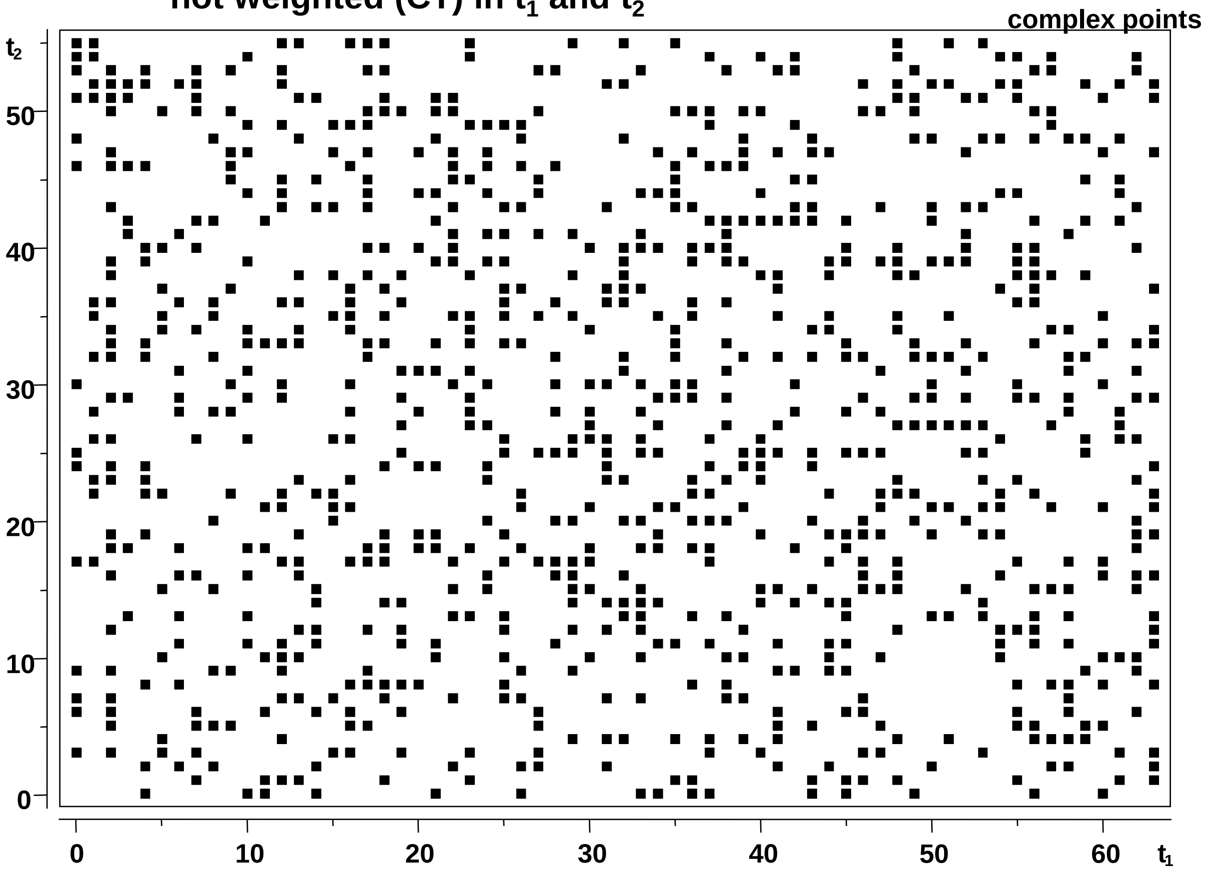
3D ($t_1 t_2$ plane)



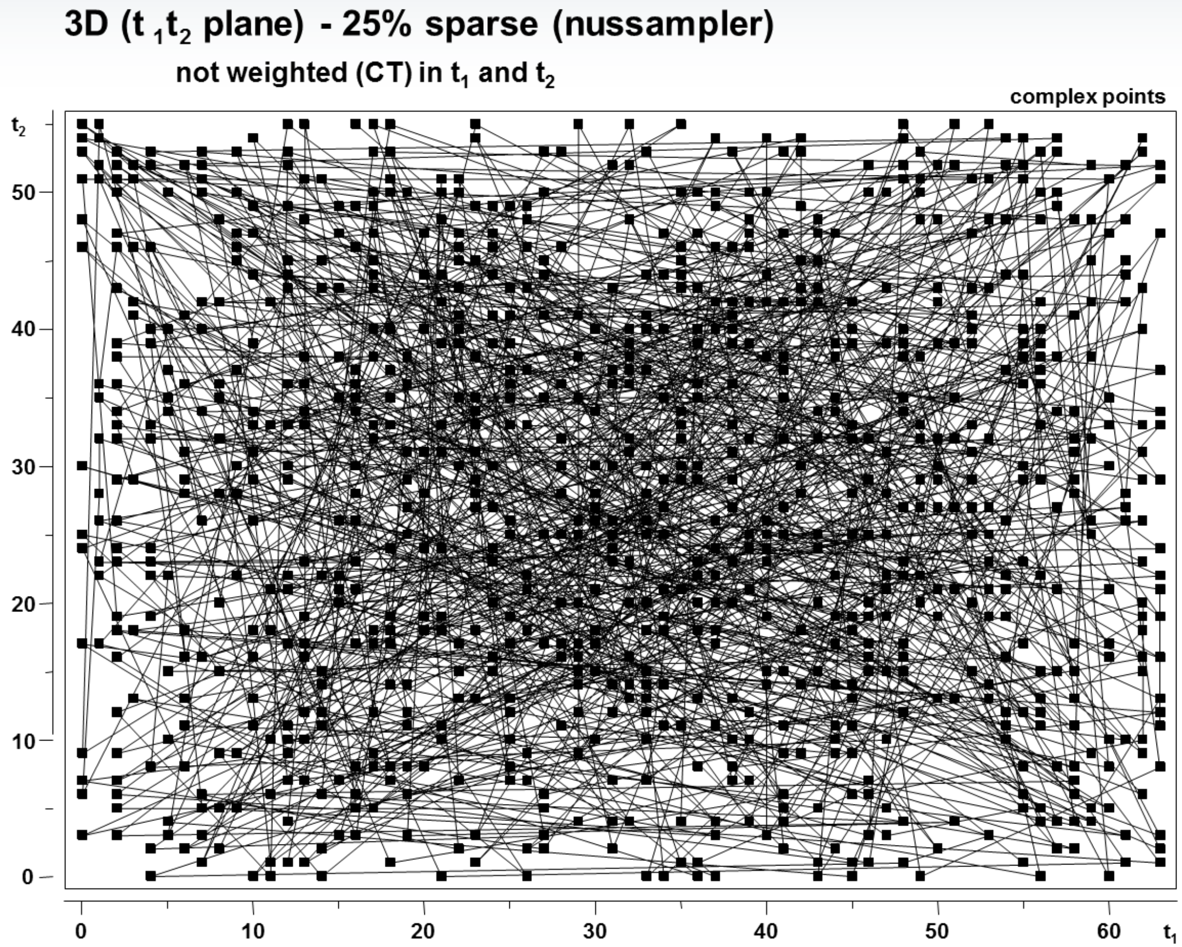
non uniform sampling

3D ($t_1 t_2$ plane) - 25% sparse (nussampler)

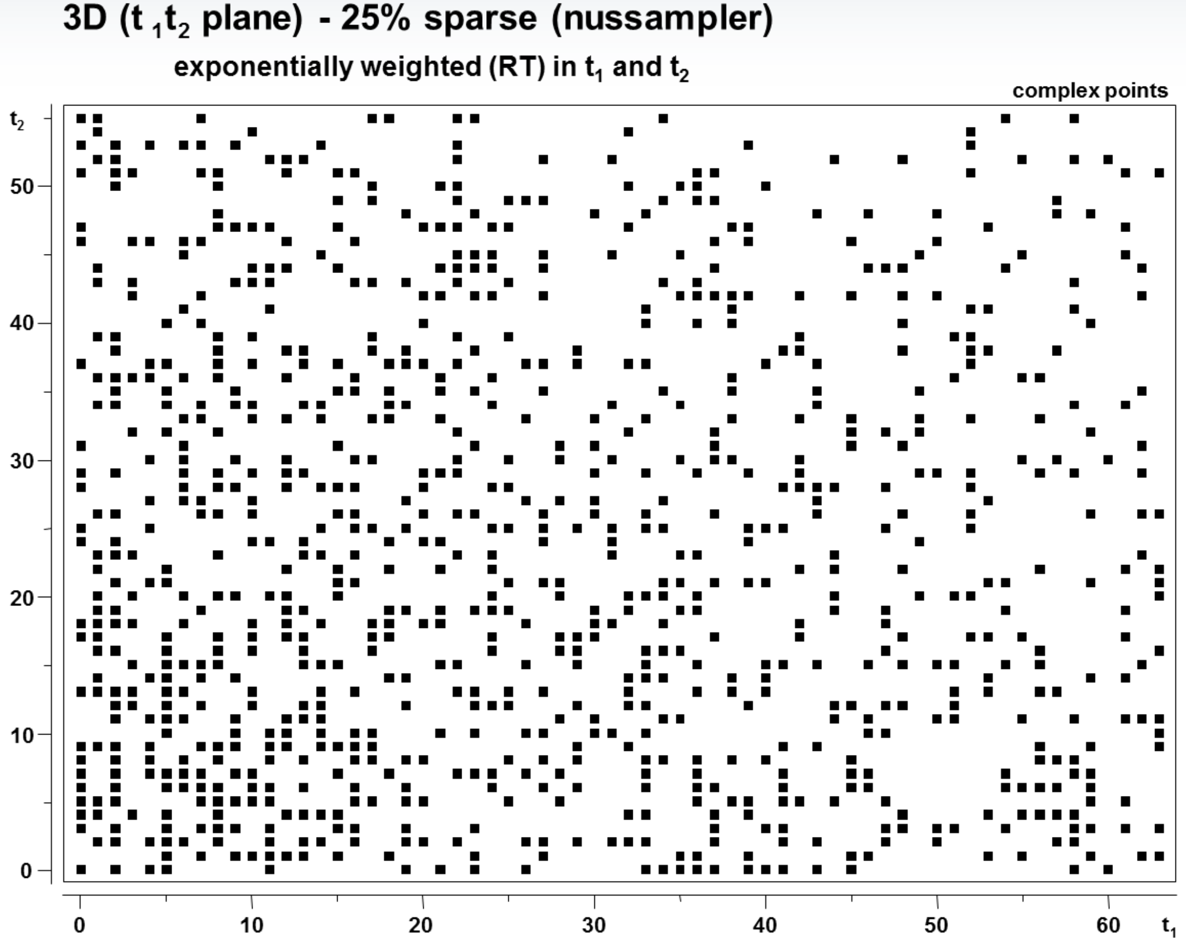
not weighted (CT) in t_1 and t_2



non uniform sampling

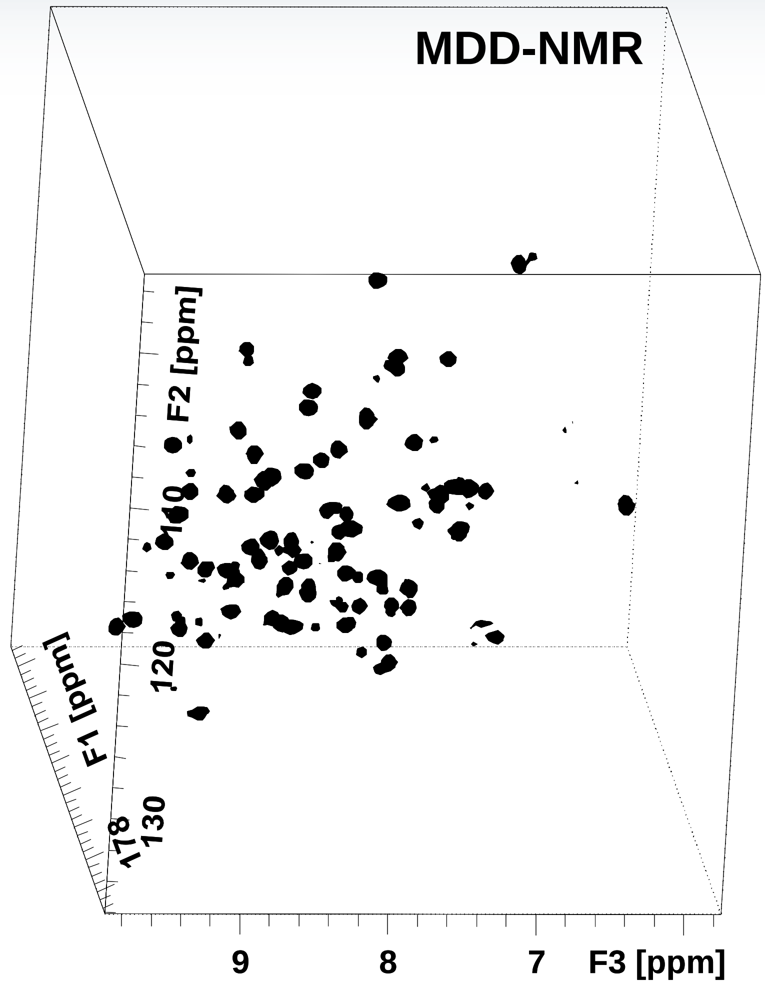


non uniform sampling



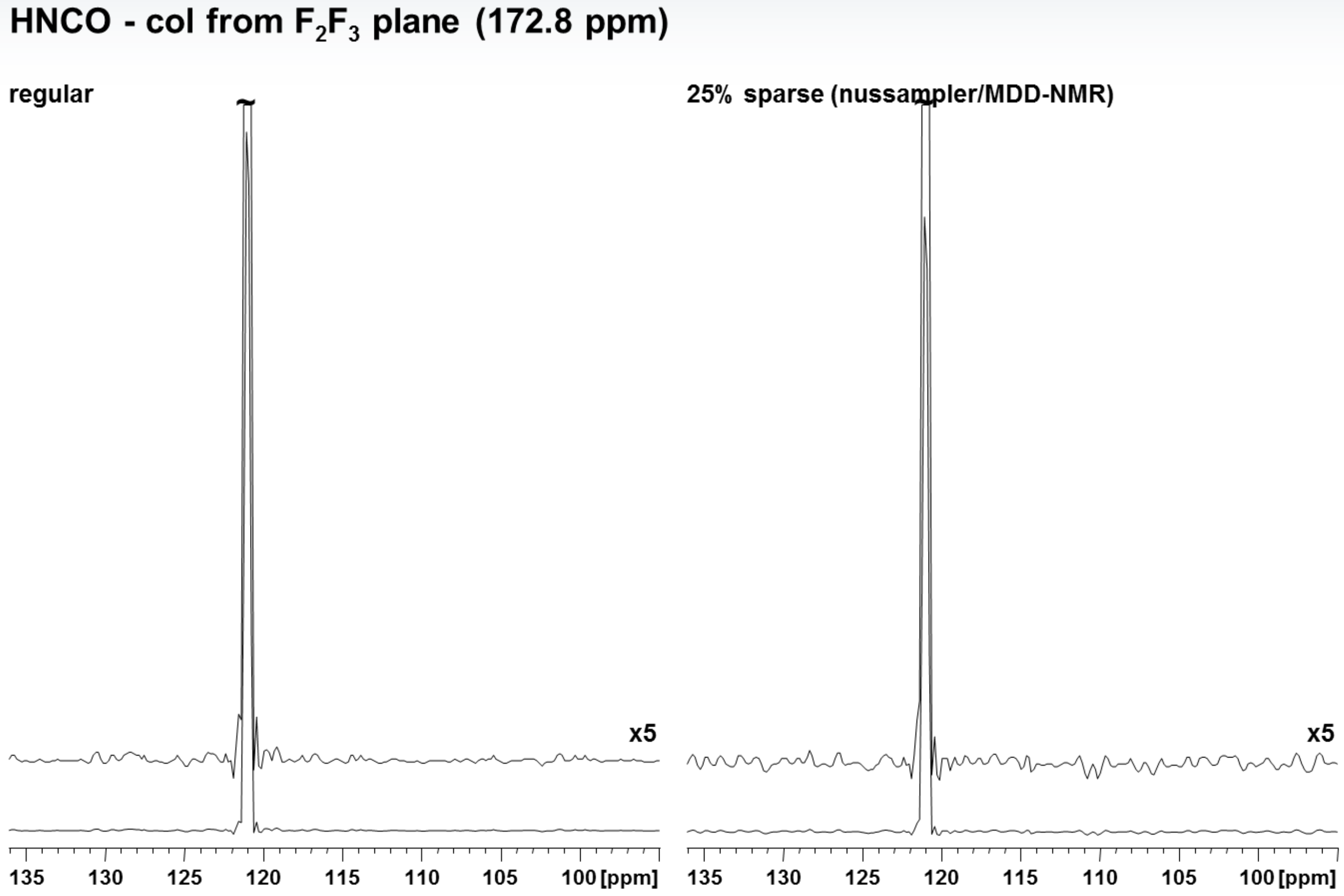
non uniform sampling

HNCO - 25% sparse (nussampler)



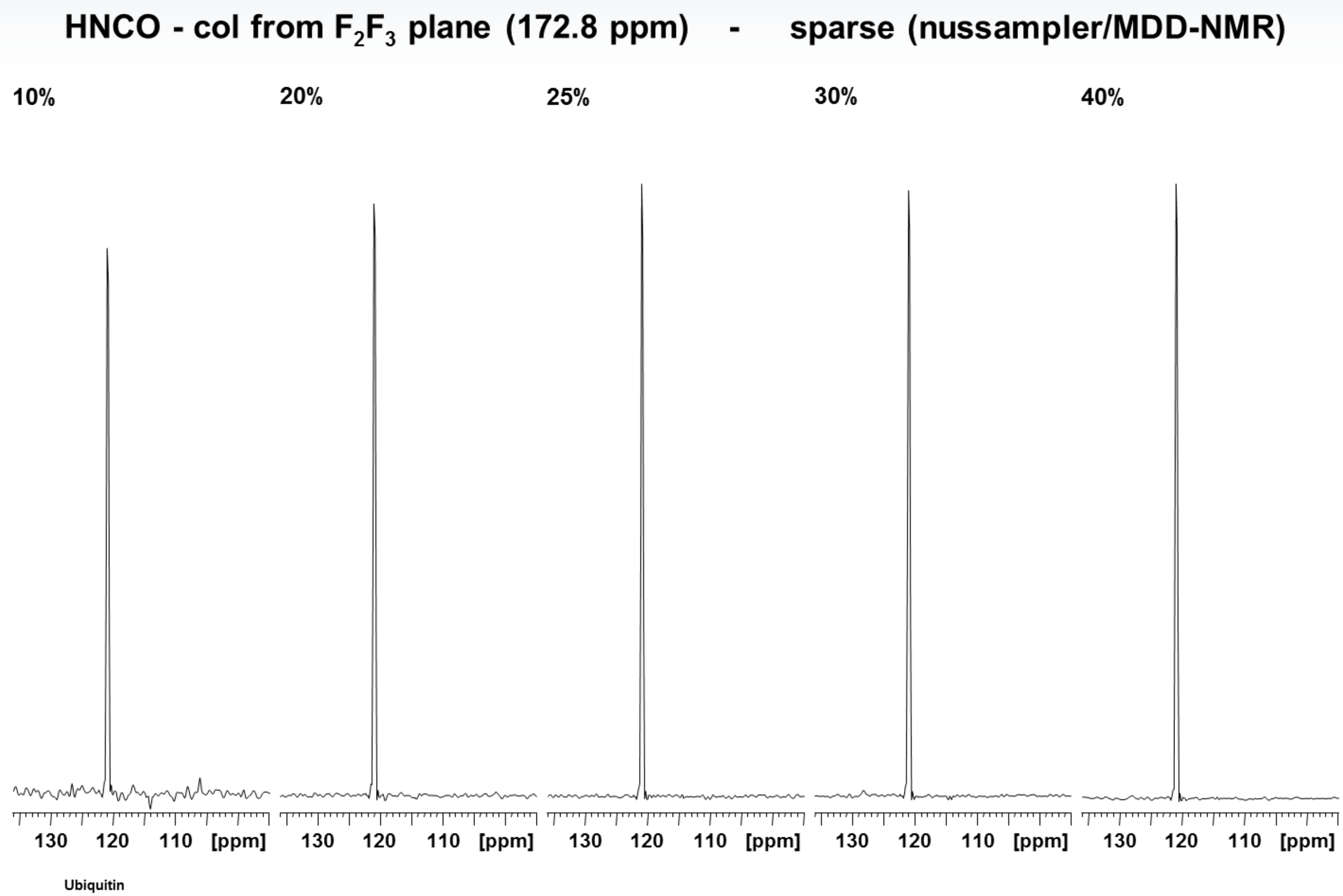
Ubiquitin

non uniform sampling



Ubiquitin

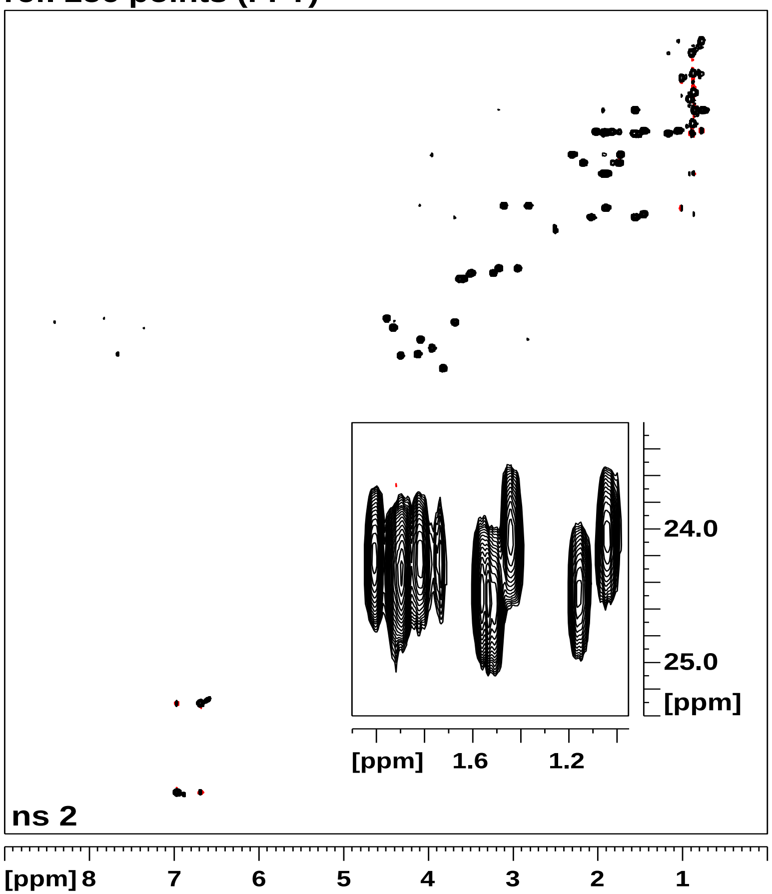
non uniform sampling



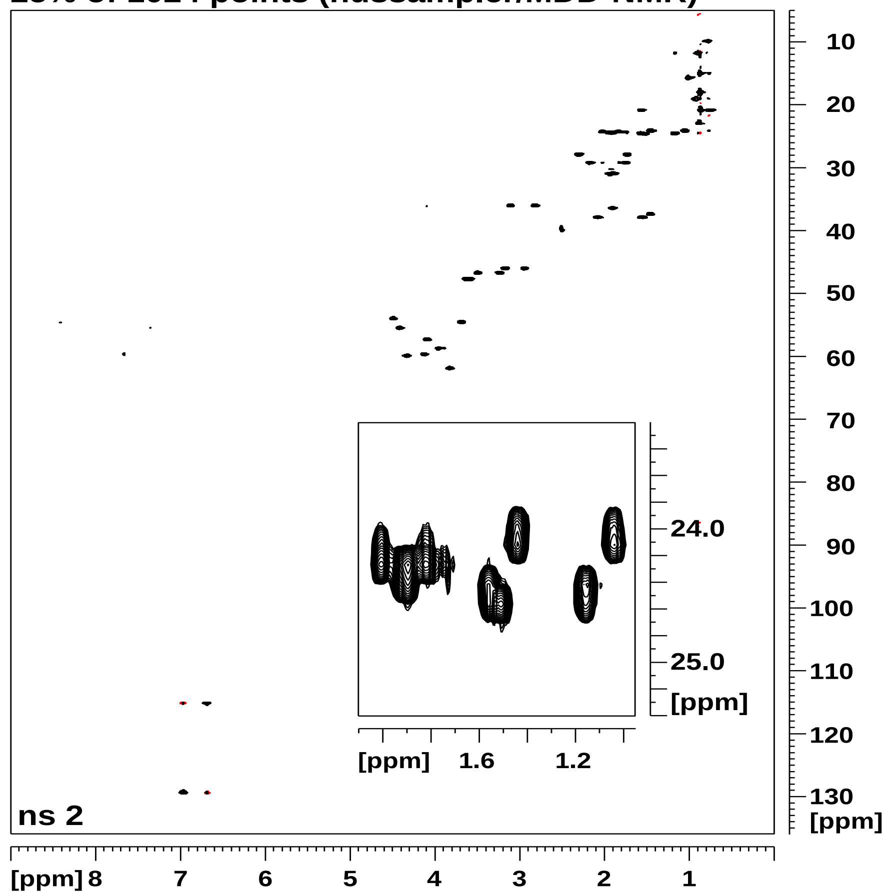
non uniform sampling

HSQC

ref. 256 points (FFT)



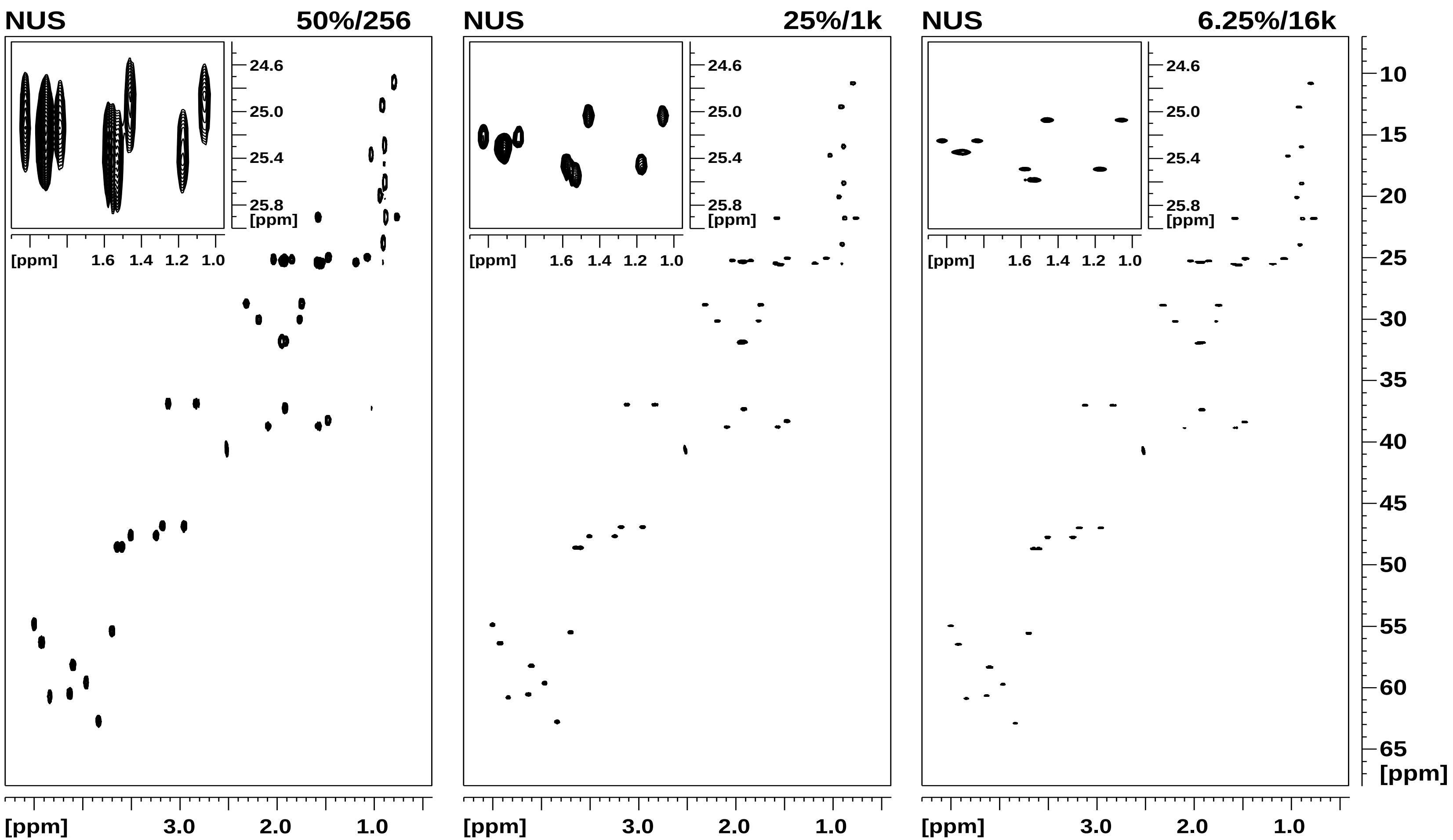
25% of 1024 points (nussampler/MDD-NMR)



20mM Hymenistatin

Non Uniform Sampling

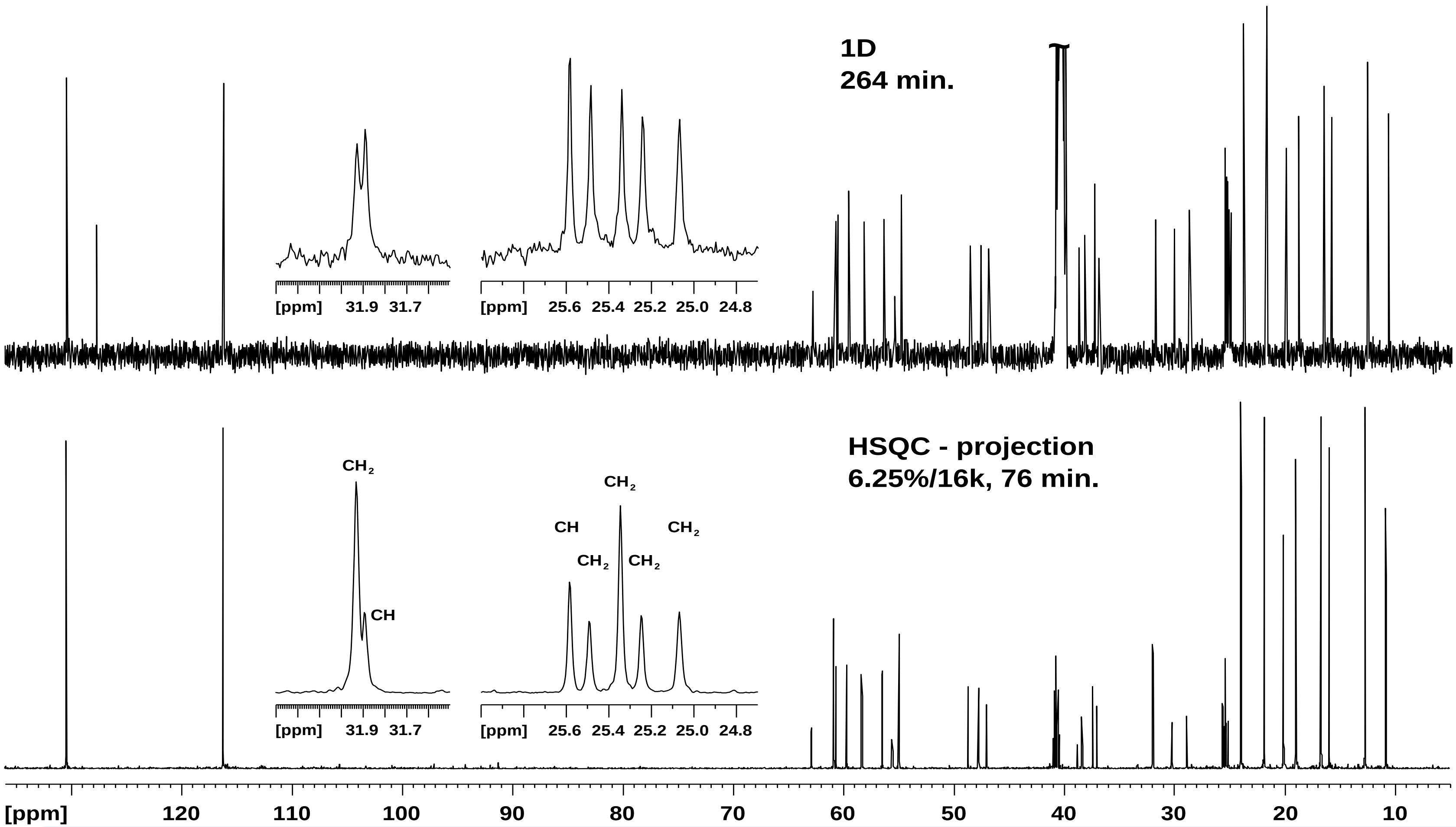
HSQC



20mM Hymenistatin

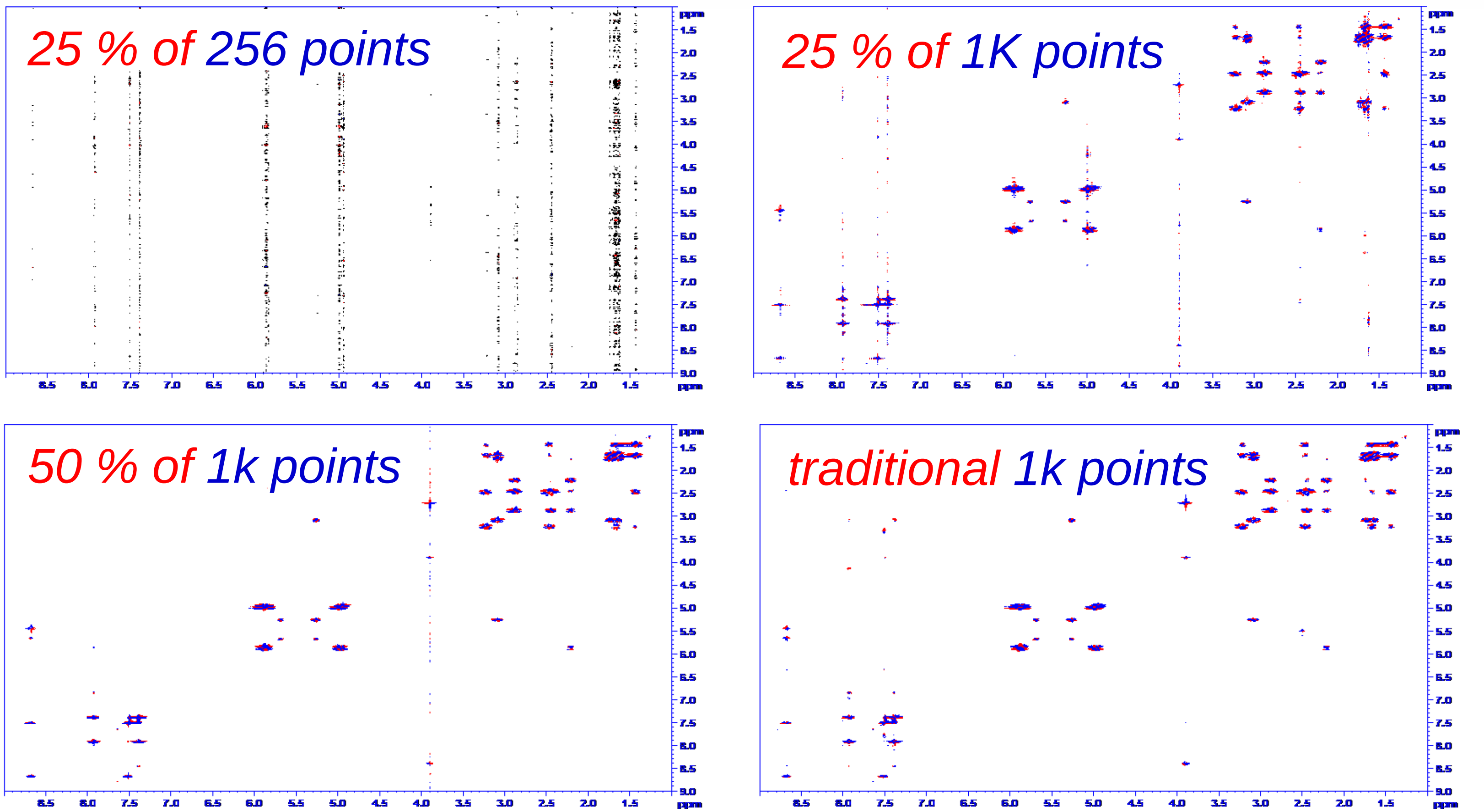
non uniform sampling

HSQC



20mM Hymenistatin

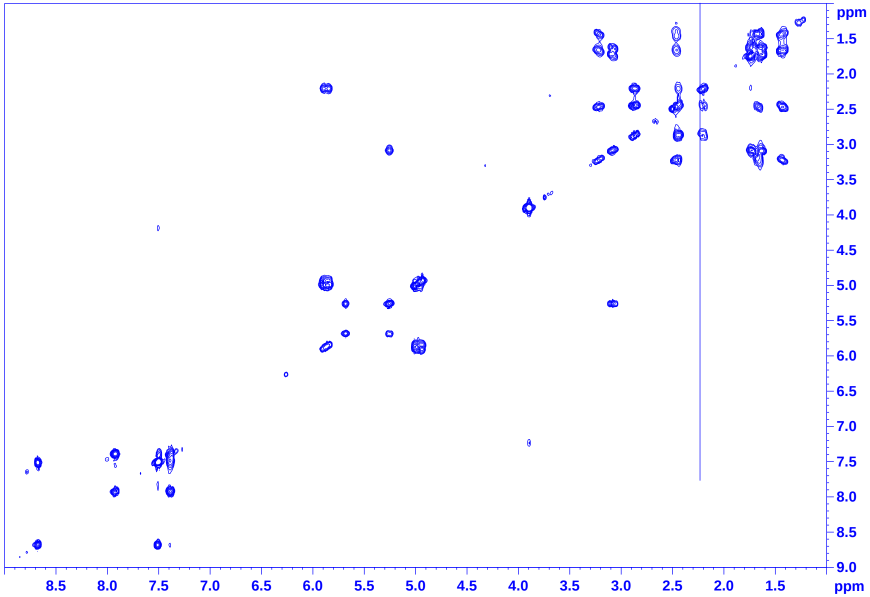
non uniform sampling
Homonuclear Experiments: DQF-COSY



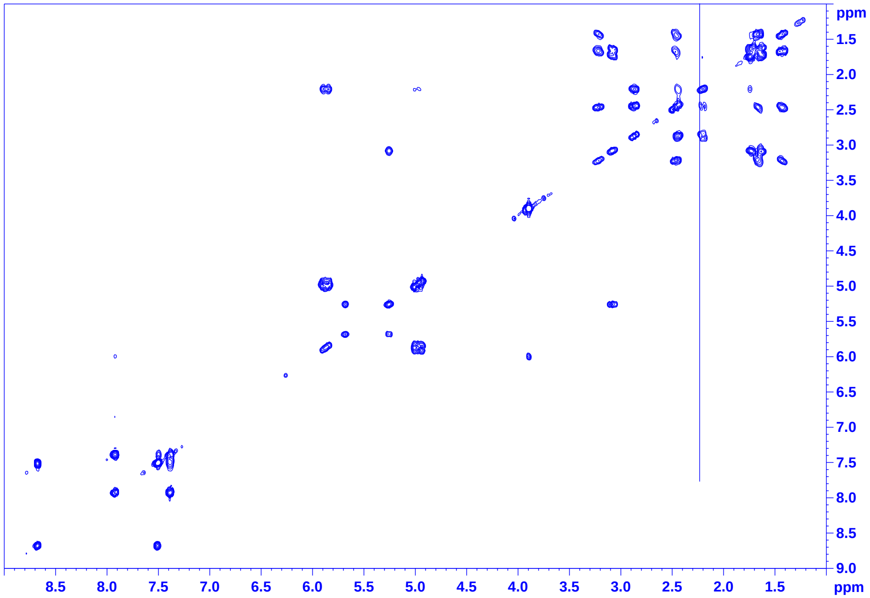
non uniform sampling

Homonuclear Experiments: Grad-COSY, NS=1

50 % of 256 points

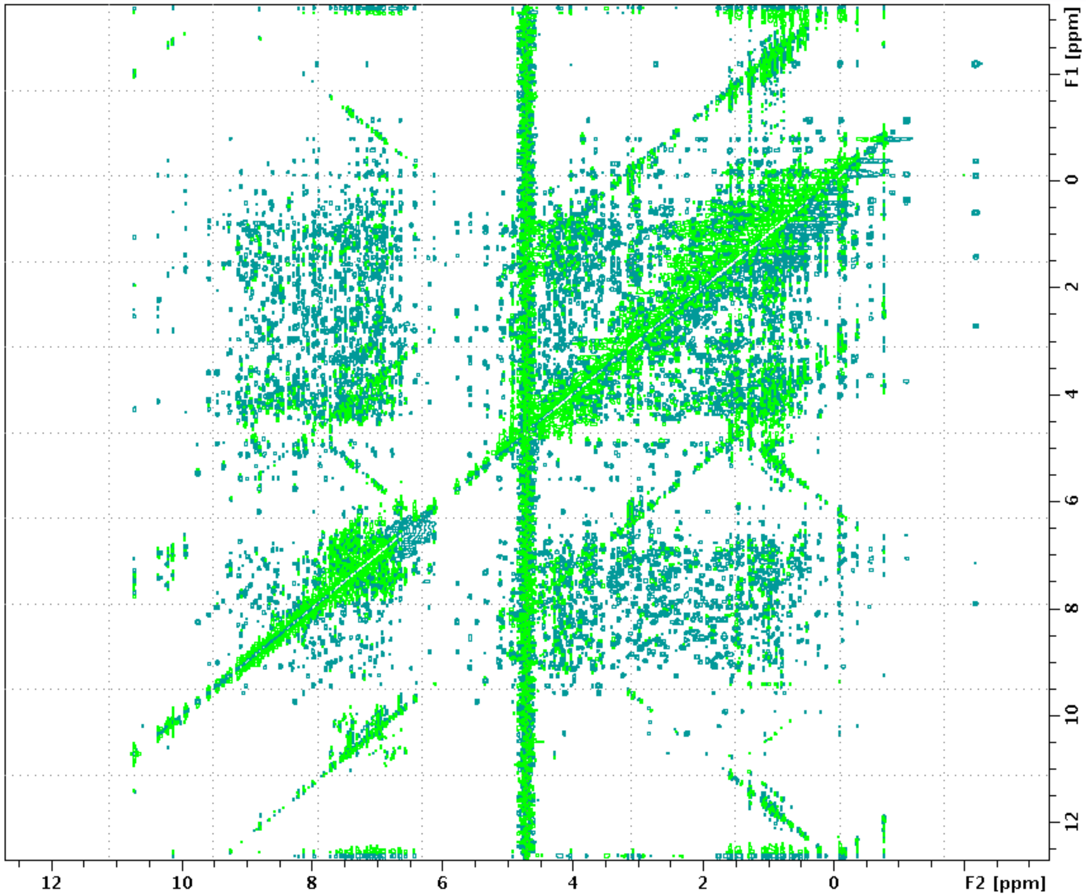


traditional 256 points



non uniform sampling
Any further applications?

NOESY
Side diagonals due to temperature oscillation of aircon

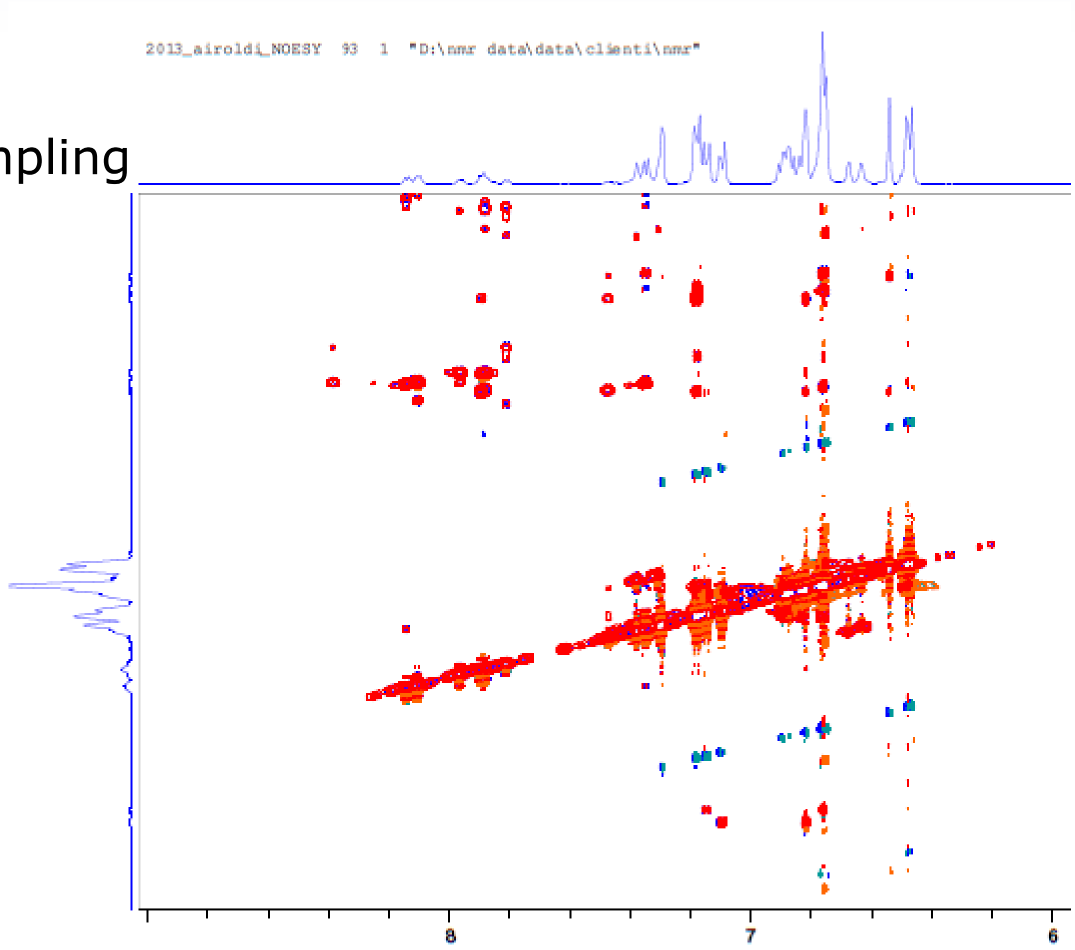


non uniform sampling
Any further applications?

NOESY

Blue: traditional sampling

Red: NUS



APSY

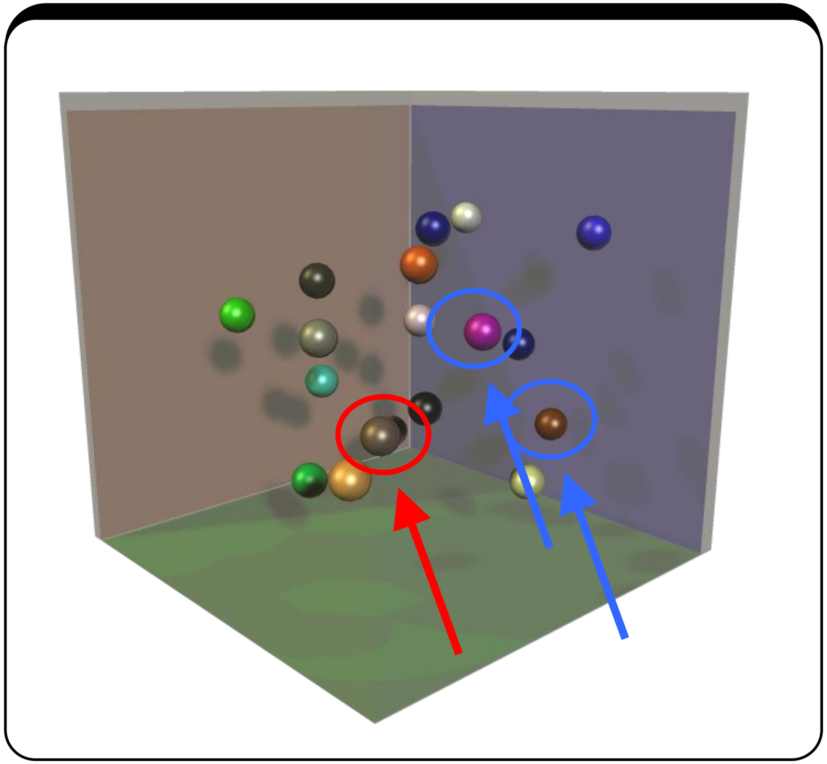
High Precision, Fast and Automated Projection Spectroscopy

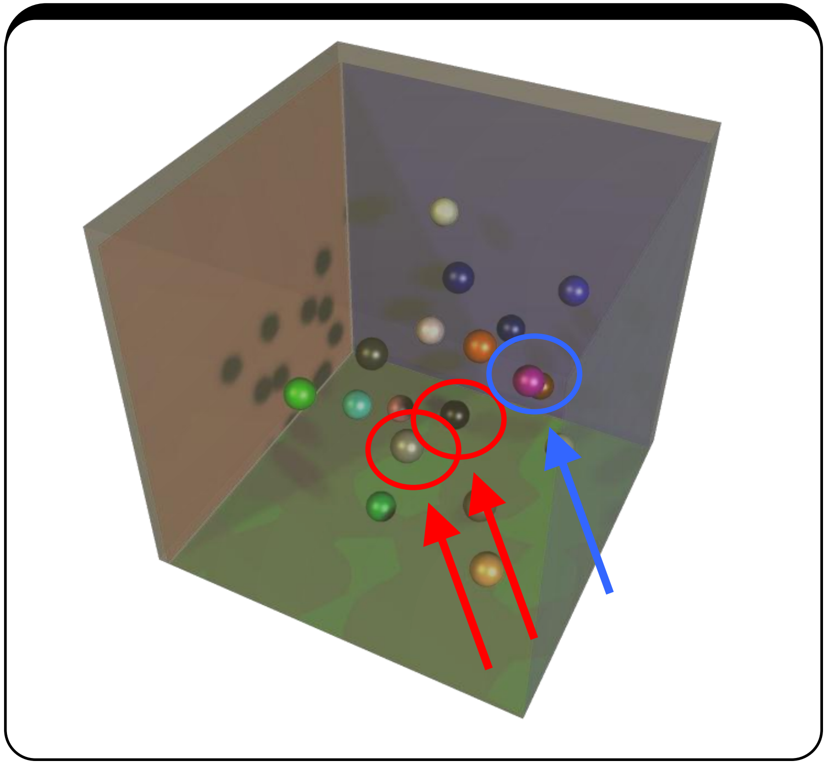
Projection Spectroscopy

Projection Spectroscopy

What is 'projection spectroscopy'?

Multiple viewpoints '***projection angles***' are required

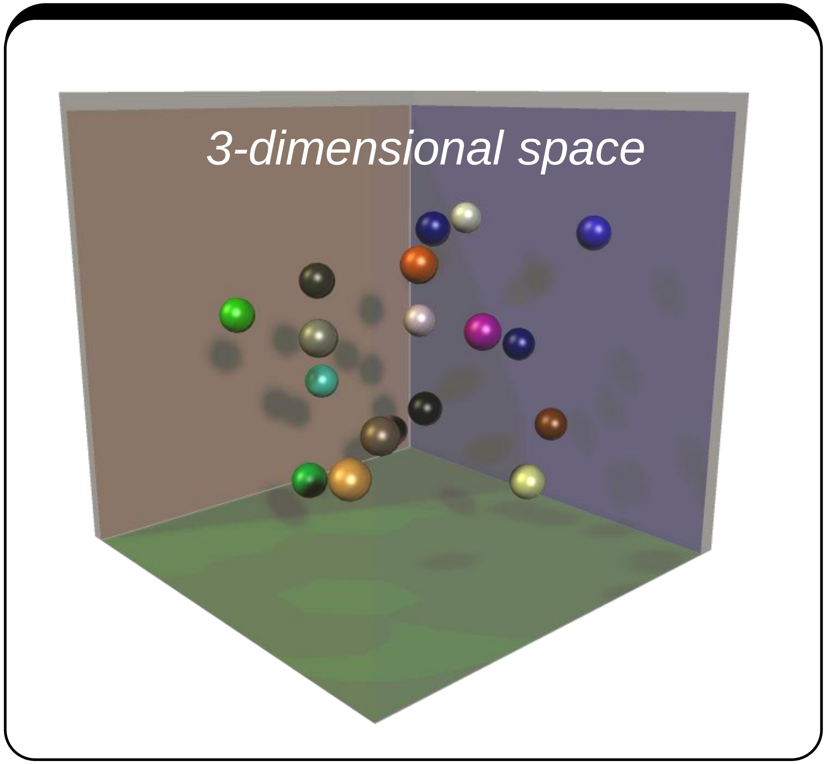


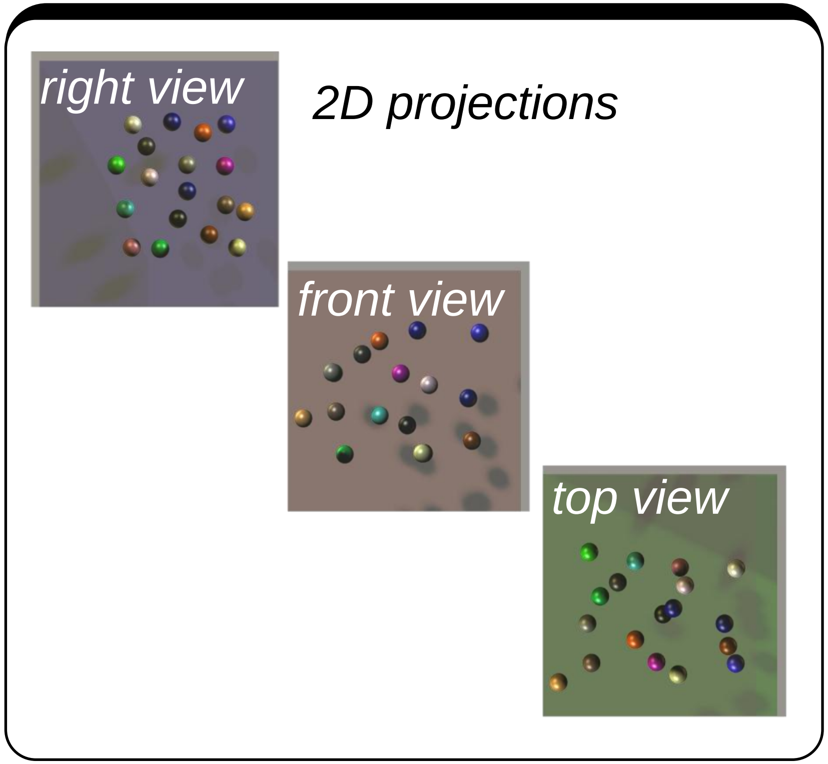


Projection Spectroscopy

What is ‘projection spectroscopy’?

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

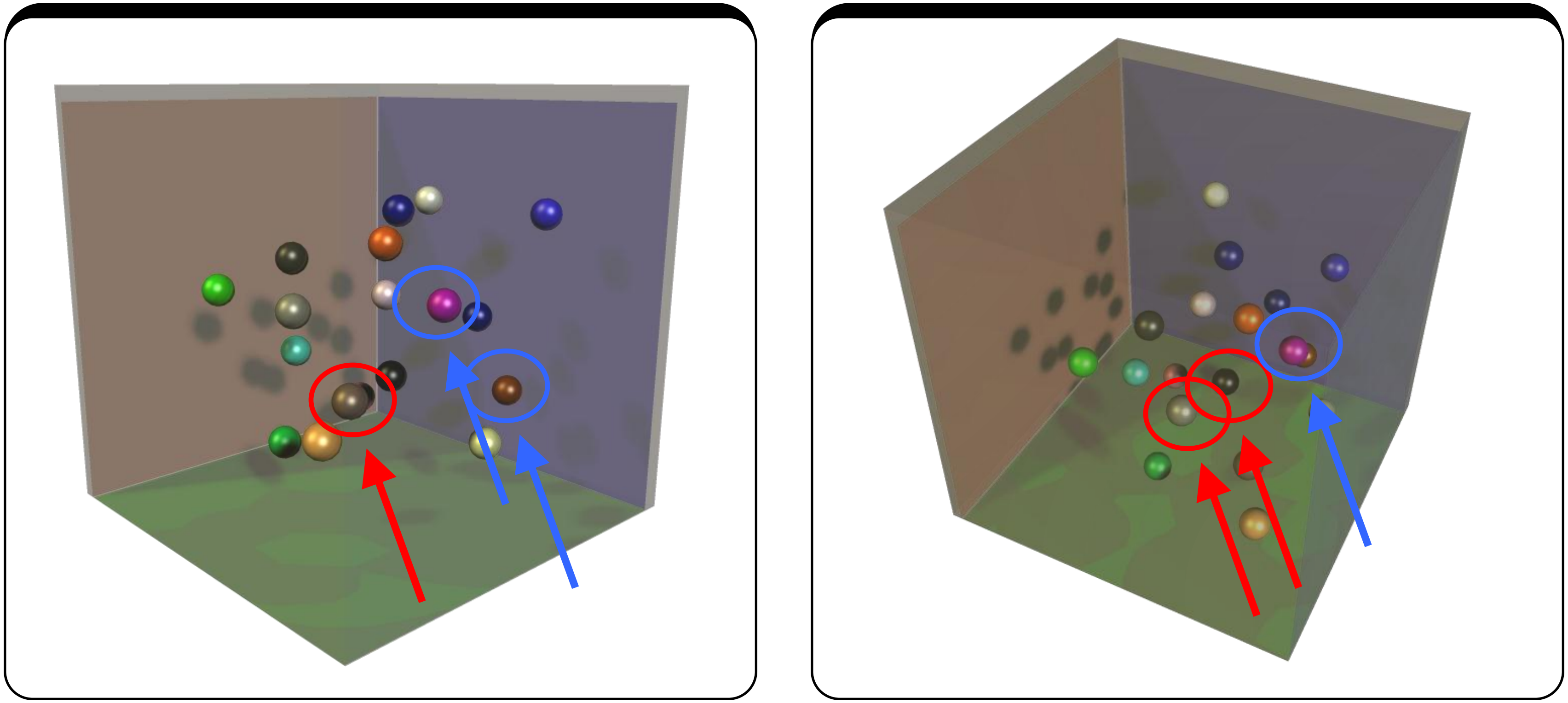




Projection Spectroscopy

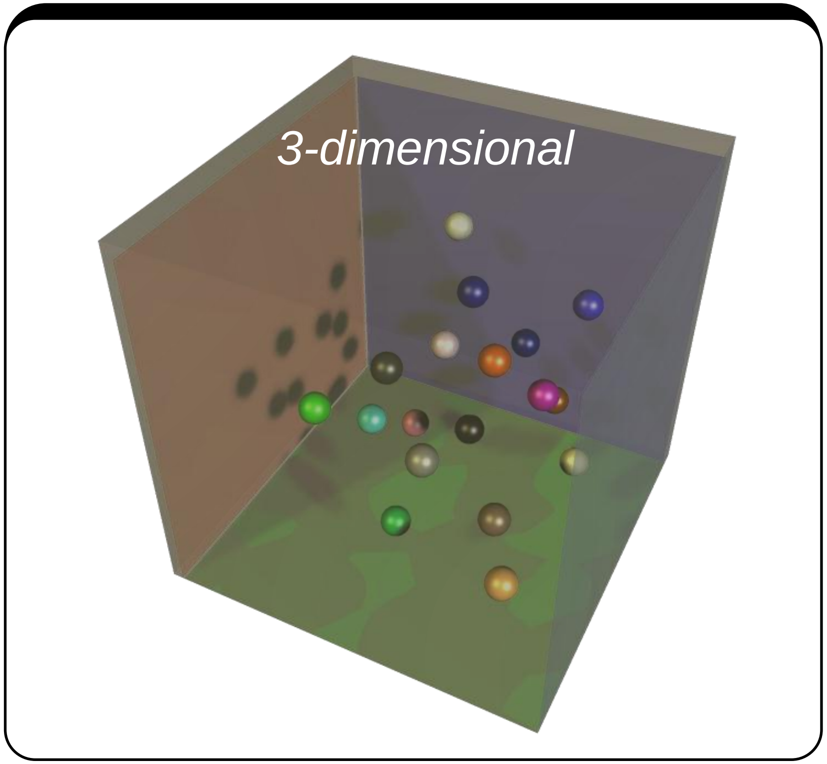
What is 'projection spectroscopy'?

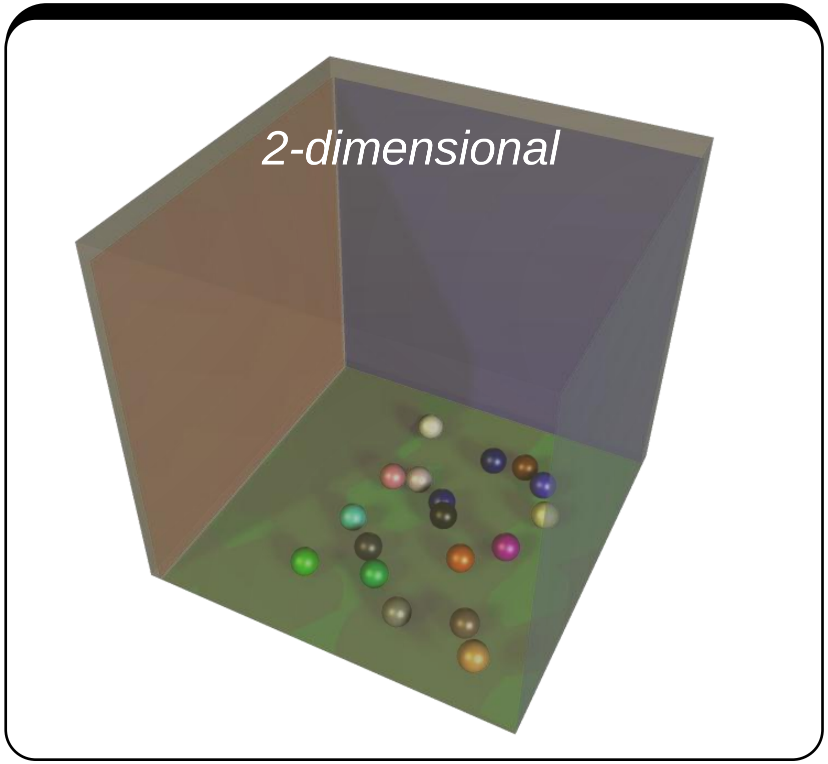
Multiple viewpoints '***projection angles***' are required



Projection Spectroscopy

Consequence of ***Reduction of dimensionality:***
Information of additional dimensions is lost

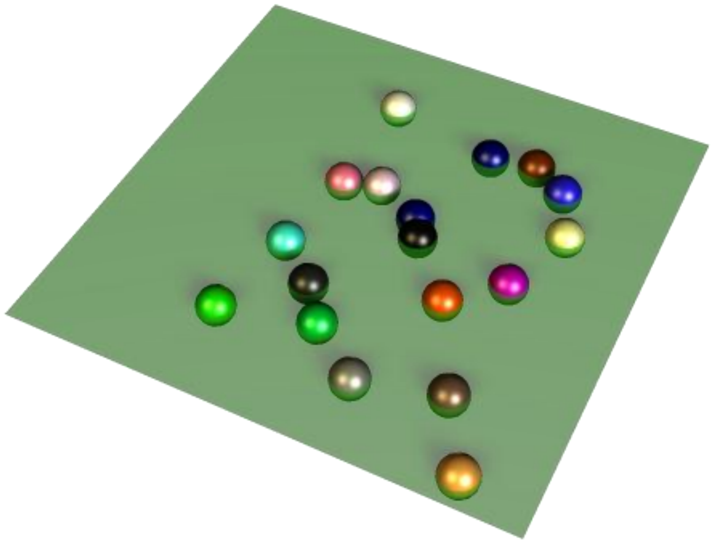




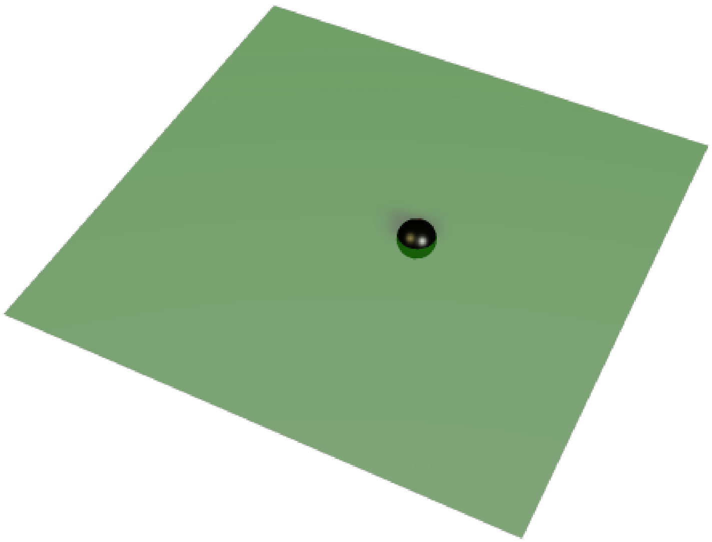
Projection Spectroscopy

Consequence of ***Reduction of dimensionality:***
Information of additional dimensions is lost

2-dimensional, all peaks



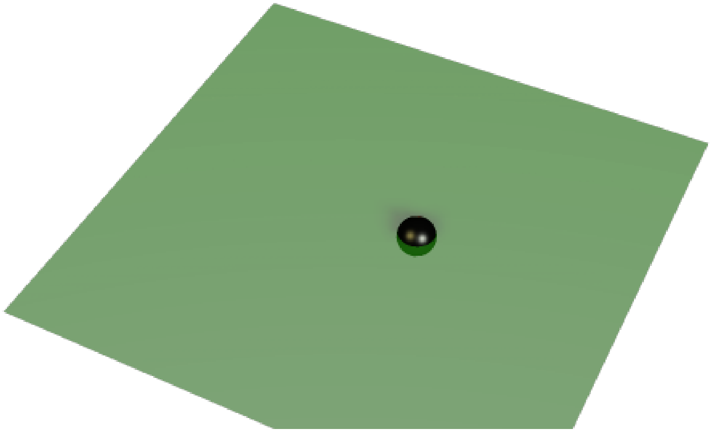
2-dimensional, one selected peak



Projection Spectroscopy

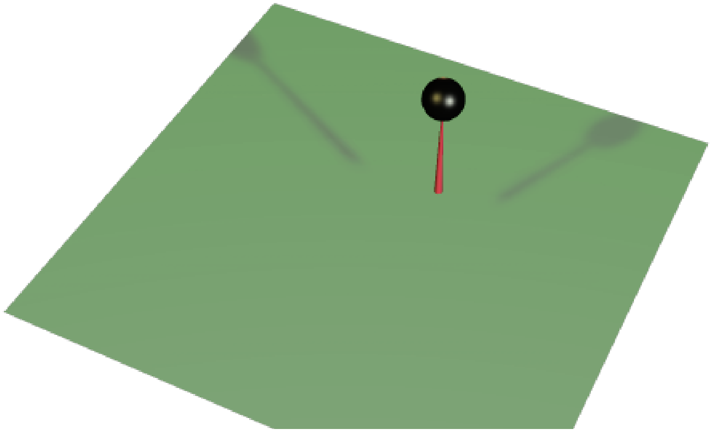
Consequence of ***Reduction of dimensionality:***
Information of additional dimensions is lost

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



dimensionality:
reduced

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



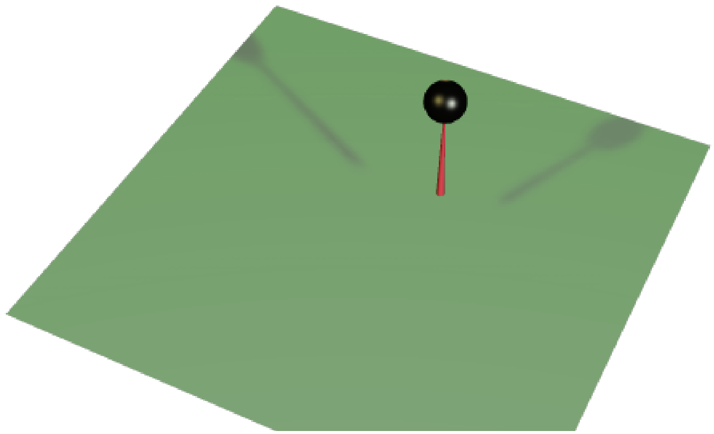
dimensionality:
full

Projection Spectroscopy

Consequence of ***Reduction of dimensionality:***
Shift information of reduced dimensions is lost, but:

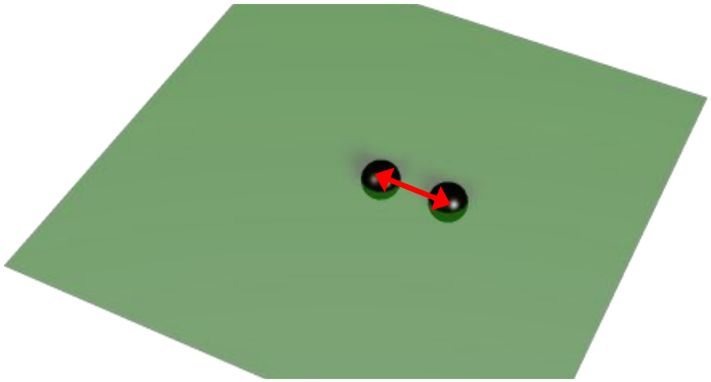
- Shift information is coded as a distance
- By additional splitting of single peaks

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

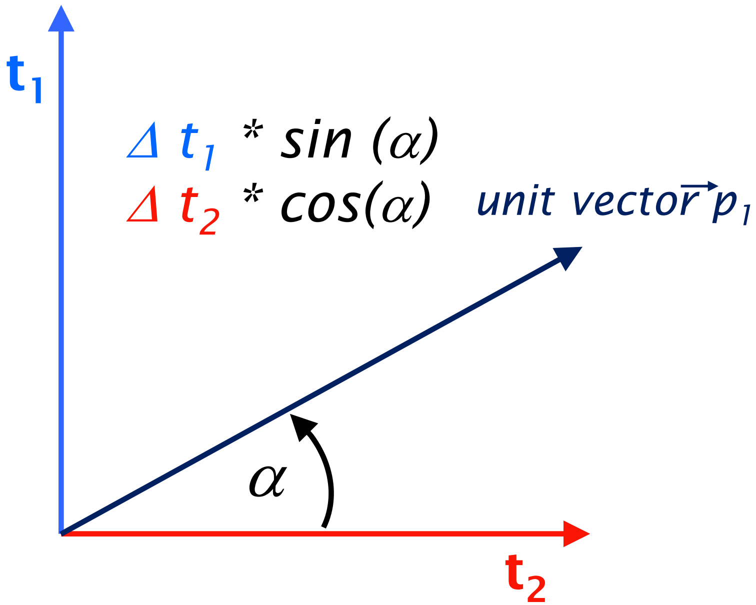
Recording of projection spectra

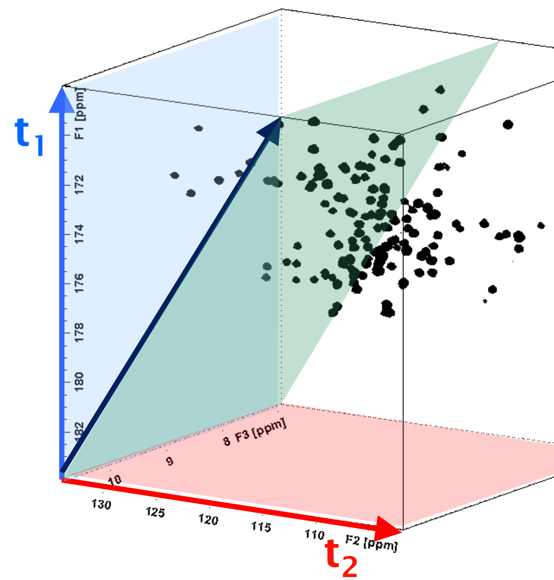
Example: **3D HNCO experiment**

single evolution during t_1 only: **2D H,C plane (α = 90°)**

single evolution during t_2 only: **2D H,N plane (α = 0°)**

simultaneous evolution during t_1 and t_2: **2D H,NC plane (α = n°)**





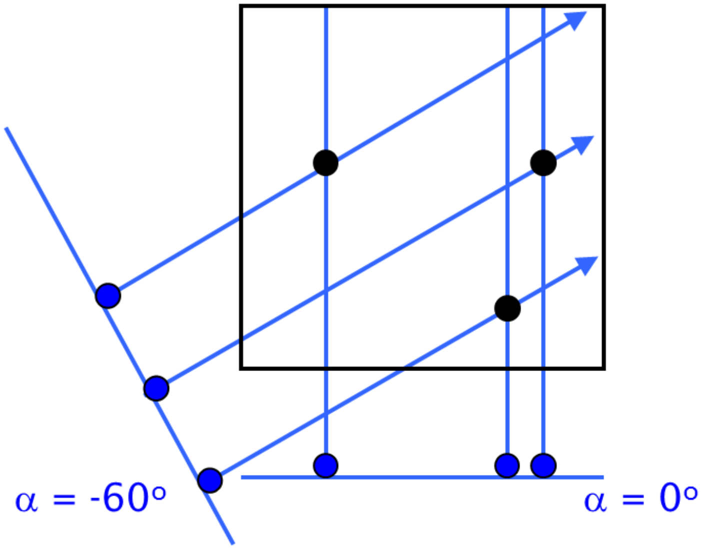
Projection Spectroscopy

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

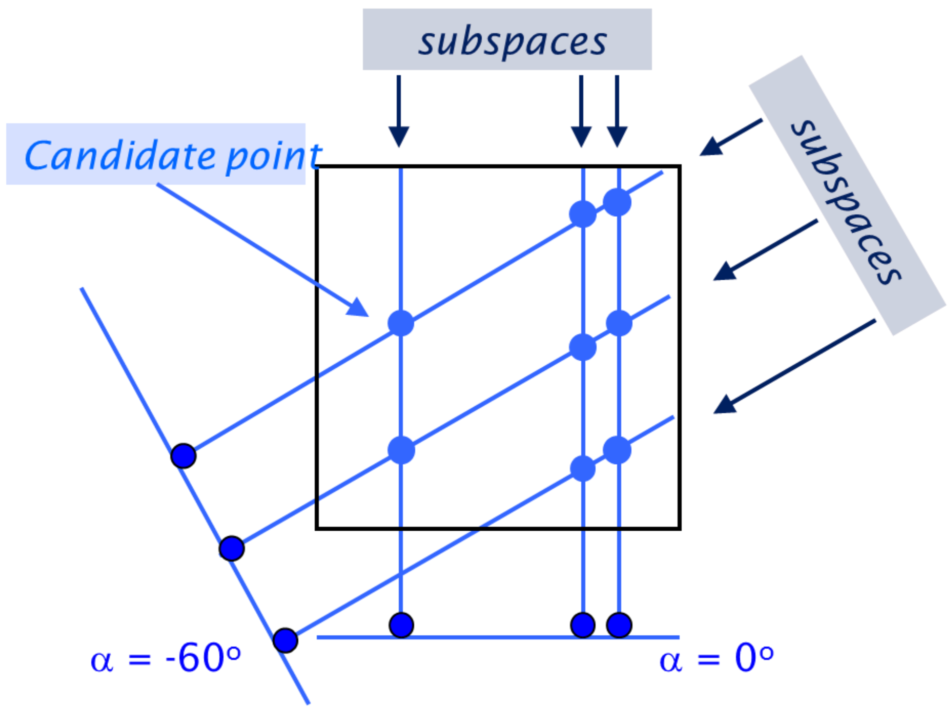
1. step: two projections are measured and selected arbitrarily:
   e.g. 0° and -60°



Principle of GAPRO

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

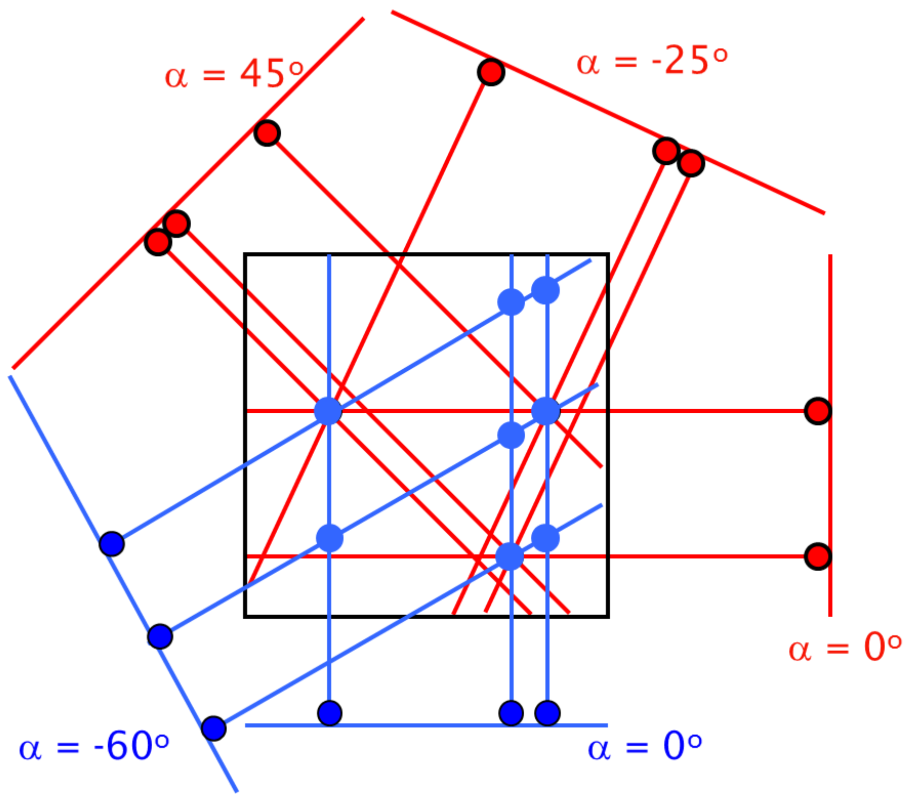
Intersection of subspaces creates candidate points



Principle of GAPRO

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

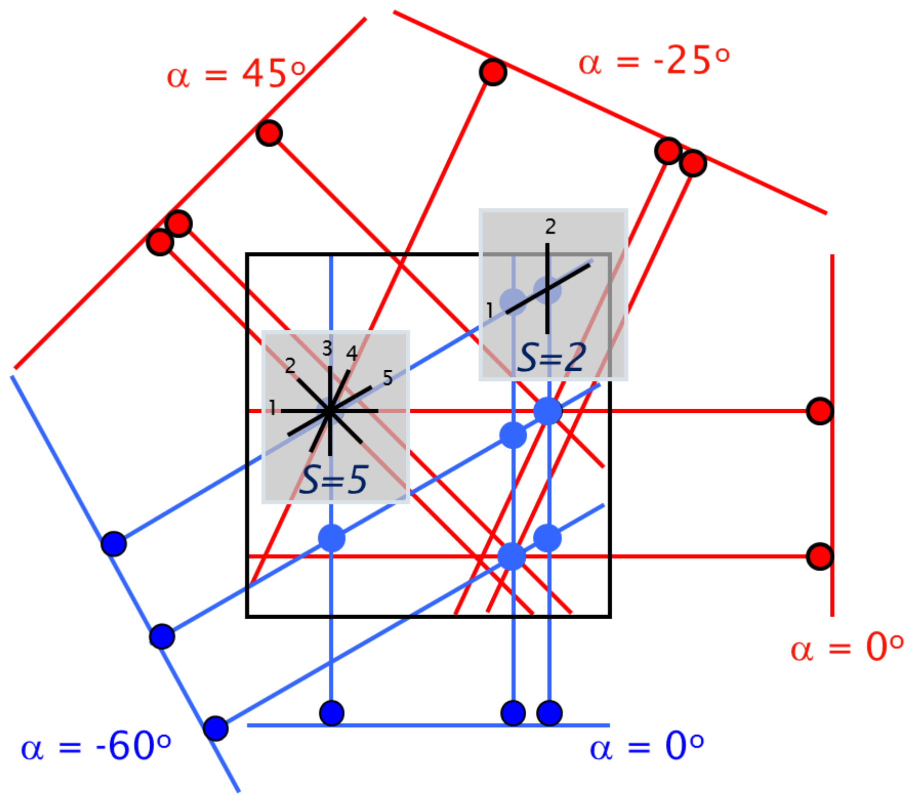
Calculate additional intersections/subspaces



Principle of GAPRO

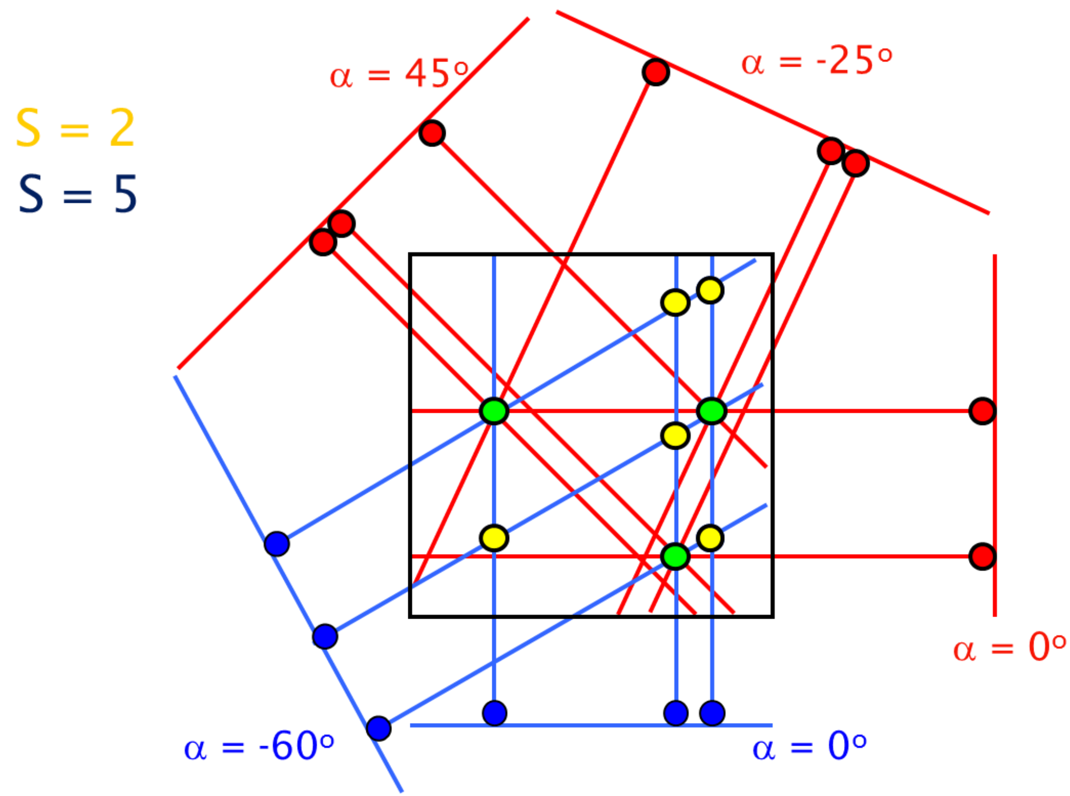
BRUKER

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



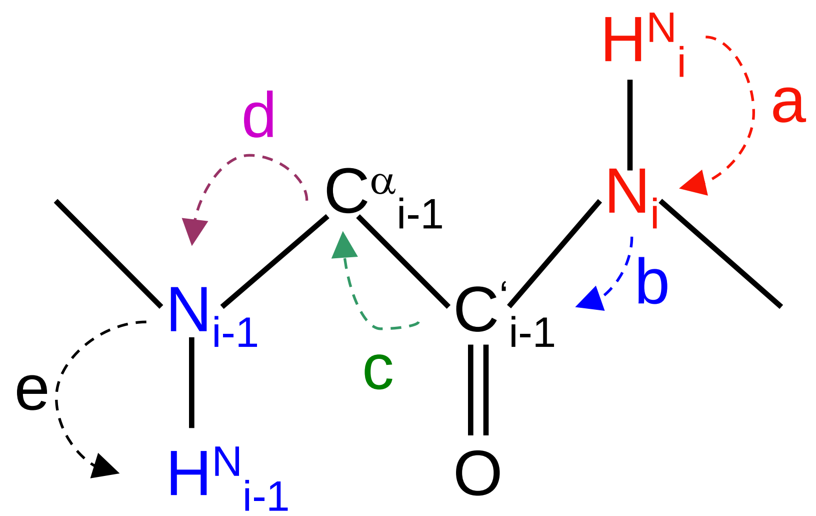
Principle of GAPRO

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



APSY experiments and software

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



APSY experiments and software

BRUKER

Key Features:

- **High Precision:** Peak lists of high precision from an optimum number of projections.

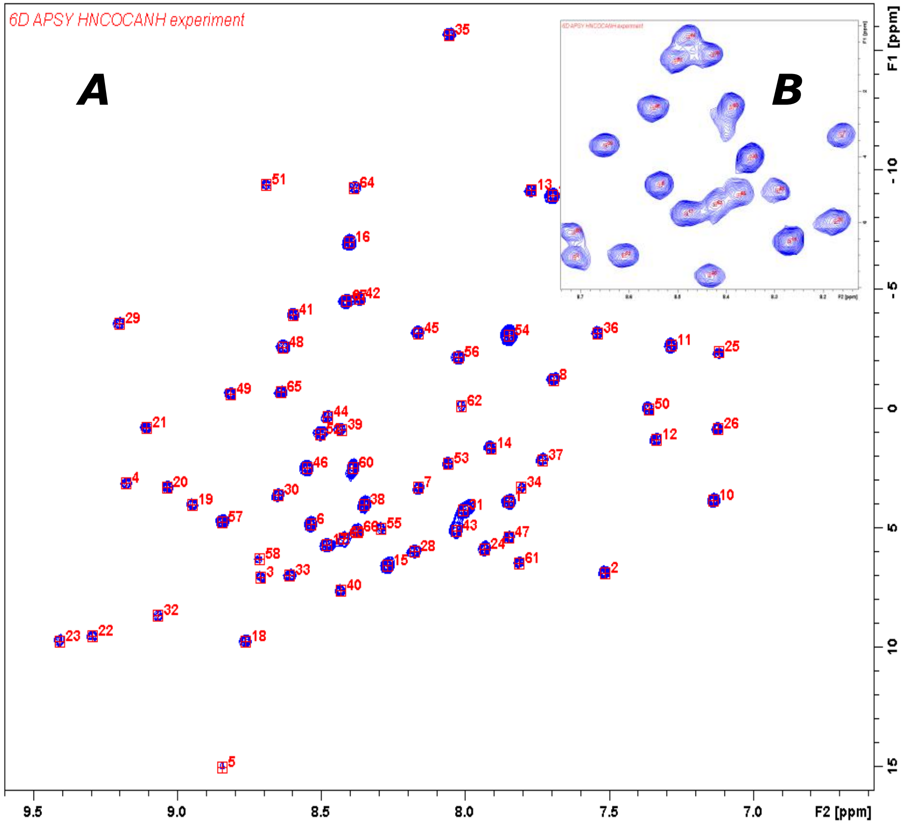
H_{i-1}	N_{i-1}	CO	CA	N_i	H_i	$\delta(N_{i-1}-N_i)$
ppm	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*Hz*
8.0613	102.4332	177.1224	52.3724	**133.0131**	**8.8405**	-4.635
8.8578	**133.1058**	174.3129	56.454	**125.1379**	**8.7061**	1.415
8.7125	**125.1096**	175.6223	58.7179	**122.0751**	**8.9428**	-1.24
8.9591	**122.0999**	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 6D-APSY-HNCOCANH experiment.

APSY experiments and software

APSY: peak list

- High Precision: Visual inspection of results.

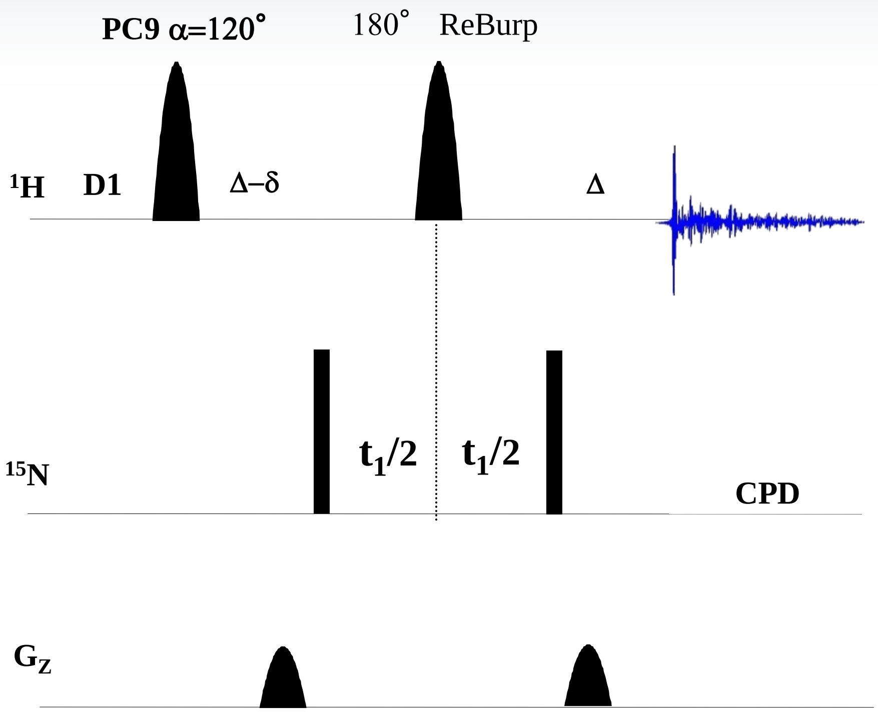


2D peak lists calculated from the 6D-APSY-HNCOCANH experiment can be displayed on any projection.

Combining two fast methods:

BEST Triple Resonance Experiments & NUS

So-Fast HMQC: Principles



Rapid Pulsing:

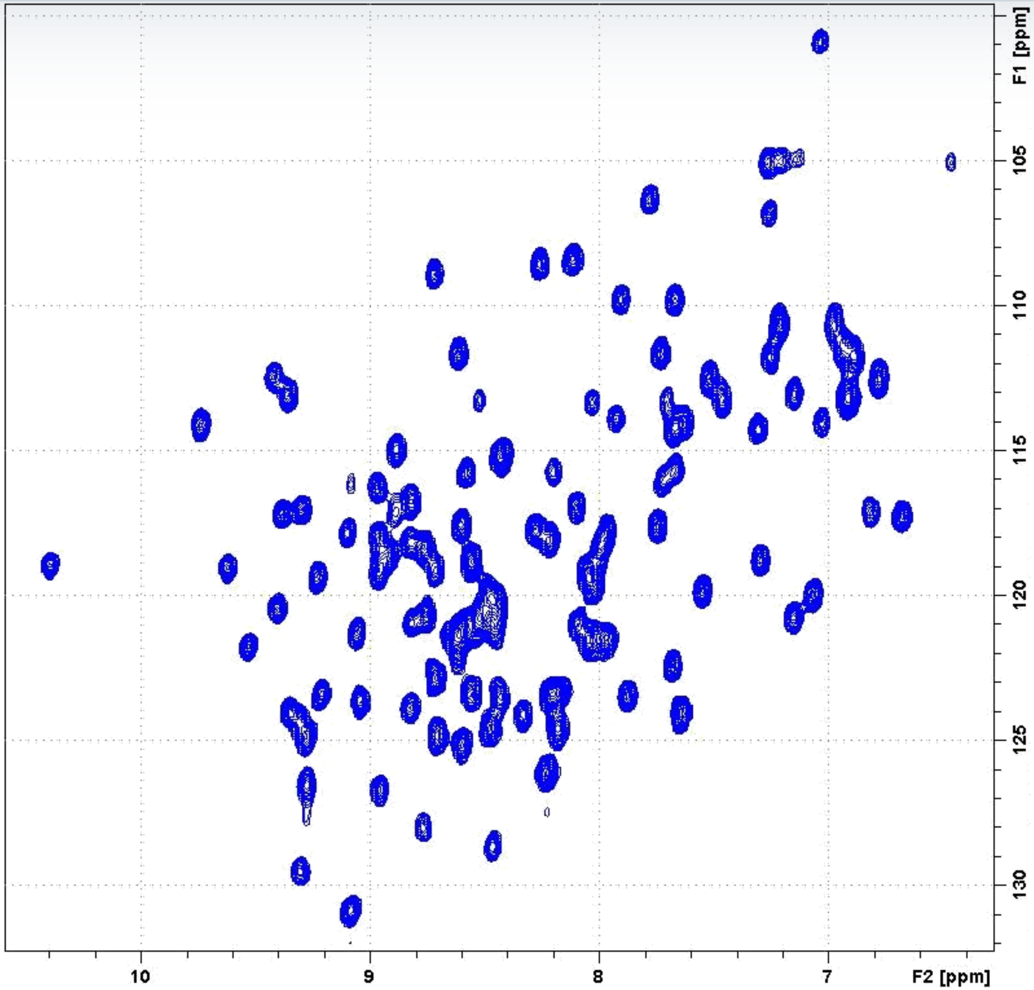
1. Experiment is based on very fast repetition rate
2. Extreme cases: D1 = 1ms
3. Selective excitation of NH protons only, keep $C_{aliphatic}$ along +Z axis
4. Enhanced T_1 relaxation of NH protons
5. Use of the Ernst angle:

Selective pulse on NH protons: 120° - 180° = -60°

P. Schanda and B. Brutscher, J. Am. Chem. Soc., 127, 8014, 2005

A. Ross, M. Salzmann and H. Senn, J. Biomol. NMR, 10, 389, 1997

So-Fast HMQC: 2 mM Ribonuclease (TCI Cryoprobe 600 MHz)



Experiment time 65s

D1= 0.3 s

AQ=41 ms

So-Fast HMQC

- Allows to study very rapid phenomena (protein folding)
- Increase the speed for HTS

- Important RF power deposited in the probe

- Required good SNR

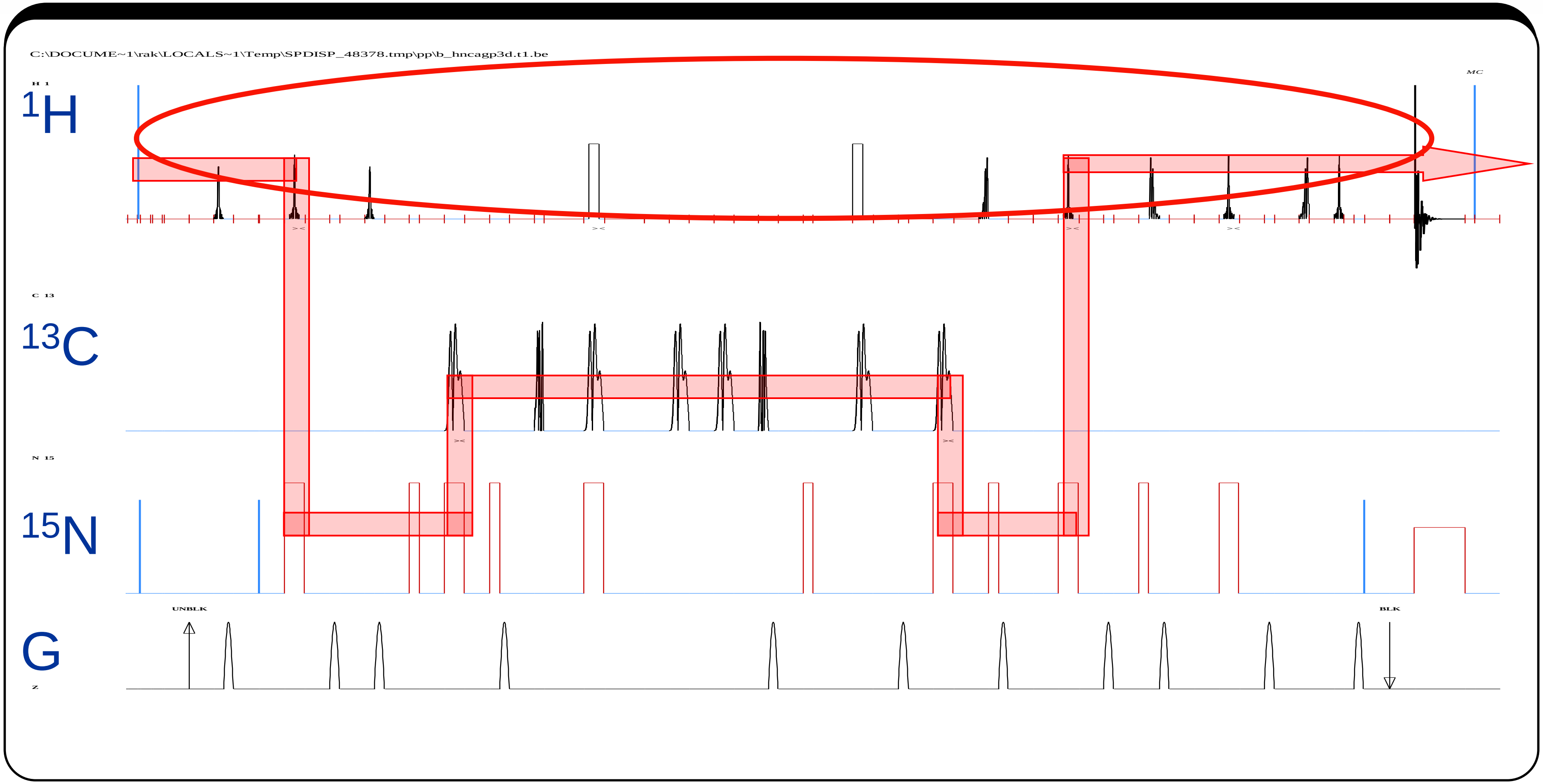
BEST triple resonance experiments

- **BEST triple resonance:**
  - Optimized for enhanced relaxation behavior of NH (bandselective excitation for amide protons, leaving H2O & aliphatics along the Z-Axis)

- **Reduced relaxation delay:**
  - D1 down to **200-1ms** instead of typical 1.5-1.0 s

BEST Triple resonance experiments

BEST-HNCA



BEST triple resonance experiments

3D-HNCO acquired with high resolution:

td 2k x 96 x 256
ns = 2

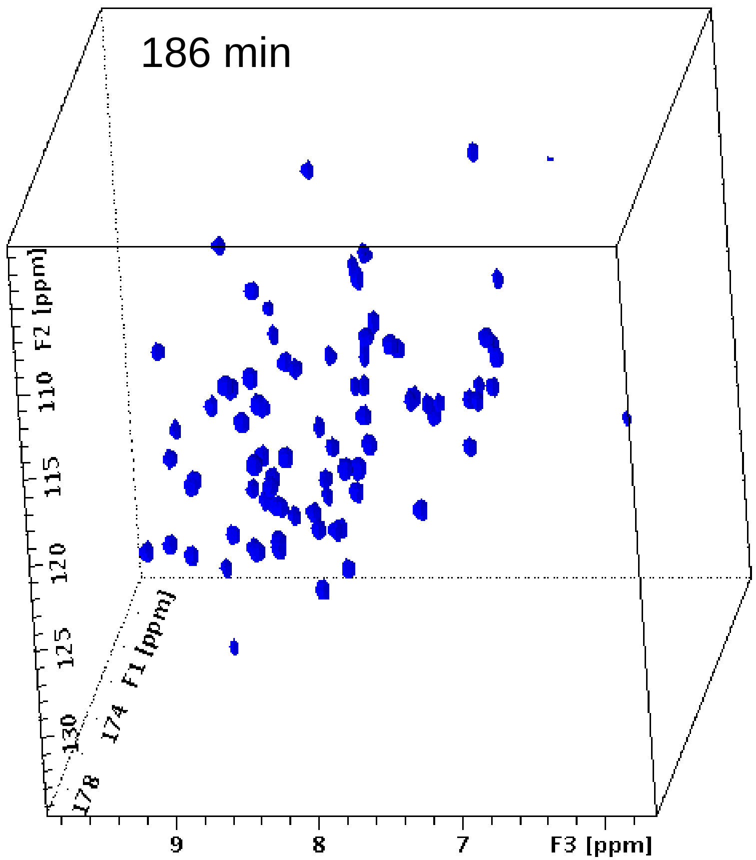
"Classical 3D HNCO"	3D BEST-HNCO	3D BEST-HNCO NUS
d1 1.0 sec	d1 10 ms	d1 10 ms
full sampling	full sampling	10% sparse sampling
997 min	186 min	17 min
1	1/5	1/50

BEST triple resonance experiments

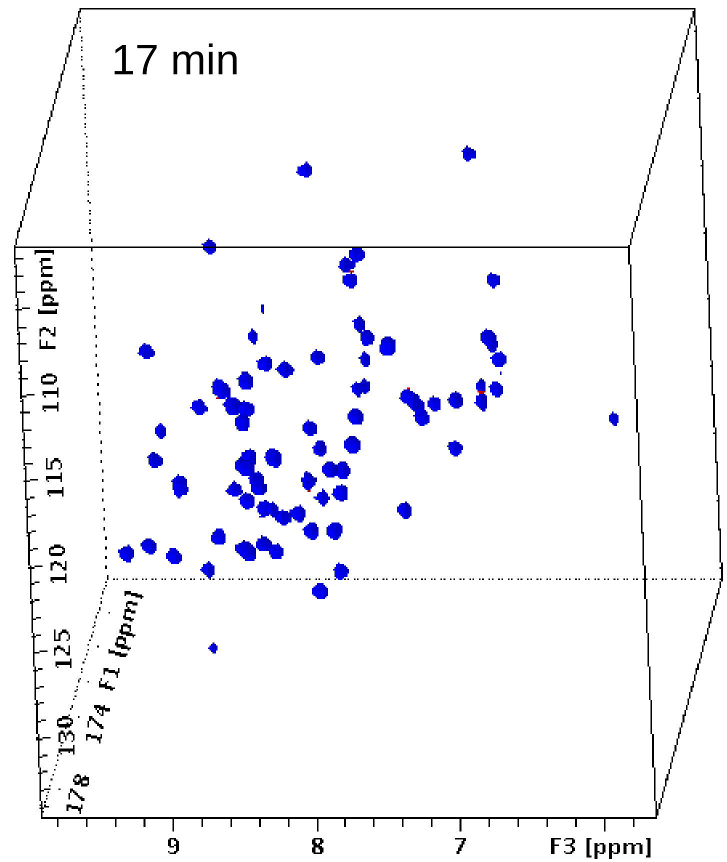
- **High duty cycle for 15N decoupling**
  - Optimize decoupling with GARP-4 to achieve **>48 ppm** bandwidth:

800 MHz	700 MHz	600 MHz	500 MHz
350us	400us	466us	560us

800 MHz BEST-HNCO traditional / NUS



3D BEST-HNCO
Traditional sampling



3D BEST-HNCO
10% sparse sampling

www.bruker.com