



# 4<sup>th</sup> Workshop on Holistic Analytical Methods for Systems Biology Studies

17-19 April 2016

Aristotle University of Thessaloniki, Greece

## Seminar 5

Protocol For NMR Based Metabolomics. Tips And Tricks

**Dr. Dimitra Benaki**

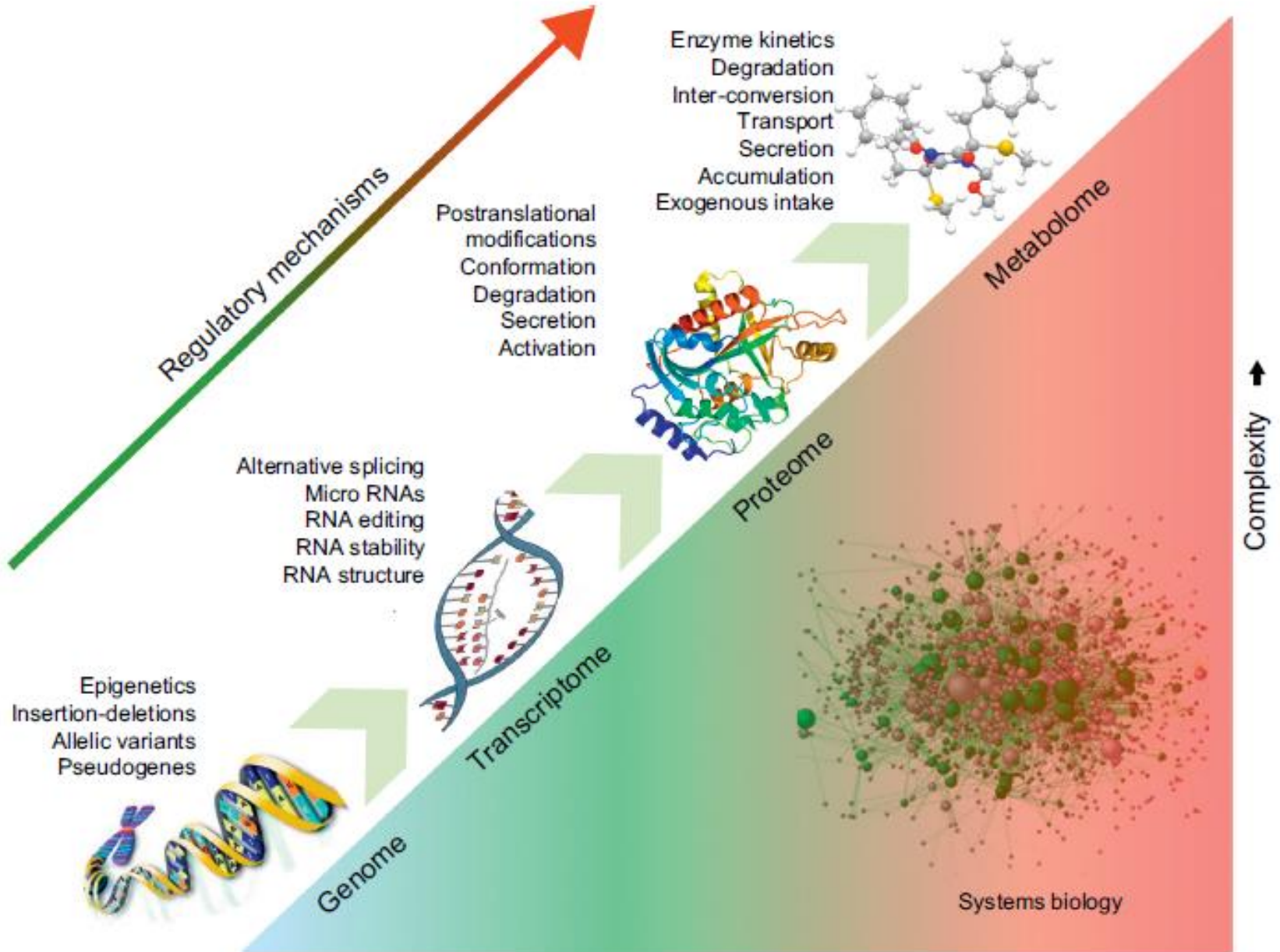
School of Pharmacy, Dept. Pharmaceutical Chemistry



HELLENIC REPUBLIC  
National and Kapodistrian  
University of Athens

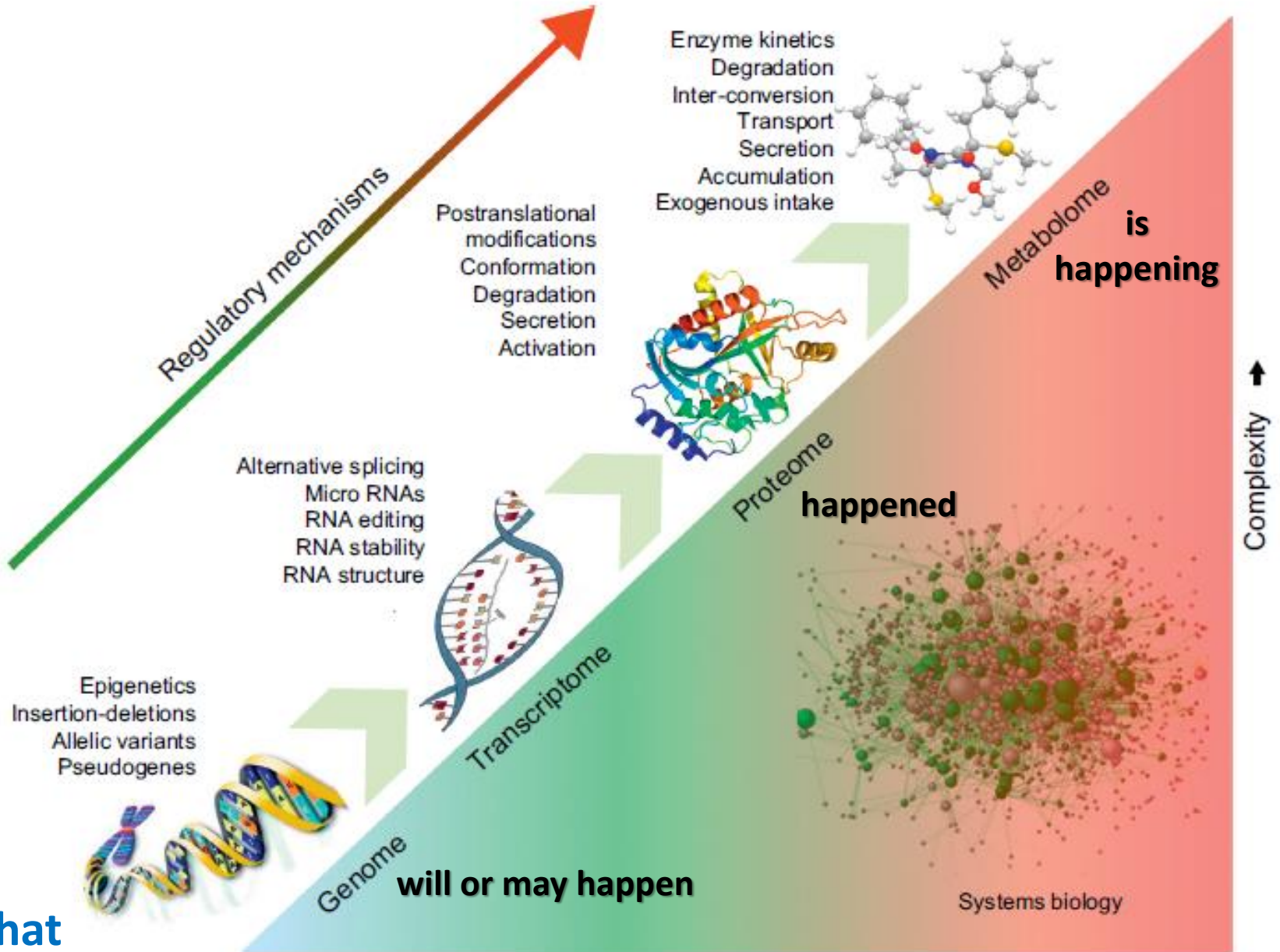
# Systems Biology

Barallobre-Barreiro et al. / Rev Esp Cardiol 2013;66(8):657-661



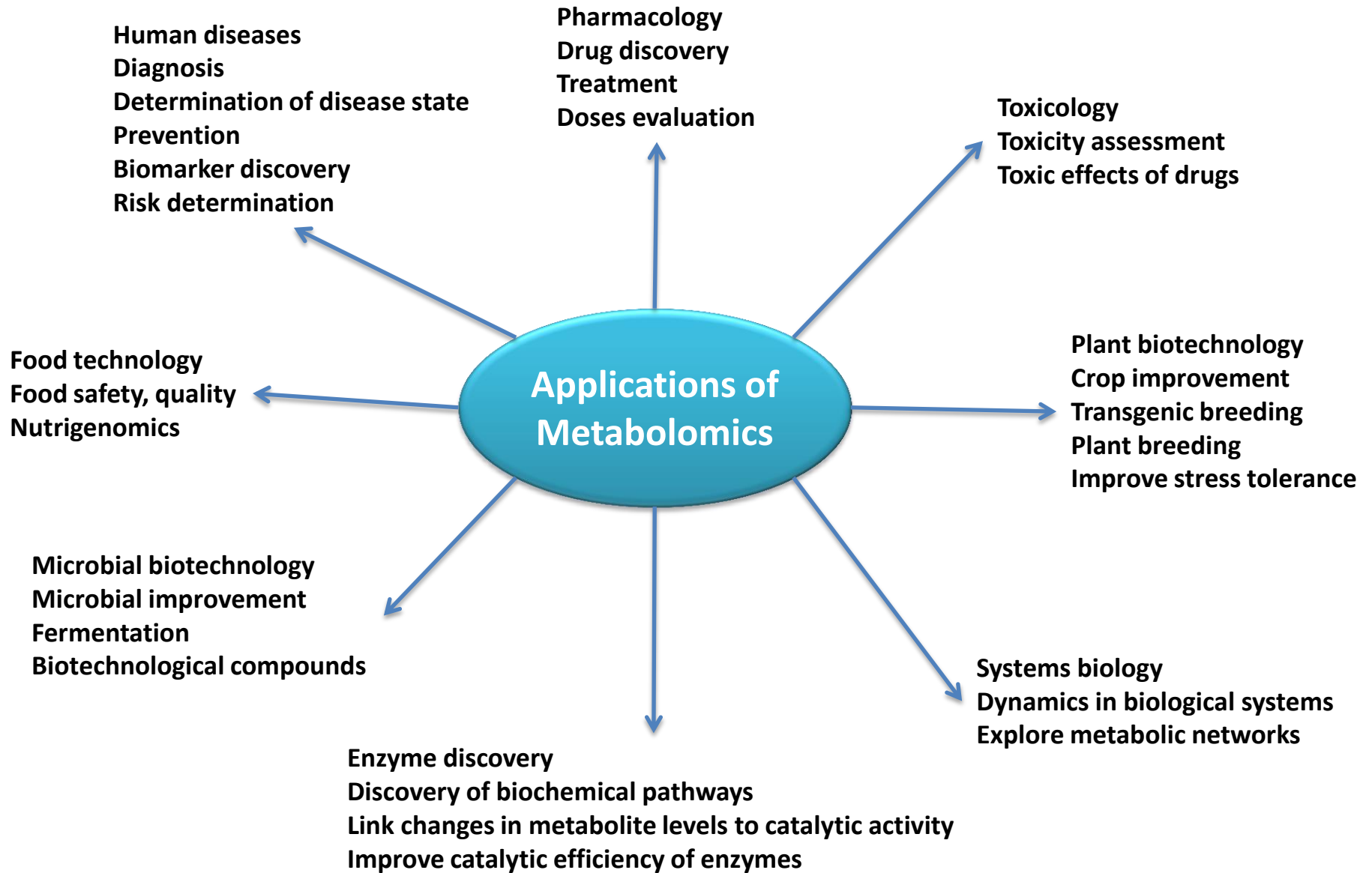
# Systems Biology

Barallobre-Barreiro et al. / Rev Esp Cardiol 2013;66(8):657-661



## What

# Applications of Metabolomics





# Metabolomics' Methods

- NMR
- GC,
- HPLC,
- UPLC,
- HPTLC
- CE (capillary electrophoresis)

## Hyphenated

- LC-MS,
- GC-MS
- GC-MS/MS
- UHPLC-MS



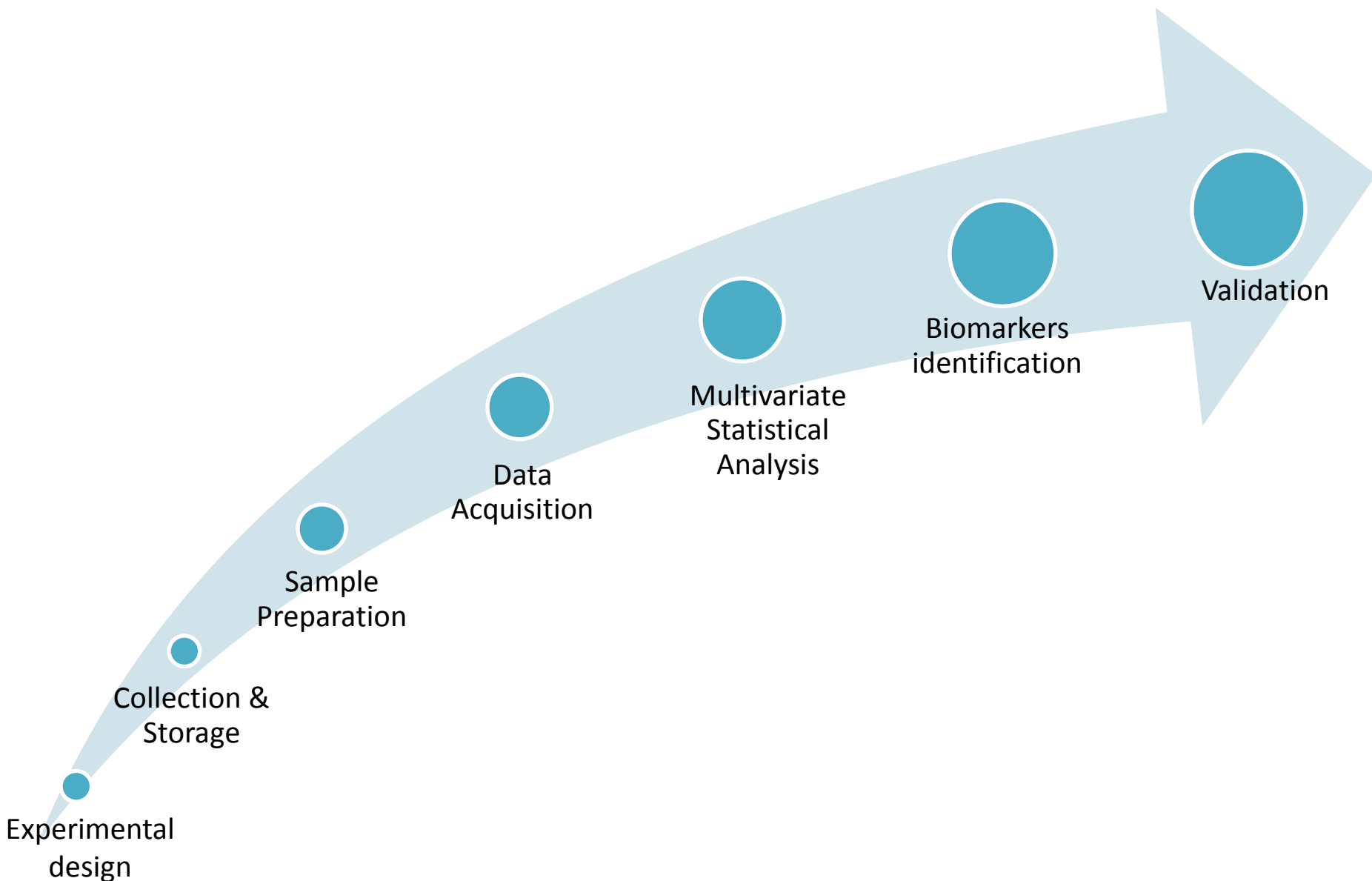
# NMR in Metabolomics



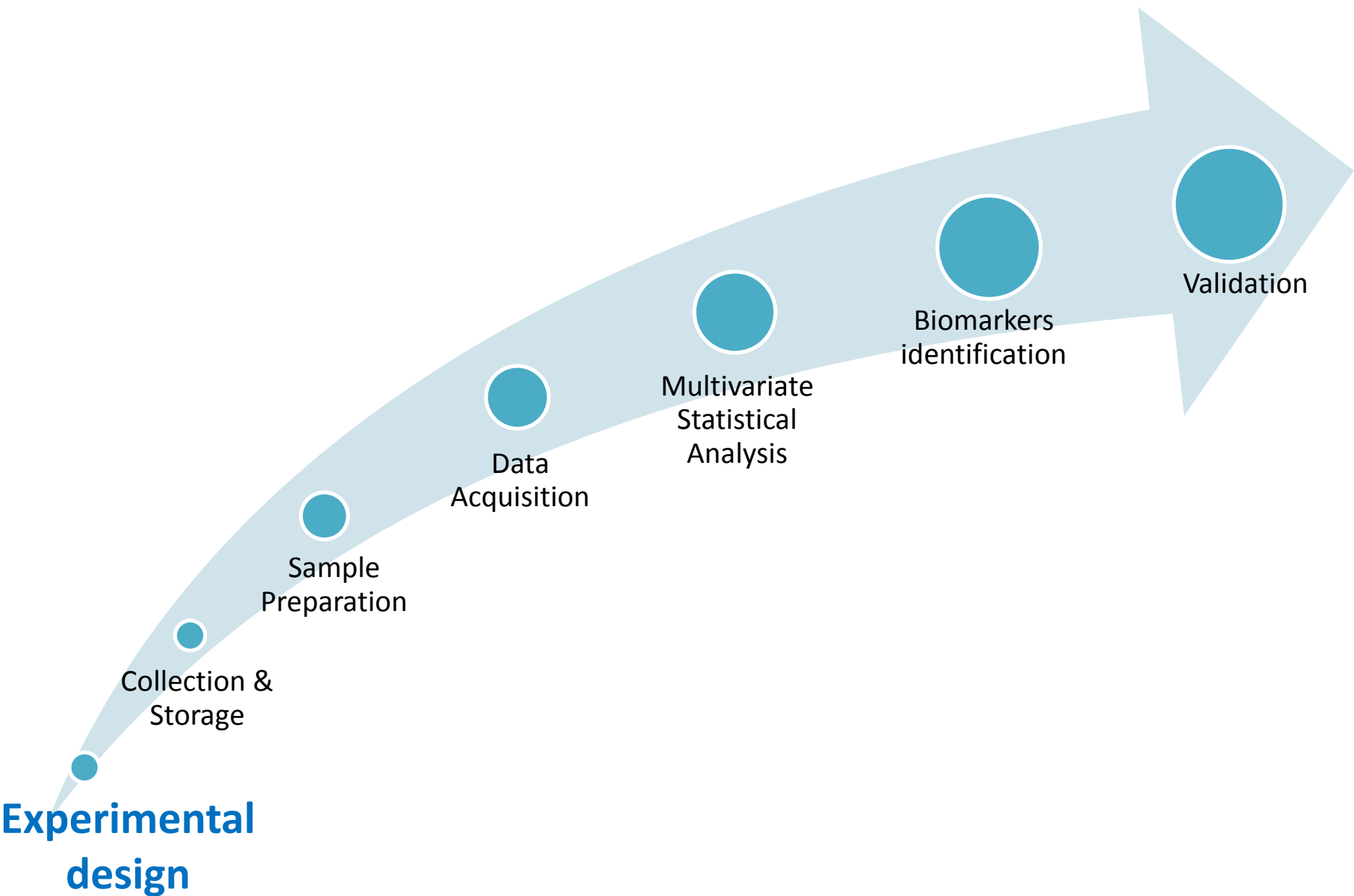
- ✓ Minimal sample preparation (biofluids)
- ✓ No extra steps, i.e. separation
- ✓ Measures multiple metabolites simultaneously
- ✓ Non-destructive
- ✓ Fast analysis
- ✓ High reproducibility
- ✓ Highly quantitative
- ✓ *High throughput*
- ✓ Steadily increasing sensitivity



# Metabolomics Workflow



# Metabolomics Workflow





# Experimental Design

## KEY ELEMENTS OF THE SCIENTIFIC METHOD



In the absence of a proper design it is essentially impossible to distinguish biological variation from technical variation. When these two sources of variation are confounded, there is no way of knowing which source is driving the observed results

# Experimental Design

- ✓ Avoid the fridge temptation

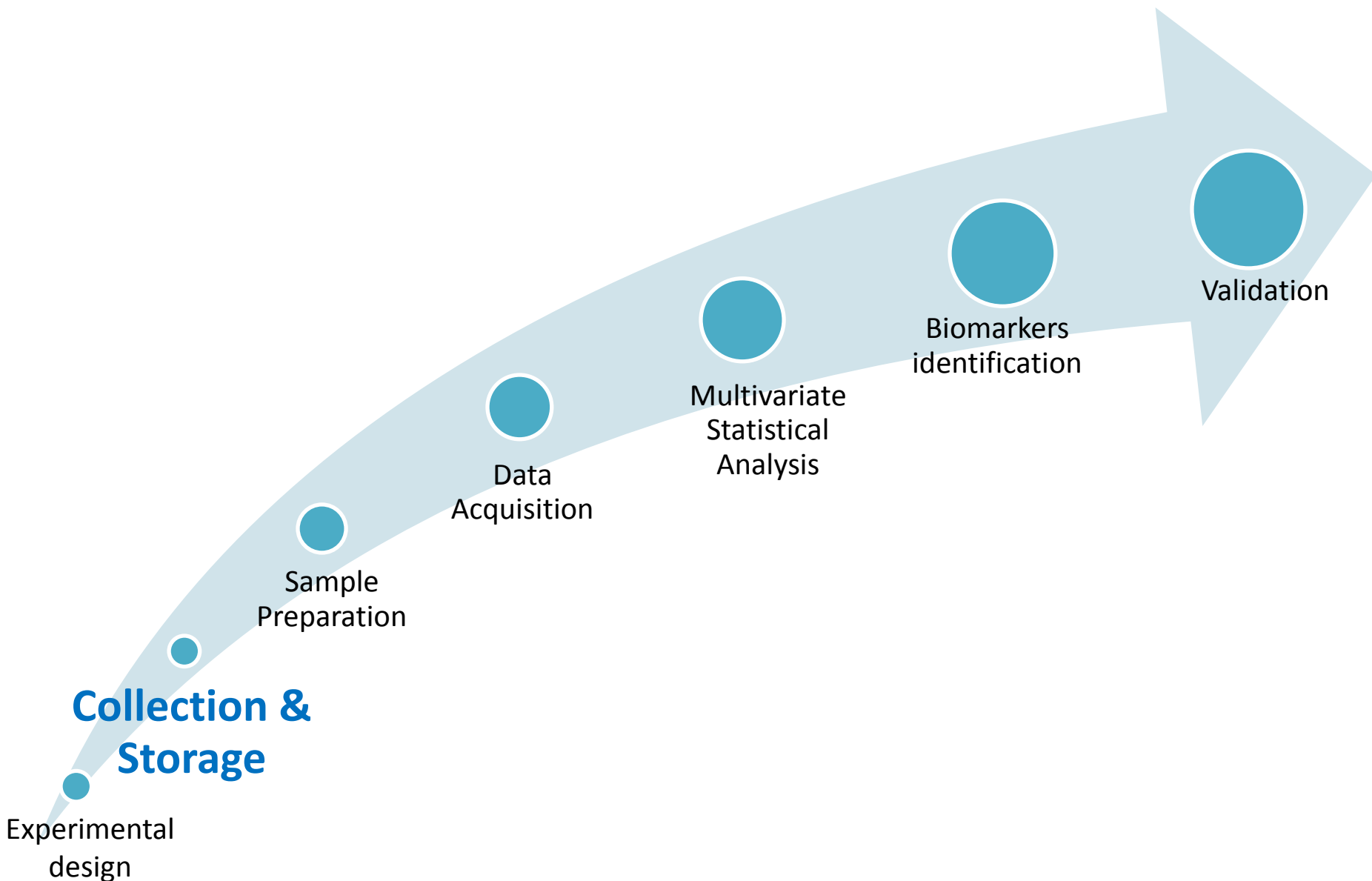


# Experimental Design

- ✓ Avoid the fridge temptation



# Metabolomics Workflow





# Collection & Storage

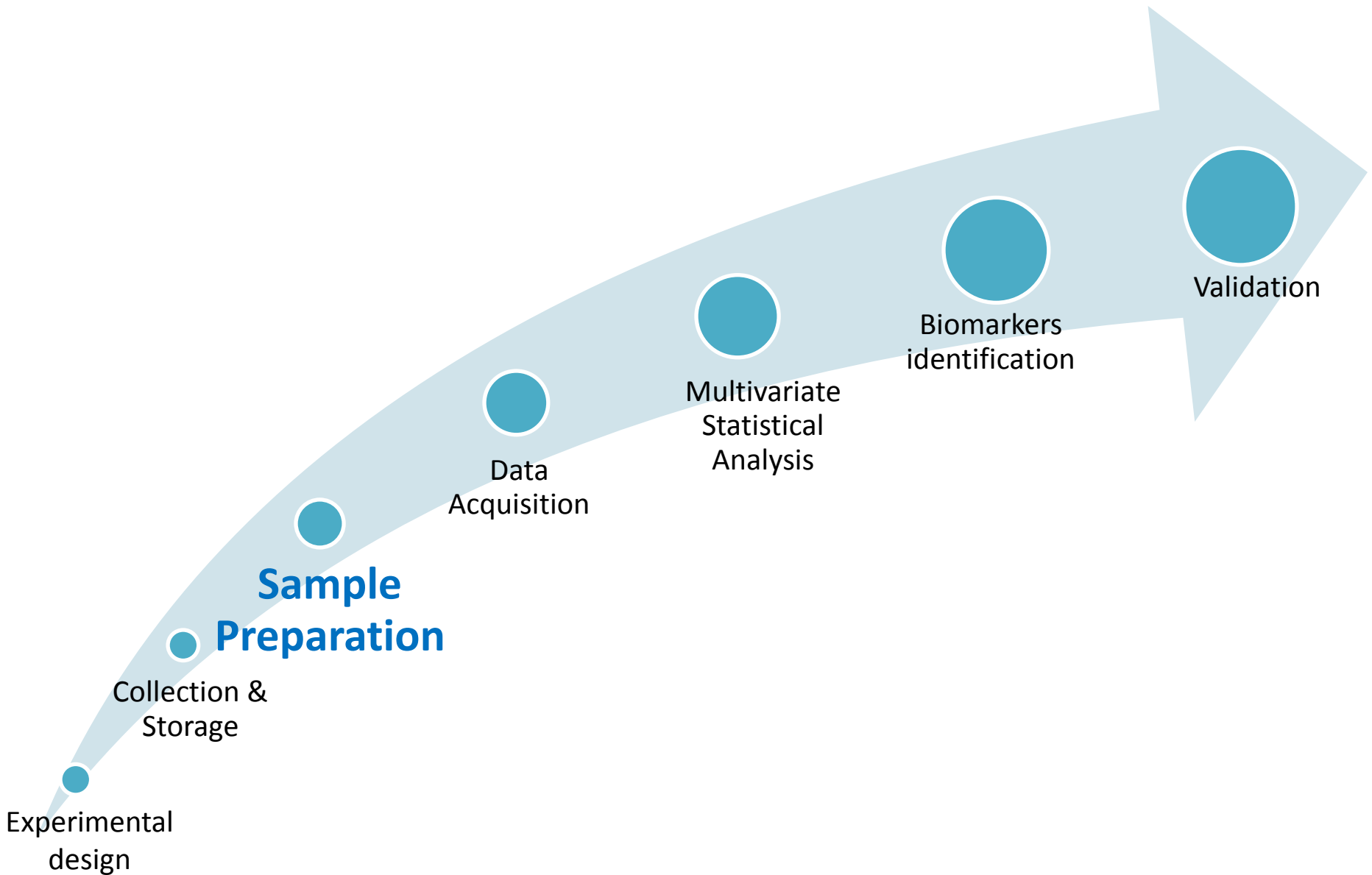
- ✓ A single person should collect/harvest the initial material
- ✓ stop enzymatic processes

transfer in dry ice

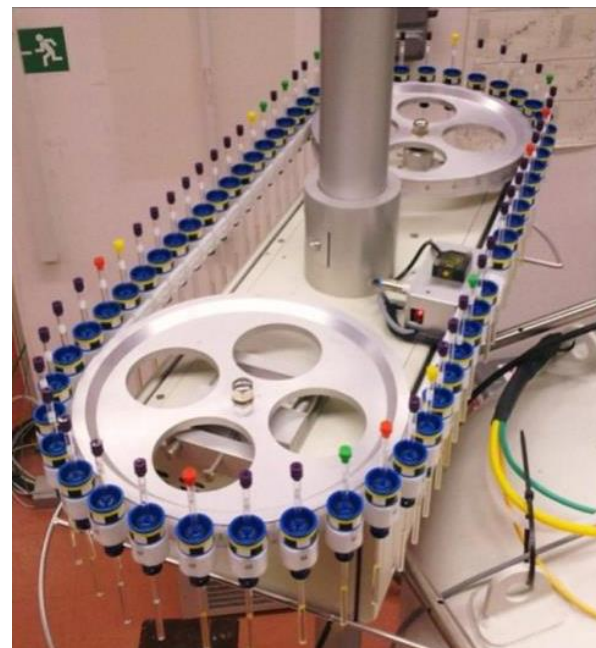
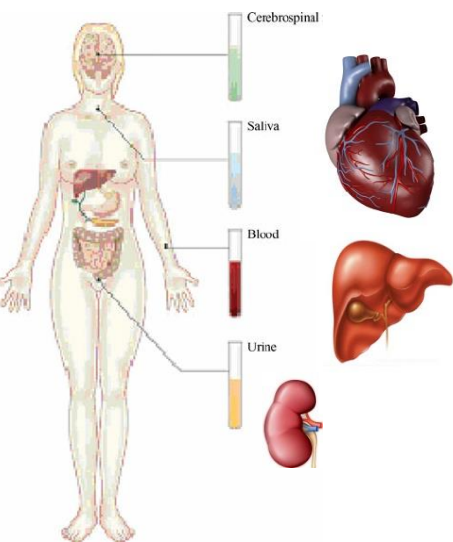
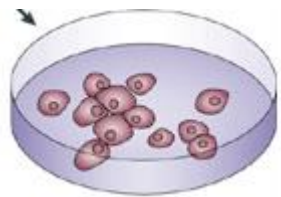


store at -80 °C

# Metabolomics Workflow



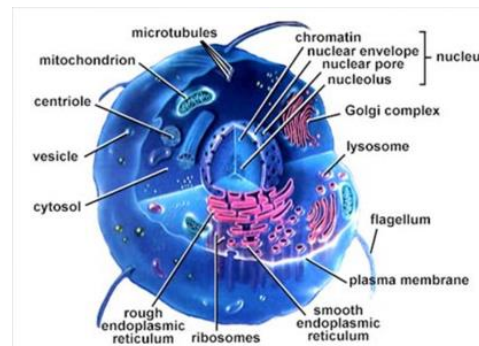
# NMR in Metabolomics



# Metabolomics sample preparation

*metabolites found within:*

- **Biofluid**
  - Urine,
  - Blood (serum, plasma),
  - Saliva,
  - Breath,
  - CSF,
  - Amniotic, etc
- **Cell**
  - Cytosolic metabolites
  - Released metabolites
- **Tissue, Organ**
  - Mammals: liver, kidney, heart, tumour, muscle, brain, fat tissue, etc.
- **Plants**
  - Leaves, roots, fruits, etc.
- **Whole organism**
  - Insects (Flies, etc)
  - Marine organisms
  - Worms, etc.

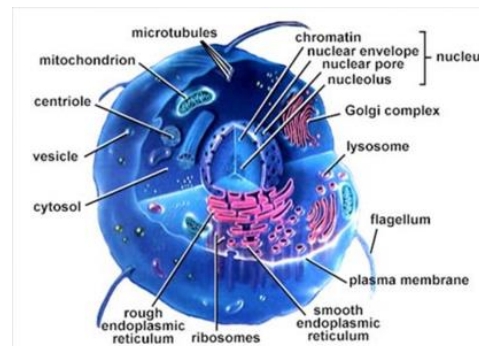




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- **Plants**
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  - Insects (Flies, etc)
  - Marine organisms
  - Worms, etc.



**Non- invasive**

# Metabolomics sample preparation

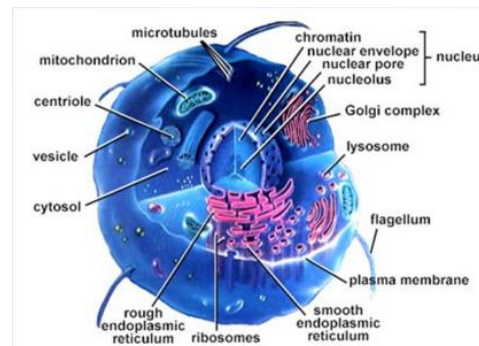
## *Standardized Protocols*

- ✓ pH adjustment: phosphate buffer pH 7.4; NaN<sub>3</sub> to eliminate bacterial growth
- ✓ axis calibration: Internal Standard (TSP, DSS; 0.01%)  
& “QUALITY CONTROL” # *Blood samples*
- ✓ field lock: deuterated solvent (10% D<sub>2</sub>O in Urine; 50% in plasma)
- ✓ Centrifuge (+4 °C) and transfer 550 μL in NMR tube
- *Plasma samples:* *gentle handling, no vortex, no centrifuge, remove protein particles with a needle*

# Metabolomics sample preparation

*metabolites found within:*

- **Biofluid**
  - Urine,
  - Blood (serum, plasma),
  - Saliva,
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- **Cell**
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- **Plants**
  - **Leaves, roots, fruits, etc.**
- **Whole organism**
  - **Insects (Flies, etc)**
  - **Marine organisms**
  - **Worms, etc.**



“tedious”

# Metabolomics sample preparation

## *Standardized Protocols*

- ✓ **Frozen sample**
- ✓ **Homogenization** in liquid N<sub>2</sub> (manually), high throughput tissue homogenizer with beads → weight (<100 mg) and store at -80 °C till extraction homogenizer probes (in extraction solvent; 1<sup>st</sup> step extract.)
- ✓ **Extraction** 3 solvent system MeOH – CHCl<sub>3</sub> – dH<sub>2</sub>O (-20 °C)

### WORK ON ICE

2 phase system; collection; repeat

- ✓ **Lyophilisation; store at -80 °C**
- ✓ **Reconstitution** axis calibration (Internal Standard; TSP, DSS; 0.01%)  
field lock (100% D<sub>2</sub>O buffered; pH 7.4; NaN<sub>3</sub>)  
centrifuge and transfer 550 µL in NMR tube



# Metabolomics sample preparation

## *Standardized Protocols*

- ✓ Frozen sample
- ✓ Homogenization in liquid N<sub>2</sub> (manually), high throughput tissue homogenizer with beads → weight (<100 mg) and store at -80 °C till extraction  
homogenizer probes (in extraction solvent; 1<sup>st</sup> step extract.)

- ✓ Extraction ✓ **CHECK SOLVENTS BEFORE EXTRACTION**

WORK INCLUDE ✓ **INCLUDE BLANK SAMPLES**

2 phase system; collection; repeat

- ✓ Lyophilisation; store at -80 °C

- ✓ Reconstitution ✓ **CHECK SOLVENTS BEFORE RECONSTITUTION**

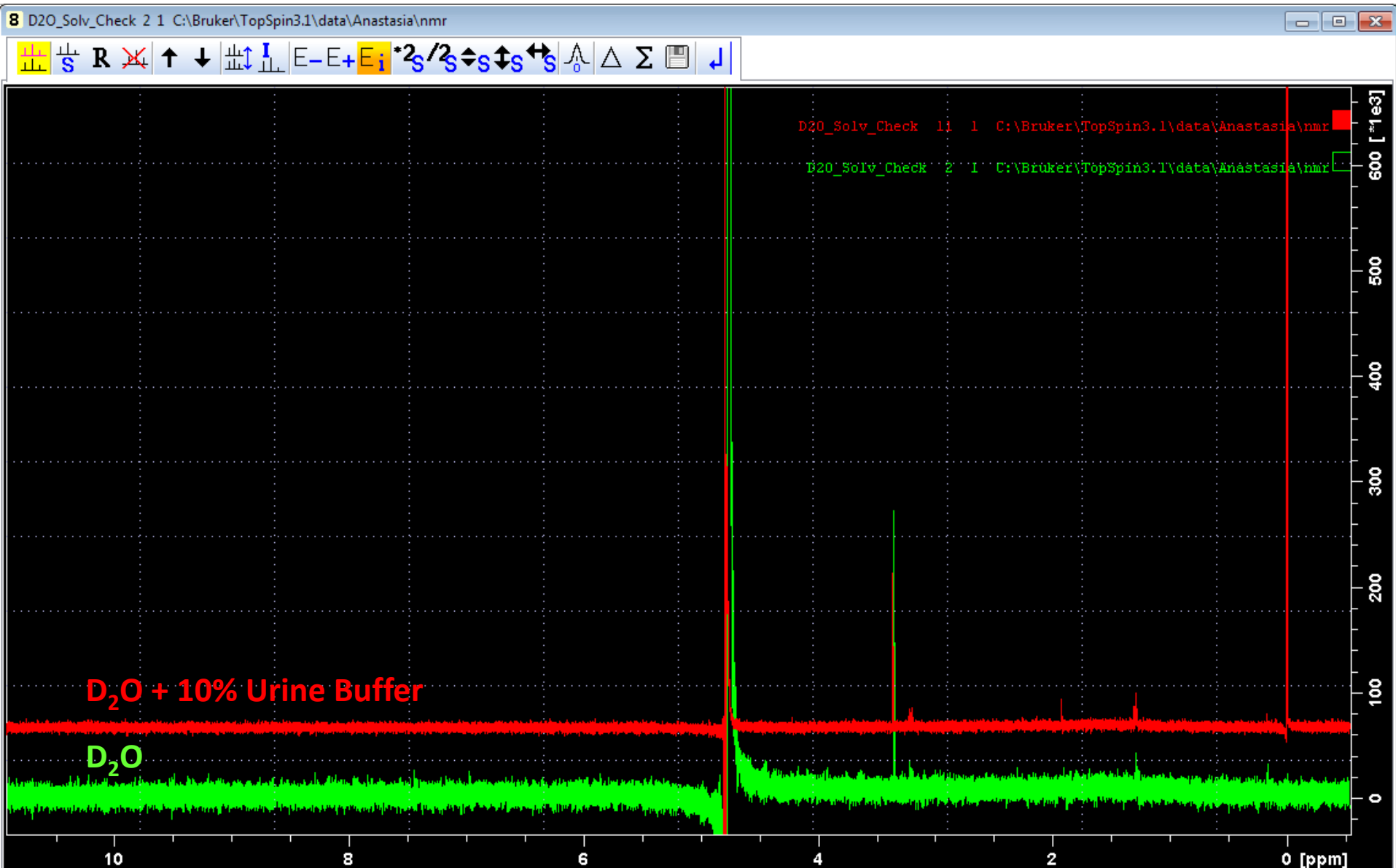
field lock (100% D<sub>2</sub>O buffered; pH 7.4; NaN<sub>3</sub>)

centrifuge and transfer 550 µL into NMR tube

- ✓ **INCLUDE BLANK SAMPLES (reconstitution buffer)**

# Metabolomics sample preparation

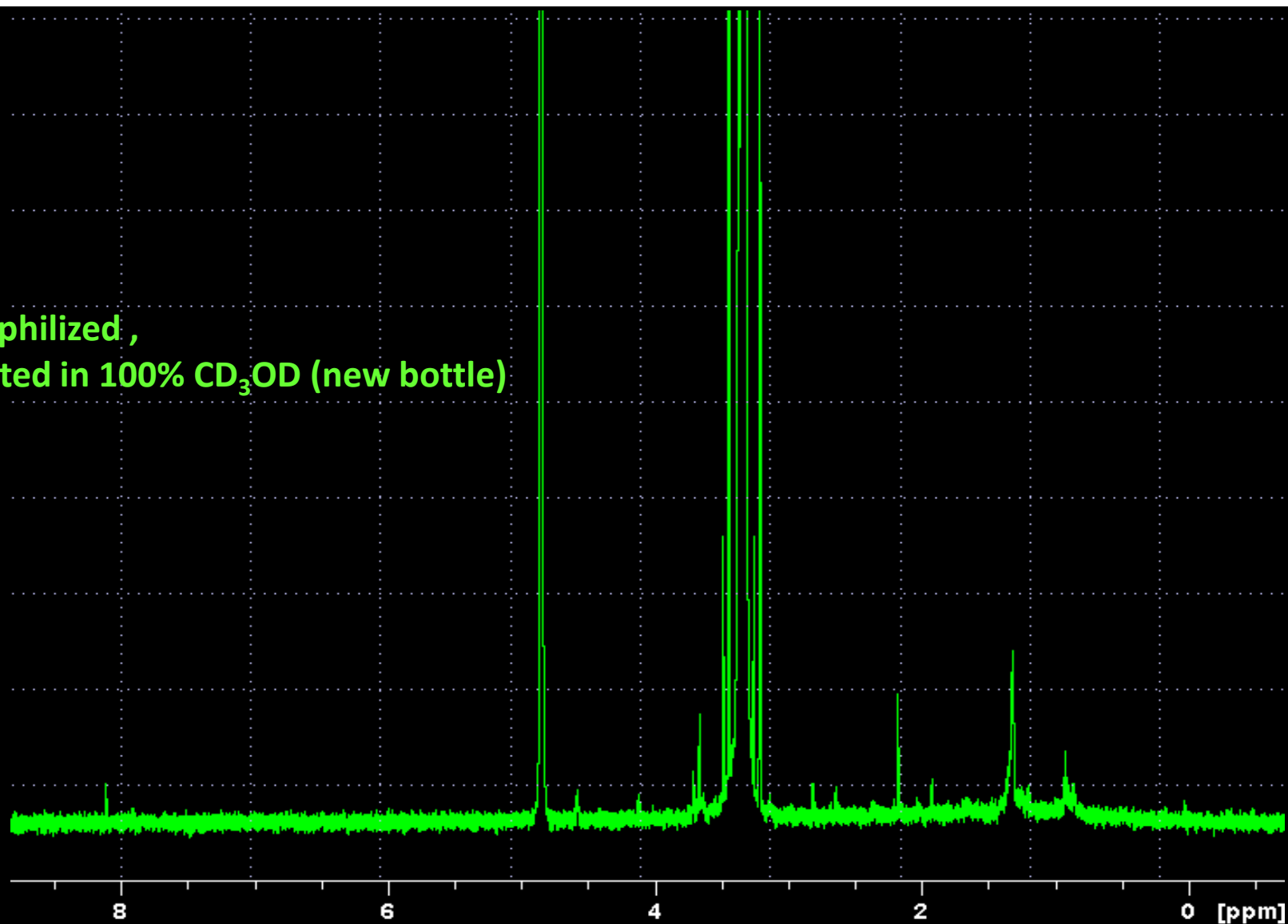
*Solvent check before NMR sample preparation*



# Metabolomics sample preparation

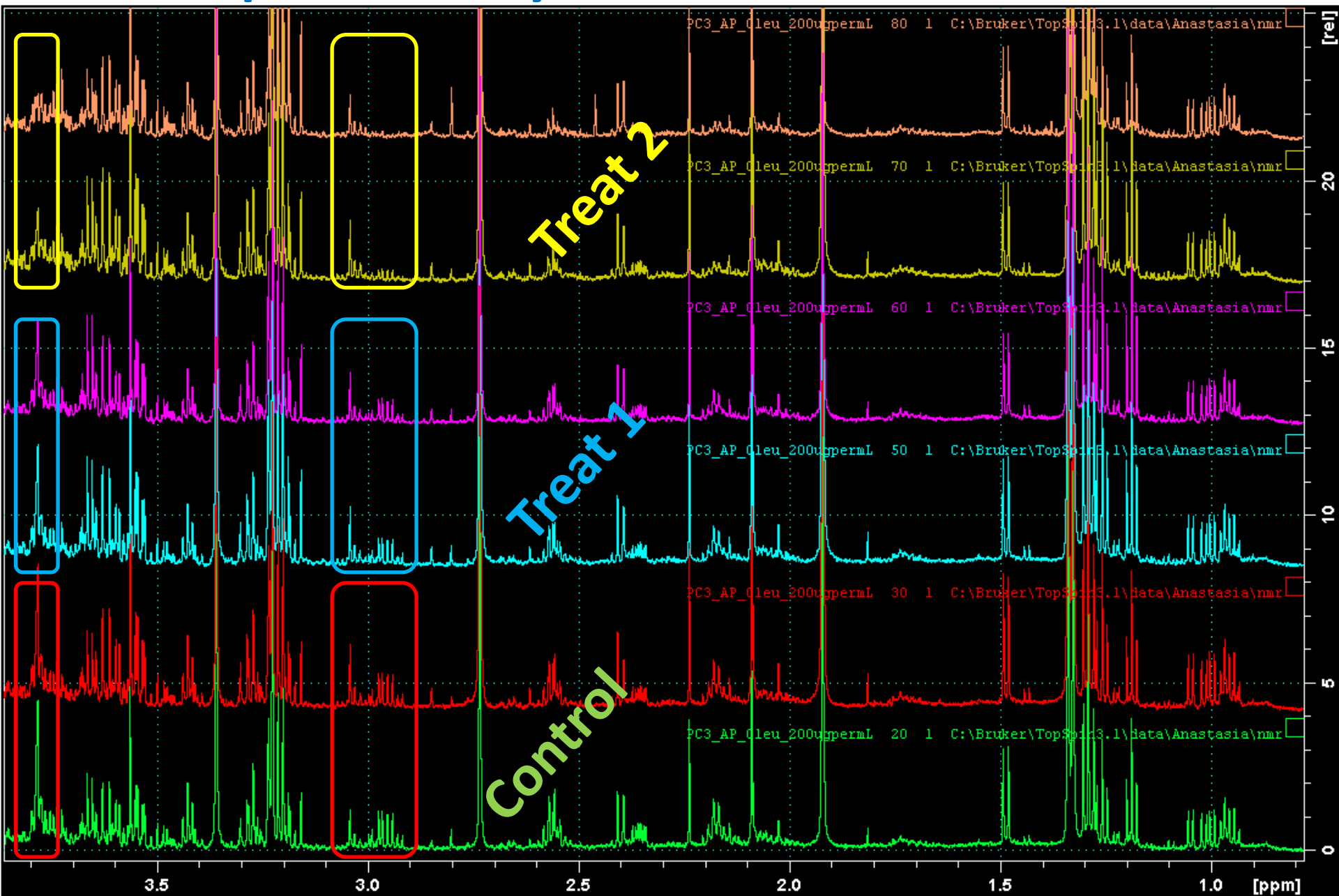
*Solvent check before extraction*

MeOH lyophilized,  
reconstituted in 100% CD<sub>3</sub>OD (new bottle)



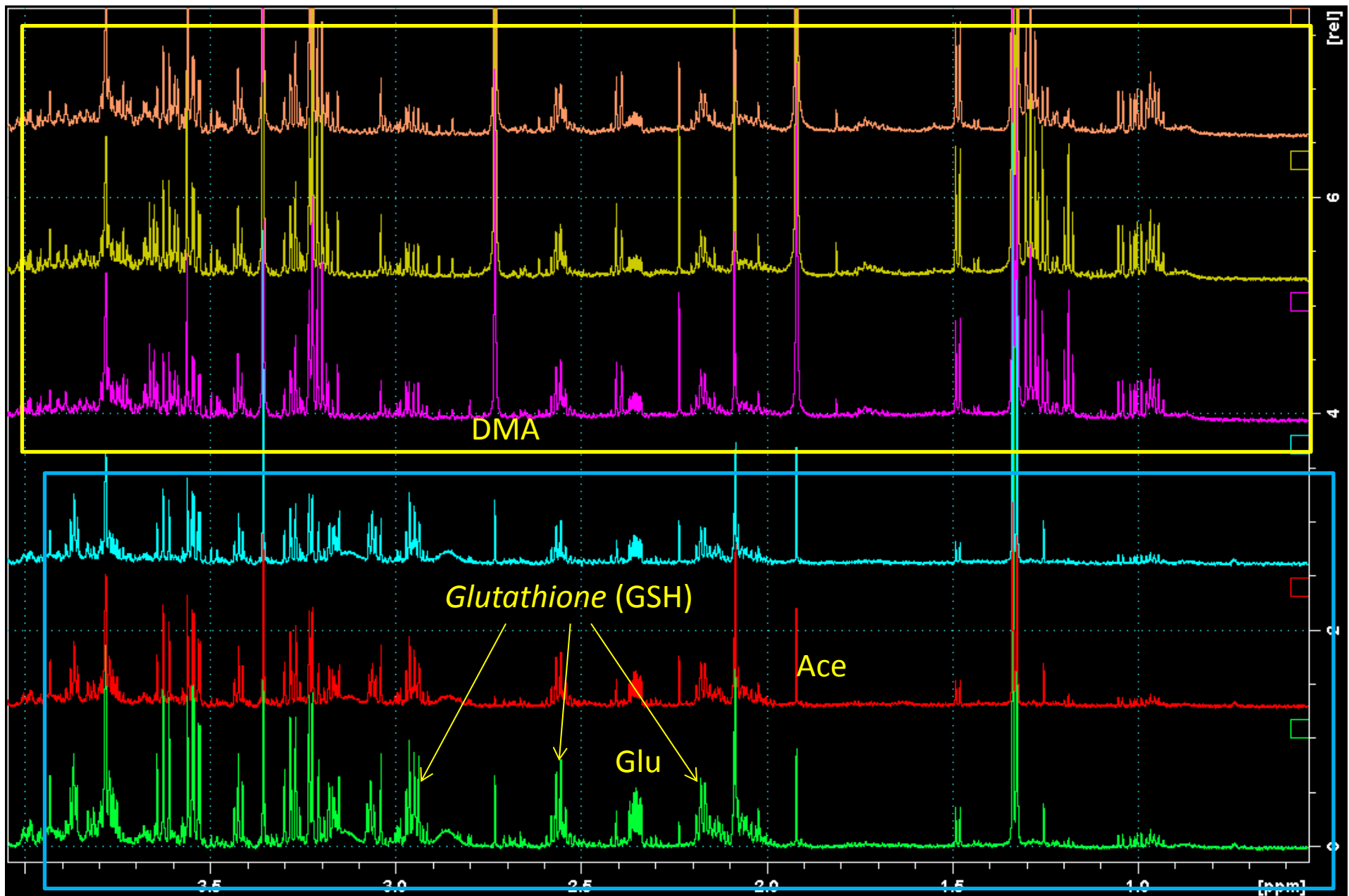
# NMR Reproducibility

PC3 cell line



# NMR Reproducibility

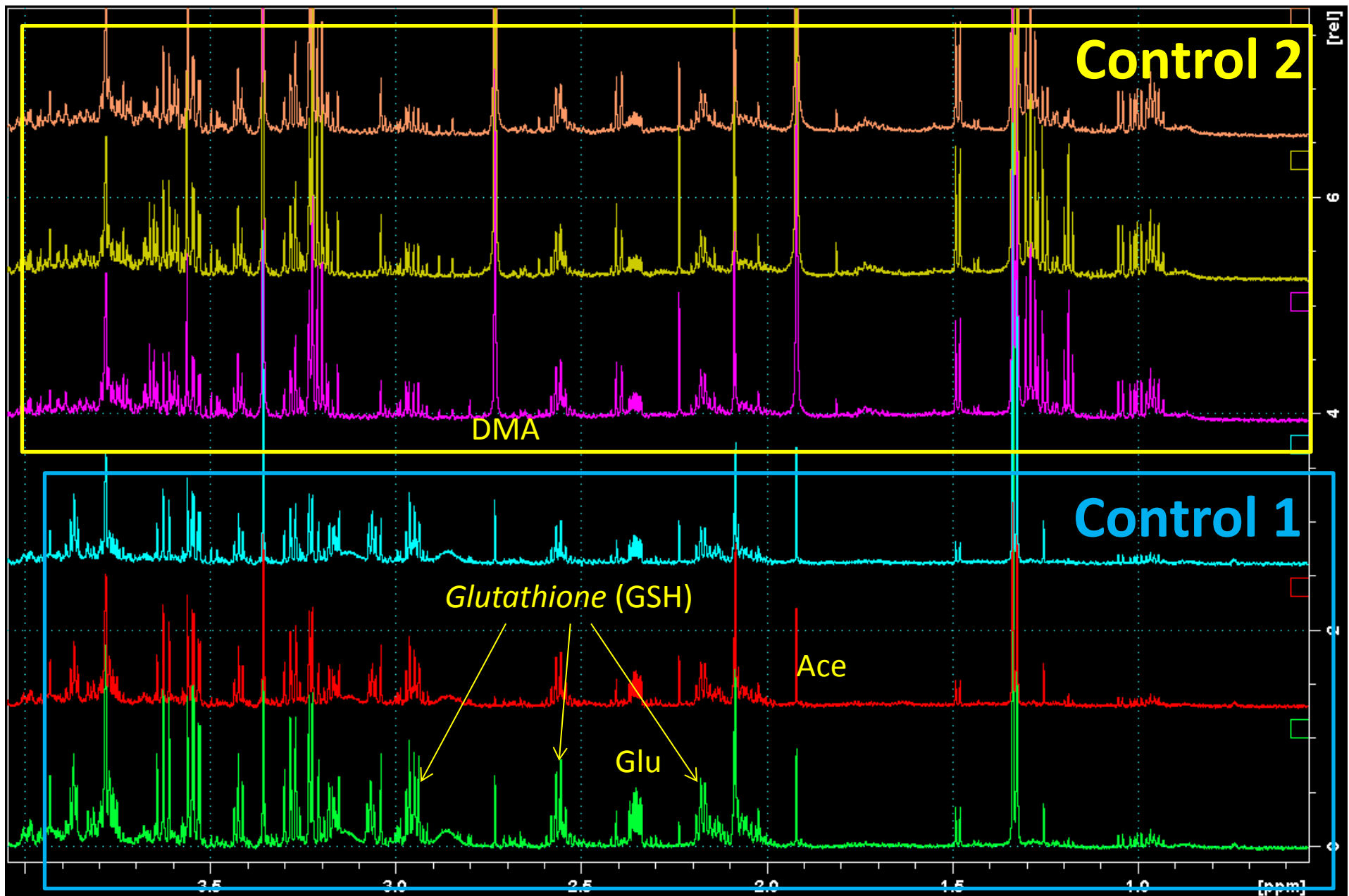
PC3 cell line





# NMR Reproducibility

PC3 cell line from 2 different persons



# NMR Reproducibility

## Urine samples recorded in 600 MHz Bruker AVANCE III, Athens

Buffer A (PBS pH=7.4): original from Bruker

Buffer B (PBS pH=7.4): local preparation

## Urine NMR samples

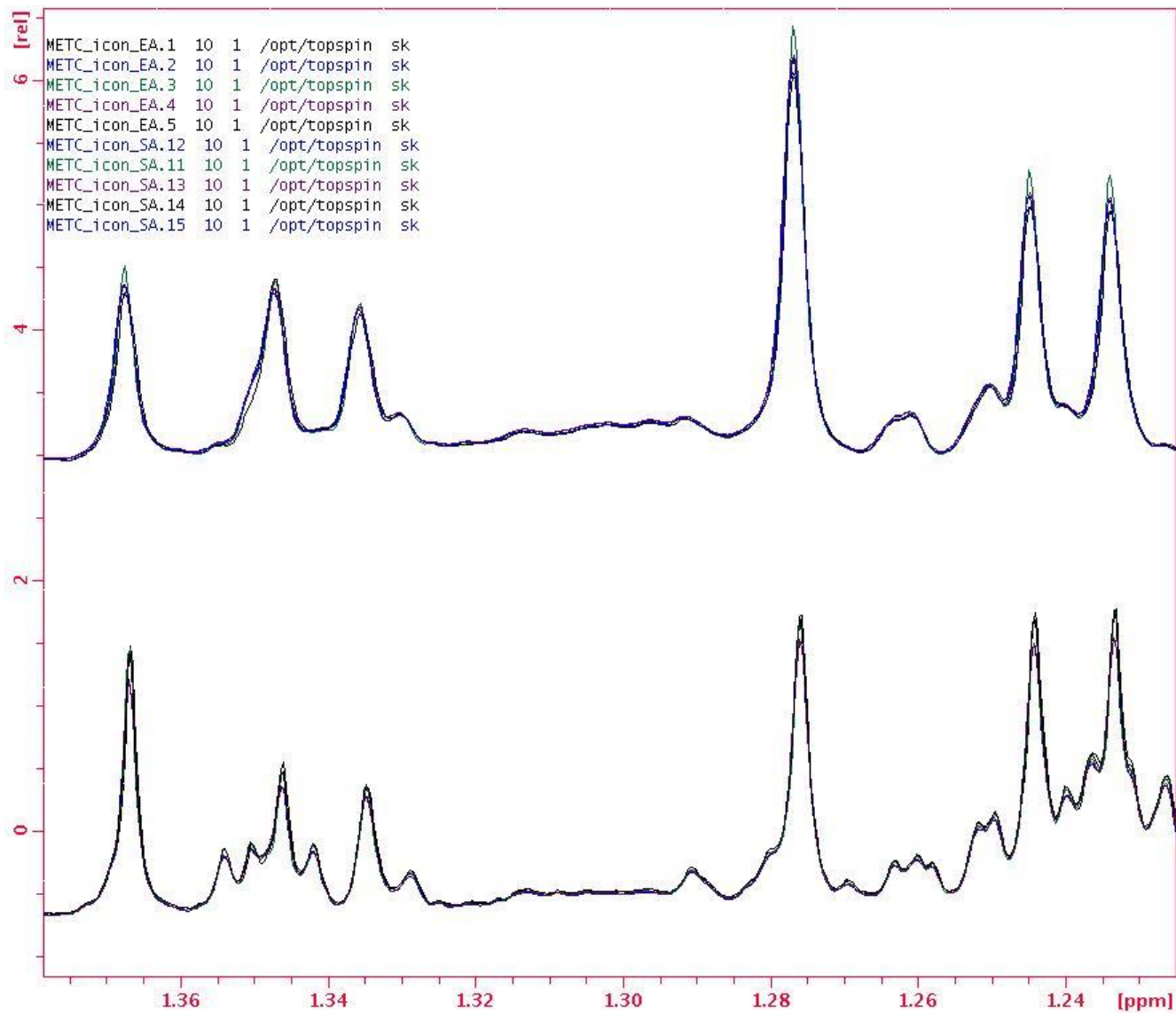
Sample 1-5 person E buffer A      1EA – 5EA

Sample 1-5 person E buffer B      1EB – 5EB

Sample 1-5 person S buffer A      1SA – 5SA

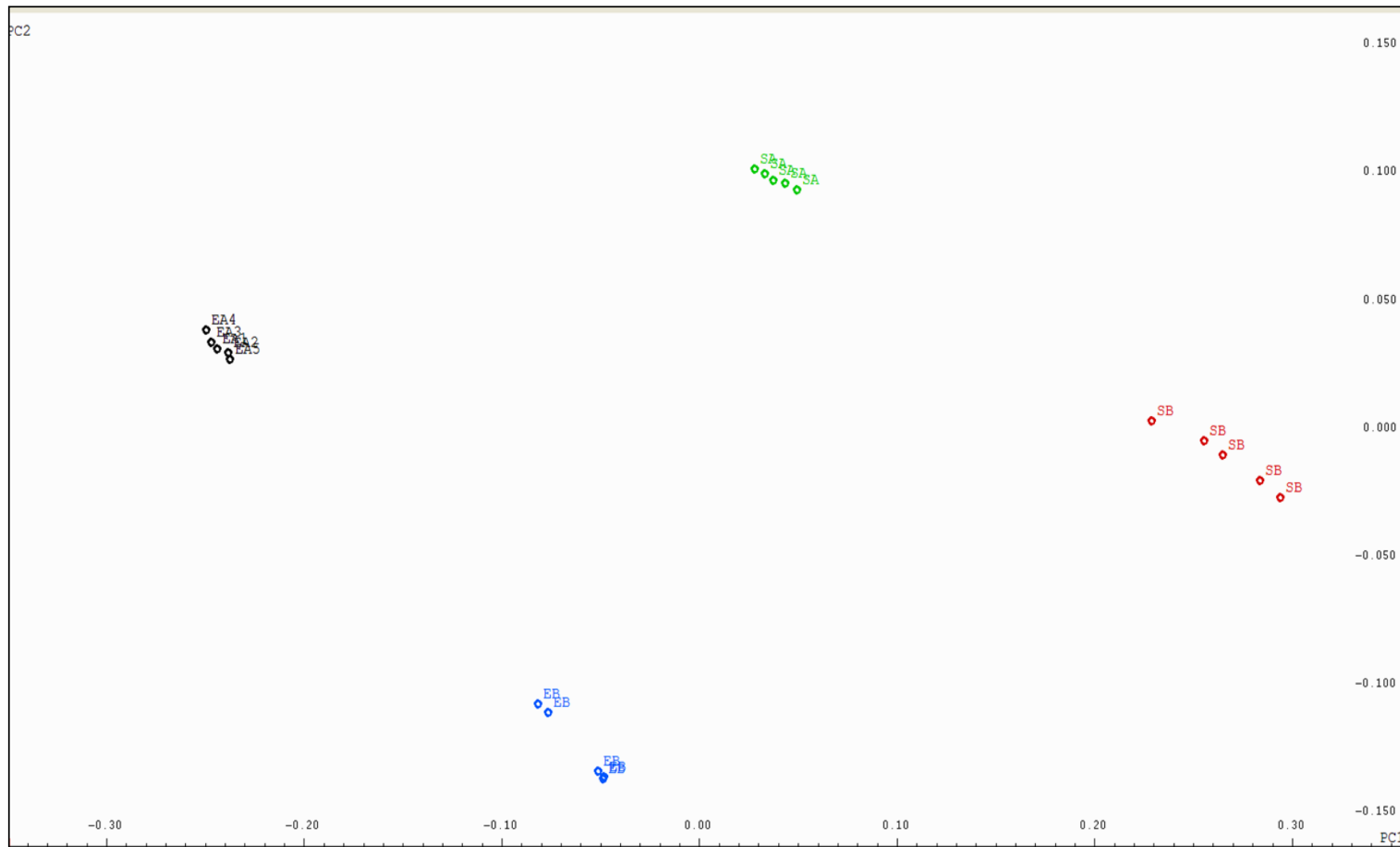
Sample 1-5 person S buffer B      1SB – 5SB

# NMR Reproducibility

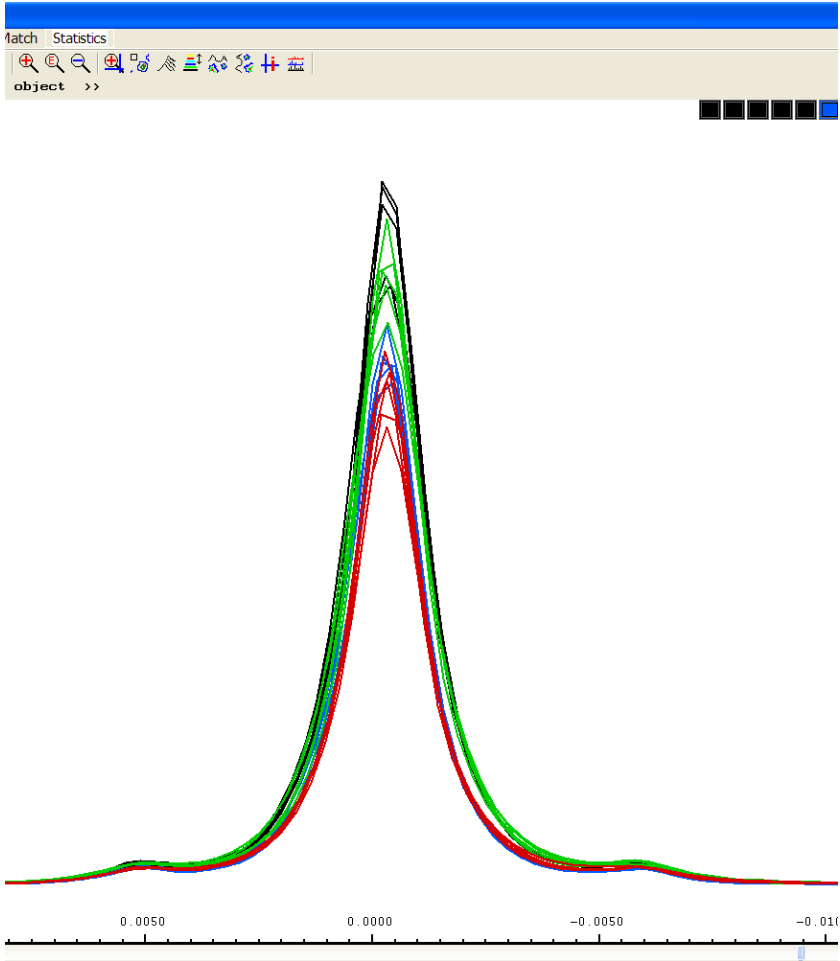


# NMR Reproducibility

PCA



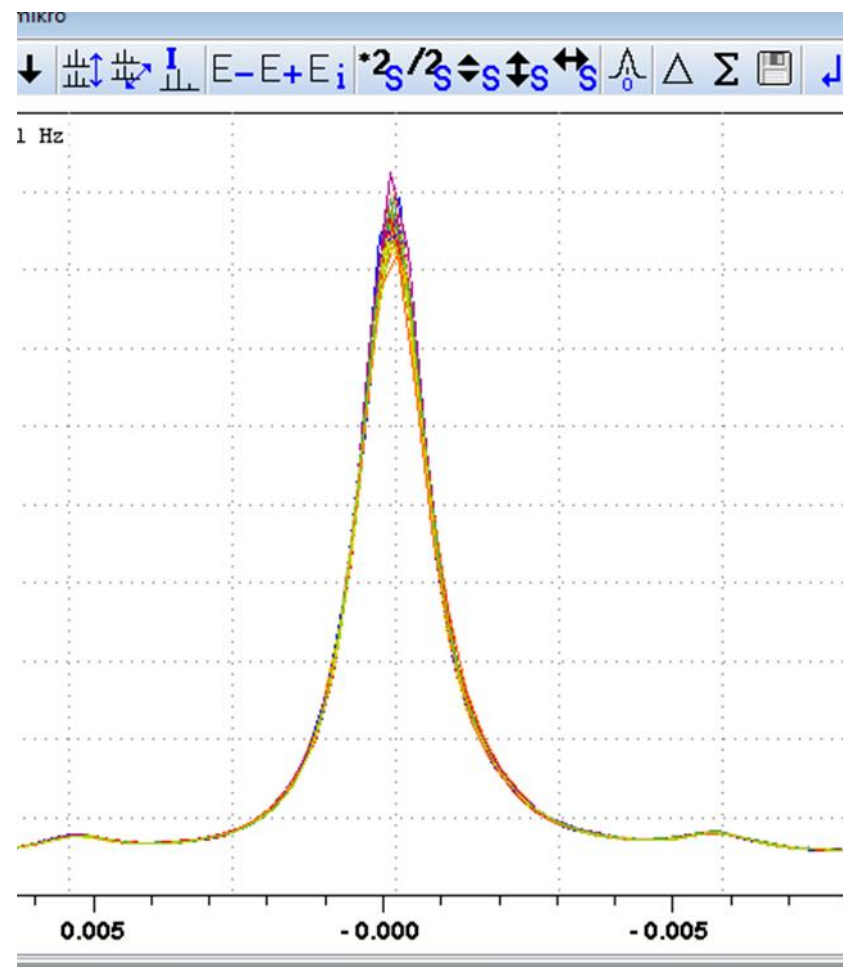
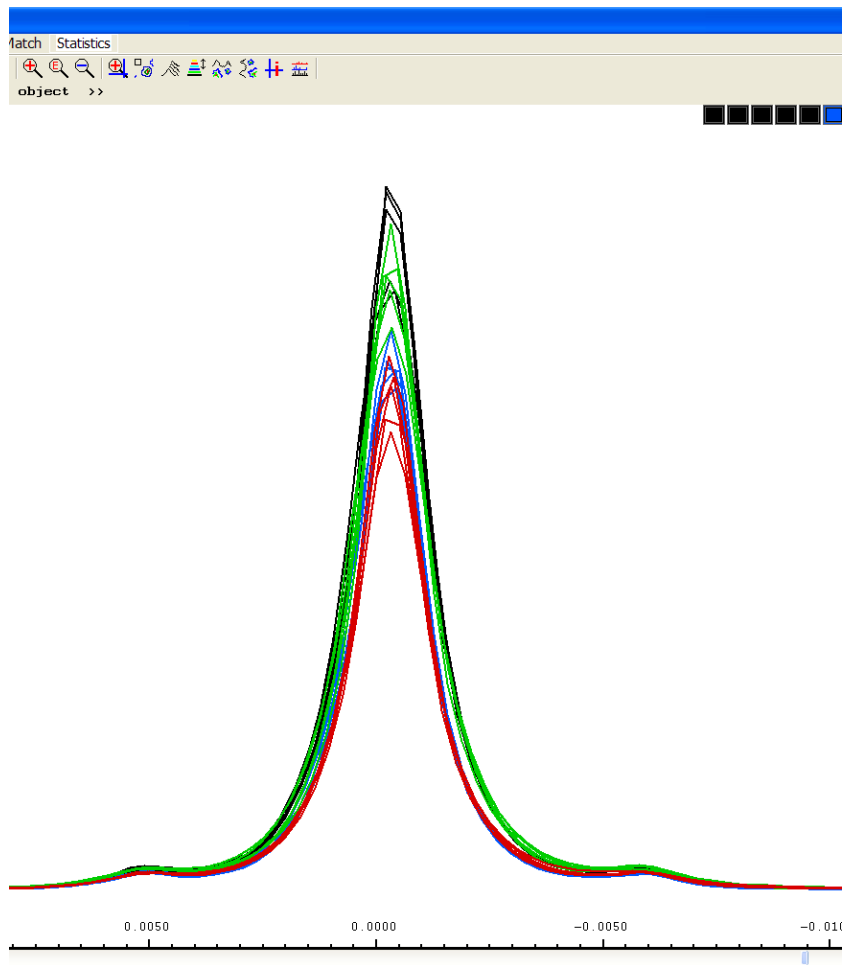
# NMR Reproducibility





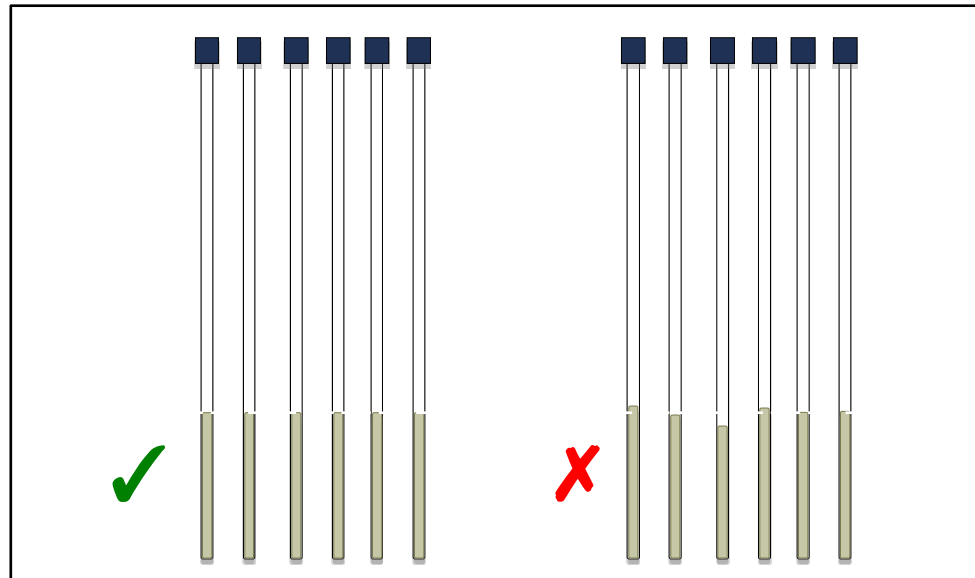
# NMR Reproducibility

✓ accepted sample preparation

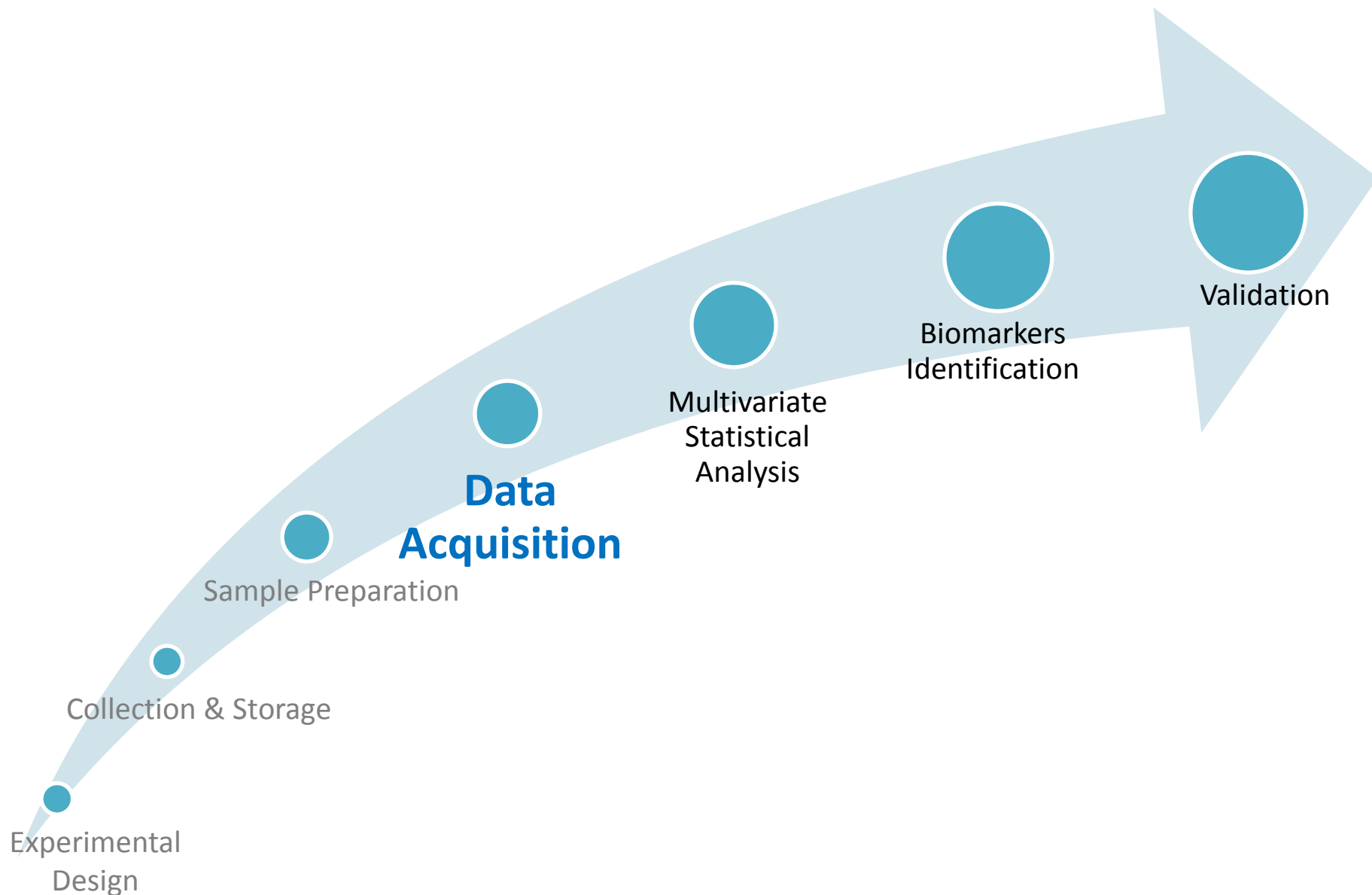


# Basic analytical issues - NMR

- ✓ Run tube and buffer blanks
- ✓ Use the same tube type for a project
- ✓ Take care = consistent sample preparation
- ✓ Sample presentation – give the spectrometer a chance !

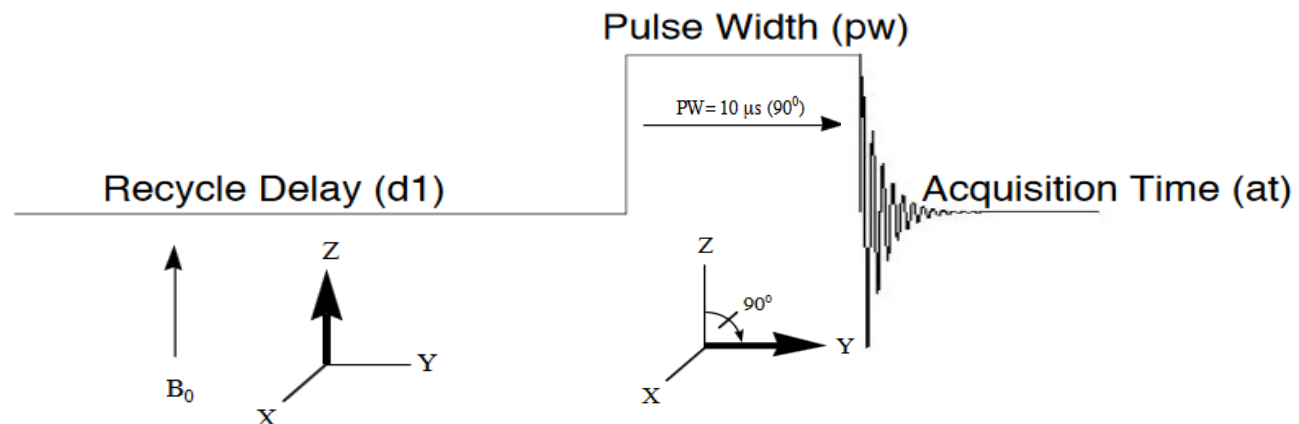


# Metabolomics Workflow



# Nuclear Magnetic Resonance

- Reproducible results on concentration critical / crucial parameters:



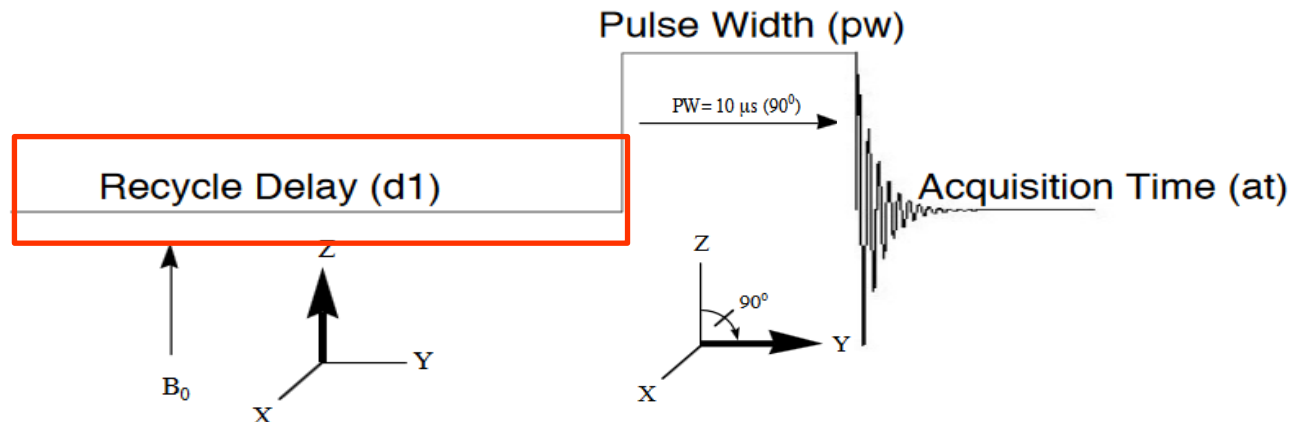
# Nuclear Magnetic Resonance

- Reproducible results on concentration critical / crucial parameters:

## Acquisition

- ✓ relaxation delay,

D[1] 4.00000000



# Nuclear Magnetic Resonance

- Reproducible results on concentration critical / crucial parameters:

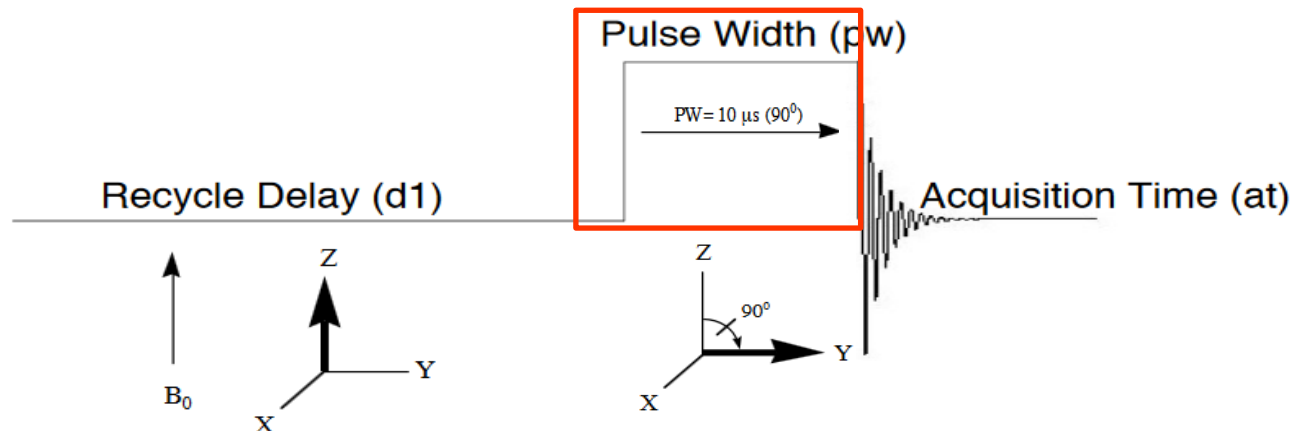
## Acquisition

✓ relaxation delay,

D[1] 4.00000000

✓ pulse width,

P[1] 11.63





# Nuclear Magnetic Resonance

- Reproducible results on concentration critical / crucial parameters:

## Acquisition

✓ relaxation delay,

D[1]

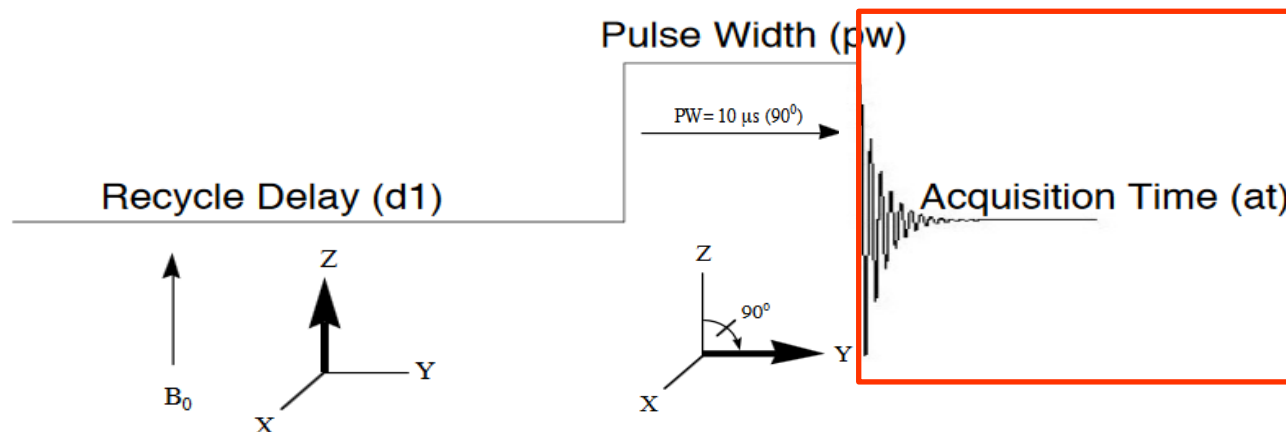
✓ pulse width,

P[1]

✓ acquisition time,

AQ [sec]

Acquisition time

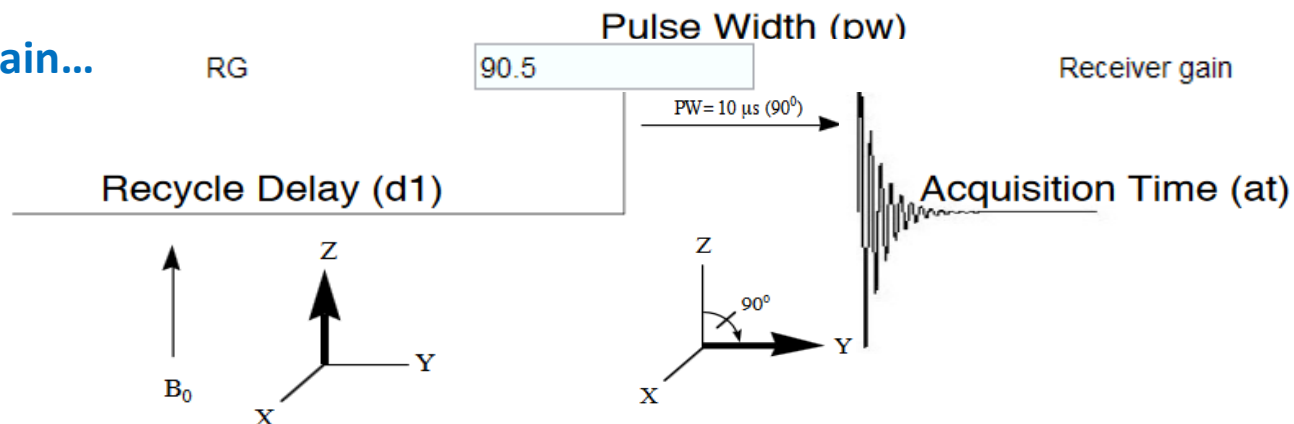


# Nuclear Magnetic Resonance

- Reproducible results on concentration critical / crucial parameters:

## Acquisition

- ✓ relaxation delay, D[1]
- ✓ pulse width, P[1]
- ✓ acquisition time, AQ [sec]  Acquisition time
- ✓ spectrum analysis, TD  Size of fid
- ✓ number of scans, NS  Number of scans
- ✓ receiver gain... RG  Receiver gain

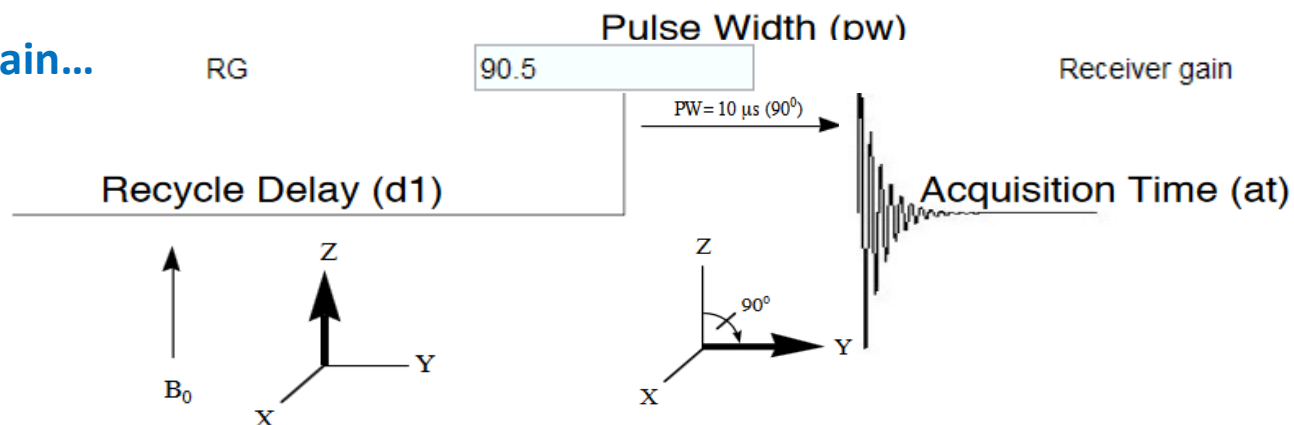


# Nuclear Magnetic Resonance

- Reproducible results on concentration critical / crucial parameters:

## Acquisition

- **RELAXATION DELAY,** D[1]
- ✓ **pulse width,** P[1]
- ✓ **acquisition time,** AQ [sec]  Acquisition time
- ✓ **spectrum analysis,** TD  Size of fid
- ✓ **number of scans,** NS  Number of scans
- ✓ **receiver gain...** RG  Receiver gain



# ✓ Acquisition Parameters

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Plot Fid

Experiment  
Width  
Receiver  
Nucleus  
Durations  
Power  
Program  
Probe  
Lists  
Wobble  
Lock  
Automation  
Miscellaneous  
User  
Routing

Experiment  
PULPROG noesygppr1d Current pulse program  
AQ\_mod DQD Acquisition mode  
TD 65536 Size of fid  
DS 4 Number of dummy scans  
NS 256 Number of scans  
TD0 1 Loop count for 'td0'

Width  
SW [ppm] 20.0283 Spectral width  
SWH [Hz] 12019.230 Spectral width  
AQ [sec] 2.7262976 Acquisition time  
FIDRES [Hz] 0.366798 Fid resolution  
FW [Hz] 625000.000 Filter width

Receiver  
RG 90.5 Receiver gain  
DW [μsec] 41.600 Dwell time  
DWOV [μsec] 0.025 Oversampling dwell time  
DECIM 1664 Decimation rate of digital filter  
DSPFIRM rectangle DSP firmware filter  
DIGTYP DRU Digitizer type  
DIGMOD baseopt Digitization mode  
DR 22 Digitizer resolution  
DDR 10 Digital digitizer resolution  
DE [μsec] 6.50 Pre-scan delay  
HPPRGN normal Preamplifier gain

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Plot Fid

Experiment  
Width  
Receiver  
Nucleus  
Durations  
Power  
Program  
Probe  
Lists  
Wobble  
Lock  
Automation  
Miscellaneous  
User  
Routing

INP [μsec] Edit... Pulse increment  
HDDUTY [%] 20.0 Homodecoupling duty cycle  
HORATE 20 Oversampling during Homode  
PCPD [μsec] Edit... CPD pulse length  
V9 [%] 5.00 Maximum variation of a delay

Power  
PLW [W] Edit... Power level in Watt  
PLdB Edit... Power level in -dBW  
PLSTRT [dB] -6 First step for PL switching  
PLSTEP 0.1 Step width for PL switching  
SHAPE Edit... Shaped pulse parameter  
GRADIENT Edit... Gradient parameters  
CAGPARS Edit... Parameters for gradient calcul  
AMP [%] Edit... Amplitude of pulse  
POWMOD low Power mode

Program parameters  
L Edit... Loop counter  
CNST Edit... Constant used in pulse progr  
CPDPRG Edit... Composite pulse decoupling  
PHCOR [degree] Edit... Correction angle for phase pr  
SUBNAM Edit... Name of subroutine  
ZGOPTNS -DFLAG\_BLK Acquisition (zg) options

Probe  
QNP 1 QNP position  
RO [Hz] 20 Rotation frequency of sample

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Plot Fid

Experiment  
Width  
Receiver  
Nucleus  
Durations  
Power  
Program  
Probe  
Lists  
Wobble  
Lock  
Automation  
Miscellaneous  
User  
Routing

WBSW [MHz] 8.0000000 Wobble sweep width  
WBST 1024 Number of wobble steps

Lock  
LOCNUC 2H Lock nucleus  
SOLVENT MeOD\_AG Sample solvent

Automation  
AUNM au\_prof1d Acquisition AU program  
PYNM acqu.py Acquisition PYTHON program  
EXP AGRO\_Fractions-1D Experiment performed

Miscellaneous  
GRDPROG Edit... Gradient program  
CHEMSTR none Molecule file for structure dis

User parameters  
USERA1 User acquisition par. 1  
USERA2 User acquisition par. 2  
USERA3 User acquisition par. 3  
USERA4 User acquisition par. 4  
USERA5 User acquisition par. 5

Routing  
RSEL Edit... Routing between FCU's and A  
RECCHAN Edit... Receiver channel  
PRECHAN Edit... Routing between Switchbox a  
RECSSEL Edit... Routing between SGU and re  
SELREC Edit... Routing between receiver and

# Acquisition Parameters

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Plot Fid

Experiment Width Receiver Nucleus Durations Power Program Probe Lists Wobble Lock Automation Miscellaneous User Routing

Experiment

PULPROG noesygppr1d Current pulse program

AQ\_mod DQD Acquisition mode

TD 65536 Size of fid

DS 4 Number of dummy scans

NS 256 Number of scans

TD0 1 Loop count for 'td0'

Width

SW [ppm] 20.0283 Spectral width

SWH [Hz] 12019.230 Spectral width

AQ [sec] 2.7262976 Acquisition time

FIDRES [Hz] 0.366798 Fid resolution

FW [Hz] 625000.000 Filter width

Receiver

RG 90.5 Receiver gain

DW [μsec] 41.600 Dwell time

DWOV [μsec] 0.025 Oversampling dwell time

DECIM 1664 Decimation rate of digital filter

DSPFIRM rectangle DSP firmware filter

DIGTYP DRU Digitizer type

DIGMOD baseopt Digitization mode

DR 22 Digitizer resolution

DDR 10 Digital digitizer resolution

DE [μsec] 6.50 Pre-scan delay

HPPRGN normal Preamplifier gain

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Plot Fid

Experiment Width Receiver Nucleus Durations Power Program Probe Lists Wobble Lock Automation Miscellaneous User Routing

INP [μsec] Edit... Pulse increment

HDDUTY [%] 20.0 Homodecoupling duty cycle

HRATE 20 Oversampling during Homode

PCPD [μsec] Edit... CPD pulse length

V9 [%] 5.00 Maximum variation of a delay

Power

PLW [W] Edit... Power level in Watt

PLdB Edit... Power level in -dBW

PLSTRT [dB] -6 First step for PL switching

PLSTEP 0.1 Step width for PL switching

SHAPE Edit... Shaped pulse parameter

GRADIENT Edit... Gradient parameters

CAGPARS Edit... Parameters for gradient calc.

AMP [%] Edit... Amplitude of pulse

POWMOD low Power mode

Program parameters

L Edit... Loop counter

CNST Edit... Constant used in pulse prog.

CPDPRG Edit... Composite pulse decoupling i

PHCOR [degree] Edit... Correction angle for phase pr

SUBNAM Edit... Name of subroutine

ZGOPTNS -DFLAG\_BLK Acquisition (zg) options

Probe

QNP 1 QNP position

RO [Hz] 20 Rotation frequency of sample

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Plot Fid

Experiment Width Receiver Nucleus Durations Power Program Probe Lists Wobble Lock Automation Miscellaneous User Routing

WBSW [MHz] 8.0000000 Wobble sweep width

WBST 1024 Number of wobble steps

Lock

LOCNUC 2H Lock nucleus

SOLVENT MeOD\_AG Sample solvent

Automation

AUNM au\_prof1d Acquisition AU program

PYNM acqu.py Acquisition PYTHON program

EXP AGRO\_Fractions-1D Experiment performed

Miscellaneous

GRDPROG Edit... Gradient program

CHEMSTR none Molecule file for structure dis

User parameters

USERA1 User acquisition par. 1

USERA2 User acquisition par. 2

USERA3 User acquisition par. 3

USERA4 User acquisition par. 4

USERA5 User acquisition par. 5

Routing

RSEL Edit... Routing between FCUs and A

RECCHAN Edit... Receiver channel

PRECHAN Edit... Routing between Switchbox a

RESEL Edit... Routing between SGU and re

SELREC Edit... Routing between receiver and

# Processing Parameters

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Plot Fid

Reference Window Phase Baseline Fourier Integration Peak Automation Miscellaneous User

Reference

SI 65536 Size of real spectrum

SF [MHz] 600.1100147 Spectrometer frequency

OFFSET [ppm] 14.85300 Low field limit of spectrum

SR [Hz] 14.68 Spectrum reference frequency

H2pPT [Hz] 0.183399 Spectral resolution

SPECTYPT UNDEFINED Type of spectrum e.g. COSY, HMQC, ...

Window function

WDW EM Window functions for trf, xtb, ...

LB [Hz] 0.30 Line broadening for em

GB 0 Gaussian max. position for gm, 0<GB<1

SSB 0 Sine bell shift SSB (0,1,2,...)

TM1 0 Left limit for tm 0<TM1<1

TM2 0 Right limit for tm 0<TM2<1

Phase correction

PHC0 [degrees] -44.138 0th order correction for pk

PHC1 [degrees] 0 1st order correction for pk

PH\_mod pk Phasing modes for trf, xtb, ...

Baseline correction

ABSG 3 Degree of polynomial for abs (0..5)

ABSF1 [ppm] 10.00000 Left limit for abst

ABSF2 [ppm] 0 Right limit for abst, abs1, abs2

BCFW [ppm] 1.00000 Filter width for bc (stlqfll)

COROFFS [Hz] 0 Correction offset for BC\_MOD=spol etc.

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Plot Fid

Reference Window Phase Baseline Fourier Integration Peak Automation Miscellaneous User

Fourier transform

TDef 0 Number of fid data points used by ft

STSR 0 First output point of strip transform

STSI 0 Total number of output points of strip transform

ME\_mod no Linear prediction for ft, xtb, ...

NCOEFF 0 Number of LP coefficients

LPBIN 0 Number of output points for LP

TDef 0 Number of back-predicted points

REVERSE FALSE Reverse spectrum during transform

FCOR 0.5 Weighting factor for first fid point

PKNL TRUE Group delay compensation

FT\_mod no Fourier transform mode for trf, xtrf

Mdd\_mod mdd MDD mode

MddCEXP FALSE RMD/MDD flag

MddCT\_SP FALSE Constant time

MddF180 FALSE Delayed sampling flag

MddNCOMP 0 Number of components

MddPHASE 0 Phase

MddRSIZE [ppm] 0 Sub region size

Integration

ABSL 5 Integral sensitivity factor for abs

AZFW [ppm] 0.10000 Min. separation between integral regions

AZFE [ppm] 0.10000 Integral region extension factor

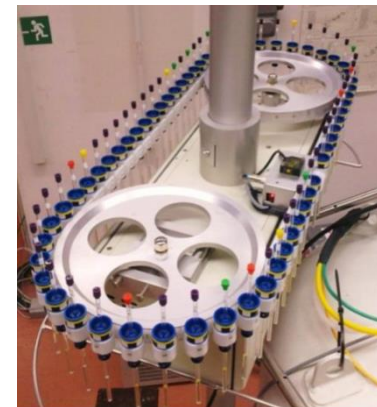
ISEN 128 Integral sens. factor w. reference to largest in

INTSCL 1 Integral scale rel. to a reference dataset

INTBC yes Auto-baseline correction of integral by abs

# NMR Standard Operation Procedure

*makes science easier*



- Water suppression test: 2m M Sucrose with 0.5mM DSS, 2 mM  $\text{NaN}_3$  in 10%  $\text{D}_2\text{O}$  and 90%  $\text{H}_2\text{O}$
- Temperature long term stability
- Temperature calibration:  $\text{CD}_3\text{OD}$  99.8%
- $^1\text{H}$  resolution test: 1%  $\text{CHCl}_3$  in Acetone- $d_6$
- Sensitivity test: 0.1% Ethylbenzene in  $\text{CDCl}_3$
- Field drift ( $\text{CDCl}_3$ )



# NMR Standard Operation Procedure

*makes science easier*

➤ Sensitivity test: 0.1% Ethylbenzene in CDCl<sub>3</sub>

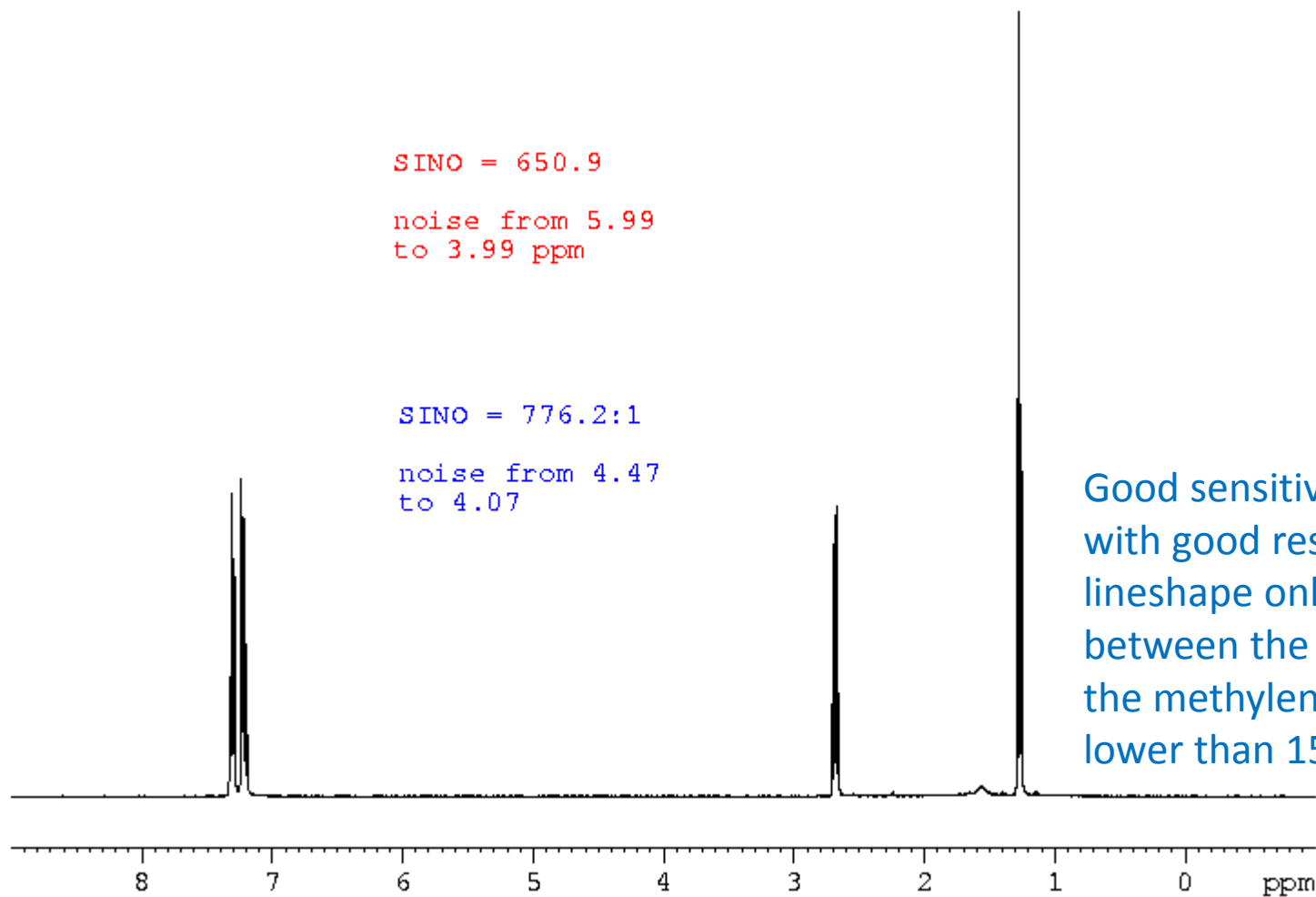
SINO = 650.9

noise from 5.99  
to 3.99 ppm

SINO = 776.2:1

noise from 4.47  
to 4.07

Good sensitivity can be obtained with good resolution and good lineshape only. The splitting between the two central lines of the methylene quartet should go lower than 15% (using a lb of 1 Hz)

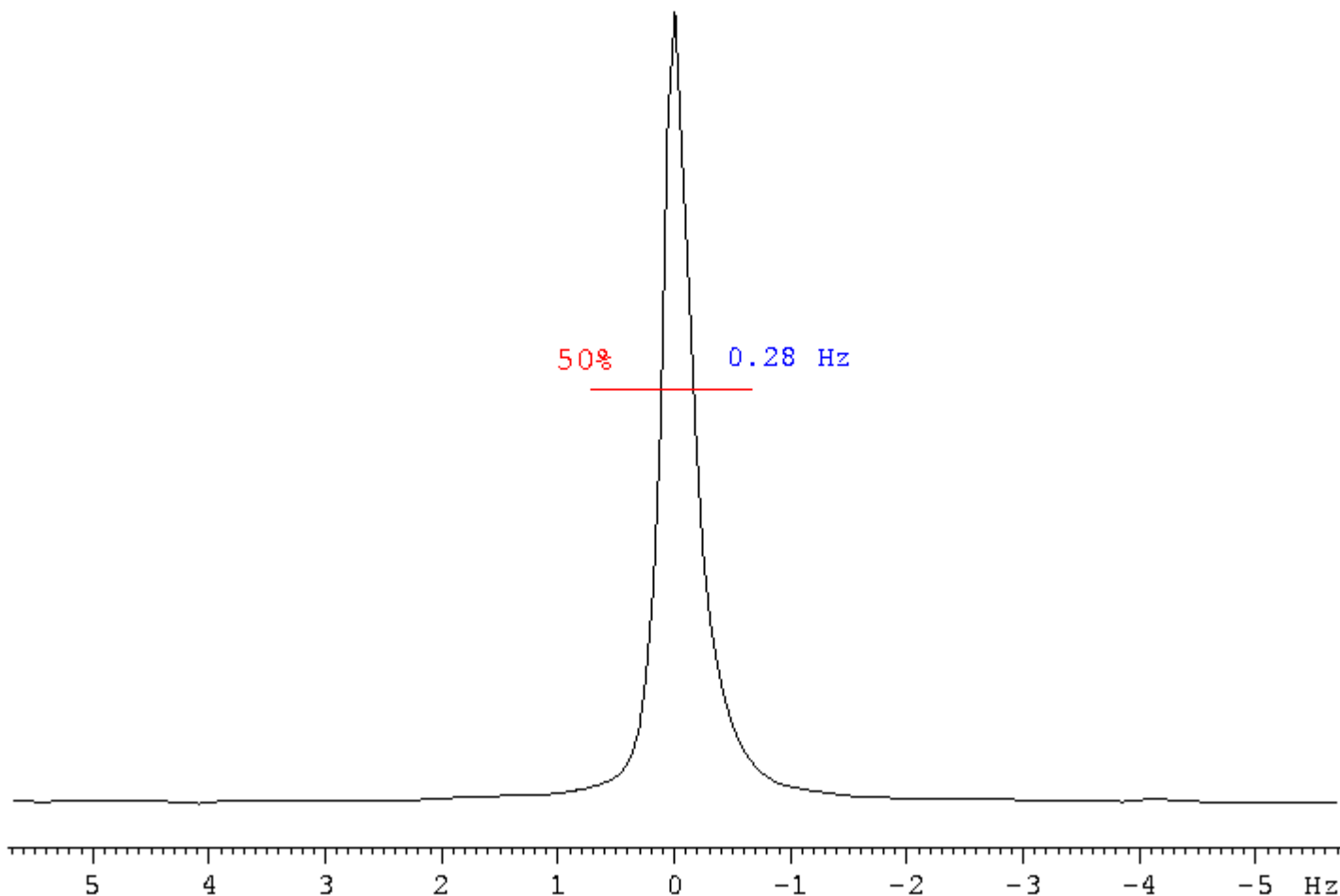


Automated S/N calculation is performed using [sinocal](#)

# NMR Standard Operation Procedure

*makes science easier*

➤  $^1\text{H}$  resolution test: 1%  $\text{CDCl}_3$  in Acetone- $d_6$

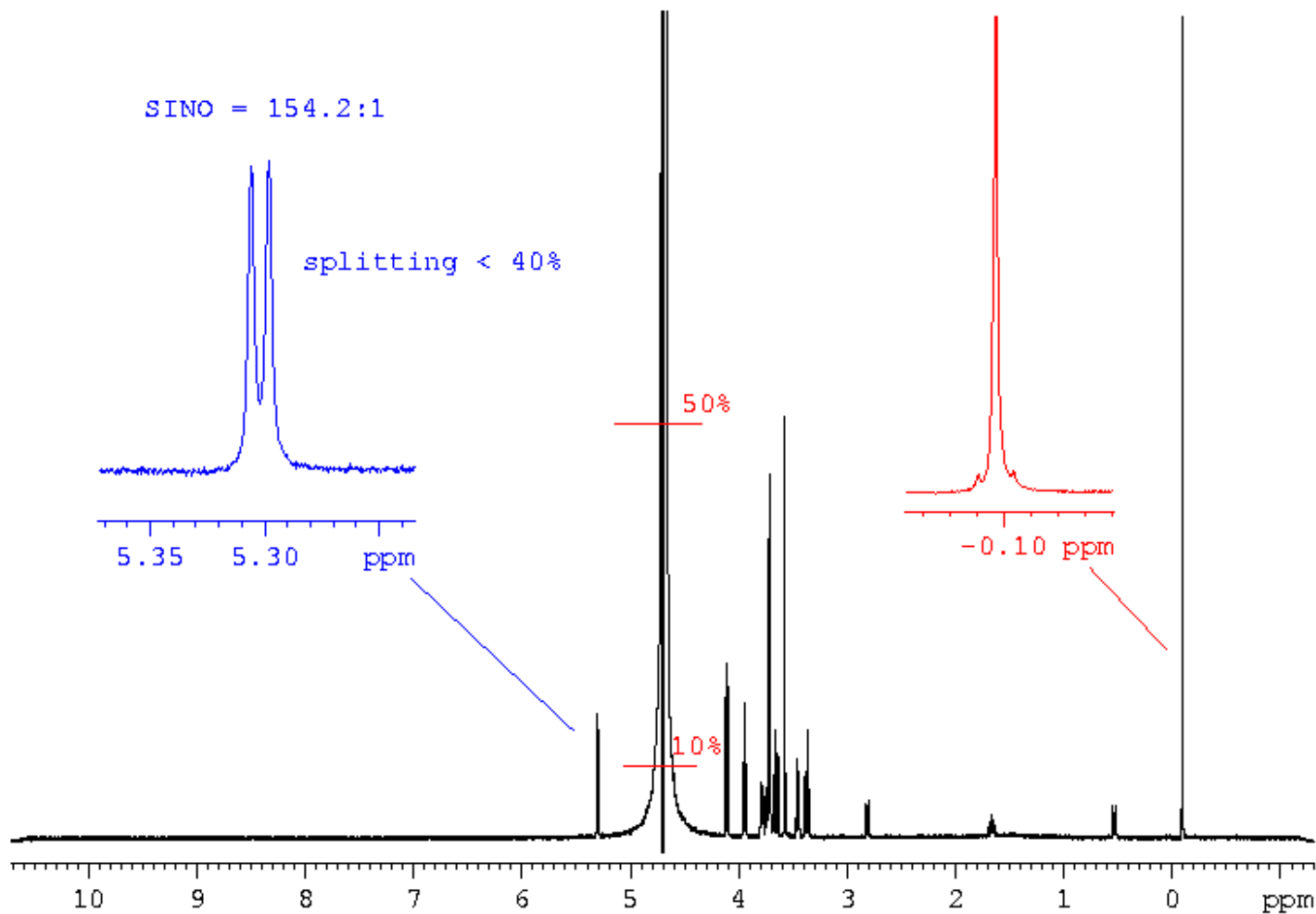


The AU [hwcal](#) program determines the resolution at the half height of the chloroform line.

# NMR Standard Operation Procedure

*makes science easier*

- 2 mM Sucrose with 0.5mM DSS, 2mM NaN<sub>3</sub> in 10% D<sub>2</sub>O and 90% H<sub>2</sub>O

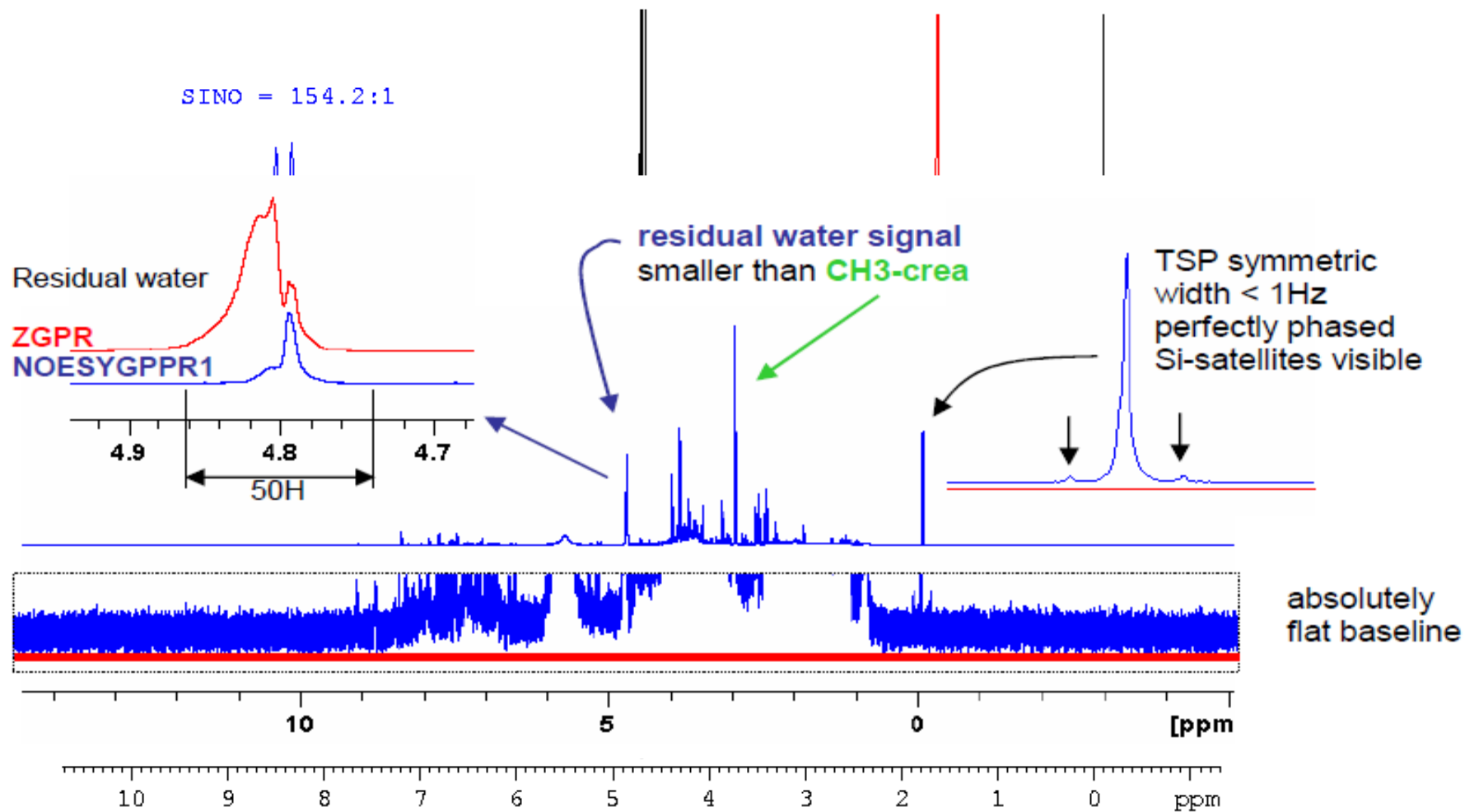


Water line width at 50% and 10% of DSS, S/N, and resolution calculations by typing [suppcal](#)

# NMR Standard Operation Procedure

*makes science easier*

- 2 mM Sucrose with 0.5mM DSS, 2mM NaN<sub>3</sub> in 10% D<sub>2</sub>O and 90% H<sub>2</sub>O



Water line width at 50% and 10% of DSS, S/N, and resolution calculations by typing [suppcal](#)

# NMR Standard Operation Procedure

## ✓ Temperature Calibration

edte window

The screenshot displays the Edte software interface with the following parameters and controls:

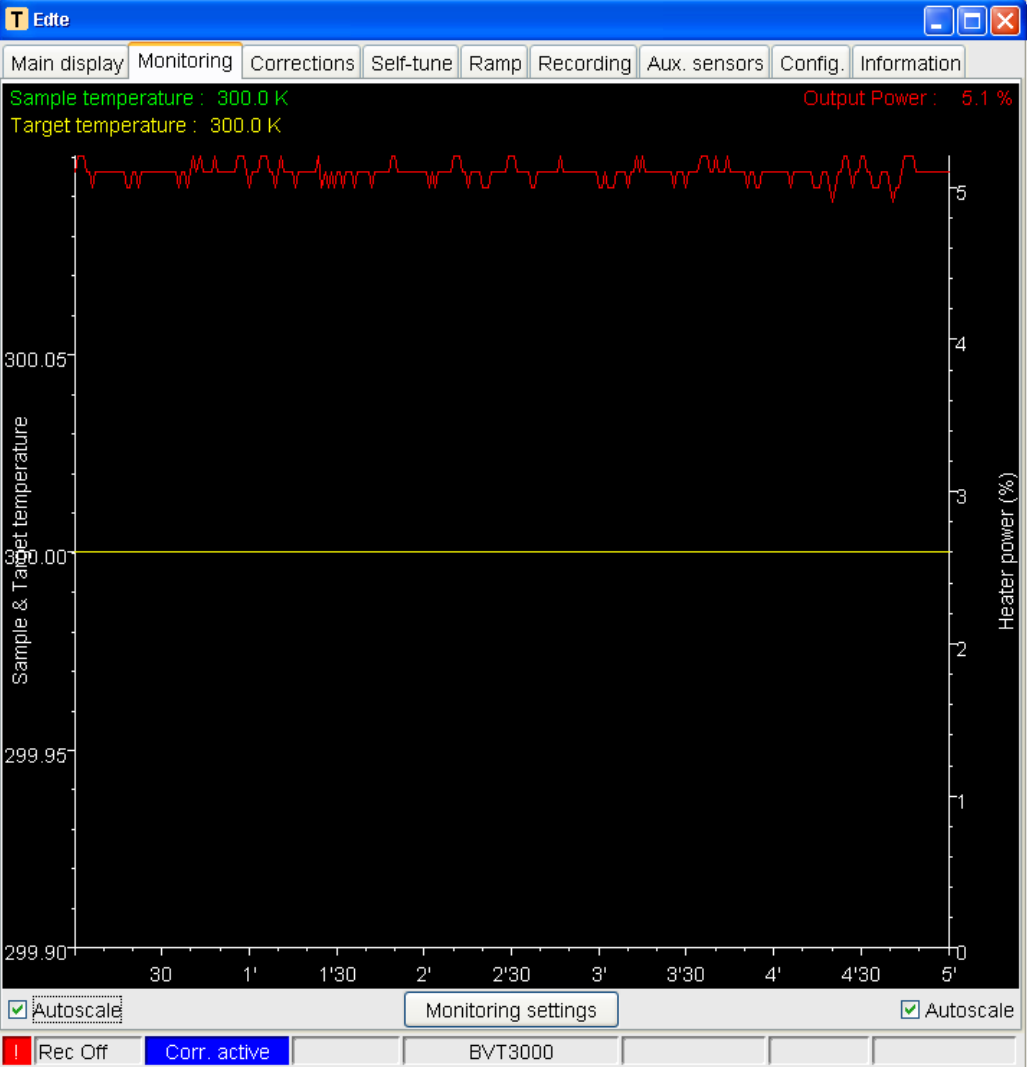
- Sample temperature:** 300.0 K
- Target temp.:** 300.0 K (with a "Change..." button)
- Probe Heater:** On (with a "Set max..." button)
- Gas flow:** 535 l/h (highlighted with a red box, with "-" and "+" buttons)
- Cooling:** Off (with a "Change..." button)

At the bottom of the interface, the status bar shows:

- Rec Off
- Corr. active
- BVT3000
- VTU [Kelvin] 300.0 (highlighted with a red box)
- Spooler (queued: 0, delayed: 0, error: 0)
- BSMS status message:  $\Delta$  XY 16, Autoshim x, Locked  $\checkmark$ , Error



- Browser Last50 Groups Alias
- 8 - zg30
  - 9 - zg30
  - 10 - zg30
  - 11 - zg30
  - 12 - zg30
  - 13 - zg30
  - 14 - zg30
  - 15 - zg30
  - 16 - zg30
  - 17 - zg30
  - 18 - zg30
  - 19 - zg30
  - 20 - zg30
  - 21 - zg30
  - 22 - zg30
  - 1 - 4Jan2016, 300.07
  - 23 - zg30
  - 1 - 4Jan2016, 300.07
  - 25 - zg30
  - 99999 - pulsecal
  - ESS29
  - ESS30-40
  - ESS32
  - ESS34-35
  - ESS46
  - Georgia\_1
  - Hyper\_KEA\_77
  - ICSN74\_44\_L\_D05
  - KHOScheck
  - KHOScheck2D
  - KSt-SolventCheck
  - Ih-NKUA026
  - Ih-NKUA083
  - Ih-test\_20120808
  - MeODTMScheck
  - 1 - noesygppr1d



Lock Display

BS status 16

Autoshim X Locked Error

Acquisition information	Fid Flash	Lock	Sample	VTU	Spooler	BSMS status message
no acquisition running		59%		[Kelvin] 300.0	queued: 0 delayed: 0 cron: 0	<b>Δ XY 16</b> Autoshim X Locked Error

# NMR Standard Operation Procedure

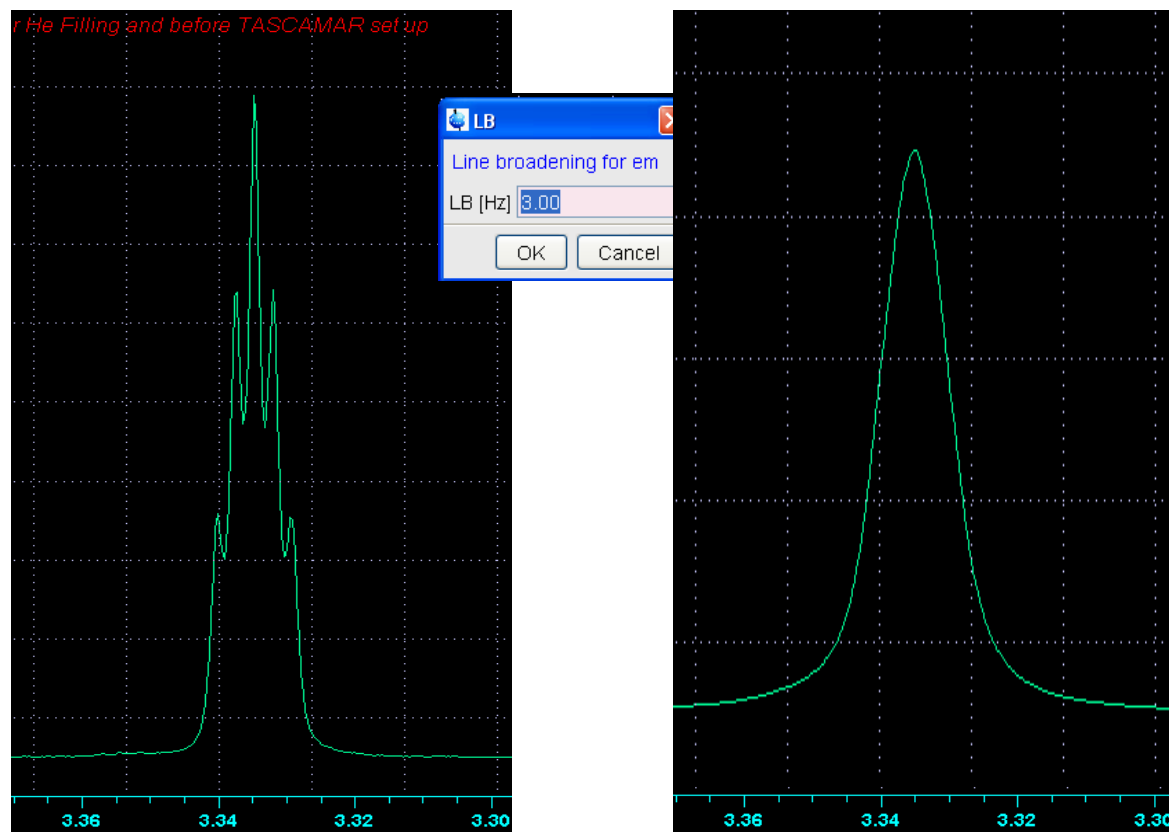
## ✓ Temperature Calibration

Temperature : 300 K  
Biological Samples

310 K  
Plasma

CD<sub>3</sub>OD, 99.8% (Bruker std)

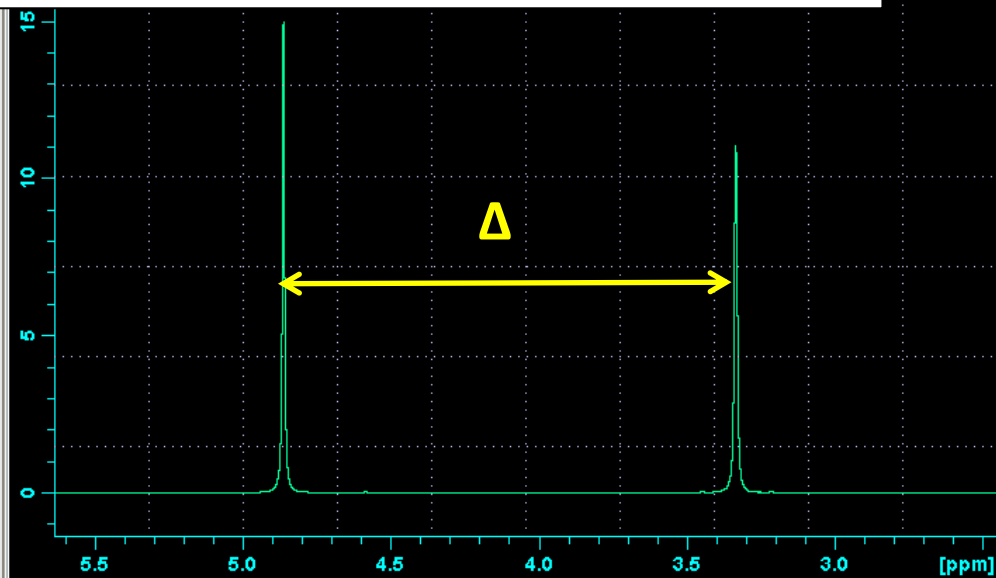
<sup>1</sup>H 1D; ns 1; ds 0; zg30



Source: Bruker Instruments, Inc. VT-Calibration Manual

$$100\% \text{ Methanol: } T = -23.832\Delta^2 - 29.46\Delta + 403.0$$

( $\Delta$  is the shift difference (ppm) between  $\text{CH}_3$  & OH peaks)



ct: finished

Acquisition information no acquisition running	Fid Flash	Lock 60%	Sample	VTU [Kelvin] 300.0	Spooler queued: 0 delayed: 0 cron: 0	BSMS status message <b>Δ XY 16</b> Autoshim x Locked ✓ Error
---------------------------------------------------	-----------	-------------	--------	--------------------------	-----------------------------------------------	--------------------------------------------------------------------

Lock Display

The Lock Display window shows a grid with a horizontal line across it. On the right side, there are several status indicators: a green bar with '3.28', a red bar with '16', and a blue bar with 'NUM'. Below these are some partially visible labels like 'S statu' and 'n x Lc'.

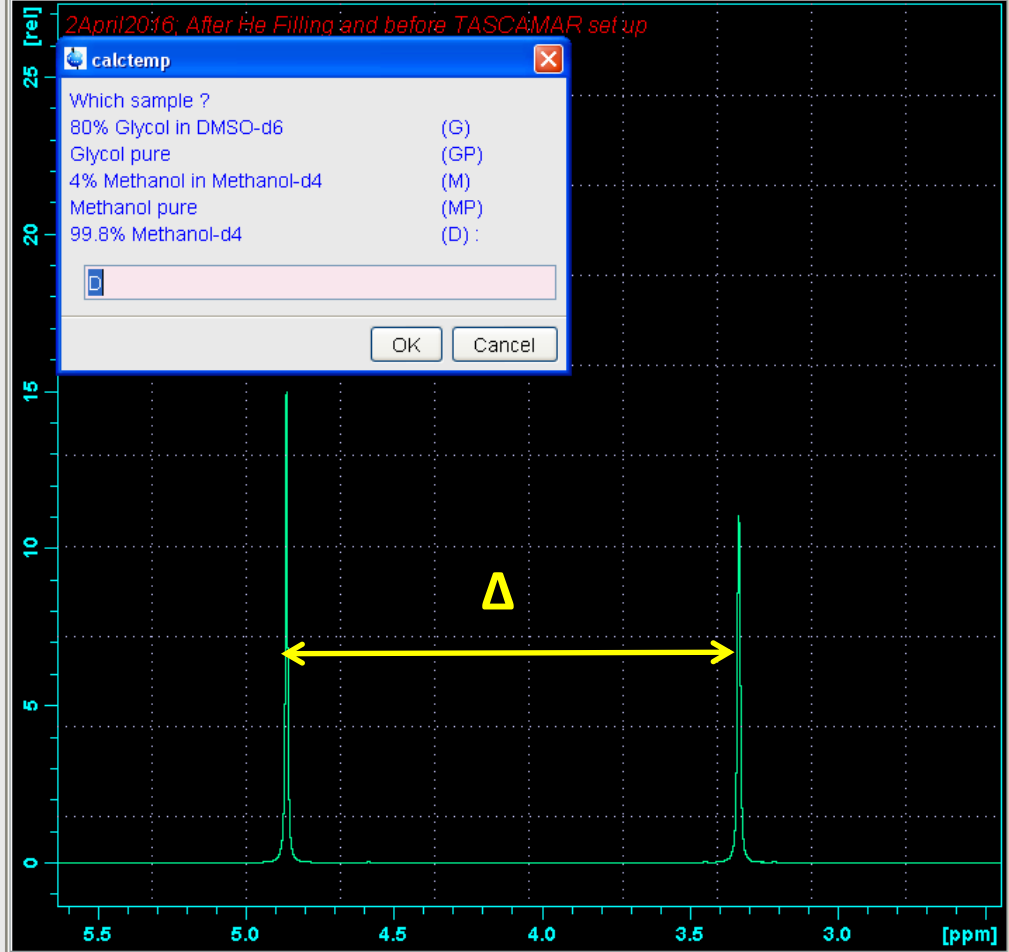




- Browse Last50 Groups Alias
- 8 - zg30
- 9 - zg30
- 10 - zg30
- 11 - zg30
- 12 - zg30
- 13 - zg30
- 14 - zg30
- 15 - zg30
- 16 - zg30
- 17 - zg30
- 18 - zg30
- 19 - zg30
- 20 - zg30
- 21 - zg30
- 22 - zg30
- 1 - 4Jan2016; 300.073
- 23 - zg30
- 1 - 4Jan2016; 300.073
- 25 - zg30
- 99999 - pulsecal
- ESS29
- ESS30-40
- ESS32
- ESS34-35
- ESS46
- Georgia\_1
- Hyper\_KEA\_77
- ICSN74\_44\_L\_D05
- KHOScheck
- KHOScheck2D
- KSt-SolventCheck
- lh-NKUA026
- lh-NKUA083
- lhstest\_20120808
- MeODTMScheck
- 1 - noesygpr1d

dbStdTests 21 1 C:\Bruker\TOPSPIN2\_pl6 AGROCOS

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Fid Acqu



3.28

status

16

Autoshim x Locked Error

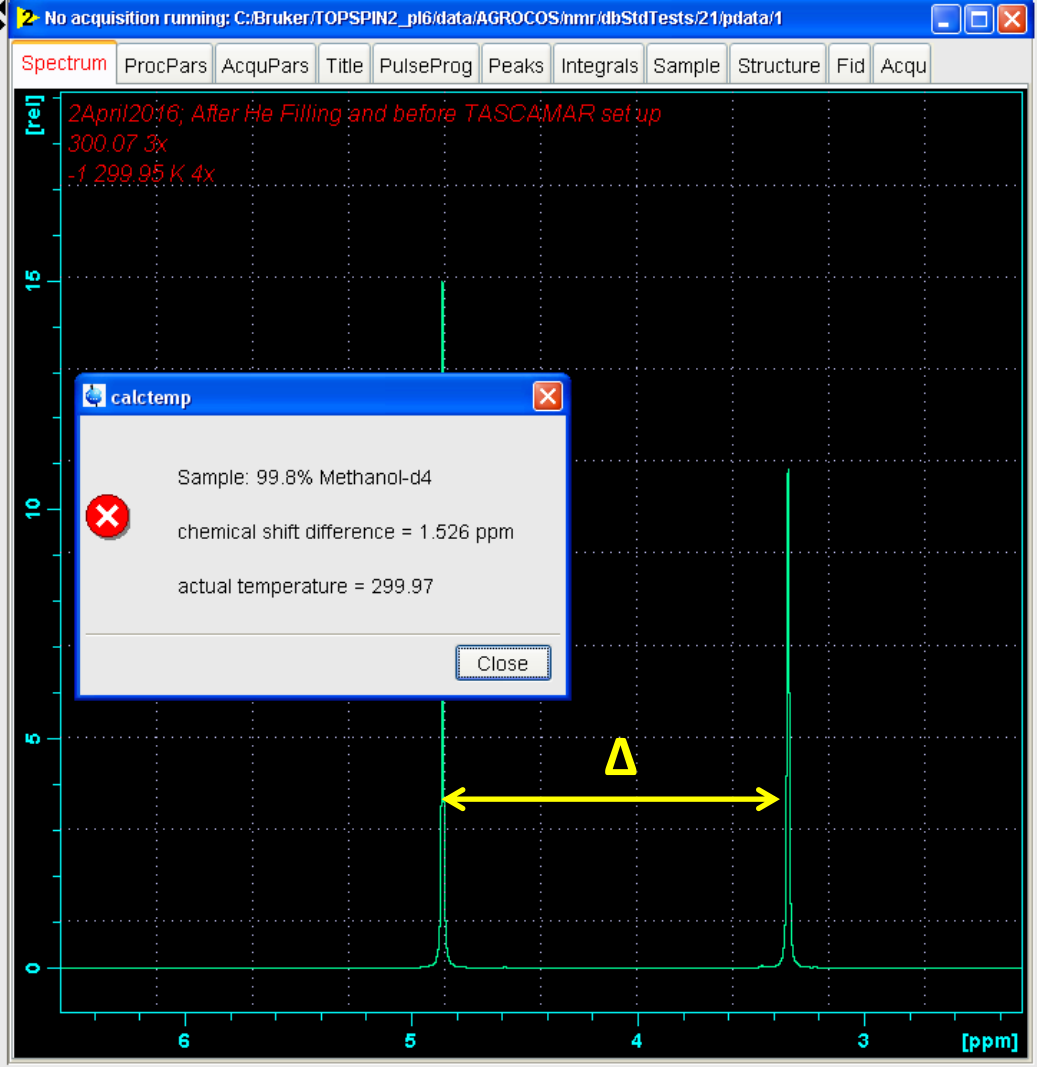
NUM

ct: finished

Acquisition information	Fid Flash	Lock	Sample	VTU	Spooler	BSMS status message
no acquisition running		60%		[Kelvin] 300.0	queued: 0 delayed: 0 cron: 0	<b>Δ XY 16</b> Autoshim x Locked Error



- Brower Last50 Groups Alias
- 8 - zg30
- 9 - zg30
- 10 - zg30
- 11 - zg30
- 12 - zg30
- 13 - zg30
- 14 - zg30
- 15 - zg30
- 16 - zg30
- 17 - zg30
- 18 - zg30
- 19 - zg30
- 20 - zg30
- 21 - zg30
- 22 - zg30
- 1 - 4Jan2016; 300.07
- 23 - zg30
- 1 - 4Jan2016; 300.07
- 25 - zg30
- 99999 - pulsecal
- ESS29
- ESS30-40
- ESS32
- ESS34-35
- ESS46
- Georgia\_1
- Hyper\_KEA\_77
- ICSN74\_44\_L\_D05
- KHOScheck
- KHOScheck2D
- KSt-SolventCheck
- lh-NKUA026
- lh-NKUA083
- lhtest\_20120808
- MeODTMScheck
- 1 - noesygppr1d



Lock Display

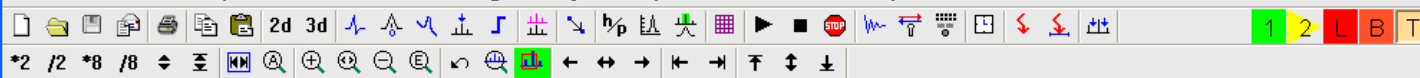
3 statu

16

NUM

pp: finished

Acquisition information	Fid Flash	Lock	Sample	VTU	Spooler	BSMS status message
no acquisition running		60%		[Kelvin] 300.0	queued: 0 delayed: 0 cron: 0	<b>Δ XY 16</b> Autoshim x Locked ✓ Error



- Browser
- Last50
- Groups
- Alias
- 8 - zg30
- 9 - zg30
- 10 - zg30
- 11 - zg30
- 12 - zg30
- 13 - zg30
- 14 - zg30
- 15 - zg30
- 16 - zg30
- 17 - zg30
- 18 - zg30
- 19 - zg30
- 20 - zg30
- 21 - zg30
- 22 - zg30
- 1 - 4Jan2016; 300.07
- 23 - zg30
- 1 - 4Jan2016; 300.07
- 25 - zg30
- 99999 - pulsecal
- ESS29
- ESS30-40
- ESS32
- ESS34-35
- ESS46
- Georgia\_1
- Hyper\_KEA\_77
- ICSN74\_44\_L\_D05
- KHOScheck
- KHOScheck2D
- KSt-SolventCheck
- lh-NKUA026
- lh-NKUA083
- lhtest\_20120808
- MeODTMScheck
- 1 - noesygppr1d

T Edte

Main display | Monitoring | **Corrections** | Self-tune | Ramp | Recording | Aux. sensors | Config. | Information

Current probehead : 5 mm PABBI 1H/D-BB Z-GRD Z814601/0044

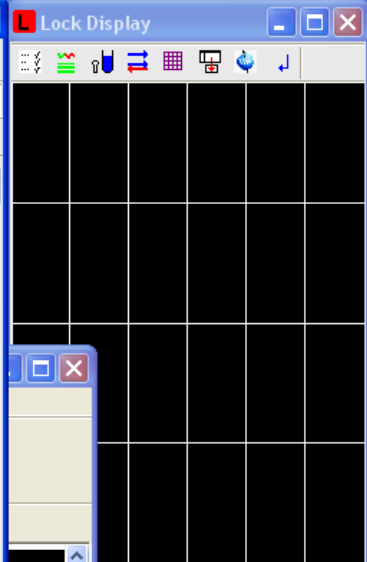
No correction Edit... Activate

No correction will be applied to the temperature entered by the user

Linear correction [active] Edit... Activate

Slope : 1.0  
Offset : -0.8

Rec Off **Corr. active** BVT3000



T Edte

Main display | Monitoring | **Corrections** | Self-tune | Ramp | Recording | Aux. sensors | Config. | Information

Sample temperature

Sample temp. 300.0 K

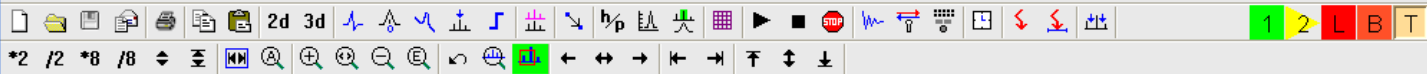
Target temp. 300.0 K Change...

Probe Heater  On 5.0 % Set max...

Gas flow 535 l/h - +

Cooling  Off Change...

Rec Off **Corr. active** BVT3000



- Browser Last50 Groups Alias
- 8 - zg30
- 9 - zg30
- 10 - zg30
- 11 - zg30
- 12 - zg30
- 13 - zg30
- 14 - zg30
- 15 - zg30
- 16 - zg30
- 17 - zg30
- 18 - zg30
- 19 - zg30
- 20 - zg30
- 21 - zg30
- 22 - zg30
- 1 - 4Jan2016; 300.07
- 23 - zg30
- 1 - 4Jan2016; 300.07
- 25 - zg30
- 99999 - pulsecal
- ESS29
- ESS30-40
- ESS32
- ESS34-35
- ESS46
- Georgia\_1
- Hyper\_KEA\_77
- ICSN74\_44\_L\_D05
- KHOScheck
- KHOScheck2D
- KSt-SolventCheck
- lh-NKUA026
- lh-NKUA083
- lhtest\_20120808
- MeODTMScheck
- 1 - noesygppr1d

T Edte

Main display | Monitoring | **Corrections** | Self-tune | Ramp | Recording | Aux. sensors | Config. | Information

Current probehead : 5 mm PABBI 1H/D-BB Z-GRD Z814601/0044

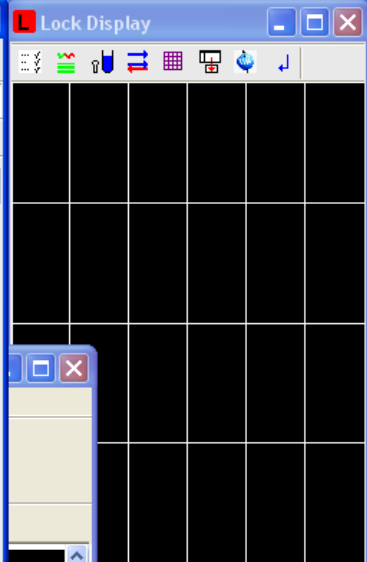
No correction Edit... Activate

No correction will be applied to the temperature entered by the user

Linear correction [active] Edit... Activate

Slope : 1.0  
Offset : -0.8

Rec Off Corr. active BVT3000



T Edte

Main display | Monitoring | **Corrections** | Self-tune | Ramp | Recording | Aux. sensors | Config. | Information

Sample temperature

Sample temp. 300.0 K

Target temp. 300.0 K Change...

Probe Heater  On 5.0 % Set max...

Gas flow 535 l/h - +

Cooling  Off Change...

Rec Off Corr. active BVT3000

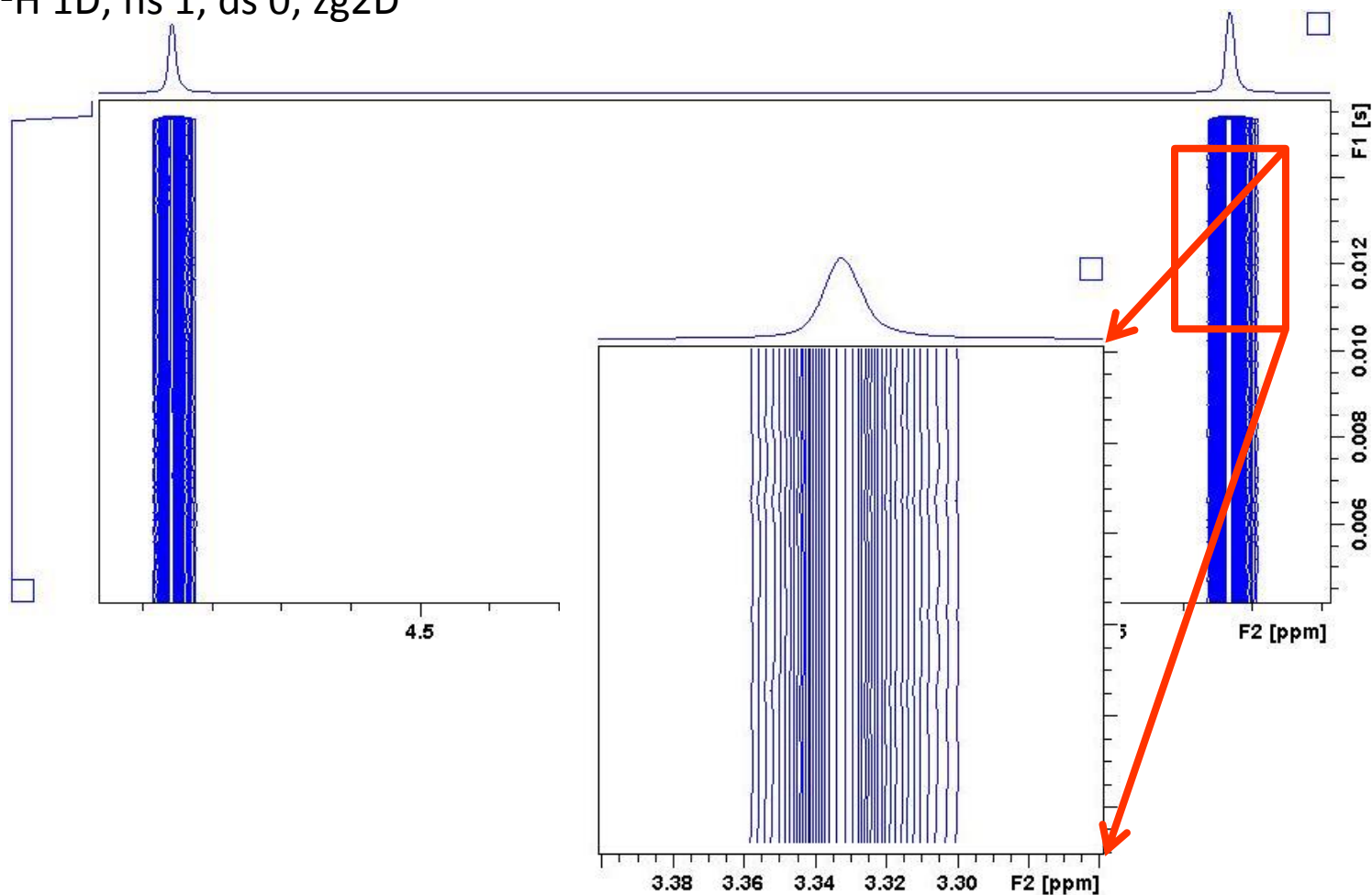
WEEKLY

# Instrument Performance

## ✓ Long Term Stability Test

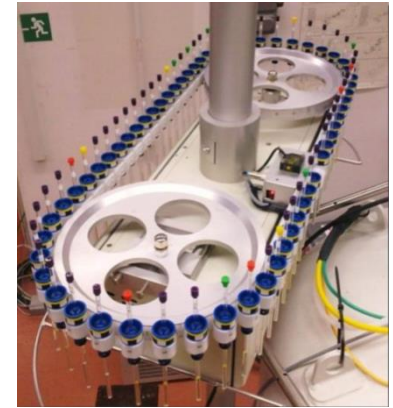
CD<sub>3</sub>OD, 99.8% (Bruker std)

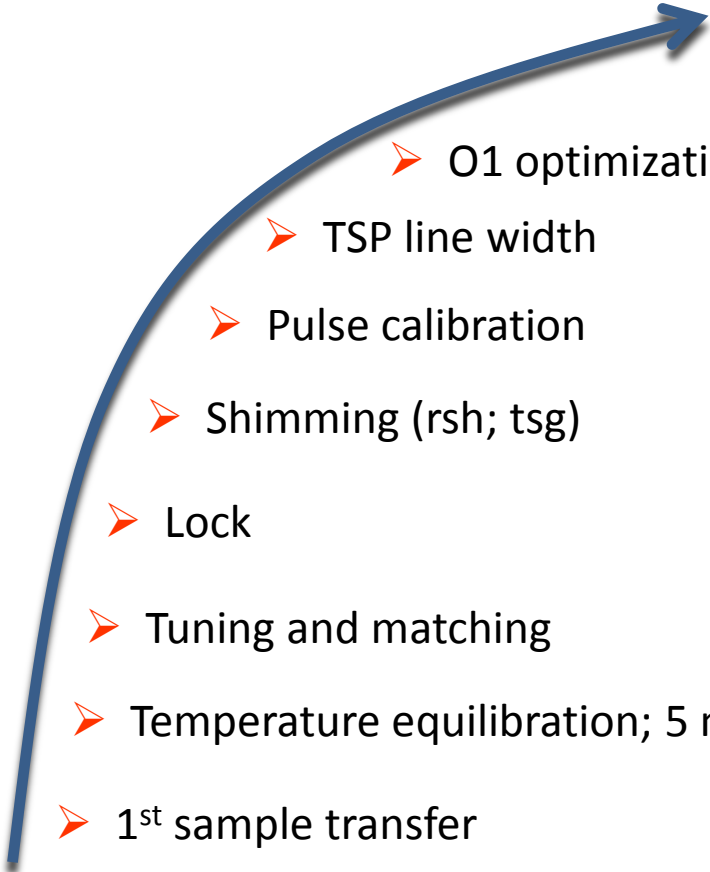
<sup>1</sup>H 1D; ns 1; ds 0; zg2D

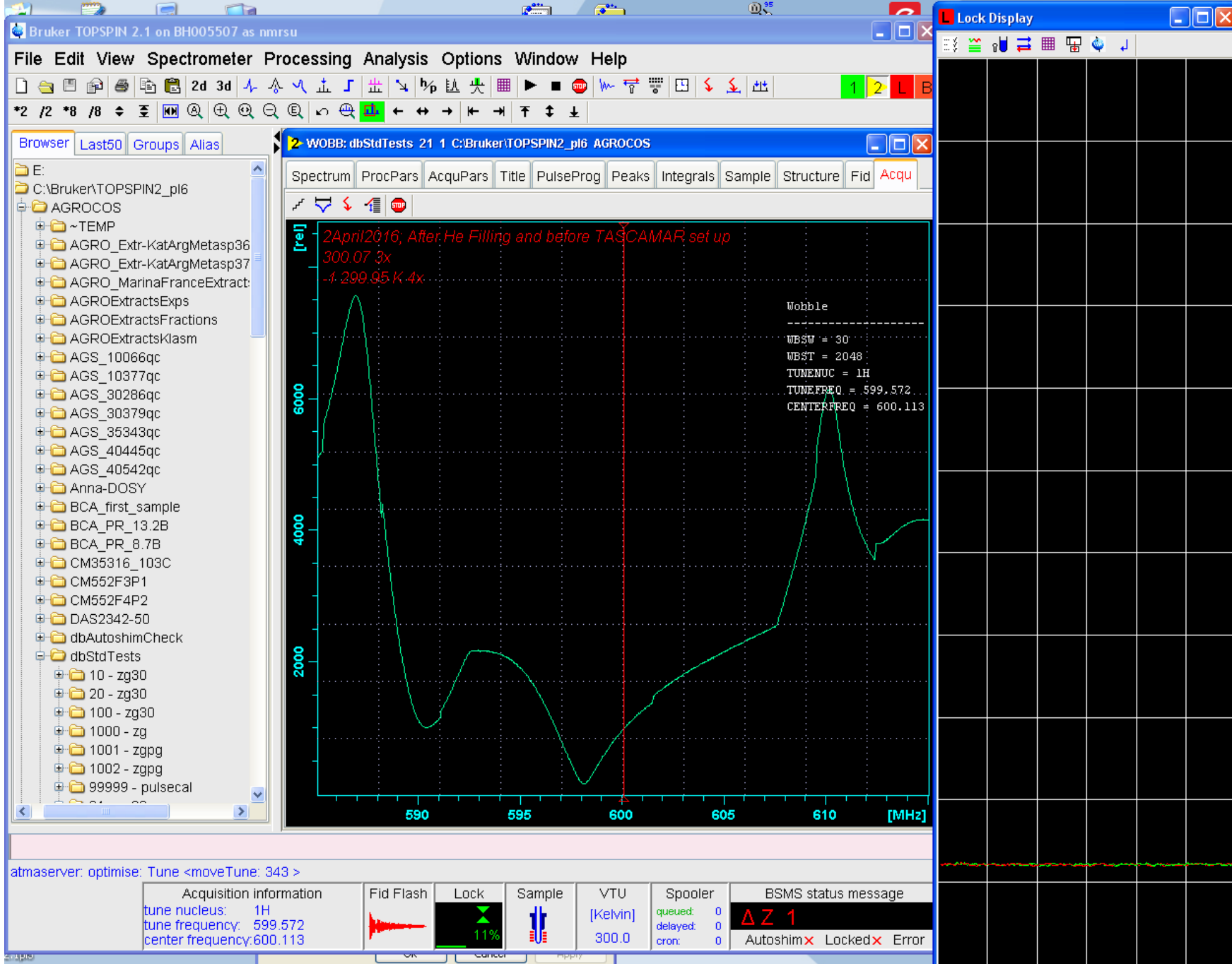


# Profiling Optimization

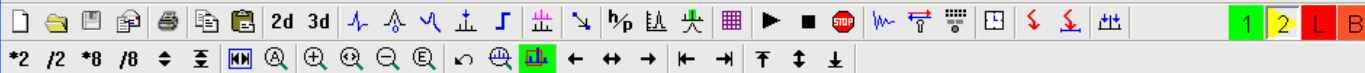
- ✓ temperature stabilization unit
  - ✓ robotic sample changer of 60 sample positions (B-ACS 60)
  - ✓ and supporting automation software



- 
- O1 optimization
  - TSP line width
  - Pulse calibration
  - Shimming (rsh; tsg)
  - Lock
  - Tuning and matching
  - Temperature equilibration; 5 min
  - 1<sup>st</sup> sample transfer







Browser Last50 Groups Alias

E:  
 C:\Bruker\TOPSPIN2\_pl6  
 AGROCOS  
 ~TEMP  
 AGRO\_Extr-KatAr  
 AGRO\_Extr-KatAr  
 AGRO\_MarinaFra  
 AGROExtractsExp  
 AGROExtractsFra  
 AGROExtractsKla  
 AGS\_10066qc  
 AGS\_10377qc  
 AGS\_30286qc  
 AGS\_30379qc  
 AGS\_35343qc  
 AGS\_40445qc  
 AGS\_40542qc  
 Anna-DOSY  
 BCA\_first\_sample  
 BCA\_PR\_13.2B  
 BCA\_PR\_8.7B  
 CM35316\_103C  
 CM552F3P1  
 CM552F4P2  
 DAS2342-50  
 dbAutoshimCheck  
 dbStdTests  
 10 - zg30  
 20 - zg30  
 100 - zg30  
 1000 - zg  
 1001 - zgpg  
 1002 - zgpg  
 99999 - pulsecal  
 21 - zg30  
 dbTempCal2013Dec

WOB: dbStdTests 21 1 C:\Bruker\TOPSPIN2\_pl6 AGROCOS

Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Fid Acqu

AtmaControl

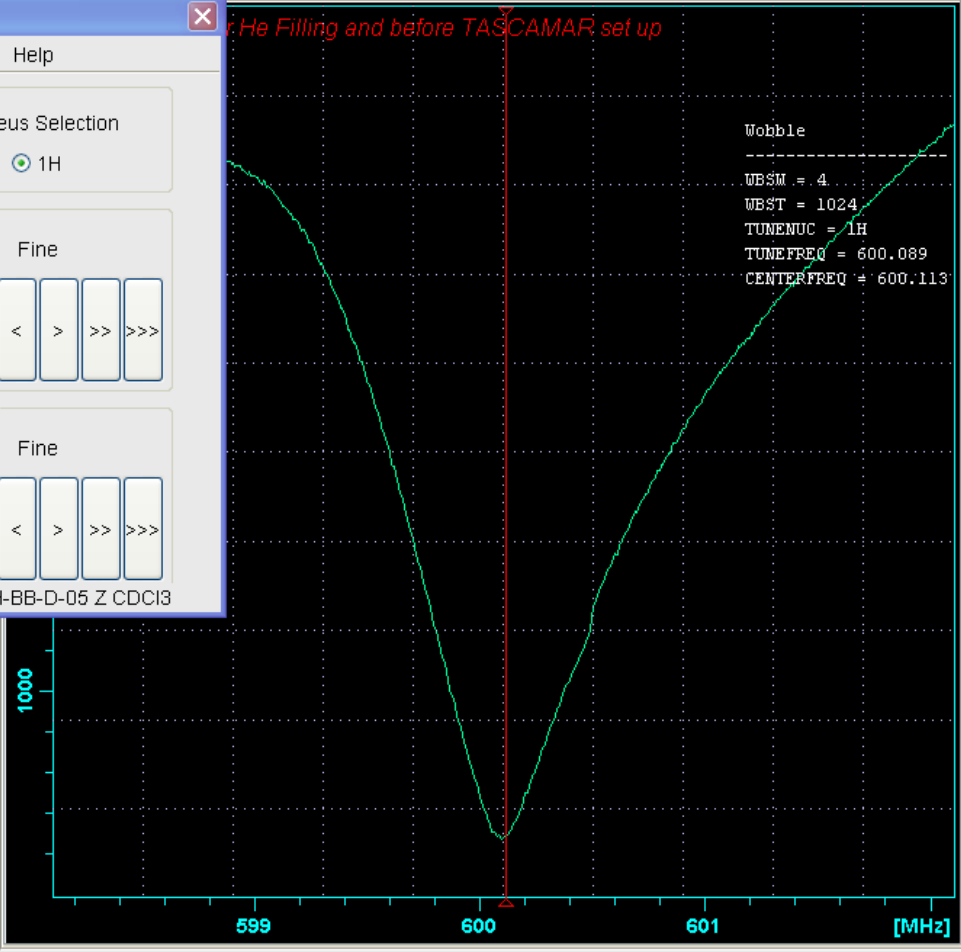
File Optimize Help

Nucleus  
 Nucleus Selection  
 1H

Tuning  
 Fine  
 <<< << < > >> >>>

Matching  
 Fine  
 <<< << < > >> >>>

PA BBI 600S3 H-BB-D-05 Z CDCI3



wbsw\_4\_atmm\_manwbsw: finished

Acquisition information tune nucleus: 1H tune frequency: 600.089 center frequency: 600.113	Fid Flash 	Lock  11%	Sample 	VTU [Kelvin] 300.0	Spooler queued: 0 delayed: 0 cron: 0	BSMS status message <b>Δ Z 1</b> Autoshim X Locked X Error
-----------------------------------------------------------------------------------------------------	---------------	-----------------	------------	--------------------------	-----------------------------------------------	------------------------------------------------------------------



Bruker TOPSPIN 2.1 on BH005507 as nmrsu

File Edit View Spectrometer Processing Analysis Options Window Help

WOB: dbStdTests 20 1 C:\Bruker\TOPSPIN2\_pl6 AGROCOS

Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Fid **Acqu**

2April2016 after He filling 1.4.2016 and before TASCAMAR setup CDC13 for field drift check;

AtmaControl

File Optimize Help

Save Position

Exit Selection

1H

Tuning

Fine

<<< << < > >> >>>

Matching

Fine

<<< << < > >> >>>

PA BBI 600S3 H-BB-D-05 Z MeOD

Wobble

WBSW = 4  
 WBST = 1024  
 TUNEMUC = 1H  
 TUNEFREQ = 600.105  
 CENTERFREQ = 600.113

599 600 601 [MHz]

**manual tuning-matching optimization**

Acquisition information  
 tune nucleus: 1H  
 tune frequency: 600.105  
 center frequency: 600.113

Fid Flash

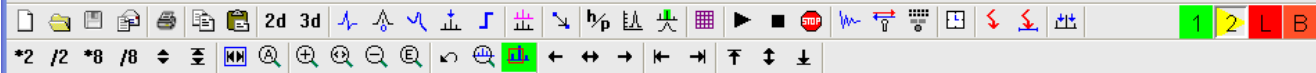
Lock  
 10%

Sample

VTU [Kelvin]  
 300.0

Spooler  
 queued: 0  
 delayed: 0  
 cron: 0

BSMS status message  
 Δ Z2 -2  
 Autoshim x Locked x Error



Browser Last50 Groups Alias

WOBBS: dbStdTests 21 1 C:\Bruker\TOPSPIN2\_pl6 AGROCOS

Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Fid Acqu

AtmaControl

File Optimize Help

Nucleus

Nucleus Selection

1H

Tuning

Fine

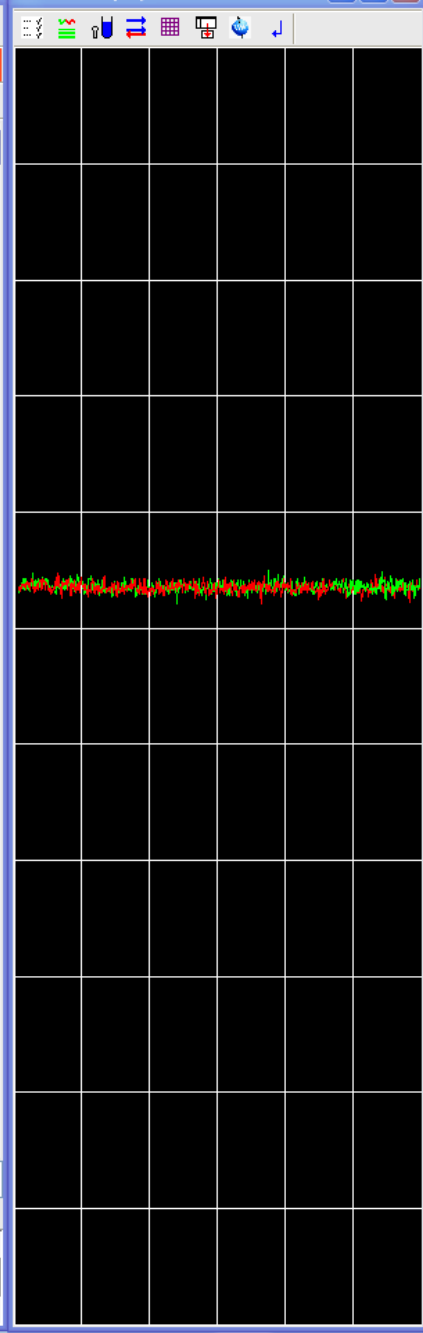
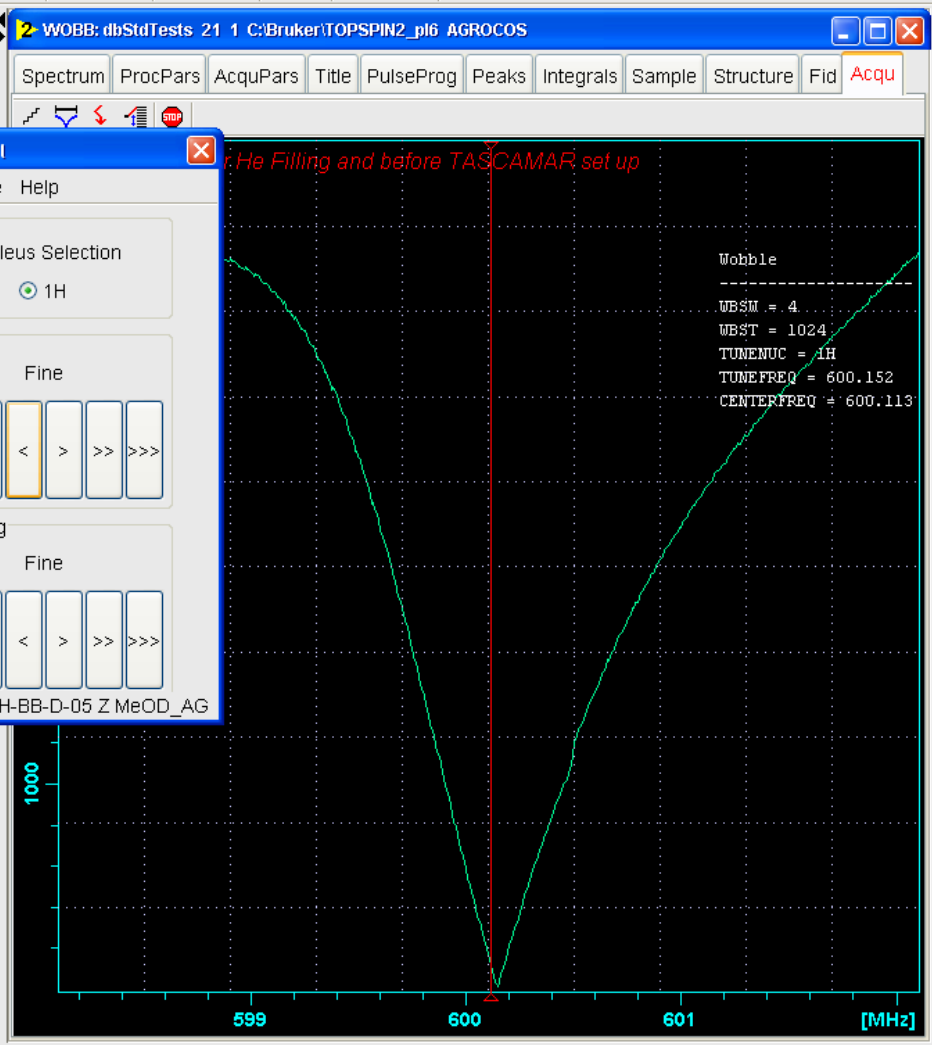
<<< << < > >> >>>

Matching

Fine

<<< << < > >> >>>

PA BBI 600S3 H-BB-D-05 Z MeOD\_AG



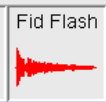
wbsw\_4\_atmm\_manwbsw: finished

Acquisition information

tune nucleus: 1H

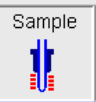
tune frequency: 600.152

center frequency: 600.113



Lock

58%



VTU

[Kelvin]

300.0

Spooler

queued: 0

delayed: 0

cron: 0

BSMS status message

**Δ Z 1**

Autoshim Locked Error

Bruker TOPSPIN 2.1 on BH005507 as nmrsu

File Edit View Spectrometer Processing Analysis Options Window Help

\*2 /2 \*8 /8

Browser Last50 Groups Alias

- AGROCOS
  - ~TEMP
  - AGRO\_Extr-KatArgMetasp36
  - AGRO\_Extr-KatArgMetasp37
  - AGRO\_MarinaFranceExtract
  - AGROExtractsExps
  - AGROExtractsFractions
  - AGROExtractsKlasm
  - AGS\_10066qc
  - AGS\_10377qc
  - AGS\_30286qc
  - AGS\_30379qc
  - AGS\_35343qc
  - AGS\_40445qc
  - AGS\_40542qc
  - Anna-DOSY
  - BCA\_first\_sample
  - BCA\_PR\_13.2B
  - BCA\_PR\_8.7B
  - CM35316\_103C
  - CM552F3P1
  - CM552F4P2
  - DAS2342-50
  - dbAutoshimCheck
  - dbStdTests
    - 10 - zg30
      - 1 - 16Sept2014 temp t
    - 100 - zg30
    - 1000 - zg
    - 1001 - zgpg
    - 1002 - zgpg
    - 99999 - pulsecal
    - 20 - zg30
  - dbTempCal2013Dec

TopShim

Shim Report Service

SHIM

Dimension  1D  3D

Optimisation solvent's default

Optimise for 1H

Use Z6

TUNE

Before off

After off

Only

PARAMETERS

[ ]

STATUS

not running

CONTROL

Start Stop Help Close

Start shimming

Gain [ ] [ ]

Gain [ ]

Rate [ ]

Z+ [ ]

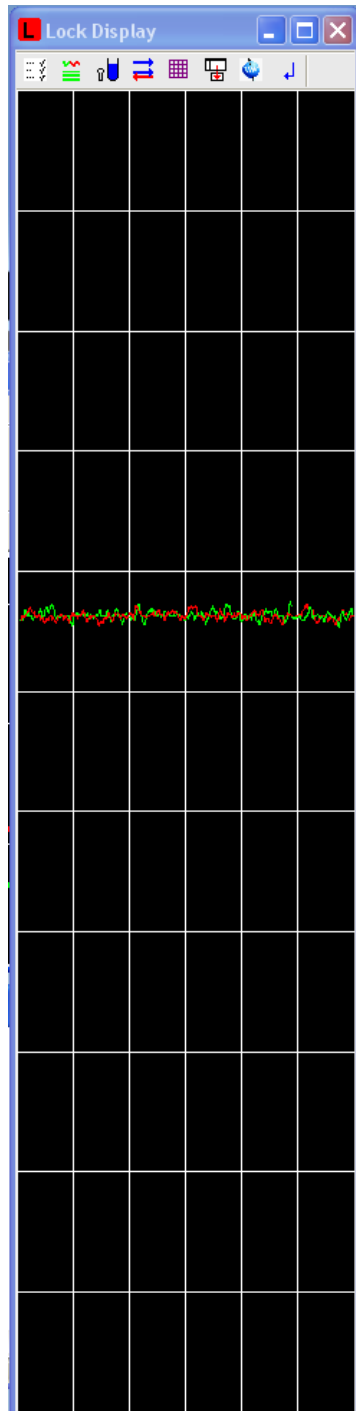
Step [ ] [ ]

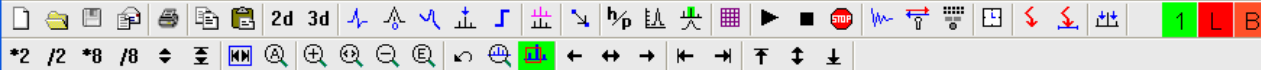
Stepsize [ ]

TOPSPIN2\_pl...

topshim: finished

Acquisition information	Fid Flash	Lock	Sample	VTU	Spooler	BSMS status message
no acquisition running		60%	[ ]	[Kelvin] 300.0	queued: 0 delayed: 0 cron: 0	<b>Δ Z6 0</b> Autoshim x Locked ✓ Error





Browser Last50 Groups Alias

- AGROCOS
  - ~TEMP
  - AGRO\_Extr-KatArgMetasp36
  - AGRO\_Extr-KatArgMetasp37
  - AGRO\_MarinaFranceExtract
  - AGROExtractsExps
  - AGROExtractsFractions
  - AGROExtractsKlasm
  - AGS\_10066qc
  - AGS\_10377qc
  - AGS\_30286qc
  - AGS\_30379qc
  - AGS\_35343qc
  - AGS\_40445qc
  - AGS\_40542qc
  - Anna-DOSY
  - BCA\_first\_sample
  - BCA\_PR\_13.2B
  - BCA\_PR\_8.7B
  - CM35316\_103C
  - CM552F3P1
  - CM552F4P2
  - DAS2342-50
  - dbAutoshimCheck
  - dbStdTests
    - 10 - zg30
      - 1 - 16Sept2014 temp...
    - 100 - zg30
    - 1000 - zg
    - 1001 - zgpg
    - 1002 - zgpg
    - 99999 - pulsecal
    - 20 - zg30
  - dbTempCal2013Dec

S TopShim

Shim Report Service

1D SHIMMING

Parameters:

- maximum order = 5
- probehead = Z814601\_0044
- solvent = CDCI3
- shim nucleus = 2H
- nucleus optimised for = 1H
- o1p (from lock) = 7.24 ppm
- optimisation parameters = ls
- linewidth 1H = 0.10 Hz
- envelope shape / strictness = 2.00 / 200

Results:

- initial B0 stdDev = 4.89 Hz
- sample size = 2.12 cm, position = -0.04 cm
- final B0 stdDev = 0.21 Hz > improvement = 23.0
- envelope width = 0.43 Hz
- shim changes:

Z	+215
Z2	+18
Z3	+52
Z4	+259
Z5	+630

duration = 1 min 42 sec  
completed successfully  
finished Sat Apr 02 13:10:24 2016

Help

ample Structure

Gain

Gain

Rate

Z+

Step

+

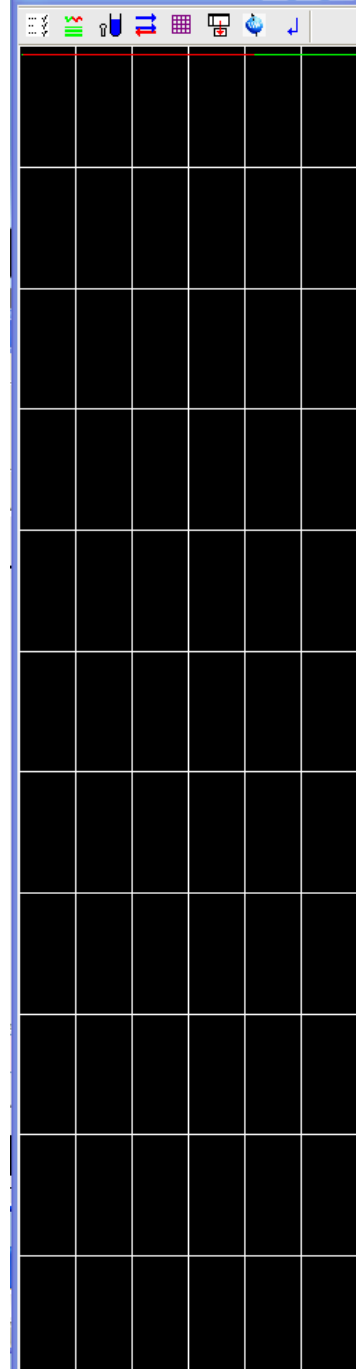
-

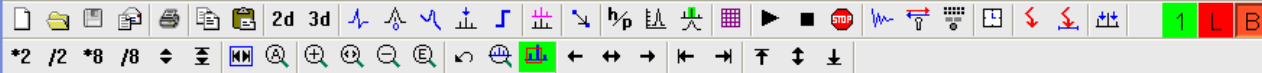
Stepsize

TOPSPIN2\_pl...

topshim: completed

Acquisition information	Fid Flash	Lock	Sample	VTU	Spooler	BSMS status message
no acquisition running		100%		[Kelvin] 300.0	queued: 0 delayed: 0 cron: 0	<b>Δ YZ5 0</b> Autoshim X Locked ✓ Error





- Browser Last50 Groups Alias
- AGROCOS
  - ~TEMP
  - AGRO\_Extr-KatArgMetasp36
  - AGRO\_Extr-KatArgMetasp37
  - AGRO\_MarinaFranceExtract
  - AGROExtractsExps
  - AGROExtractsFractions
  - AGROExtractsKlasm
  - AGS\_10066qc
  - AGS\_10377qc
  - AGS\_30286qc
  - AGS\_30379qc
  - AGS\_35343qc
  - AGS\_40445qc
  - AGS\_40542qc
  - Anna-DOSY
  - BCA\_first\_sample
  - BCA\_PR\_13.2B
  - BCA\_PR\_8.7B
  - CM35316\_103C
  - CM552F3P1
  - CM552F4P2
  - DAS2342-50
  - dbAutoshimCheck
  - dbStdTests
    - 10 - zg30
      - 1 - 16Sept2014 temp
    - 100 - zg30
    - 1000 - zg
    - 1001 - zgpg
    - 1002 - zgpg
    - 99999 - pulsecal
    - 20 - zg30
  - dbTempCal2013Dec

**BSMS Control Suite**

Main Lock/Level Shim Autoshim Service Log Help

AUTO

Lock Phase Power Gain Shim

LOCK

On-Off Phase Power Gain

SAMPLE

LIFT SPIN Measure Rate

SHIM

Spin. Z Z<sup>2</sup> Z<sup>3</sup> Z<sup>4</sup>

NonSpin. X XZ

Y YZ

XY

X<sup>2</sup>-Y<sup>2</sup>

**STD BY**

Previous Actual Step

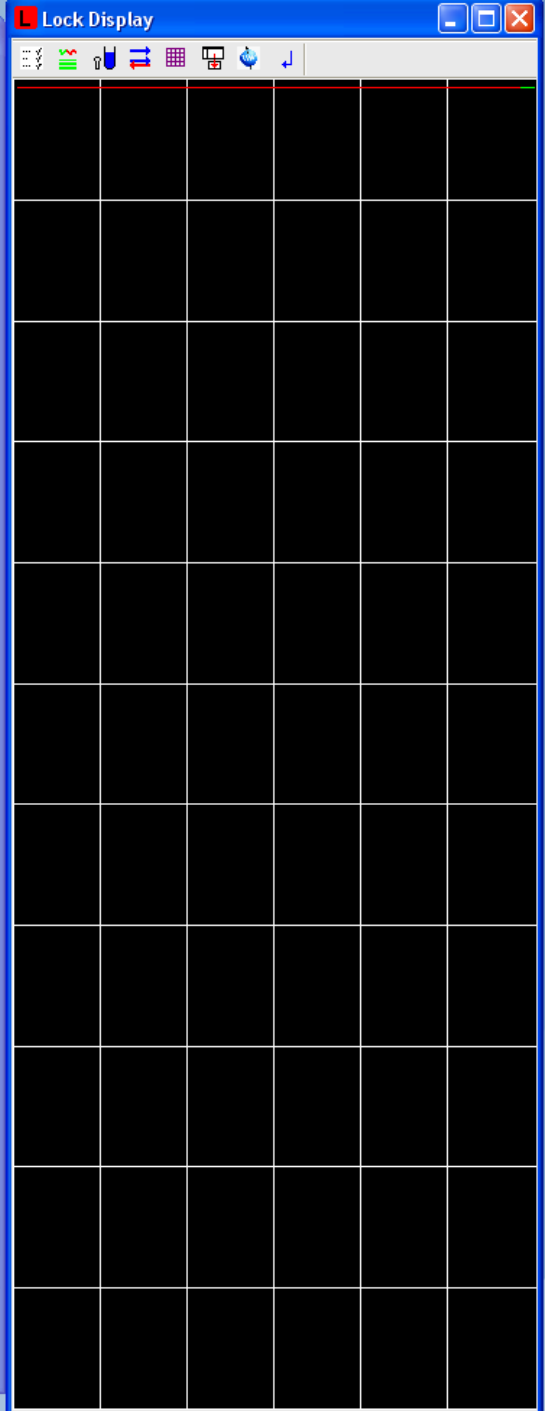
Absolute [ ] [ ] + Reset

Difference [ ] [ ] -

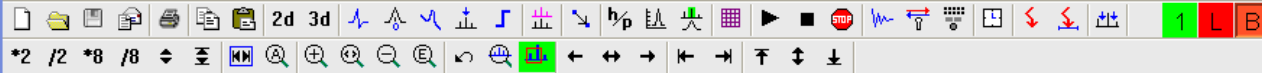
Stepsize [ ]

Sample: down missing up

down missing up



Acquisition information no acquisition running	Fid Flash	Lock 100%	Sample [Icon]	VTU [Kelvin] 300.0	Spooler queued: 0 delayed: 0 cron: 0	BSMS status message <b>Δ YZ5 0</b> Autoshim x Locked ✓ Error
---------------------------------------------------	-----------	--------------	------------------	--------------------------	-----------------------------------------------	--------------------------------------------------------------------



- Browser Last50 Groups Alias
- AGROCOS
  - ~TEMP
  - AGRO\_Extra-KatArgMetasp36
  - AGRO\_Extra-KatArgMetasp37
  - AGRO\_MarinaFranceExtract
  - AGROExtractsExps
  - AGROExtractsFractions
  - AGROExtractsKlasm
  - AGS\_10066qc
  - AGS\_10377qc
  - AGS\_30286qc
  - AGS\_30379qc
  - AGS\_35343qc
  - AGS\_40445qc
  - AGS\_40542qc
  - Anna-DOSY
  - BCA\_first\_sample
  - BCA\_PR\_13.2B
  - BCA\_PR\_8.7B
  - CM35316\_103C
  - CM552F3P1
  - CM552F4P2
  - DAS2342-50
  - dbAutoshimCheck
  - dbStdTests
    - 10 - zg30
      - 1 - 16Sept2014 temp
    - 100 - zg30
    - 1000 - zg
    - 1001 - zgpg
    - 1002 - zgpg
    - 99999 - pulsecal
    - 20 - zg30
  - dbTempCal2013Dec

**BSMS Control Suite**

Main Lock/Level Shim Autoshim Service Log Help

AUTO

Lock Phase Power Gain Shim

LOCK

On-Off Phase Power Gain

SAMPLE

LIFT SPIN Measure Rate

SHIM

Spin. Z Z<sup>2</sup> Z<sup>3</sup> Z<sup>4</sup>

NonSpin. X XZ

Y YZ

XY

X<sup>2</sup>-Y<sup>2</sup>

**STD BY**

Previous Actual Step

Absolute + Reset

Difference -

Stepsize

Sample: down missing up

STD BY

Acquisition information: no acquisition running

Fid Flash: 100%

Lock: 100%

Sample: [Icon]

VTU: [Kelvin] 300.0

Spooler: queued: 0, delayed: 0, cron: 0

BSMS status message: **Δ YZ5 0**  
Autoshim x Locked ✓ Error



Bruker TOPSPIN 2.1 on BH005507 as nmrsu

File Edit View Spectrometer Processing Analysis Options Window Help

2d 3d [Icons]

\*2 /2 \*8 /8 [Icons]

Browser Last50 Groups Alias

- AGROCOS
  - ~TEMP
  - AGRO\_Extr-KatArgMetasp36
  - AGRO\_Extr-KatArgMetasp37
  - AGRO\_MarinaFranceExtract
  - AGROExtractsExps
  - AGROExtractsFractions
  - AGROExtractsKlasm
  - AGS\_10066qc
  - AGS\_10377qc
  - AGS\_30286qc
  - AGS\_30379qc
  - AGS\_35343qc
  - AGS\_40445qc
  - AGS\_40542qc
  - Anna-DOSY
  - BCA\_first\_sample
  - BCA\_PR\_13.2B
  - BCA\_PR\_8.7B
  - CM35316\_103C
  - CM552F3P1
  - CM552F4P2
  - DAS2342-50
  - dbAutoshimCheck
  - dbStdTests
    - 10 - zg30
      - 1 - 16Sept2014 temp [Selected]
      - 100 - zg30
      - 1000 - zg
      - 1001 - zgpg
      - 1002 - zgpg
      - 99999 - pulsecal
      - 20 - zg30
    - dbTempCal2013Dec

**BSMS Control Suite**

Main Lock/Level Shim Autoshim Service Log Help

AUTO

Lock Phase Power **Gain** Shim

LOCK

**On-Off** Phase Power Gain

SAMPLE

LIFT SPIN Measure Rate

SHIM

Spin. Z Z<sup>2</sup> Z<sup>3</sup> Z<sup>4</sup>

NonSpin. X XZ

Y YZ

XY

X<sup>2</sup>-Y<sup>2</sup>

**STD BY**

Absolute Previous Actual Step + Reset

Difference Previous Actual Step -

Stepsize

**STD BY**

Sample: down missing up

down missing up

Lock Display

Acquisition information

no acquisition running

Fid Flash

Lock

100%

Sample

VTU [Kelvin] 300.0

Spooler

queued: 0  
delayed: 0  
cron: 0

BSMS status message

**Δ YZ5 0**

Autoshim x Locked ✓ Error



- Browser
- Last50
- Groups
- Alias
- AGROCOS
  - ~TEMP
    - AGRO\_Extr-KatArgMetasp36
    - AGRO\_Extr-KatArgMetasp37
    - AGRO\_MarinaFranceExtract
    - AGROExtractsExps
    - AGROExtractsFractions
    - AGROExtractsKlasm
    - AGS\_10066qc
    - AGS\_10377qc
    - AGS\_30286qc
    - AGS\_30379qc
    - AGS\_35343qc
    - AGS\_40445qc
    - AGS\_40542qc
    - Anna-DOSY
    - BCA\_first\_sample
    - BCA\_PR\_13.2B
    - BCA\_PR\_8.7B
    - CM35316\_103C
    - CM552F3P1
    - CM552F4P2
    - DAS2342-50
    - dbAutoshimCheck
    - dbStdTests
      - 10 - zg30
        - 1 - 16Sept2014 temp
        - 100 - zg30
        - 1000 - zg
        - 1001 - zgpg
        - 1002 - zgpg
        - 99999 - pulsecal
        - 20 - zg30
      - dbTempCal2013Dec

### S TopShim

Shim Report Service

SHIM

Dimension  1D  3D

Optimisation solvent's default

Optimise for 1H

Use Z6

TUNE

Before off

After Z-X-Y-XZ-YZ-Z

Only

PARAMETERS

[ ]

STATUS

not running

CONTROL

Start Stop Help Close

Start shimming

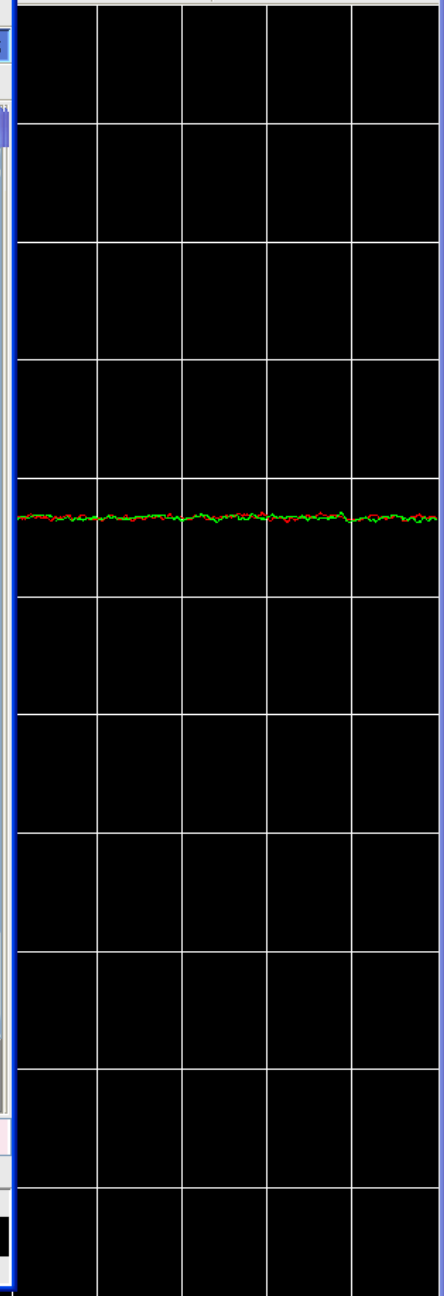
Shim

Step

+ - Reset

Stepsize

[ ]



Acquisition information no acquisition running	Fid Flash	Lock 61%	Sample [Icon]	VTU [Kelvin] 300.0	Spooler queued: 0 delayed: 0 cron: 0	BSMS status message <b>Δ YZ5 0</b> Autoshim x Locked ✓ Error
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Bruker TOPSPIN 2.1 on BH005507 as nmrsu  
 File Edit View Spectrometer Processing Analysis Options Window Help

\*2 /2 \*8 /8  
 1 L B S

Browser Last50 Groups Alias

- AGROCOS
  - ~TEMP
  - AGRO\_Extr-KatArgMetasp36
  - AGRO\_Extr-KatArgMetasp37
  - AGRO\_MarinaFranceExtract
  - AGROExtractsExps
  - AGROExtractsFractions
  - AGROExtractsKlasm
  - AGS\_10066qc
  - AGS\_10377qc
  - AGS\_30286qc
  - AGS\_30379qc
  - AGS\_35343qc
  - AGS\_40445qc
  - AGS\_40542qc
  - Anna-DOSY
  - BCA\_first\_sample
  - BCA\_PR\_13.2B
  - BCA\_PR\_8.7B
  - CM35316\_103C
  - CM552F3P1
  - CM552F4P2
  - DAS2342-50
  - dbAutoshimCheck
  - dbStdTests
    - 10 - zg30
      - 1 - 18Sept2014 temp
      - 100 - zg30
      - 1000 - zg
      - 1001 - zgpg
      - 1002 - zgpg
      - 99999 - pulsecal
      - 20 - zg30
  - dbTempCal2013Dec

S TopShir  
 BSMS Control Suite

Main Lock/Level Shim Autoshim Service Log Help

TUNE  
 LOCK & SPIN  
 On-Off Phase Power Gain SPIN

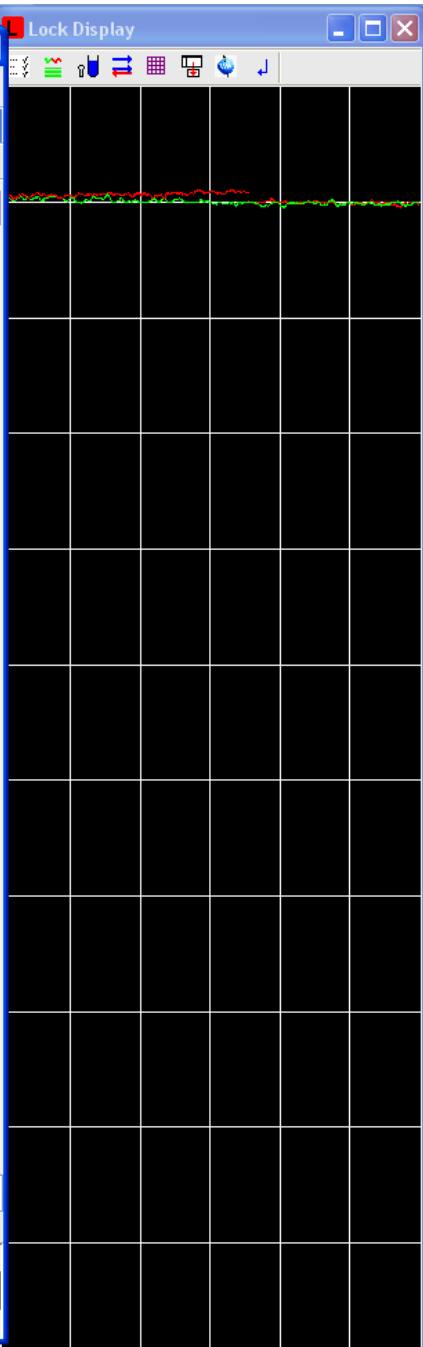
SHIM  
 Spin: Z Z<sup>2</sup> Z<sup>3</sup> Z<sup>4</sup> Z<sup>5</sup> Z<sup>6</sup>  
 NonSpin: X XZ XZ<sup>2</sup> XZ<sup>3</sup> XZ<sup>4</sup> XZ<sup>5</sup>  
 Y YZ YZ<sup>2</sup> YZ<sup>3</sup> YZ<sup>4</sup> YZ<sup>5</sup>  
 XY XYZ XYZ<sup>2</sup> XYZ<sup>3</sup> XYZ<sup>4</sup> XYZ<sup>5</sup>  
 X<sup>2</sup>-Y<sup>2</sup> (X<sup>2</sup>-Y<sup>2</sup>)Z (X<sup>2</sup>-Y<sup>2</sup>)Z<sup>2</sup> (X<sup>2</sup>-Y<sup>2</sup>)Z<sup>3</sup> (X<sup>2</sup>-Y<sup>2</sup>)Z<sup>4</sup> (X<sup>2</sup>-Y<sup>2</sup>)Z<sup>5</sup>  
 X<sup>3</sup> X<sup>3</sup>Z  
 Y<sup>3</sup> Y<sup>3</sup>Z

Results: tune lock shim cha Z X Y YZ duration complete finished

**Phase [degree]**  
 Previous Actual Step  
 Absolute 47.0 30.6 + Reset  
 Difference 0.0 -16.4 -  
 Stepsize 0.1

STD BY

Sample: down missing up



Acquisition information: no acquisition running  
 Fid Flash: [icon]  
 Lock: 91% [icon]  
 Sample: [icon]  
 VTU: [Kelvin] 300.0  
 Spooler: queued: 0, delayed: 0, cron: 0  
 BSMS status message: **Δ YZ5 0**  
 Autoshim x Locked ✓ Error

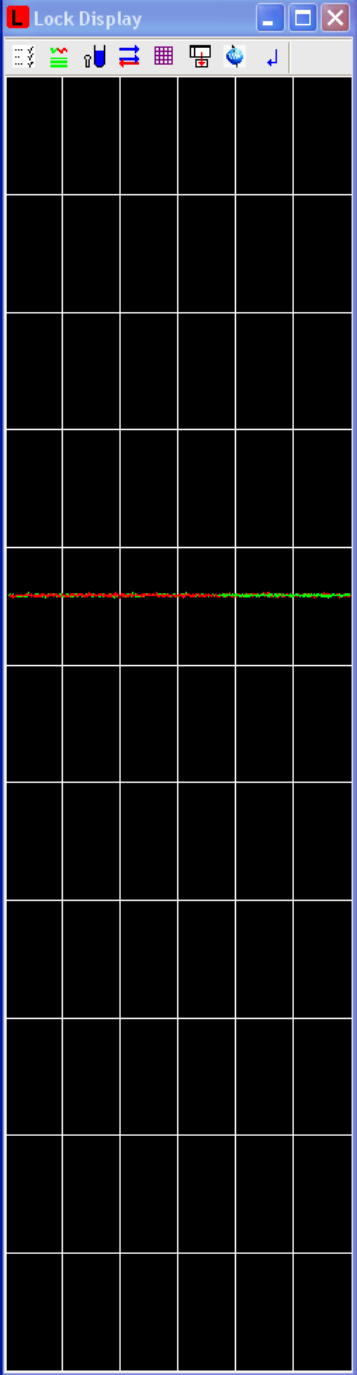


- Browser Last50 Groups Alias
- E:
  - C:\Bruker\TOPSPIN2\_pl6
    - AGROCOS
      - ~TEMP
      - AGRO\_Extr-KatArgMetasp36
      - AGRO\_Extr-KatArgMetasp37
      - AGRO\_MarinaFranceExtract
      - AGROExtractsExps
      - AGROExtractsFractions
      - AGROExtractsKlasm
      - AGS\_10066qc
      - AGS\_10377qc
      - AGS\_30286qc
      - AGS\_30379qc
      - AGS\_35343qc
      - AGS\_40445qc
      - AGS\_40542qc
      - Anna-DOSY
      - BCA\_first\_sample
      - BCA\_PR\_13.2B
      - BCA\_PR\_8.7B
      - CM35316\_103C
      - CM552F3P1
      - CM552F4P2
      - DAS2342-50
      - dbAutoshimCheck
      - dbStdTests
        - 10 - zg30
        - 20 - zg30
        - 100 - zg30
        - 1000 - zg
        - 1001 - zgpg
        - 1002 - zgpg
        - 99999 - pulsecal
        - 21 - zg30
      - dbTempCal2013Dec

2 No acquisition running: C:\Bruker\TOPSPIN2\_pl6\data\AGROCOS/nmr/dbStdTests/21/pdata/1

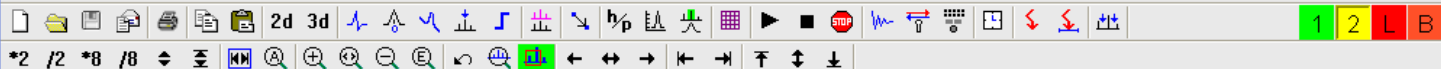
Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Fid **Acqu**

Start acquisition on: dbStdTests 21 1 C:\Bruker\TOPSPIN2\_pl6 AGROCOS



pulsecal  
rga: setting RG to 20.20

Acquisition information no acquisition running	Fid Flash 	Lock 60%	Sample 	VTU [Kelvin] 300.0	Spooler queued: 0 delayed: 0 cron: 0	BSMS status message <b>Δ XY 16</b> Autoshim x Locked ✓ Error
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Browser Last50 Groups Alias

- E:
- C:\Bruker\TOPSPIN2\_pl6
- AGROCOS
  - ~TEMP
  - AGRO\_Extr-KatArgMetasp36
  - AGRO\_Extr-KatArgMetasp37
  - AGRO\_MarinaFranceExtract:
  - AGROExtractsExps
  - AGROExtractsFractions
  - AGROExtractsKlasm
  - AGS\_10066qc
  - AGS\_10377qc
  - AGS\_30286qc
  - AGS\_30379qc
  - AGS\_35343qc
  - AGS\_40445qc
  - AGS\_40542qc
  - Anna-DOSY
  - BCA\_first\_sample
  - BCA\_PR\_13.2B
  - BCA\_PR\_8.7B
  - CM35316\_103C
  - CM552F3P1
  - CM552F4P2
  - DAS2342-50
  - dbAutoshimCheck
  - dbStdTests
    - 10 - zg30
    - 20 - zg30
    - 100 - zg30
    - 1000 - zg
    - 1001 - zgpg
    - 1002 - zgpg
    - 99999 - pulsecal
    - 21 - zg30
  - dbTempCal2013Dec

2 No acquisition running: C:\Bruker\TOPSPIN2\_pl6\data\AGROCOS\mmr\dbStdTests\21\pdata\1

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Fid Acqu

Start

1H:

gammaB1/2pi at 15.0 dB : 6254.1 Hz

90o pulse at 15.0 dB : 39.97 us

90o pulse at 3.0 dB : 10.04 us

Close

Bruker\TOPSPIN2\_pl6 AGROCOS

pulsecal  
pp: finished

Acquisition information no acquisition running	Fid Flash	Lock 60%	Sample	VTU [Kelvin] 300.0	Spooler queued: 0 delayed: 0 cron: 0	BSMS status message <b>Δ XY 16</b> Autoshim x Locked ✓ Error
---------------------------------------------------	-----------	-------------	--------	--------------------------	-----------------------------------------------	--------------------------------------------------------------------

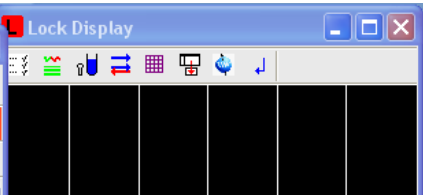
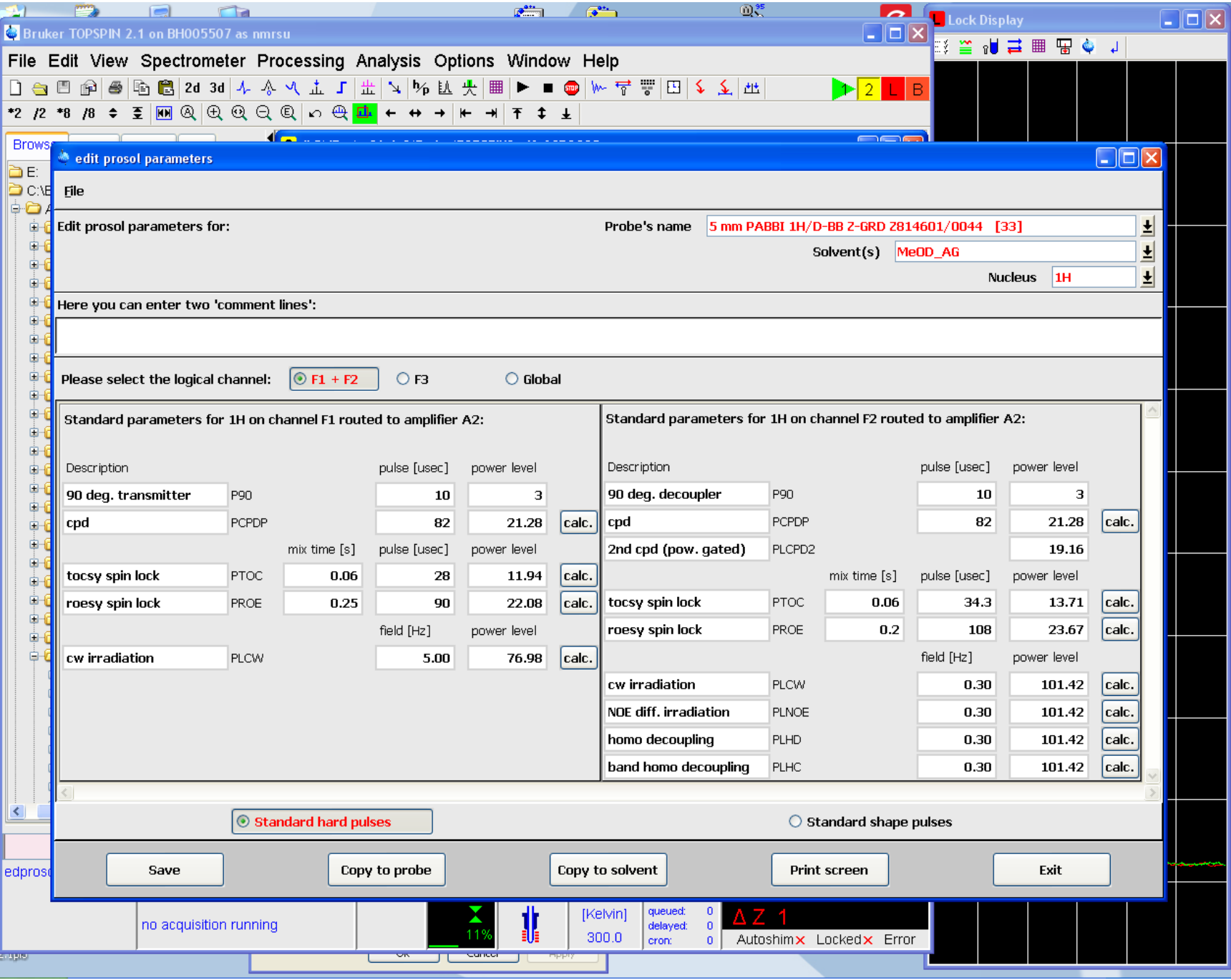
Lock Display

6 Ac

3 statu

16

NUM



Brows



**edit prosol parameters**

File

Edit prosol parameters for: Probe's name **5 mm PABBI 1H/D-BB 2-GRD 2814601/0044 [33]** Solvent(s) **MeOD-AG** Nucleus **1H**

Here you can enter two 'comment lines':

Please select the logical channel:  F1 + F2  F3  Global

Standard parameters for 1H on channel F1 routed to amplifier A2:				Standard parameters for 1H on channel F2 routed to amplifier A2:					
Description		pulse [usec]	power level	Description		pulse [usec]	power level		
90 deg. transmitter	P90	10	3	90 deg. decoupler	P90	10	3		
cpd	PCPDP	82	21.28	cpd	PCPDP	82	21.28		
				2nd cpd (pow. gated)	PLCPD2		19.16		
		mix time [s]	power level			mix time [s]	power level		
tocsy spin lock	PTOC	0.06	28	11.94	tocsy spin lock	PTOC	0.06	34.3	13.71
roesy spin lock	PROE	0.25	90	22.08	roesy spin lock	PROE	0.2	108	23.67
			field [Hz]	power level			field [Hz]	power level	
cw irradiation	PLCW	5.00	76.98	cw irradiation	PLCW	0.30	101.42		
				NOE diff. irradiation	PLNOE	0.30	101.42		
				homo decoupling	PLHD	0.30	101.42		
				band homo decoupling	PLHC	0.30	101.42		

Standard hard pulses  Standard shape pulses

Save Copy to probe Copy to solvent Print screen Exit

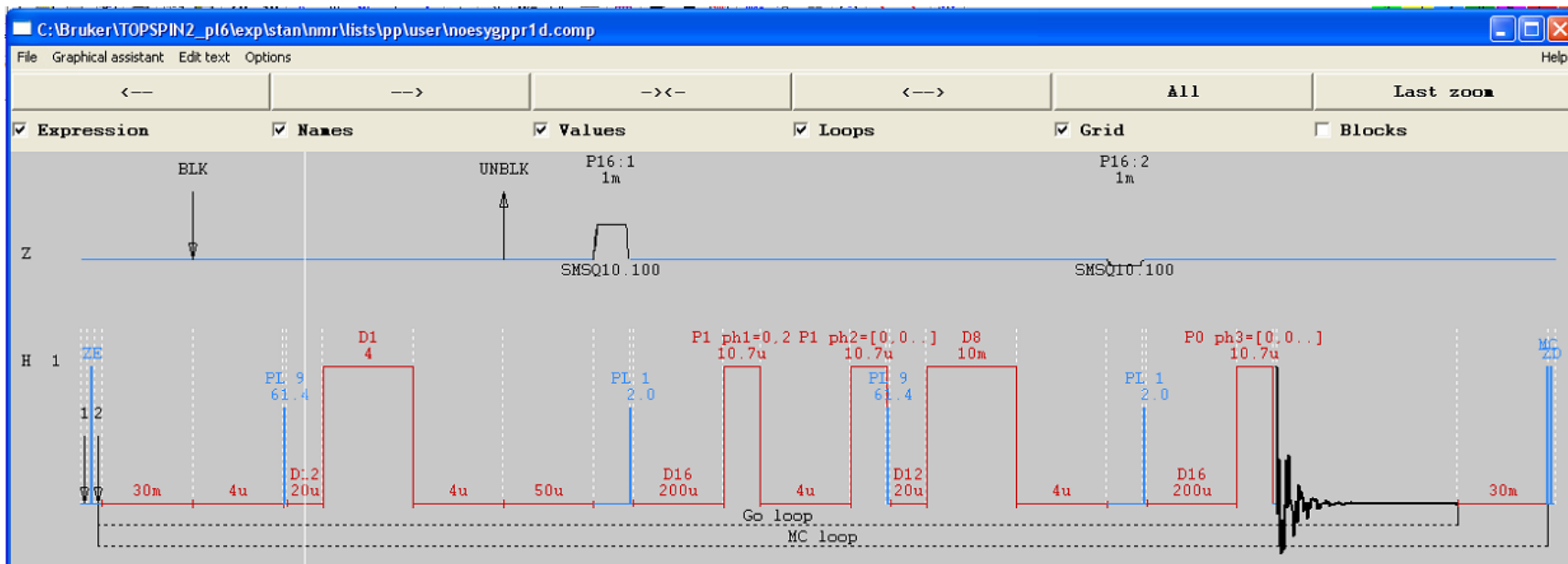
no acquisition running



[Kelvin] 300.0

queued: 0  
delayed: 0  
cron: 0

**Δ Z 1**  
Autoshim x Locked x Error



AcquPars Title PulseProg Peaks Integrals Sample Structure Fid Acqu

Installed probe: 5 mm PABBI 1H/D-BB Z-GRD Z814601/0044

General

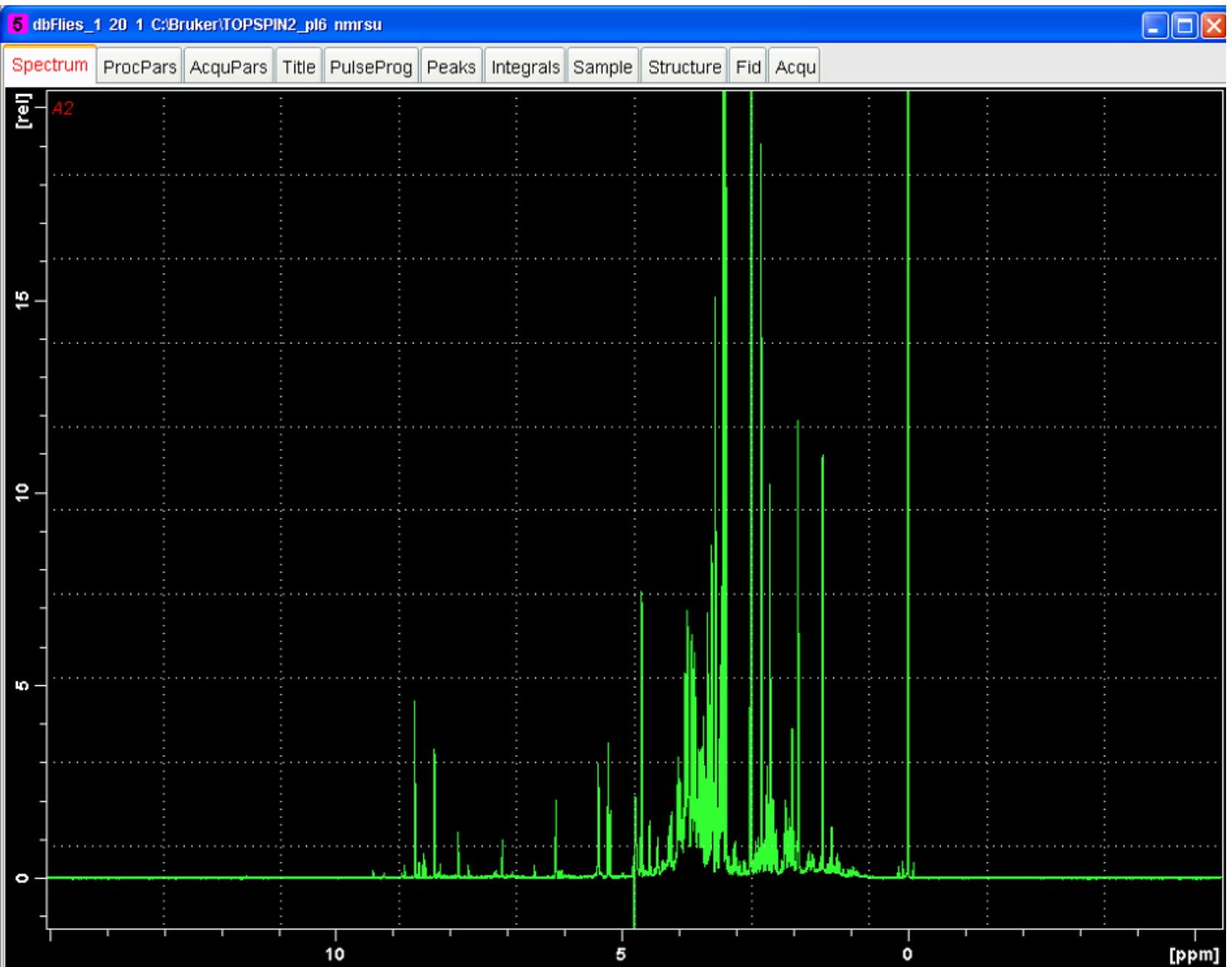
PULPROG	noesygppr1d.comp	Pulse program for acquisition
TD	65536	Time domain size
NS	192	Number of scans
DS	4	Number of dummy scans
SWH [Hz]	12335.53	Sweep width in Hz
AQ [s]	2.6564426	Acquisition time
RG	90.5	Receiver gain
DW [μs]	40.533	Dwell time
DE [μs]	10.00	Pre-scan-delay
D1 [s]	4.00000000	Relaxation delay, 1-5 * T1
D8 [s]	0.01000000	Mixing time
d12 [s]	0.00002000	D12=20u
D16 [s]	0.00020000	Delay for homospoil/gradients recovery
TD0	1	Dimension of accumulation loop

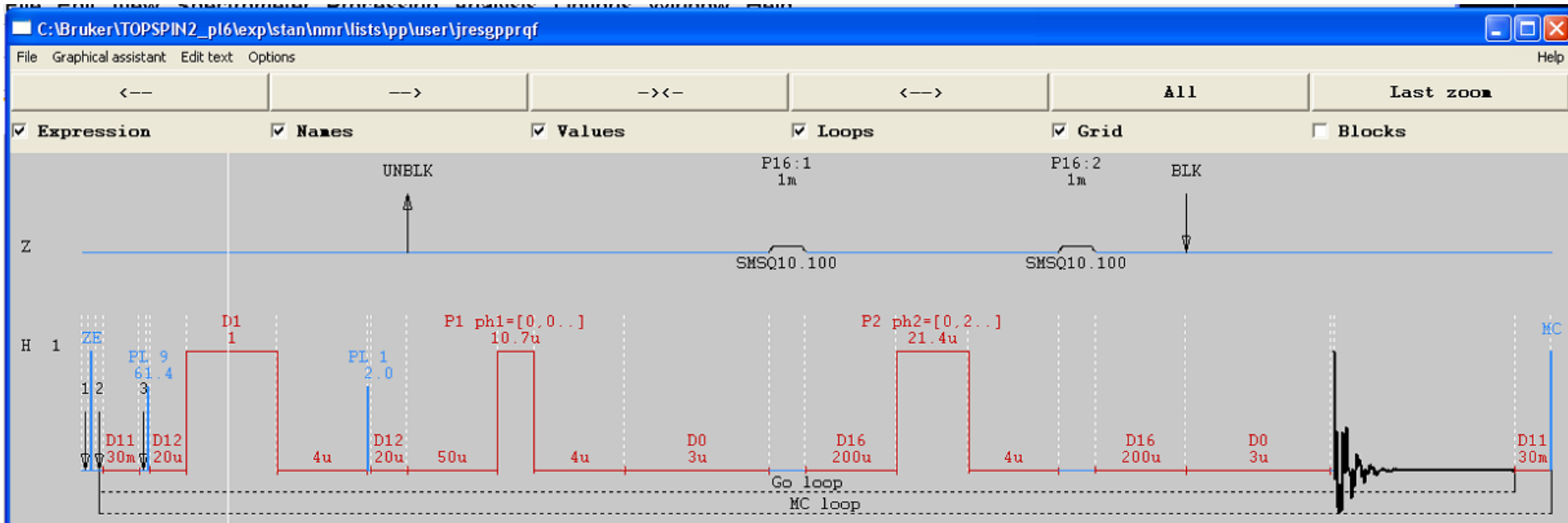
Channel f1

NUC1	1H	Nucleus for channel 1
P0 [μs]	10.70	For any flip angle
P1 [μs]	10.70	F1 channel - 90 degree high power pulse
PL1 [dB]	2.00	F1 channel - power level for pulse (default)
PL1W [W]	16.95835686	F1 channel - power level for pulse (default)
PL9 [dB]	61.41	F1 channel - power level for presaturation
PL9W [W]	0.00001941	F1 channel - power level for presaturation
SFO1 [MHz]	600.1128270	Frequency of observe channel

Gradient channel

GPNAM1	SMSQ10.100	SINE.100
GPNAM2	SMSQ10.100	SINE.100
GPZ1 [%]	50.00	50%
GPZ2 [%]	-10.00	-10%
P16 [μs]	1000.00	Homospoil/gradients pulse

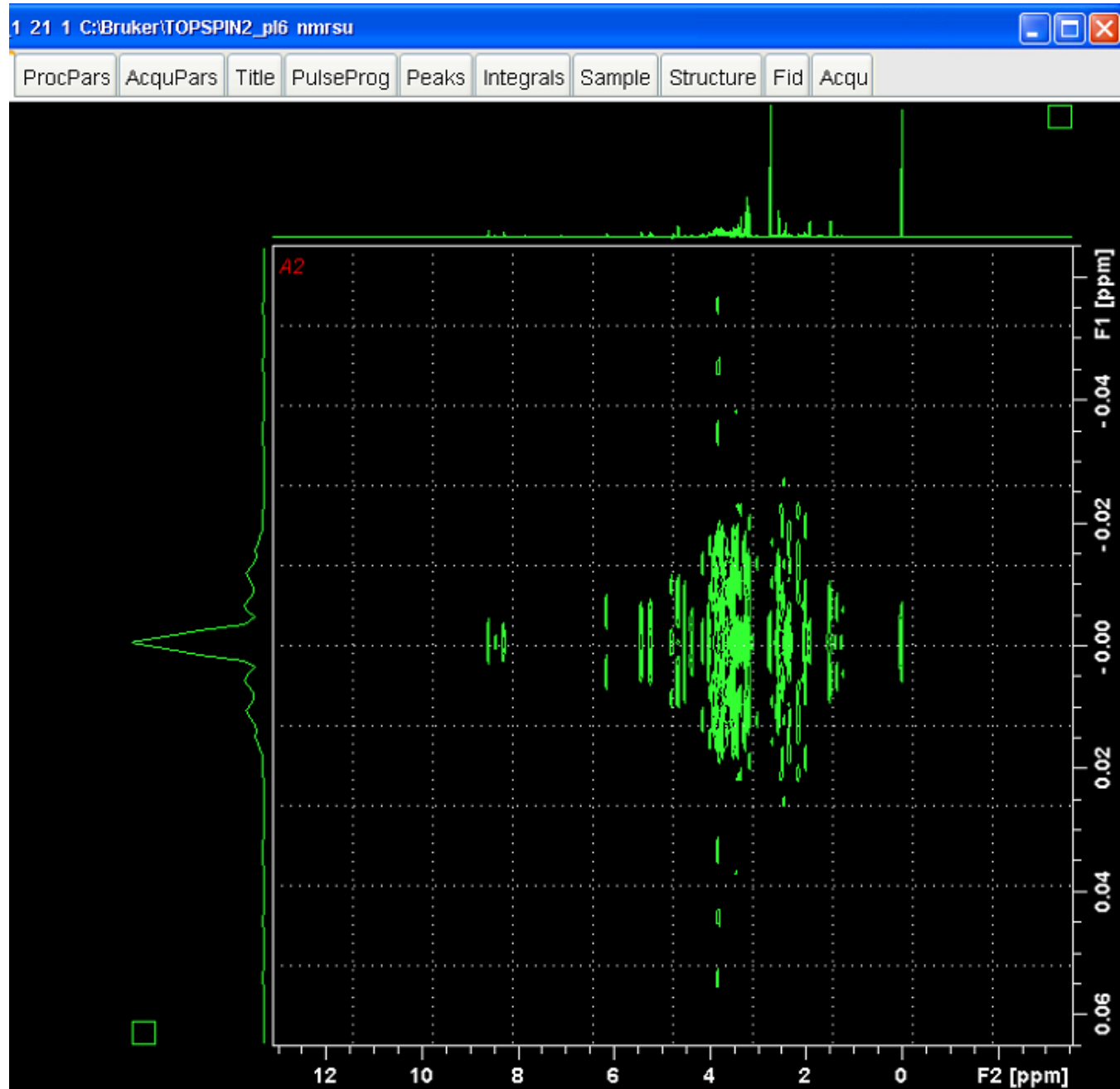



**General**

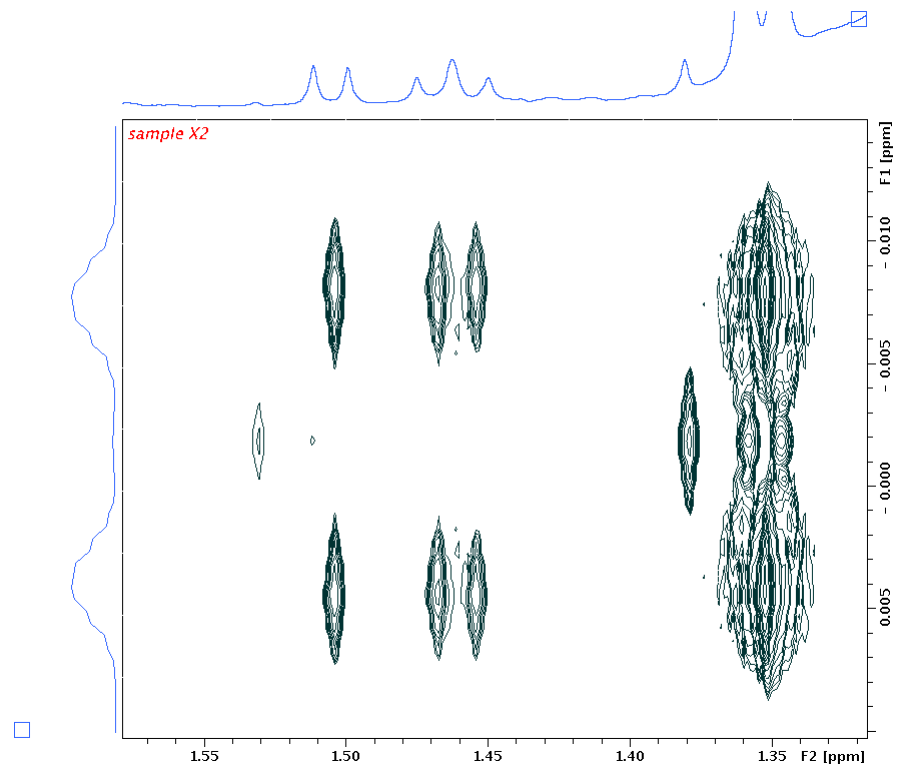
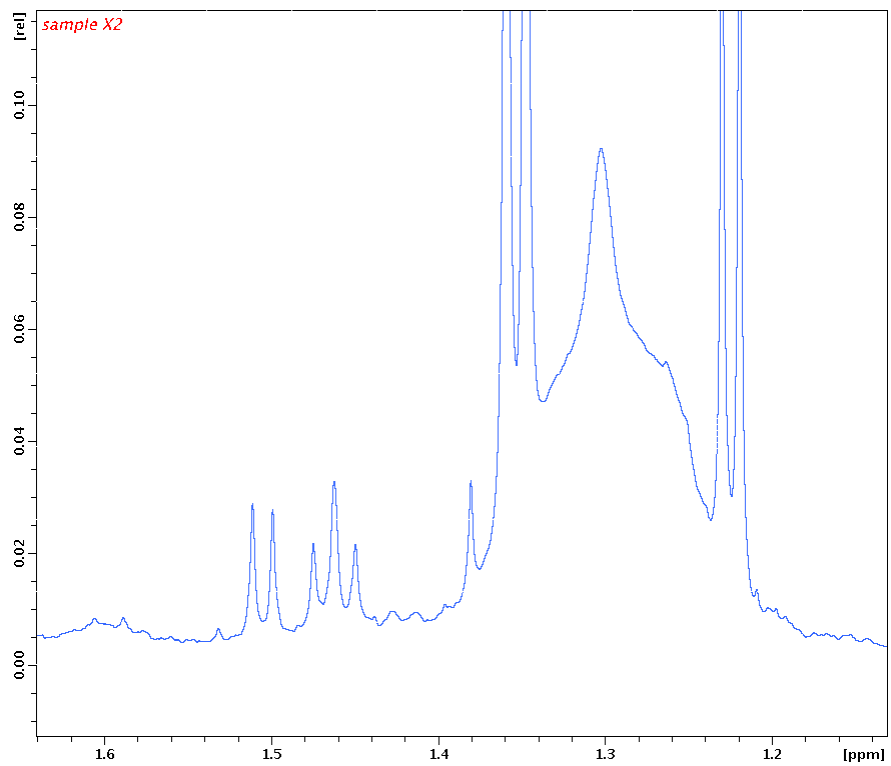
PULPROG	jresgpprqf	Pulse program for acquisition
TD	8192	Time domain size
NS	4	Number of scans
DS	16	Number of dummy scans
SWH [Hz]	10000.00	Sweep width in Hz
AQ [s]	0.4096500	Acquisition time
RG	90.5	Receiver gain
DW [μs]	50.000	Dwell time
DE [μs]	10.00	Pre-scan-delay
d0 [s]	0.00000300	D0=3u
D1 [s]	1.00000000	Relaxation delay, 1-5 * T1
d11 [s]	0.03000000	D11=30m
d12 [s]	0.00002000	D12=20u
D16 [s]	0.00020000	Delay for homospoil/gradient recovery
IND [s]	0.00640000	1/(2 * w), w = max. width of multiplet

**Channel f1**

NUC1	1H	Nucleus for channel 1
P1 [μs]	10.70	F1 channel - 90 degree high power pulse
p2 [μs]	21.39	P2=p1*2
PL1 [dB]	2.00	F1 channel - power level for pulse (default)
PL1W [W]	16.95835686	F1 channel - power level for pulse (default)
PL9 [dB]	61.41	F1 channel - power level for presaturation
PL9W [W]	0.00001941	F1 channel - power level for presaturation
SFO1 [MHz]	600.1128270	Frequency of observe channel
<b>Gradient channel</b>		
GP1 [μs]	SMSQ10.100	SINE.100
GP2 [μs]	SMSQ10.100	SINE.100
GPZ1 [%]	10.00	10%
GPZ2 [%]	10.00	10%
P16 [μs]	1000.00	Homospoil/gradient pulse







Bruker TOPSPIN 2.1 on BH005507 as nmr

File Edit View Spectrometer Processing Analysis Options Window Help

\*2 /2 \*8 /8

Browser Last50 Groups Alias

- dbCH-UrineBufferCheck
- dbCH-UrineNb2D
- dbD2OCheck
- dbFiles\_1\_Lips
- dbFiles\_1
  - 10 - noesygppr1d.comp
  - 11 - jresgpprpf
  - 20 - noesygppr1d.comp
    - 1 - A1
    - 21 - jresgpprpf
      - 1 - A2
    - 30 - noesygppr1d.comp
    - 31 - jresgpprpf
    - 40 - noesygppr1d.comp
    - 41 - jresgpprpf
    - 50 - noesygppr1d.comp
    - 51 - jresgpprpf
    - 60 - noesygppr1d.comp
    - 61 - jresgpprpf
    - 62 - hsqcedetgpcisp2.3
      - 1 - A6
    - 63 - dpsi2phpr
      - 1 - A6
    - 110 - noesygppr1d.comp
      - 1 - B1
    - 111 - jresgpprpf
      - 1 - B1
    - 120 - noesygppr1d.comp
    - 121 - jresgpprpf
    - 130 - noesygppr1d.comp
    - 131 - jresgpprpf
    - 140 - noesygppr1d.comp
    - 141 - jresgpprpf
    - 150 - noesygppr1d.comp
    - 151 - jresgpprpf
    - 160 - noesygppr1d.comp

5 dbFiles\_1 20 1 C:\Bruker\TOPSPIN2\_pl6\nmrsu

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample St

[rel] A2

80

60

40

20

0

10 5 0

AUNM

Acquisition AU program

AUNM au\_prof1d

OK Cancel

au\_prof1d (C:\Bruker\TOPSPIN2\_pl6\explstan\nmr\au\src)

File Edit Search

Compile Execute

```

5 /* AU program for 1D data acquisition. */
6 /******
7 /* Keywords :
8 /* zg,profiling
9 /******
10 /* Description/Usage :
11 /* AU program for data acquisition with baselinefilter.
12 /* The lockphase is optimized, a pulsecalibration is performed.*/
13 /******
14 /* Author(s) :
15 /* Name : Ulrich Braumann
16 /* Organisation : Bruker BioSpin GmbH
17 /* Email : ulrich.braumann@bruker-biospin.de
18 /******
19 /* Name Date Modification:
20 /* eub 070110 created
21 /* rke 070716 save parameters
22 /******
23 /*
24 $Id: au_prof1d,v 1.4 2007/08/20 11:51:46 rke Exp $
25 */
26
27 GETCURDATA
28
29 // optimize lockphase
30 AUTOPHASE;
31
32 // determine 90deg pulse automatically, no display of results
33 // ATTENTION: pulse calibration starts with PROSOL values
34 // current values are IGNORED
35 XCMD("pulsecal fast quiet");
36 XCMD("saveprofpars");
37
38 // run experiment
39 ZG
40
41 QUIT
42
  
```

1 : 1

Bruker TOPSPIN 2.1 on BH005507 as nmr

File Edit View Spectrometer Processing Analysis Options Window Help

2d 3d

5 dbFiles\_1 20 1 C:\Bruker\TOPSPIN2\_pl6\exp\stan\nmr\au\src

Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample

9.77 ppm / 5864.75 Hz  
Index = 16827 - 16931  
Value = 0.01 rel

AUNMP

Processing AU program

AUNMP:  E

OK Cancel

proc\_prof1d (C:\Bruker\TOPSPIN2\_pl6\exp\stan\nmr\au\src)

File Edit Search

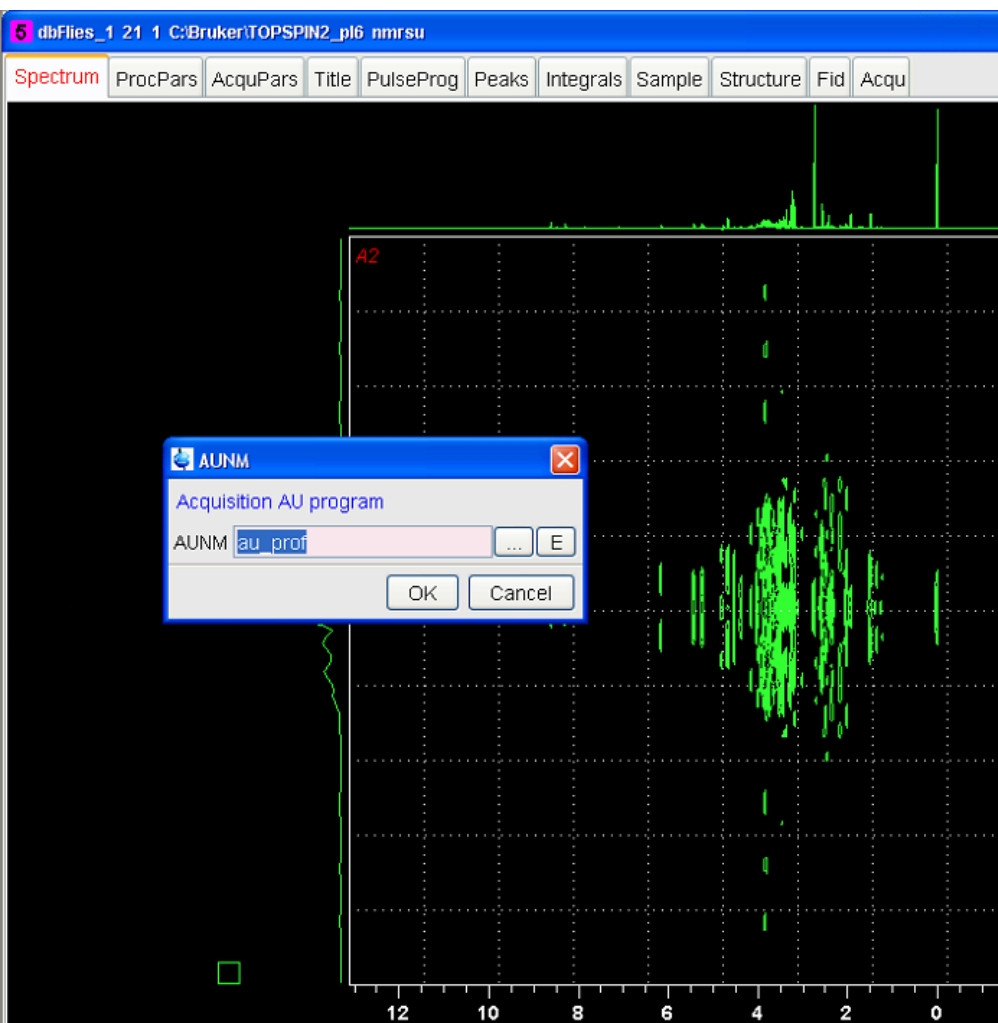
Compile Execute

```

1 /*****/
2 /* proc_prof1d      16.07.2007      */
3 /*****/
4 /* Short Description :      */
5 /* AU program for 1D data processing.      */
6 /*****/
7 /* Keywords :      */
8 /* profiling, processing      */
9 /*****/
10 /* Description/Usage :      */
11 /* AU program for data acquisition with baselinefilter.      */
12 /* The lockphase is optimized, a pulsecalibration is performed.*/
13 /*****/
14 /* Author(s) :      */
15 /* Name           : Ulrich Braumann      */
16 /* Organisation  : Bruker BioSpin GmbH      */
17 /* Email          : ulrich.braumann@bruker-biospin.de      */
18 /*****/
19 /* Name      Date      Modification:      */
20 /* eub       070110   created      */
21 /* rke       070716   save param SR      */
22 /*****/
23 /*
24 $Id: proc_prof1d,v 1.2.2.1 2008/06/16 14:25:17 rke Exp $
25 */
26
27 GETCURDATA
28
29 XCMD ("apk0.noe 1");
30 XCMD ("saveprofpars sr");
31
32 QUIT
33

```

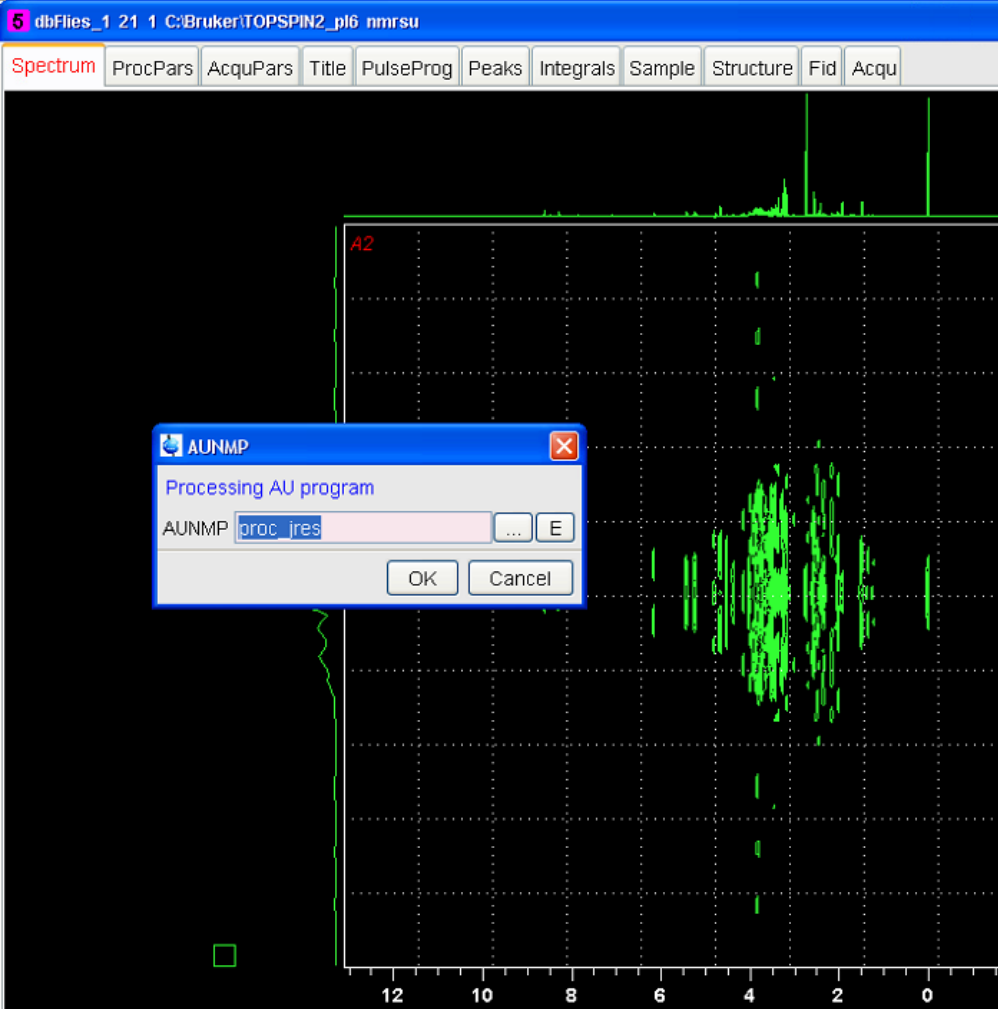
1 : 1



```

au_prof (C:\Bruker\TOPSPIN2_pl6\exp\stan\nmr\au\src)
File Edit Search
Compile Execute

1 /*****
2 /* au_prof          03.04.2007          */
3 /*****
4 /* Short Description :
5 /* AU program for profiler data acquisition.
6 /*****
7 /* Keywords :
8 /* zg,profiling
9 /*****
10 /* Description/Usage :
11 /* Gets P1/P11 from experiment number below current dataset
12 /* RG is also taken, works only for JRES and COSY
13 /*****
14 /* Author(s) :
15 /* Name           : Ulrich Braumann
16 /* Organisation   : Bruker BioSpin
17 /* Email          : ulrich.braumann@bruker-biospin.de
18 /*****
19 /* Name      Date      Modification:
20 /* eub      070305    created
21 /* rke      070403    separation into external AU
22 /*****
23 /*
24 $Id: au_prof,v 1.2 2007/08/20 11:46:44 rke Exp $
25 */
26
27
28 GETCURDATA
29
30 XCMD("getprofpars");
31 ERRORABORT
32
33 // run experiment, no RGA !!
34 ZG
35 ERRORABORT
36
37 QUIT
38
  
```



proc\_jres (C:\Bruker\TOPSPIN2\_pl6\exp\stan\nmr\au\src)

File Edit Search

Compile Execute

```

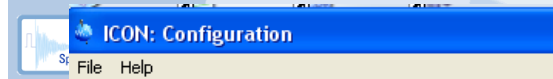
3 /*****/
4 /* Short Description : */
5 /* AU program for data processing of 2D JRES in automation. */
6 /*****/
7 /* Keywords : */
8 /* automation, profiling */
9 /*****/
10 /* Description/Usage : */
11 /* Gets PI from experiment number below current dataset */
12 /*****/
13 /* Author(s) : */
14 /* Name : Ulrich Braumann */
15 /* Organisation : Bruker BioSpin GmbH */
16 /* Email : ulrich.braumann@bruker-biospin.de */
17 /*****/
18 /* Name Date Modification: */
19 /* eub 070309 created */
20 /* rke 070403 calculation of SR changed */
21 /* hasc 091118 ABS1/ABS2 changed */
22 /*****/
23 /*
24 $Id: proc_jres,v 1.3.2.1 2009/11/19 06:46:44 wem Exp $
25 */
26
27 double o1;
28
29 FETCHPARS ("o1", &o1)
30 STOREPAR1 ("SR", o1)
31
32 XFB
33 ERRORABORT
34
35 TILT
36 SYMJ
37 ABS2
38
39 QUIT
40

```

1:1

# Metabolic Profiling Automation





Routine Spectrosc

- User Settings
  - User Manager
  - Composite Experiments**
  - Additional Users
  - Originator Items
- Automation
  - Master Switches
  - Automation Window
  - Lock/Shim Options
    - Solvent/Probe
    - Dependencies
  - Tuning/Matching
  - Priority
  - Temperature Handling
  - LC-NMR Options
  - SampleTrack Options
  - Fail Safe / Error Handling
  - Web Interface
- General Options
- ToolBox Setup
- Accounting

### Composite Experiments

```
NOESY - 1d 1H and NOESY with gradients
Homonuclear_Presaturation - ProtonPR; COSYPR; NOESYPR; TOCSYPR;
Homonuclear - COSY; COSY1r; NOESY;
Full_Set - proton; cosy; cosy1r; qc-dept; bc;
NOESYGPPR - NOESY with presat and Z gradients; by nmrsu
NOESYGPPH - NOESY phase sensitive with Z gradients; by nmrsu
URINE - urine 1H, Jres and SQF1D
Natural_Products - routine
URINE_set - urine 1H and Jres
NOESYESGPPH.sar - Rainer
TEFA_noesygppr1d.comp - noesy for serum
Serum_TEFA - full set of serum experiments
COSY45SW - sw opt. COSY45 (magn. mode)
COSY90SW - sw opt. COSY90 (magn. mode)
COSYGPSW - sw opt. COSY with gradients (magn. mode)
COSYDOPHSW - sw opt. COSY with dg filter (States-TPPI)
```

### Component Experiment Viewer

Experiments	F2 Reference	F1 Reference

### Commands

Status Line



ICON: Configuration

File Help

User Settings

- User Manager
- Composite Experiments
- Additional Users
- Originator Items

Automation

- Master Switches
- Automation Window
- Lock/Shim Options
  - Solvent/Probe Dependencies
- Tuning/Matching
- Priority
- Temperature Handling
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- SampleTrack Options
- Fail Safe / Error Handling
- Web Interface

- General Options
- ToolBox Setup
- Accounting

Users

User ID	User's Full Name
AGROCOS	Sarantos Kostidis
ASPNET	ASP.NET Machine Account
Administrator	
Fokial	Nicolas Fokialakis
Guest	
Halampalaki	Halampalaki Maria
HelpAssistant	Remote Desktop Help Assista
Kostakis	Kostakis Giannis
METC_ICON	METC_ICON
Magiatis	Magiatis Prokopis
Marakos	Marakos Panagiotis
Nectaros	Aligiannis Nectaros
SUPPORT_388945a0	CN=Microsoft Corporation

Experiment List

Mode	Name	Experiment Comment
●	N SAR.13C	13C zg;
●	N db_TissueBC_1D	BCM33 EXON20; 12.2.14
●	N db_TissueBC_JRES	
●	C Full_Set	proton; cosy; cosy1r; qc-dept; bc;
●	C Homonuclear	COSY; COSY1r; NOESY;
●	N db_STD_Tyr	PB1 w 785+1336+144+18 STD wi
●	N TISSUE_BC_AQ_1D	
●	C Homonuclear_Presaturation	ProtonPR; COSYPR; NOESYPR; TC
●	C S.COSY	1D 1H and COSY with gradients
●	C S.COSYPR	1D 1H and COSY with presaturat
●	C S.COSYPR	1D 1H and COSYPR

Permissions

- |                                                      |                                                        |
|------------------------------------------------------|--------------------------------------------------------|
| <input checked="" type="checkbox"/> Priority         | <input checked="" type="checkbox"/> Parameter Edit     |
| <input type="checkbox"/> Archive Data                | <input checked="" type="checkbox"/> Exit (ICON-NMR)    |
| <input checked="" type="checkbox"/> Supervisor       | <input checked="" type="checkbox"/> Data Set Name Edit |
| <input type="checkbox"/> Essential Originator        | <input checked="" type="checkbox"/> Originator         |
| <input checked="" type="checkbox"/> Manual Lock/Shim | <input type="checkbox"/> Mail Spectrum PDF/PS          |
| <input type="checkbox"/> E-mail Notification         | <input type="checkbox"/> JDX DataMail                  |
| <input type="checkbox"/> JDX Copy                    | <input type="checkbox"/> Edit Lock/Shim/ATM            |
| <input type="checkbox"/> ZIP Copy                    | <input type="checkbox"/> ZIP DataMail                  |

Data Set Names

```
$DATE
$DATEUSER
$NUMERICDATE
$HOLDER-%d-%m-%$data(UserName)
%d%m%Y-$data(UserName)
```

Data Directories

User Specific Originator Info

User Specific Parameters/Command

```
ns
td
sw
o1p
d1
```

Spectrum Number Filename



Archiving Directory



Target E-mail Address

Umask for this user

Commands

-





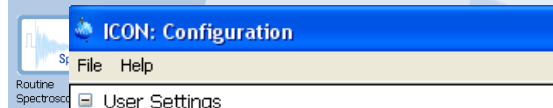
- File Help
- [-] User Settings
  - User Manager
  - Composite Experiments
  - Additional Users
  - Originator Items
- [-] Automation
  - Master Switches
  - Automation Window
  - [-] Lock/Shim Options**
    - Solvent/Probe Dependencies
  - Tuning/Matching
  - Priority
  - Temperature Handling
  - LC-NMR Options
  - SampleTrack Options
  - Fail Safe / Error Handling
  - Web Interface
- General Options
- ToolBox Setup
- Accounting

### Locking

Lock Program    
 Lock only after a solvent change

### Shimming

Shim Program    
 Abort Acquisition on Shim Failure  
Shim the sample    
Shim after this number of experiments    
 Always shim after a QNP change  
Total time allocated for tune command (minutes)



User Settings

- User Manager
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Automation

- Master Switches
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- SampleTrack Options
- Fail Safe / Error Handling
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- Accounting

Solvent/Probe Dependencies

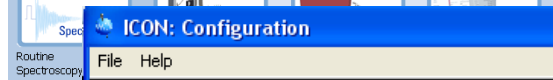
Enable Shim file Loading

Probe Type to associate shim files with  [33]

Copy this shimfile to all entries for this Probe

Use standard shimfiles where available (e.g. Acetic.01) for this probe

No.	Solvent	Comment	Lock	Lock		
			<input checked="" type="checkbox"/>	<input type="checkbox"/>	METC_EXTRACT	
			<input type="checkbox"/>	<input checked="" type="checkbox"/>	dbCellsD2O	TOPSHIM_WITH_AUT
9	Cell+D2O	Cell+D2O	<input type="checkbox"/>	<input checked="" type="checkbox"/>		
10	CH3CN+D2O	HPLC Solvent (Acetonitril/D2O)	<input checked="" type="checkbox"/>	<input type="checkbox"/>		
11	CH3OH+D2O	HPLC Solvent (Methanol/D2O)	<input checked="" type="checkbox"/>	<input type="checkbox"/>		
12	D2O	deuteriumoxide	<input type="checkbox"/>	<input checked="" type="checkbox"/>	db_D2O	TOPSHIM_WITH_AUT
13	DEE	diethylether-d10	<input type="checkbox"/>	<input type="checkbox"/>		
14	Dioxane	dioxane-d8	<input type="checkbox"/>	<input type="checkbox"/>		
15	DME	dimethylether-d6	<input type="checkbox"/>	<input type="checkbox"/>		
16	DMF	dimethylformamide-d7	<input type="checkbox"/>	<input type="checkbox"/>		
17	DMSO	dimethylsulfoxide-d6	<input type="checkbox"/>	<input checked="" type="checkbox"/>	bbi.dms0	
18	DMSO+D2O	DMSO+D2O 9:1	<input type="checkbox"/>	<input checked="" type="checkbox"/>	DMSO+H2O	
19	EtOD	ethanol-d6	<input checked="" type="checkbox"/>	<input type="checkbox"/>		
20	H2O+D2O	90%H2O and 10%D2O	<input type="checkbox"/>	<input type="checkbox"/>	dbSucr-535lh	TOPSHIM_WITH_AUT
21	MeOD	methanol-d4	<input checked="" type="checkbox"/>	<input type="checkbox"/>	dbMeODsolvent	TOPSHIM_WITH_AUT
22	MeOD_AG	methanol-d4 for AGROCOS	<input checked="" type="checkbox"/>	<input type="checkbox"/>	dbMeOD-AG-535lh	TOPSHIM_WITH_AUT
23	None	no Solvent	<input type="checkbox"/>	<input type="checkbox"/>		
24	PLASMA+D2O	Plasma and Serum 50:50 Puffe	<input type="checkbox"/>	<input checked="" type="checkbox"/>	dbPlasma-310K	TOPSHIM_WITH_AUT
25	But	butanol-d5	<input type="checkbox"/>	<input type="checkbox"/>		



- [-] User Settings
  - User Manager
  - Composite Experiments
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  - Originator Items
- [-] Automation
  - Master Switches
  - Automation Window
  - [-] Lock/Shim Options
    - Solvent/Probe Dependencies
    - Tuning/Matching**
  - Priority
  - Temperature Handling
  - LC-NMR Options
  - SampleTrack Options
  - Fail Safe / Error Handling
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- ToolBox Setup
- Accounting

### Automatic Probe Tuning and Matching (ATM) Setup

- Enable ATM Optimization (ATM PROBE INSTALLED)     Use Fast (Coarse) Optimization     Abort Acquisition on ATM Failure

#### Experiment Dependent ATM Optimization Settings

Set individual entries by double-clicking on the appropriate entry or use the Set/Execute combination below

Experiment Name	1H Channel Tuning/Matching	X Channel Tuning/Matching
db_CELLIP_1H	<input checked="" type="checkbox"/> Always	<input type="checkbox"/> Never
db_CELLIP_COSY	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_CELLIP_HSQCed	<input checked="" type="checkbox"/> Always	<input checked="" type="checkbox"/> Always
db_CELLIP_JRES	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_CELLIP_TOCSY	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_cosygppr_TreatAD	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_MK_extrMeOD_1D	<input checked="" type="checkbox"/> Always	<input type="checkbox"/> Never
db_MK_extrMeOD_JRes	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_noesygpprTreatAD	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_PB1_2Dnoe_es	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_PB1STD	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_PB1zges	<input checked="" type="checkbox"/> Always	<input type="checkbox"/> Never
db_PLASMA_CPMG	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_PLASMA_DIFF	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_PLASMA_JRES	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_PLASMA_NOE	<input checked="" type="checkbox"/> Always	<input type="checkbox"/> Never
db_STD_Tyr	<input checked="" type="checkbox"/> Always	<input checked="" type="checkbox"/> Always
db_STDdiffesgp_TreatAD	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_TisKidn_1D	<input checked="" type="checkbox"/> Always	<input type="checkbox"/> Never
db_TisKidn_1H	<input checked="" type="checkbox"/> Always	<input type="checkbox"/> Never
db_TisKidn_HSQC	<input checked="" type="checkbox"/> Always	<input checked="" type="checkbox"/> Always
db_TisKidn_JRes	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_TisKidn_TOCSY	<input type="checkbox"/> Never	<input type="checkbox"/> Never
db_TisLiv_1D	<input checked="" type="checkbox"/> Always	<input type="checkbox"/> Never
db_TisLiv_HSQC	<input checked="" type="checkbox"/> Always	<input checked="" type="checkbox"/> Always
db_TisLiv_JRES	<input type="checkbox"/> Never	<input type="checkbox"/> Never

Set  to all

Can't find your experiment? [Click here](#)

Update Experiment List



- File Help
- ICON: Configuration
  - File Help
  - User Settings
    - User Manager
    - Composite Experiments
    - Additional Users
    - Originator Items
  - Automation
    - Master Switches
    - Automation Window
    - Lock/Shim Options
      - Solvent/Probe Dependencies
    - Tuning/Matching
    - Priority
    - Temperature Handling**
    - LC-NMR Options
    - SampleTrack Options
    - Fail Safe / Error Handling
    - Web Interface
  - General Options
  - ToolBox Setup
  - Accounting

### Temperature Handling

Temperature Handling (On/Off)  
 Valid only for Standard BACS, Manual Mode, LC, MAS and SampleJet  
(Post Insertion available on SixPack and NMR Case)

#### PRE INSERTION Set/Check

Temperature Setting before Sample Insertion

Set & Check Temperature before Sample Insertion

Pre-Insertion Temperature Set/Check Routine

#### POST INSERTION Set/Check

Set & Check Temperature after Sample Insertion

Temperature Setting after Sample Insertion

Post-Insertion Temperature Set/Check Routine

Save

Do Acquisition

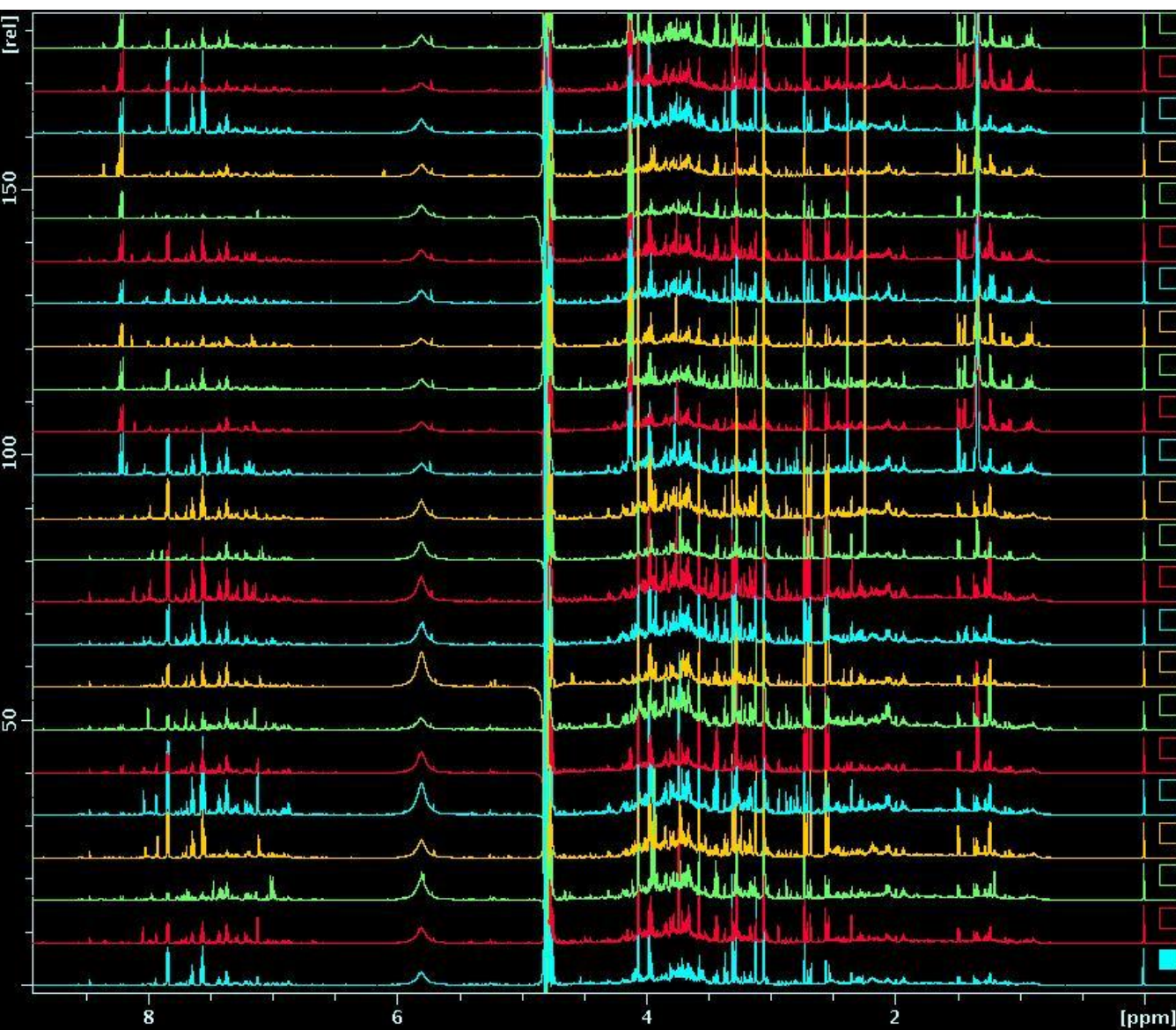
Holder	Type	Status	Disk	Name	No.	Solvent	Experiment	Par	Title / Orig	Pri	Time	User
		Queued	C:\Bruker\TOPSPIN2_pl6	TASCMAR	520	MeOD_AG	AGRO_Fractions-1D		XML-190 in Methan noesypr1d		00:28:39	nmrsu
		Queued	C:\Bruker\TOPSPIN2_pl6	TASCMAR	521	MeOD_AG	AGRO_Fractions-J-res		XML-190 in Methan jres-presat, artefac		00:08:32	nmrsu
37		Queued	C:\Bruker\TOPSPIN2_pl6	TASCMAR	330	MeOD_AG	AGRO_Fractions-1D		XML-254 in Methan noesypr1d		00:28:39	nmrsu
		Queued	C:\Bruker\TOPSPIN2_pl6	TASCMAR	331	MeOD_AG	AGRO_Fractions-J-res		XML-254 in Methan jres-presat, artefac		00:08:32	nmrsu
38		Queued	C:\Bruker\TOPSPIN2_pl6	TASCMAR	140	MeOD_AG	AGRO_Fractions-1D		XML-137 in Methan noesypr1d		00:28:39	nmrsu
		Queued	C:\Bruker\TOPSPIN2_pl6	TASCMAR	141	MeOD_AG	AGRO_Fractions-J-res		XML-137 in Methan jres-presat, artefac		00:08:32	nmrsu
39		Queued	C:\Bruker\TOPSPIN2_pl6	TASCMAR	530	MeOD_AG	AGRO_Fractions-1D		XML-199 in Methan noesypr1d		00:28:39	nmrsu
		Queued	C:\Bruker\TOPSPIN2_pl6	TASCMAR	531	MeOD_AG	AGRO_Fractions-J-res		XML-199 in Methan jres-presat, artefac		00:08:32	nmrsu
40		Queued										

Change User

Preceding Experiments

#	Date	Holder	Name	No.	Experiment	Load	ATM	R	Lock	Shim	Acq	Proc	User	Disk	Title /
52	2016-04-03 11:00:34	26	TASCMAR	100	AGRO_Fractions-1D	✓	✓		✓	✓			nmrsu	C:\Bruker\TOPSPIN2_pl6	XML-1-Methan AV600
51	2016-04-03 10:51:48	25	TASCMAR	291	AGRO_Fractions-J-res						✓	✓	nmrsu	C:\Bruker\TOPSPIN2_pl6	XML-2-Methan AV600 artefac
50	2016-04-03 10:15:00	25	TASCMAR	290	AGRO_Fractions-1D	✓	✓		✓	✓	✓	✓	nmrsu	C:\Bruker\TOPSPIN2_pl6	XML-2-Methan AV600
49	2016-04-03 10:06:15	24	TASCMAR	481	AGRO_Fractions-J-res						✓	✓	nmrsu	C:\Bruker\TOPSPIN2_pl6	XML-9-Methan AV600 artefac
48	2016-04-03 09:29:35	24	TASCMAR	480	AGRO_Fractions-1D	✓	✓		✓	✓	✓	✓	nmrsu	C:\Bruker\TOPSPIN2_pl6	XML-9-Methan AV600
47	2016-04-03 09:20:49	23	TASCMAR	91	AGRO_Fractions-J-res						✓	✓	nmrsu	C:\Bruker\TOPSPIN2_pl6	XML-1-Methan AV600 artefac

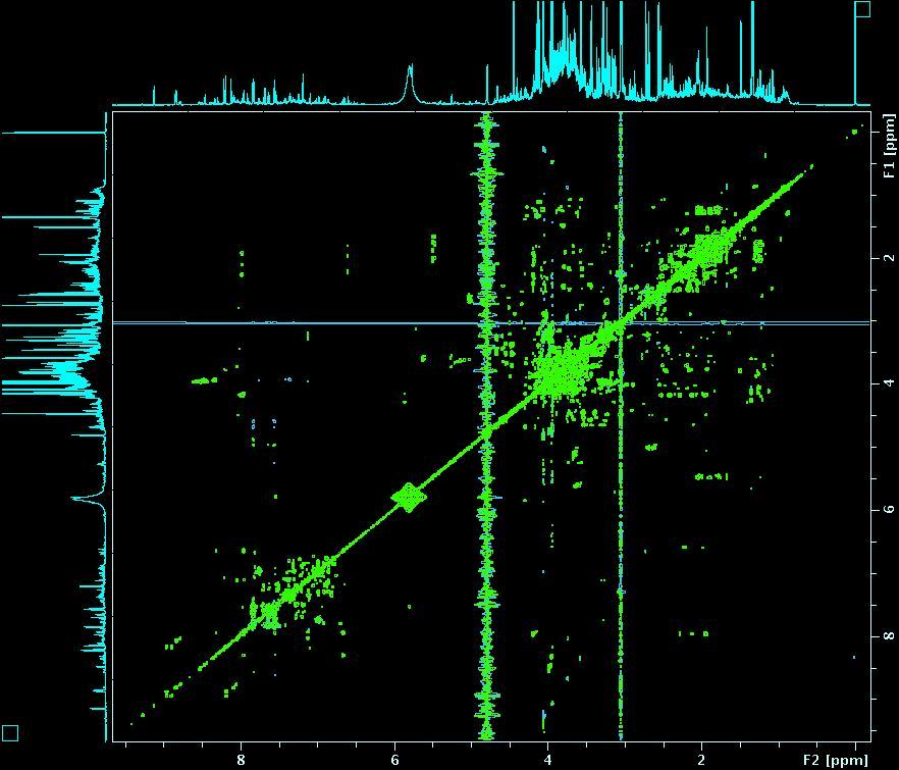
## Resulting 1D NOESY spectra of urine



✓ Calibrated to TSP  
signal at  $\delta$  0.0 ppm

✓ Phase and Baseline  
corrected





600MHz 2D TOCSY

## Assignment process

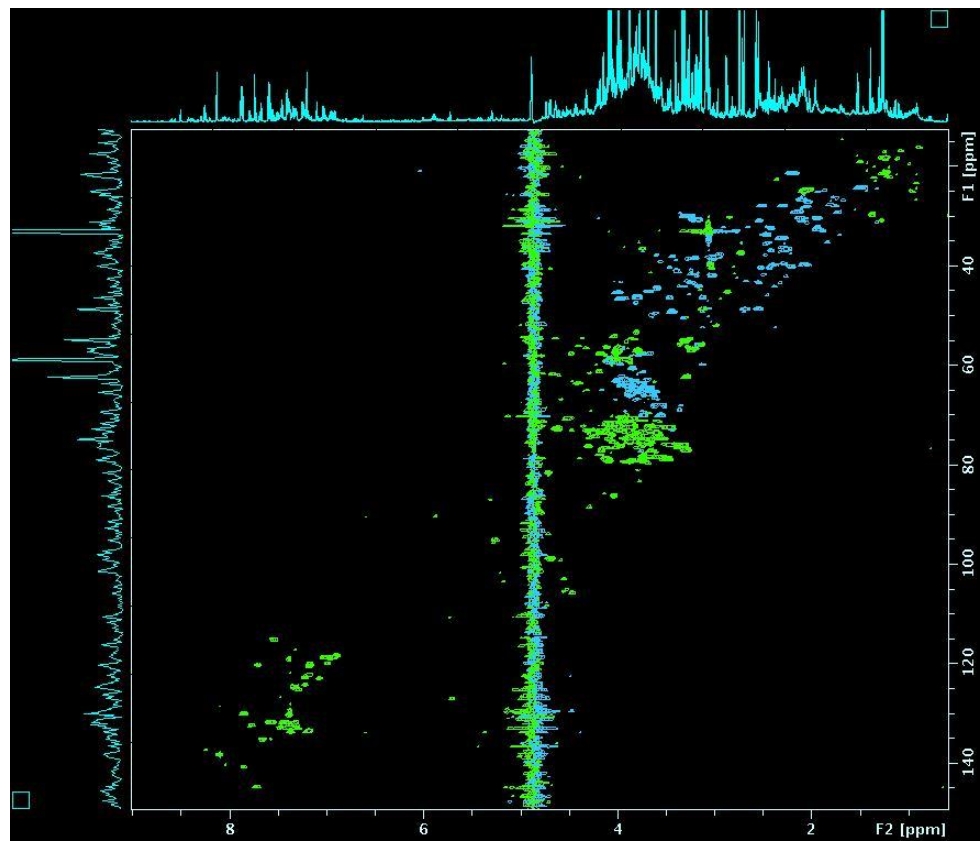
2D spectra

Literature and web databases

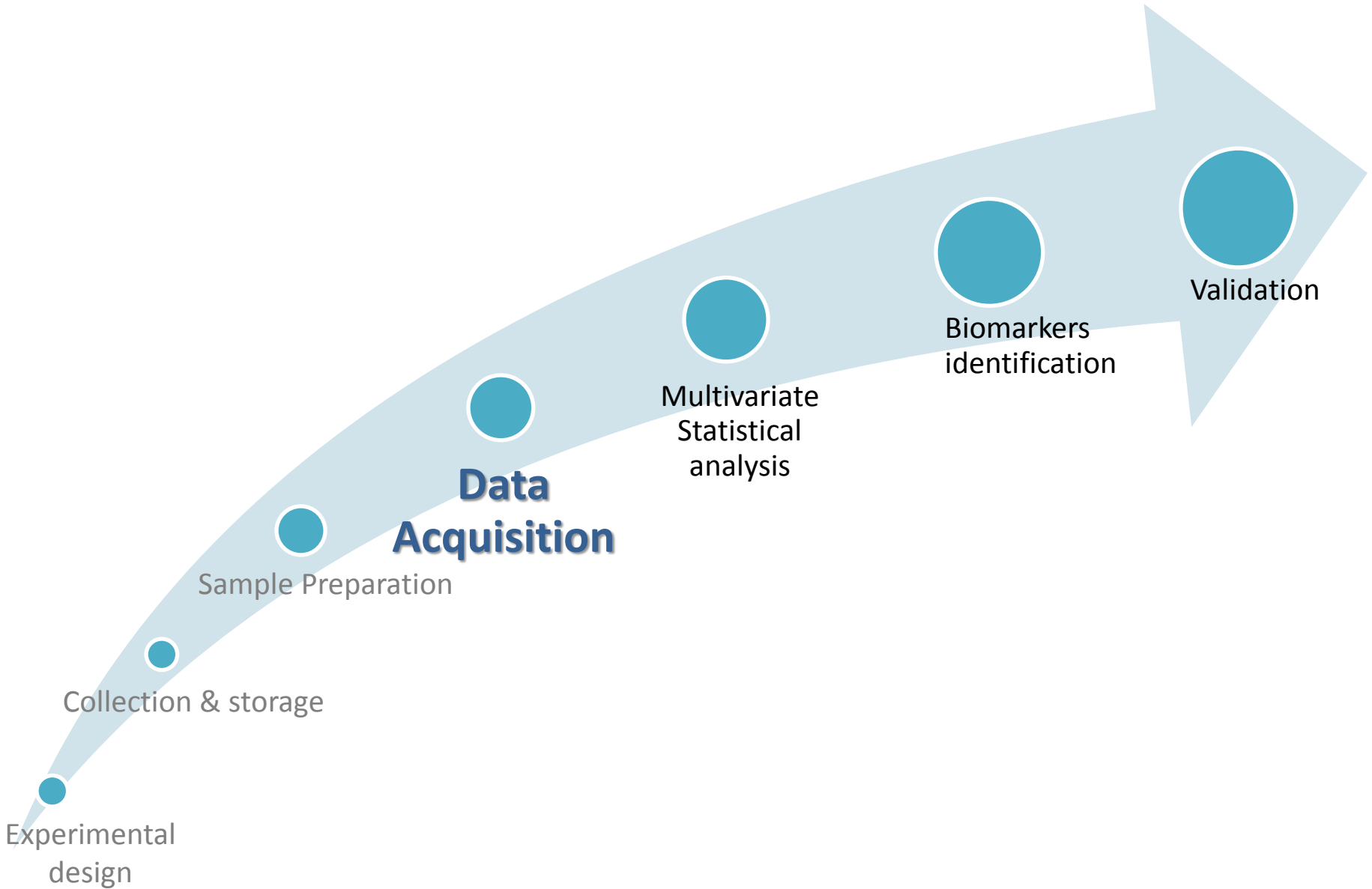
(HMDB, BMRB)

Chenomx

600MHz  $^1\text{H}$ - $^{13}\text{C}$  DEPT135-HSQC



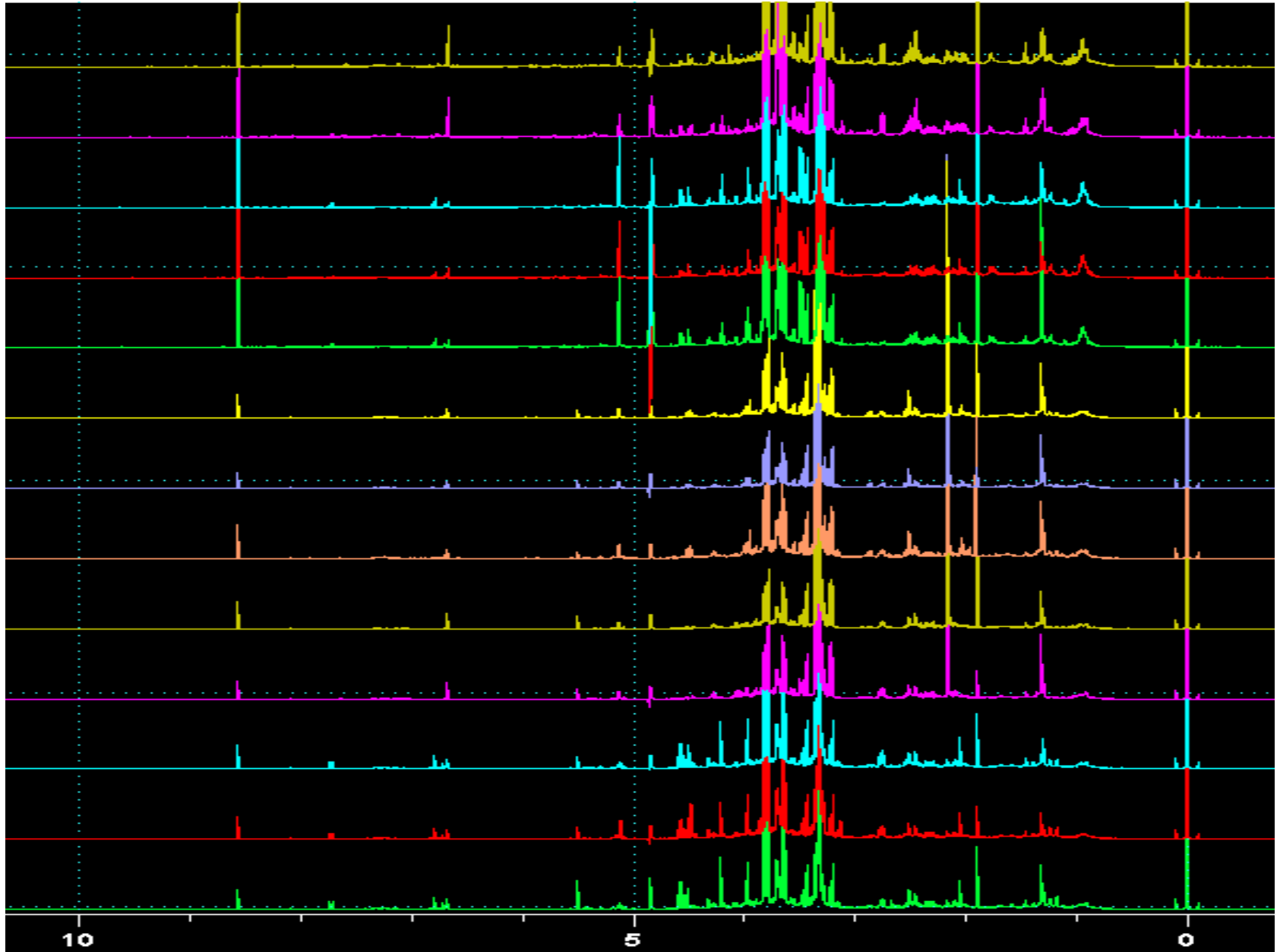
# Metabolomics Workflow





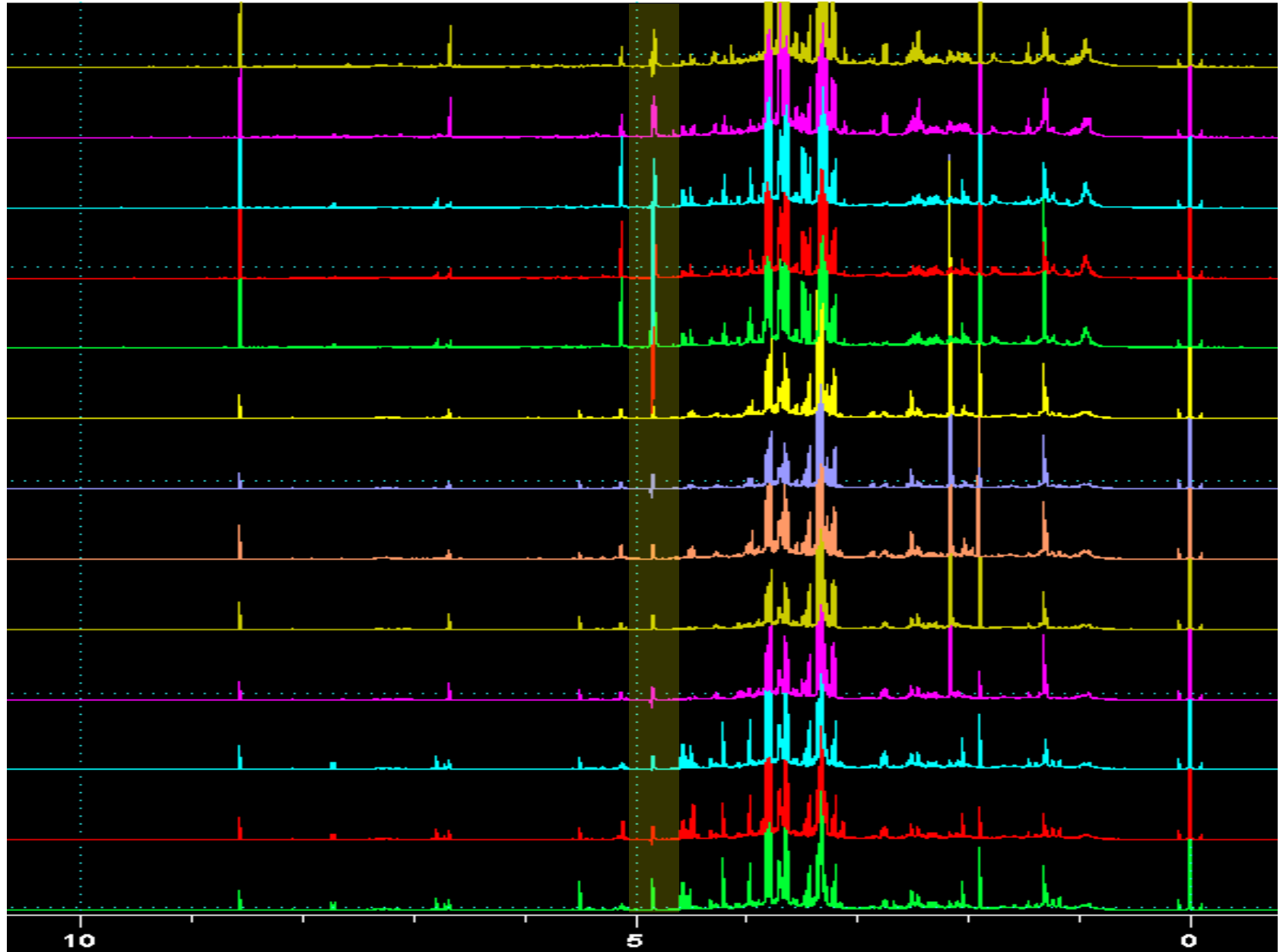
# Batch Effects

Cladobotryum genus species



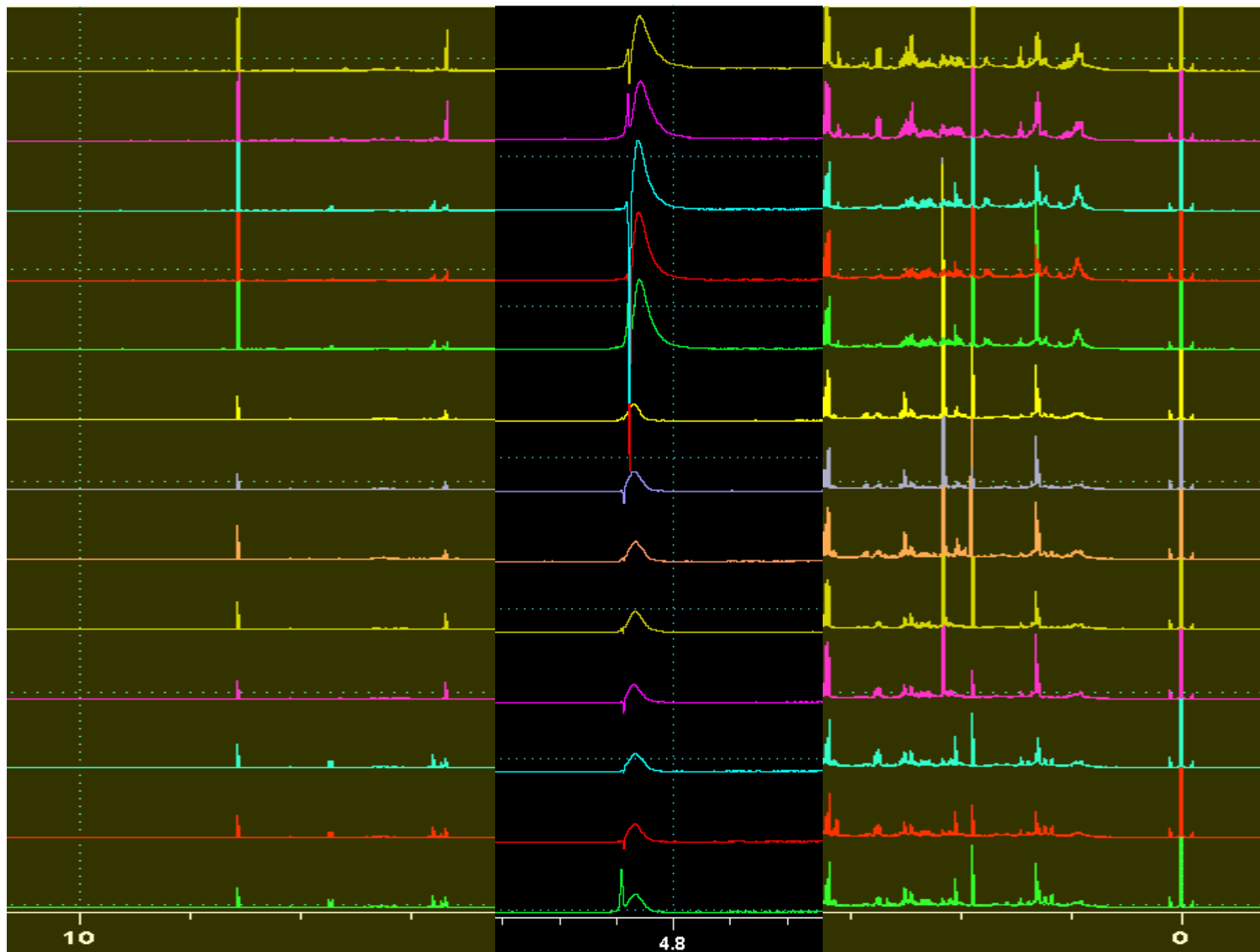
# Batch Effects

Cladobotryum genus species



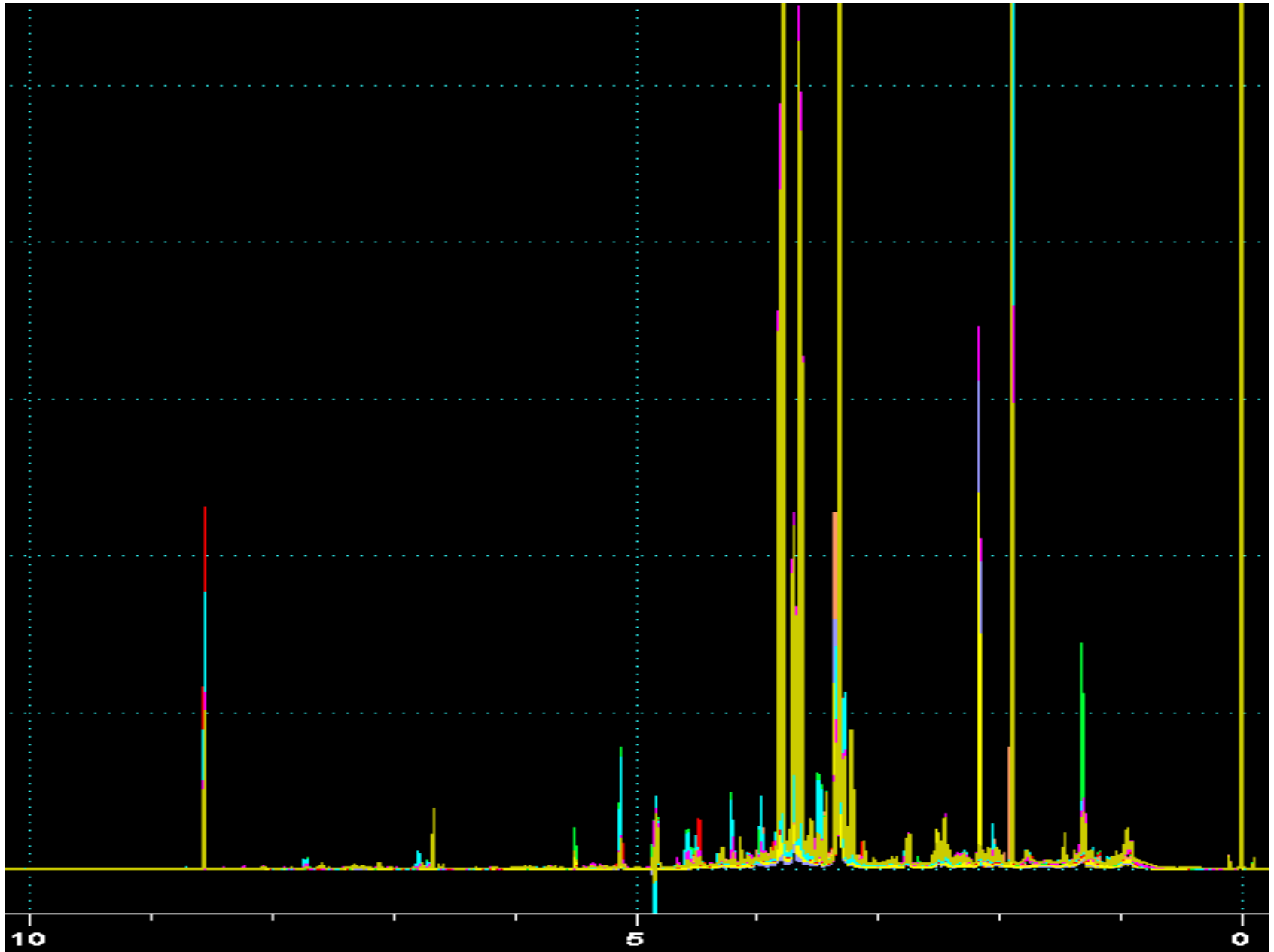
# Batch Effects

Cladobotryum genus species



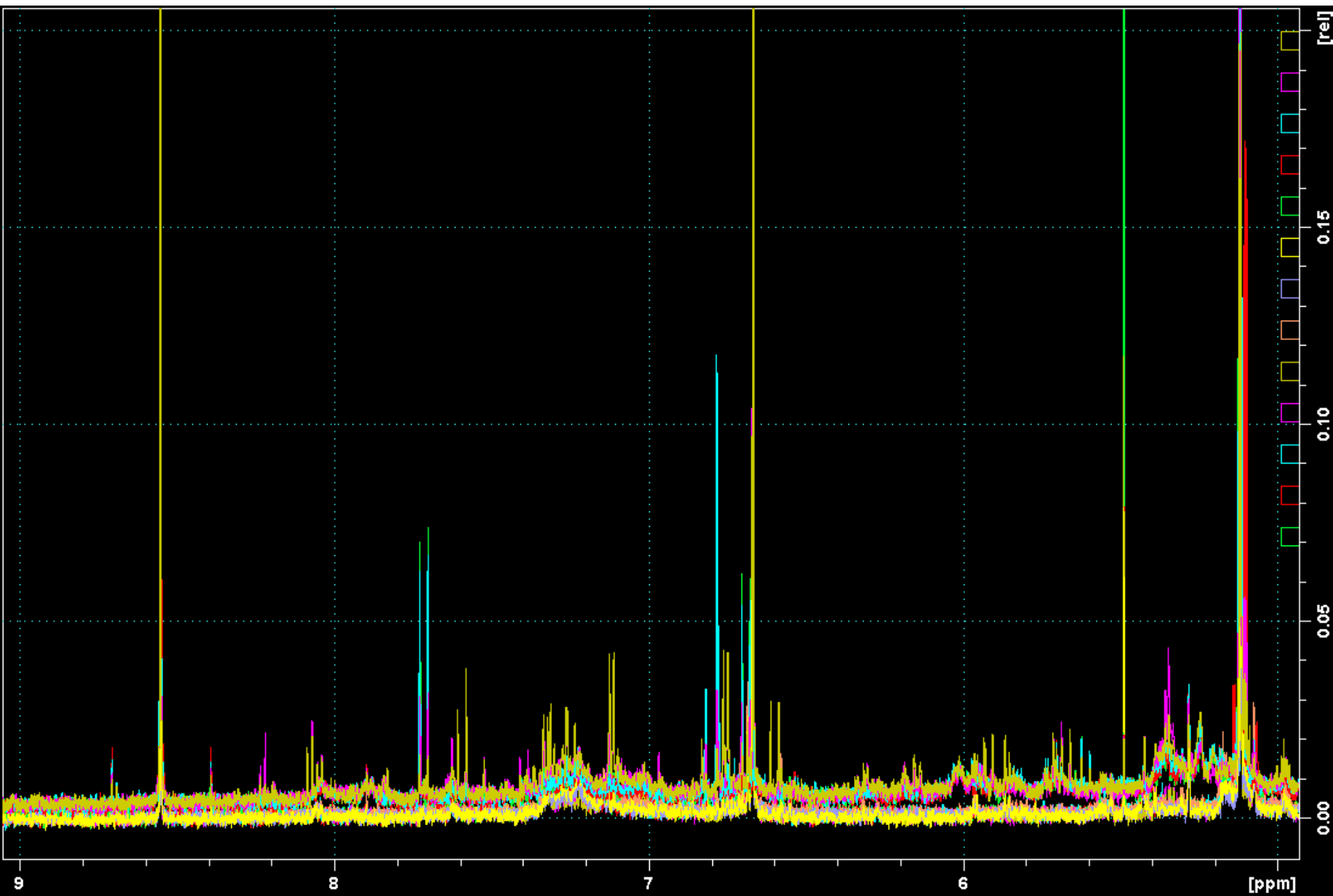
# Batch Effects

Cladobotryum genus species



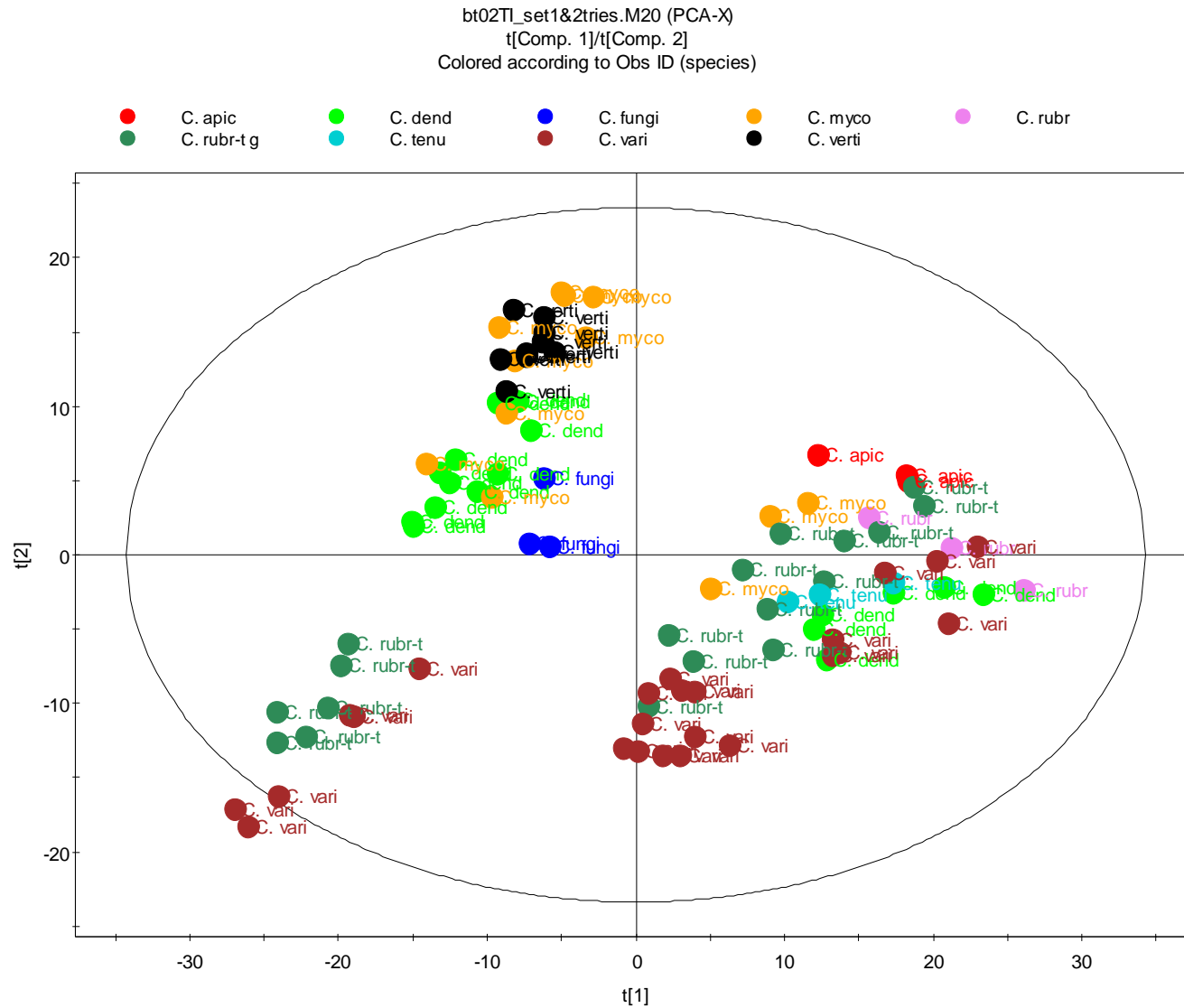
# Batch Effects

Cladobotryum genus species



# Batch Effects

## Cladobotryum genus species

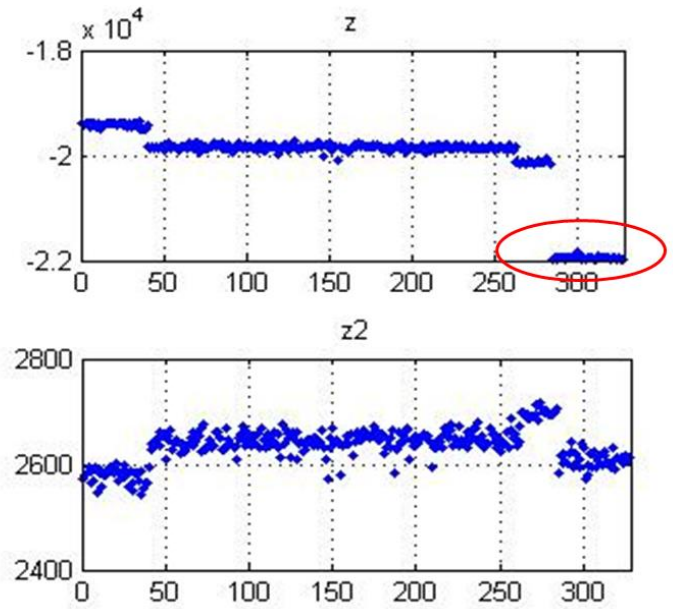
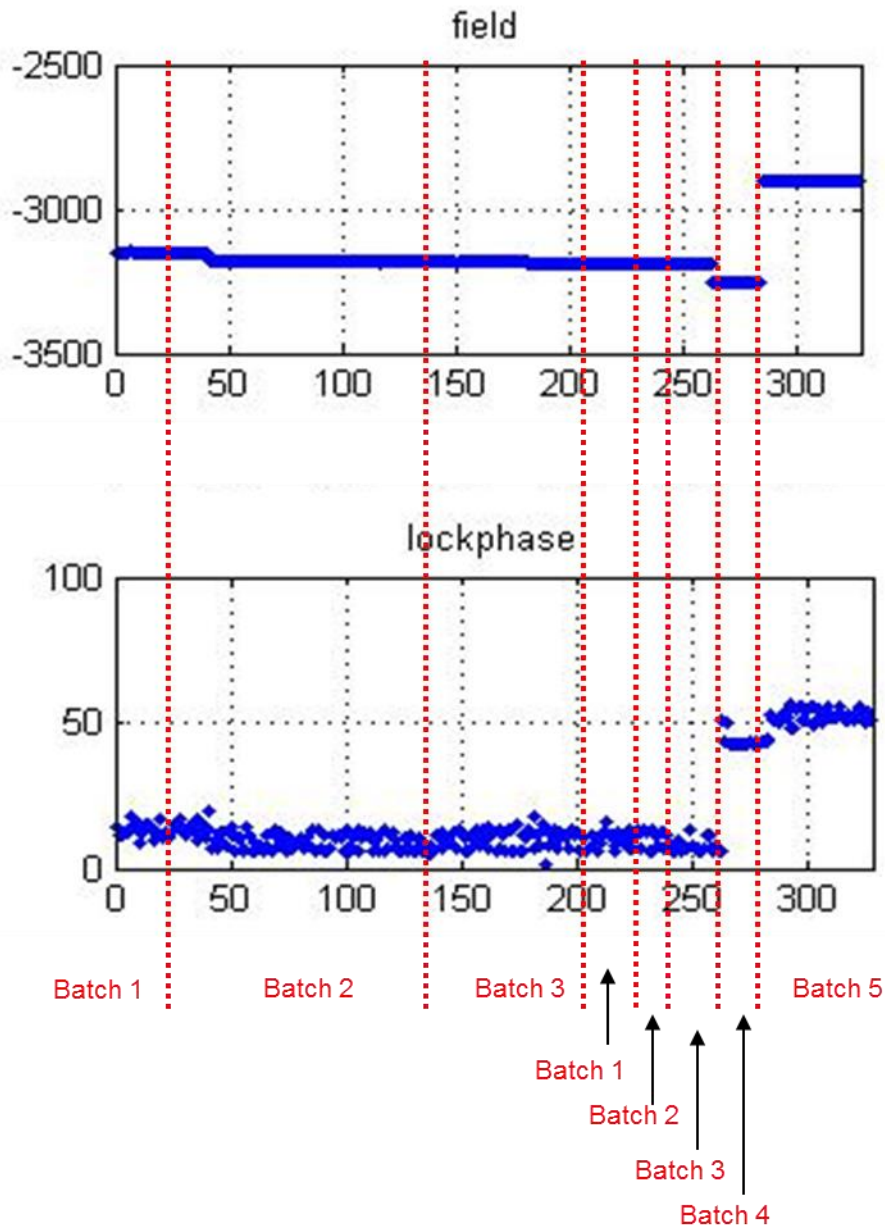


R2X[1] = 0.426144

R2X[2] = 0.198183

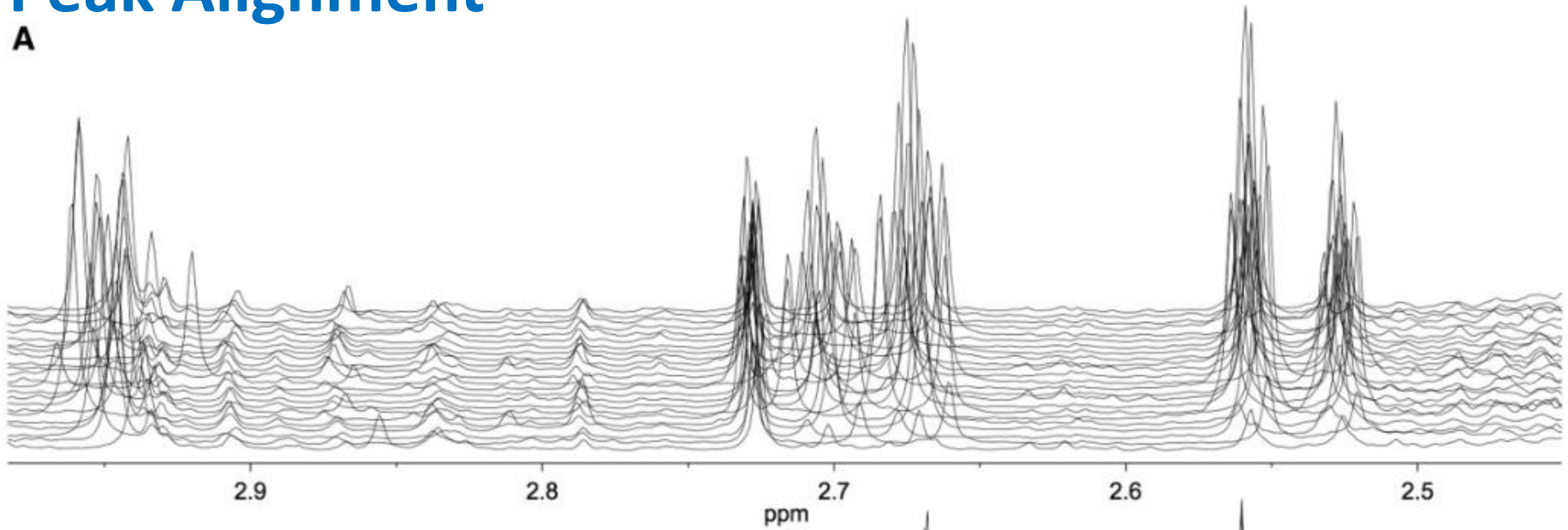
Ellipse: Hotelling T2 (0.95)

# Batch Effects

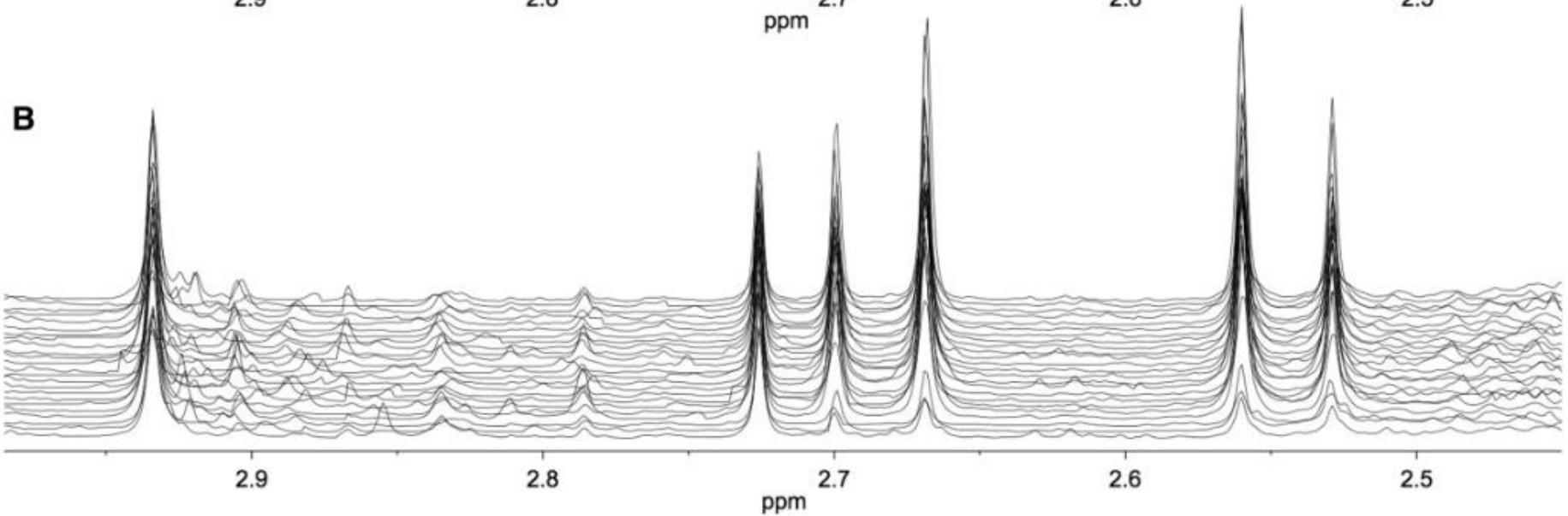


# Peak Alignment

**A**



**B**

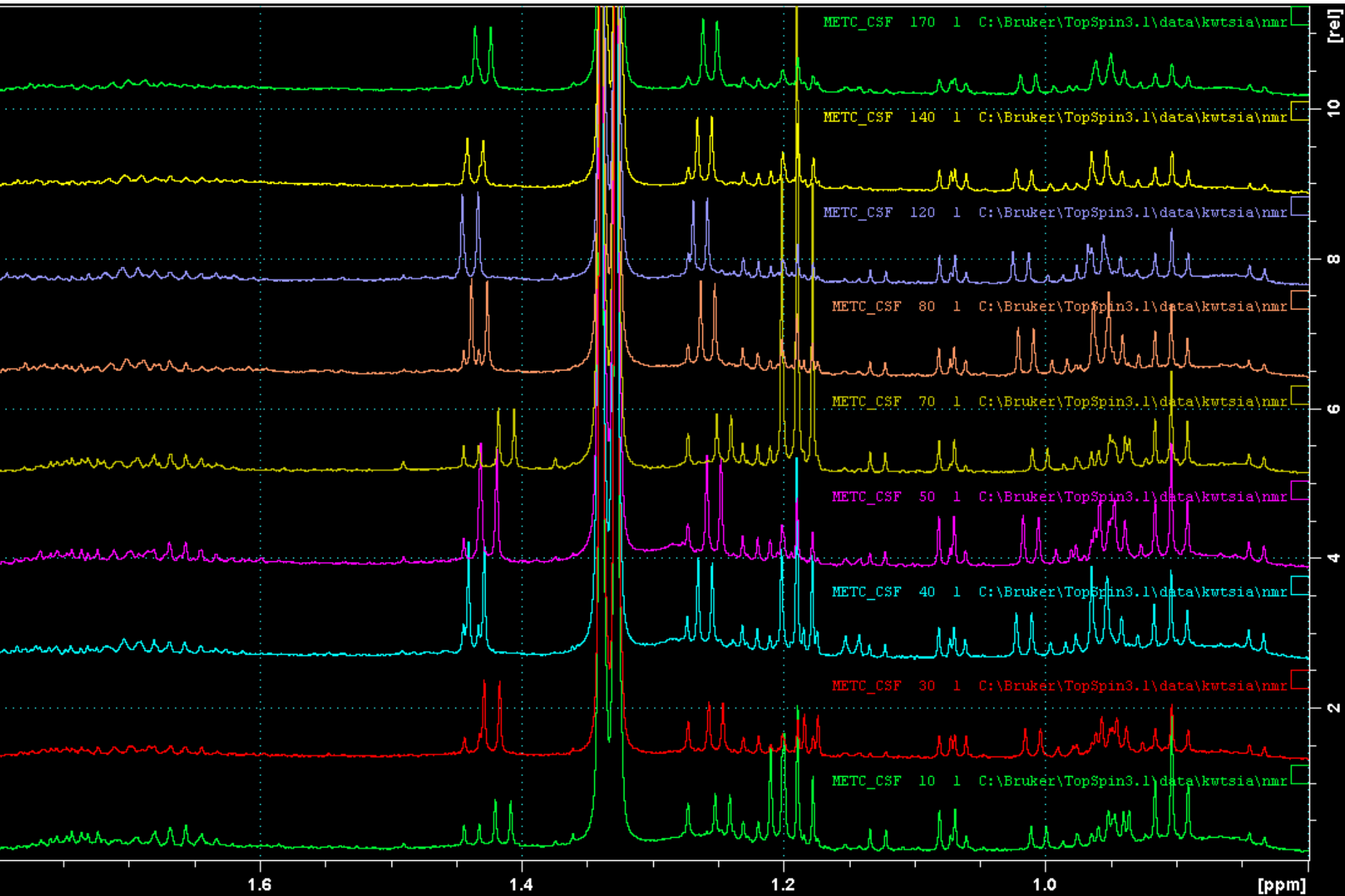


**Figure 2.** Alignment of NMR spectra is critical for the comparison of the data. (A) Collection of 1D NMR spectra corresponding to a set of urine samples; (B) same set of NMR spectra after the application of speaq [20]. The application of this bioinformatics tool translates into a better alignment of the spectra, thus overcoming the impact of chemical and physical variations on the chemical shifts of the metabolites present in those samples.



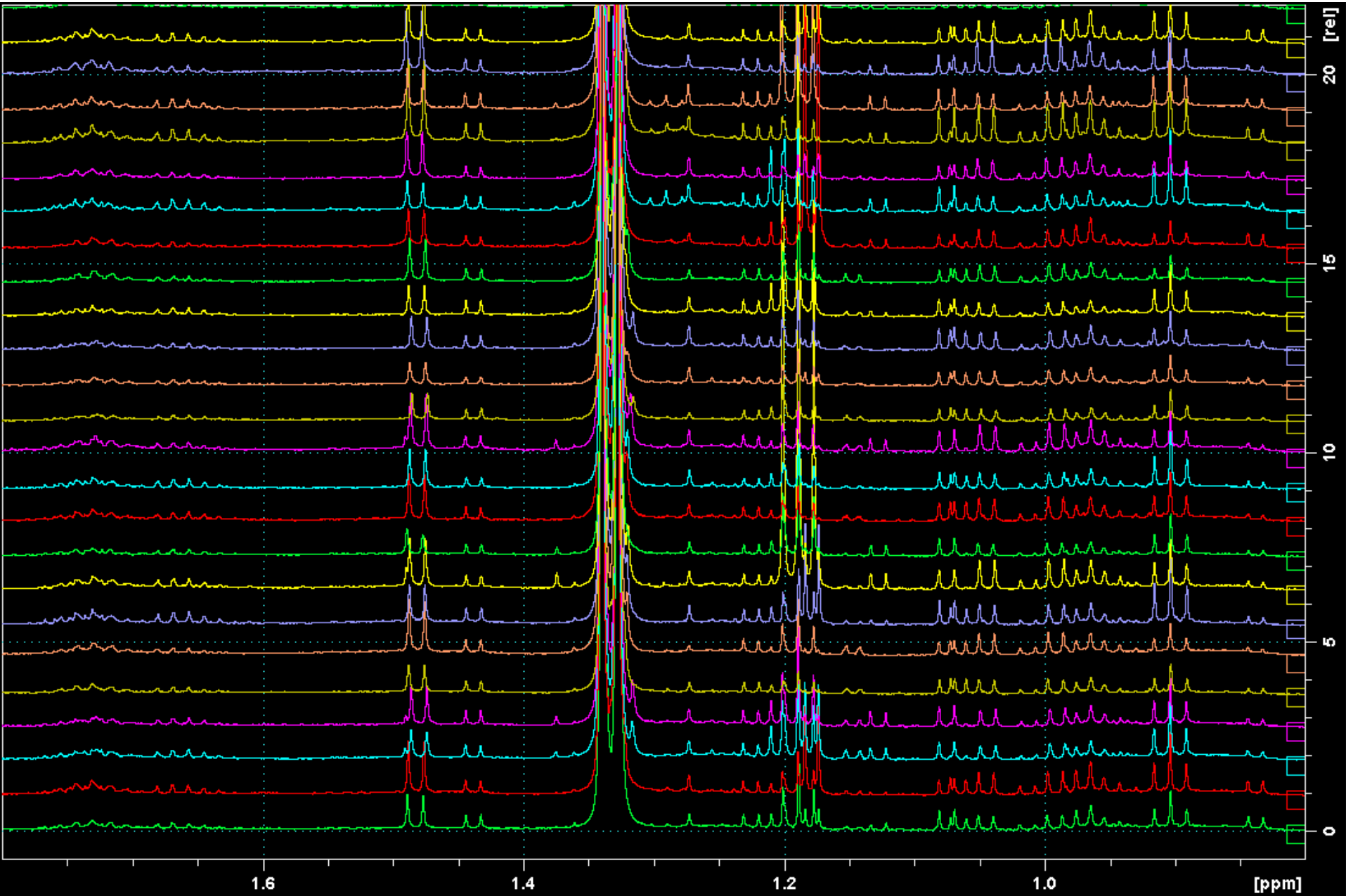
# Peak Alignment

CSF, MS patients

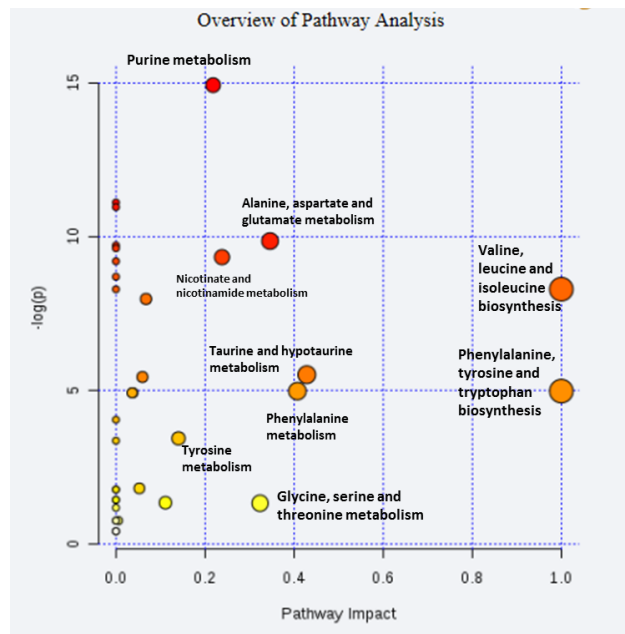
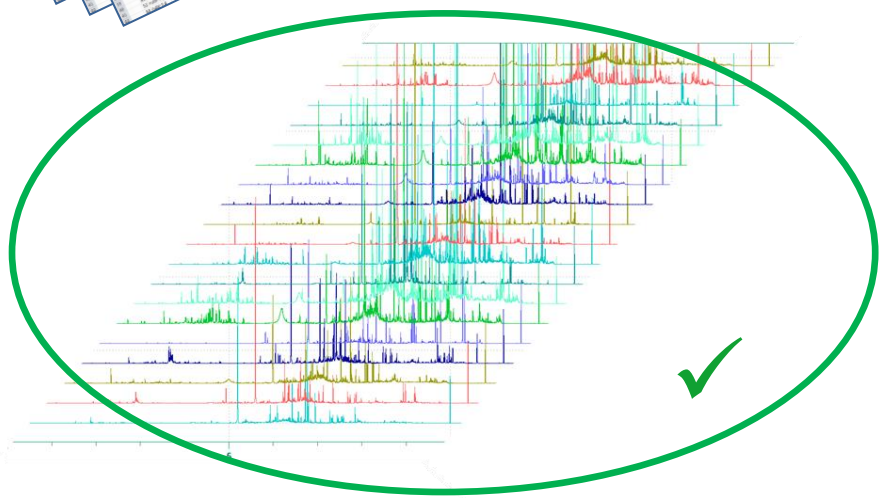
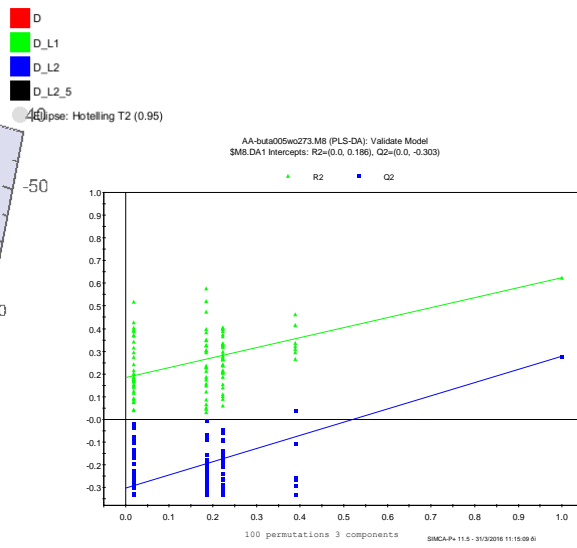
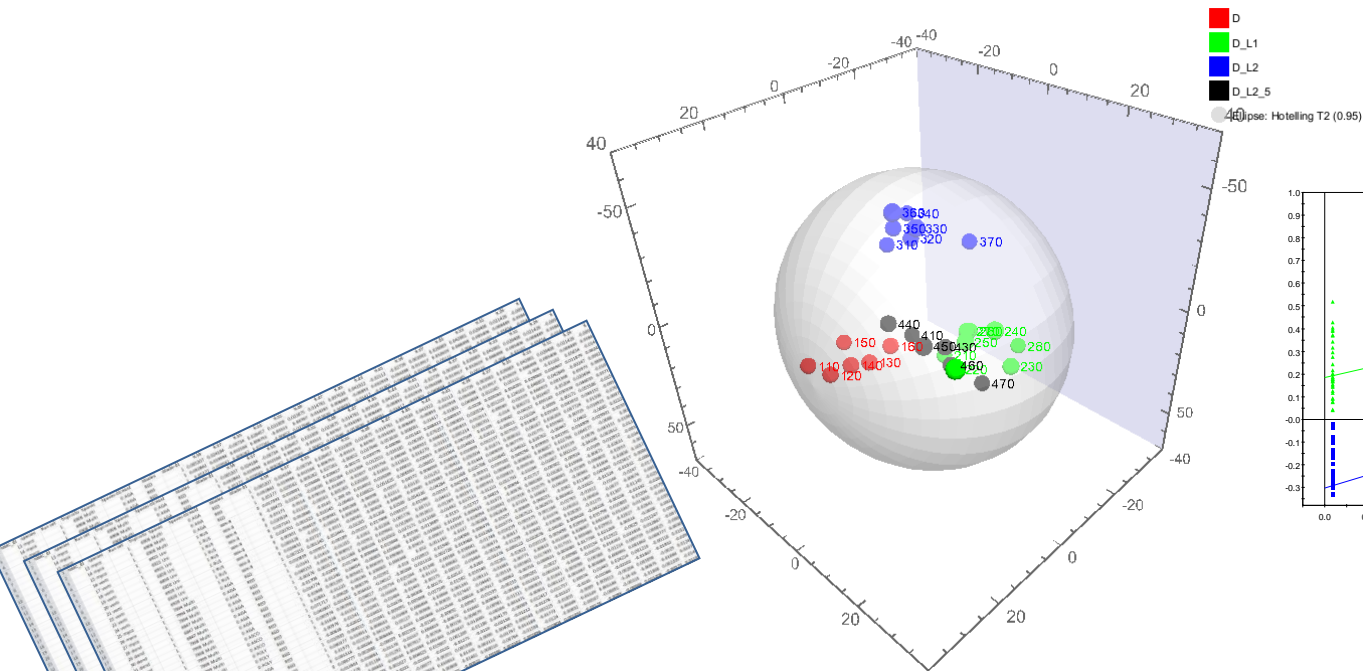


# Peak Alignment

CSF, MS patients









# Heterocovariance based metabolomics

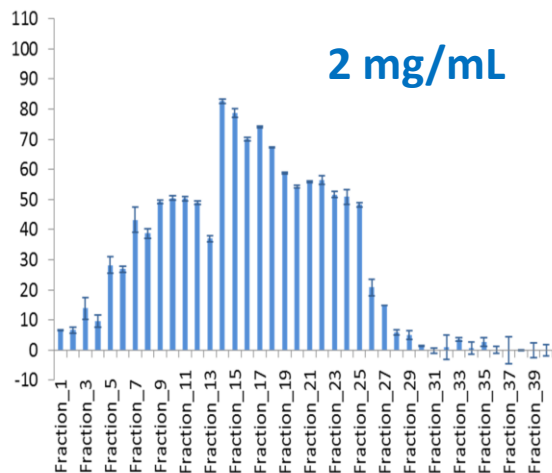
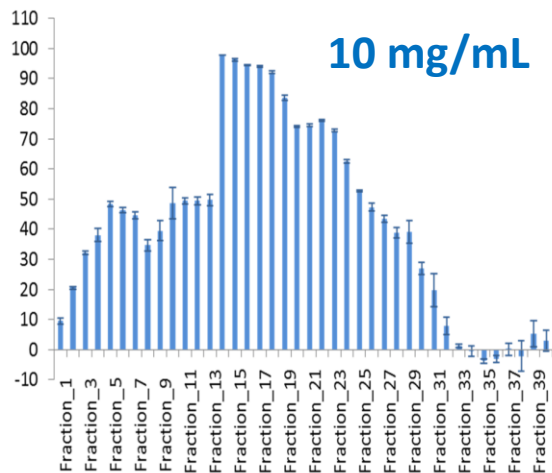
a powerful tool accelerating bioactive natural products identification



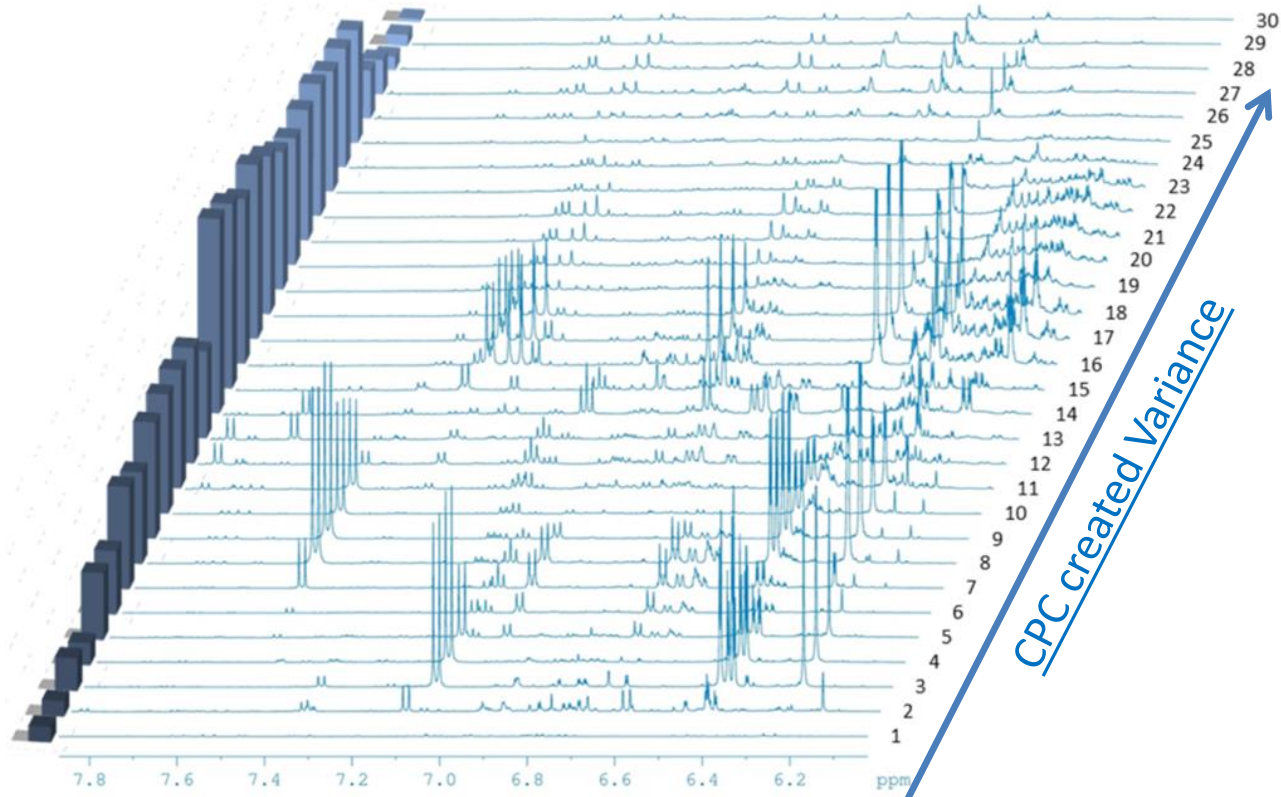
*Morus alba* case

# Heterocovariance based metabolomics

✓ Tyrosinase Inhibition Activity



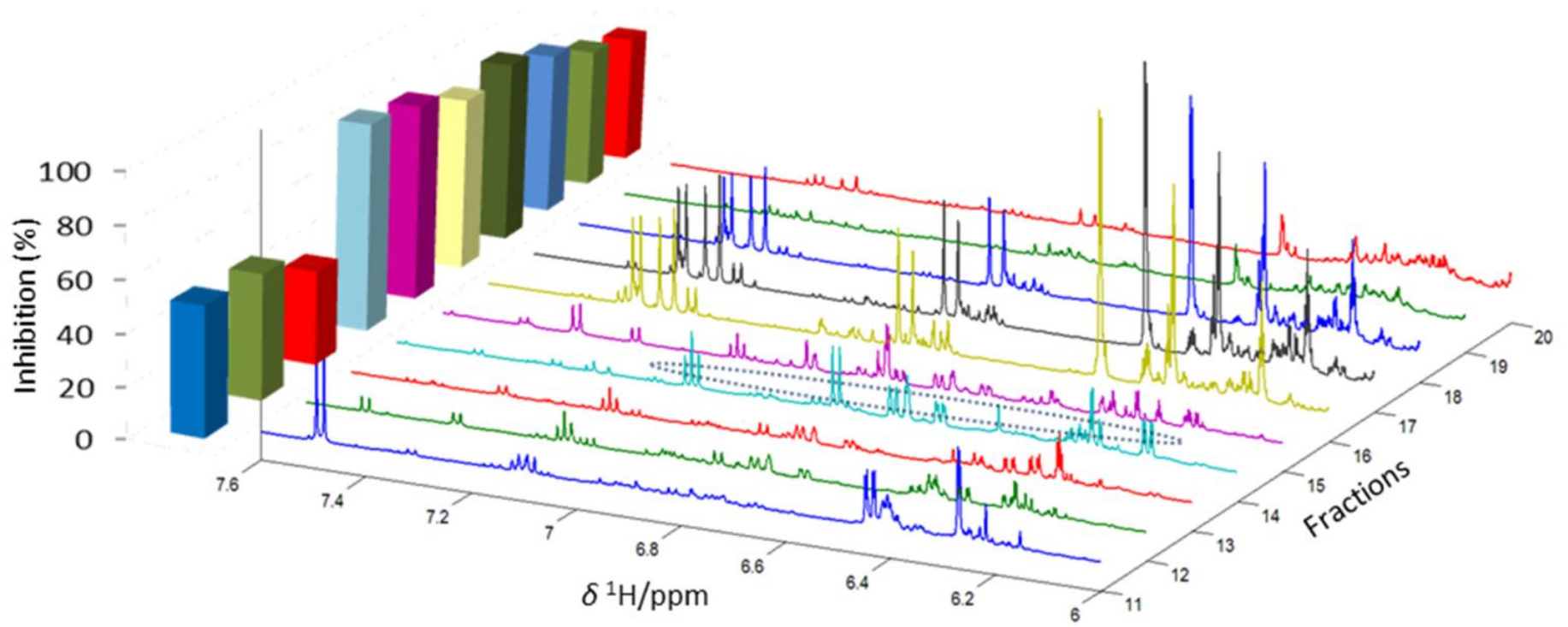
✓ <sup>1</sup>H NMR profile of *Morus alba* fractions 1-30



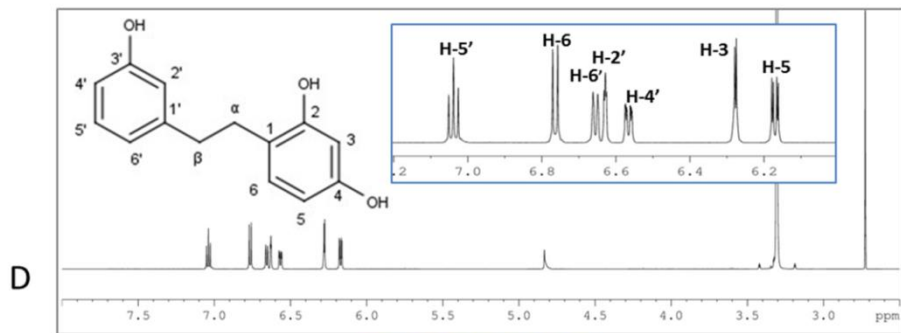
30 fractions from *Morus alba* by CPC



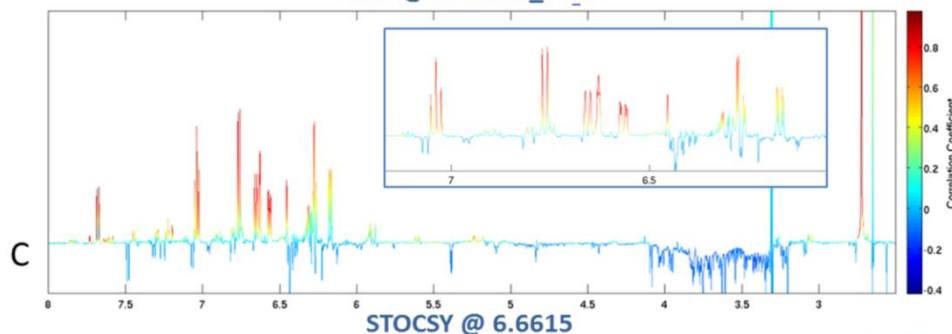
# Heterocovariance based metabolomics



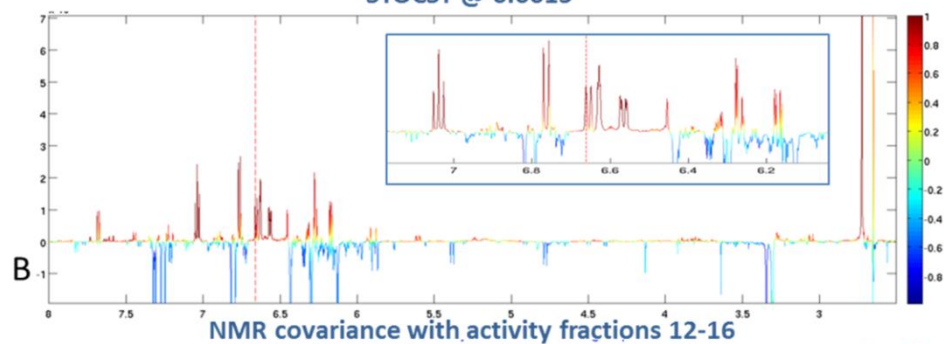
$^1\text{H}$  NMR spectrum of purified **2,4,3'-trihydroxydihydrostilbene**



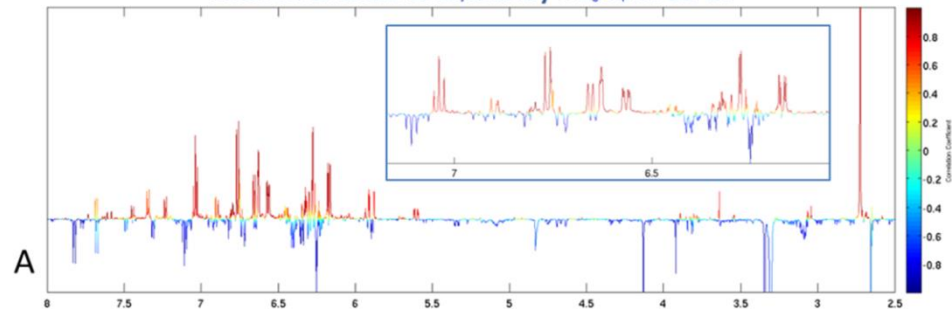
SHY plot  
correlation of mass at  $m/z$  229.086  
at RT = 7.89 min and NMR



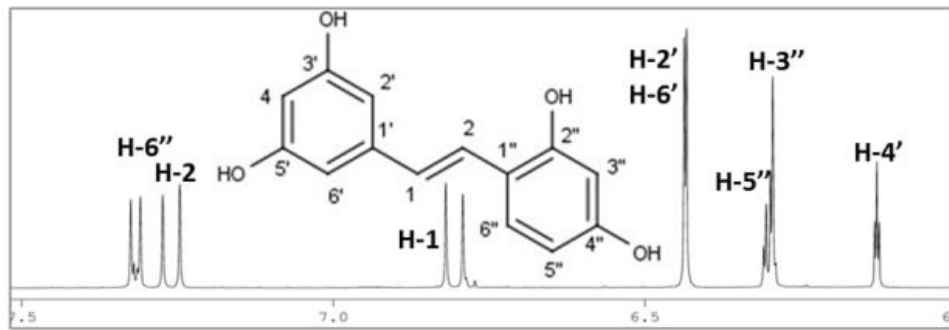
STOCSY  
NMR peak correlation



HETCA  
covariance of biological activity  
with corresponding NMR data

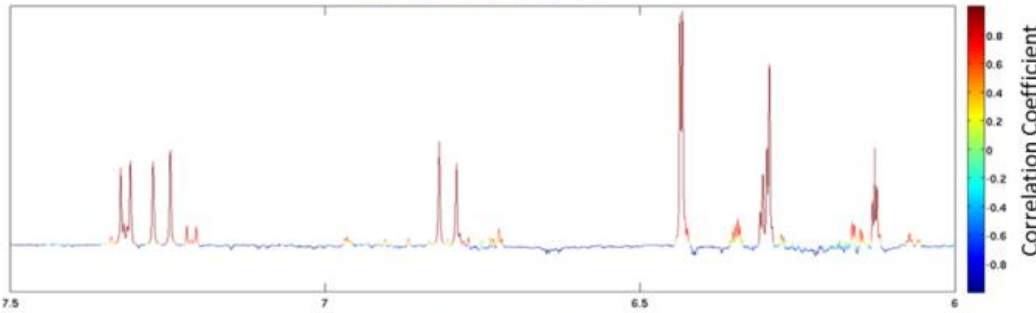






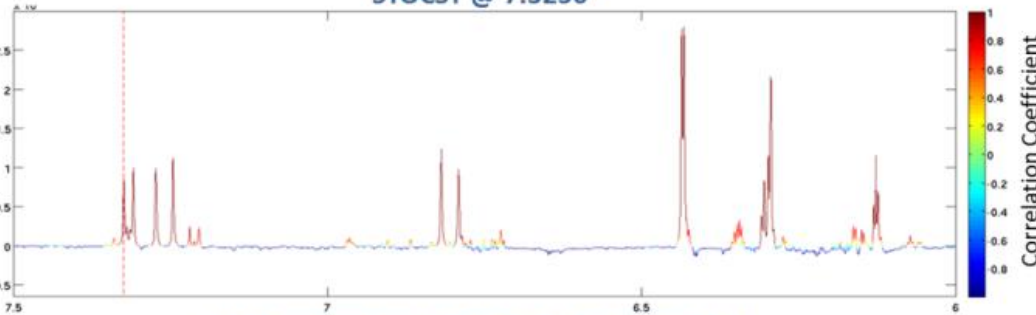
<sup>1</sup>H NMR spectrum of purified oxyresveratrol

SHY @ 243.066\_7.70



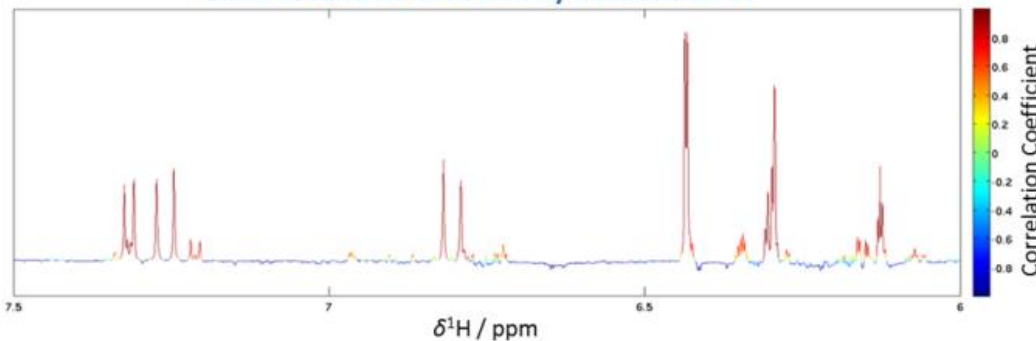
SHY plot  
correlation of mass at m/z 243.066  
at RT = 7.70 min and NMR

STOCSY @ 7.3250



STOCSY  
NMR peak correlation

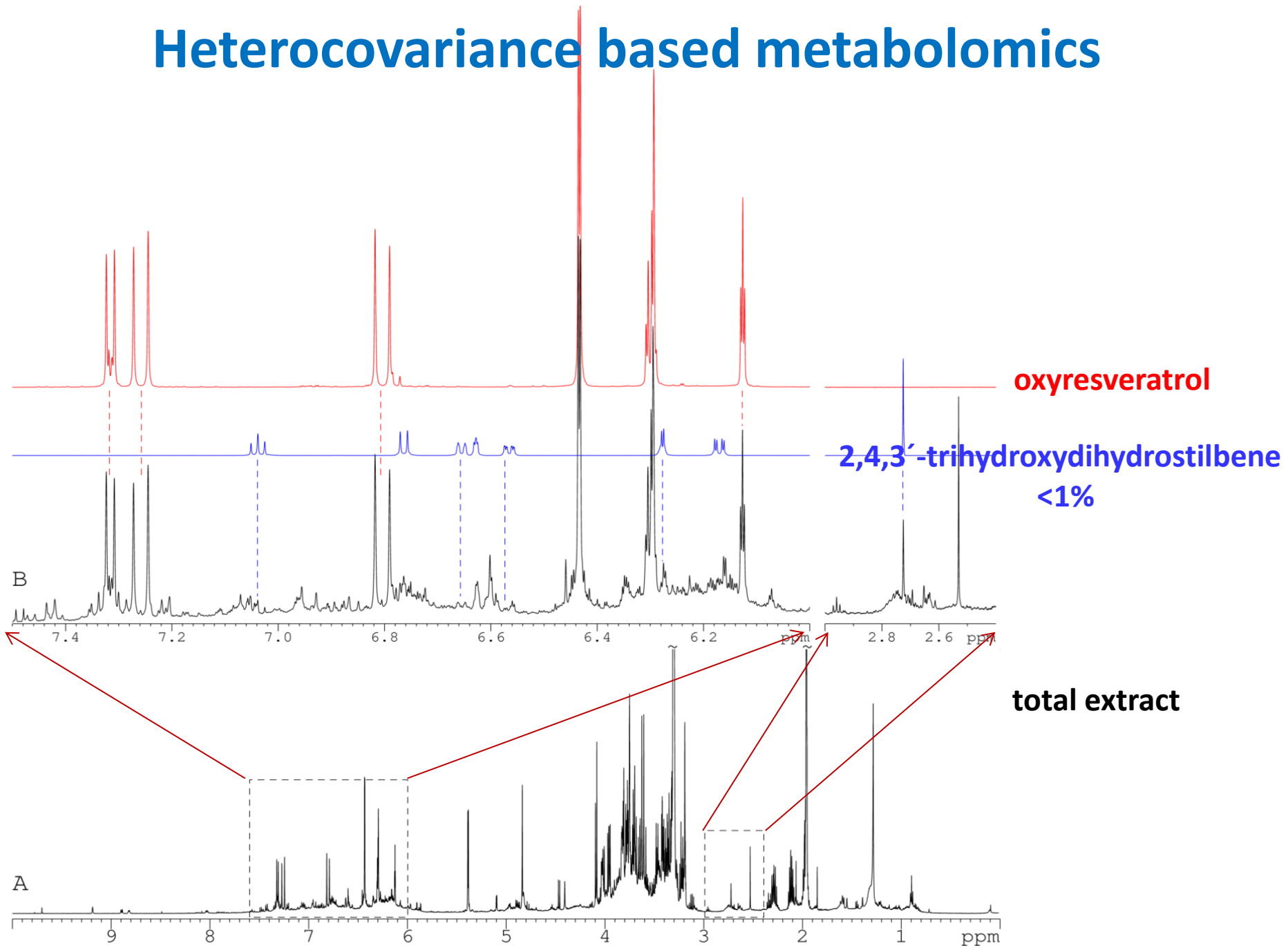
NMR covariance with activity fractions 16-19



HETCA  
covariance of biological activity  
with corresponding NMR data

$\delta^1\text{H} / \text{ppm}$

# Heterocovariance based metabolomics



# **Heterocovariance based metabolomics as a powerful tool accelerating bioactive natural product identification**

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**THANK YOU**

*for your attention*