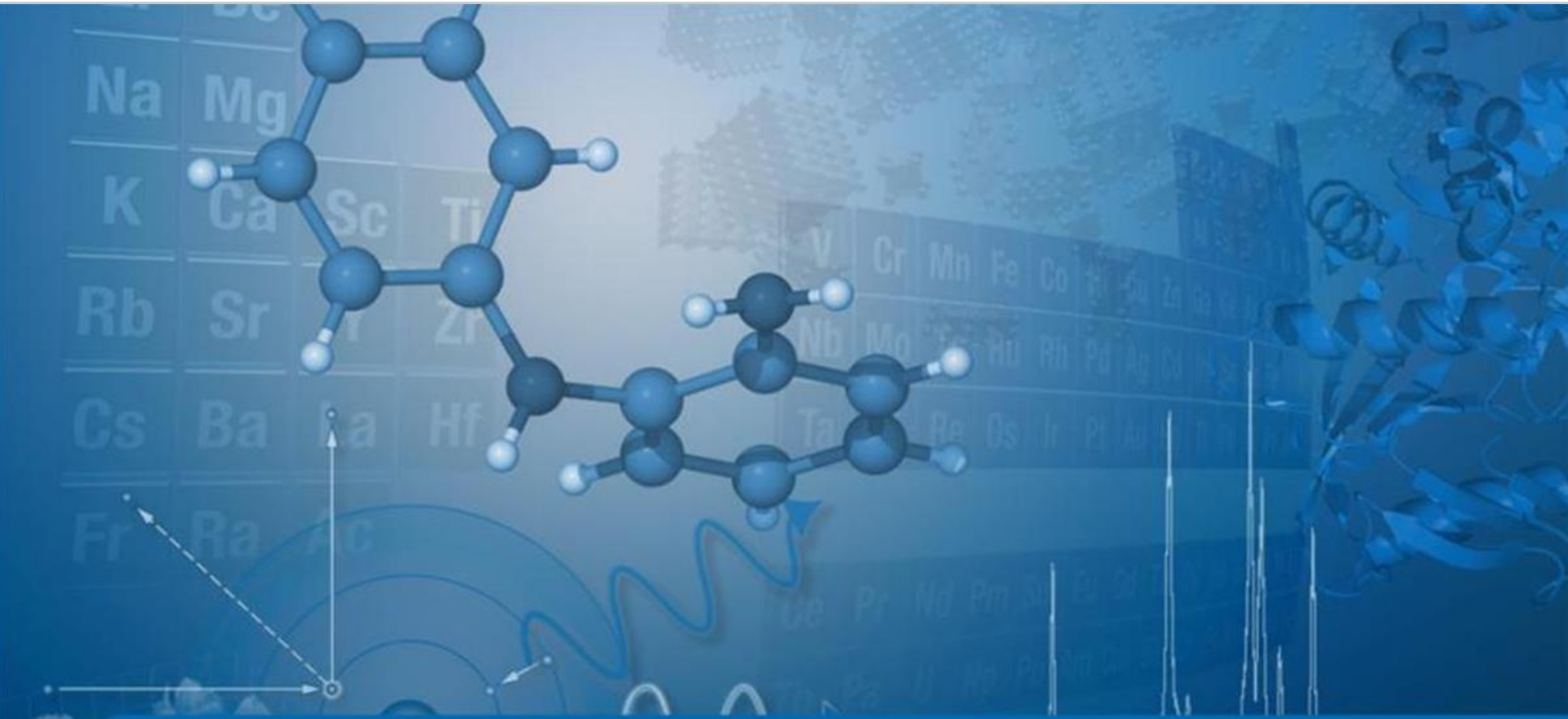
2D Acquisition and Processing

Dr. Benjamin Görling



1D Experiments

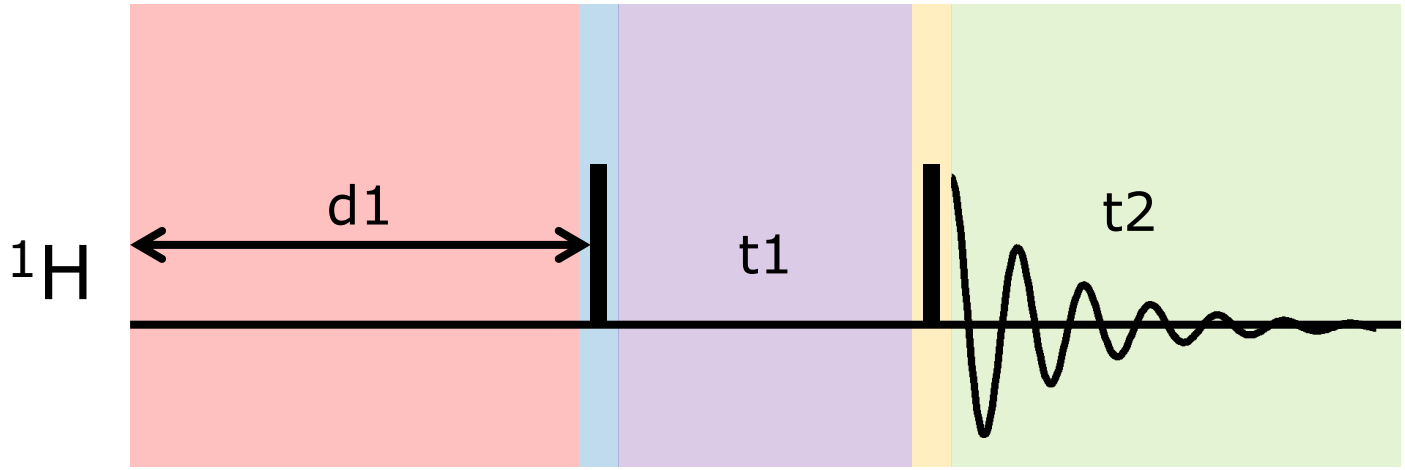
- **Relaxation delay**: time needed for relaxation
- **Preparation**: spins are excited by one or more pulses
- **Acquisition**: Signal is detected as a function of time t2

2D Experiments

Relaxation delay	Preparation	Evolution	Mixing	Acquisition

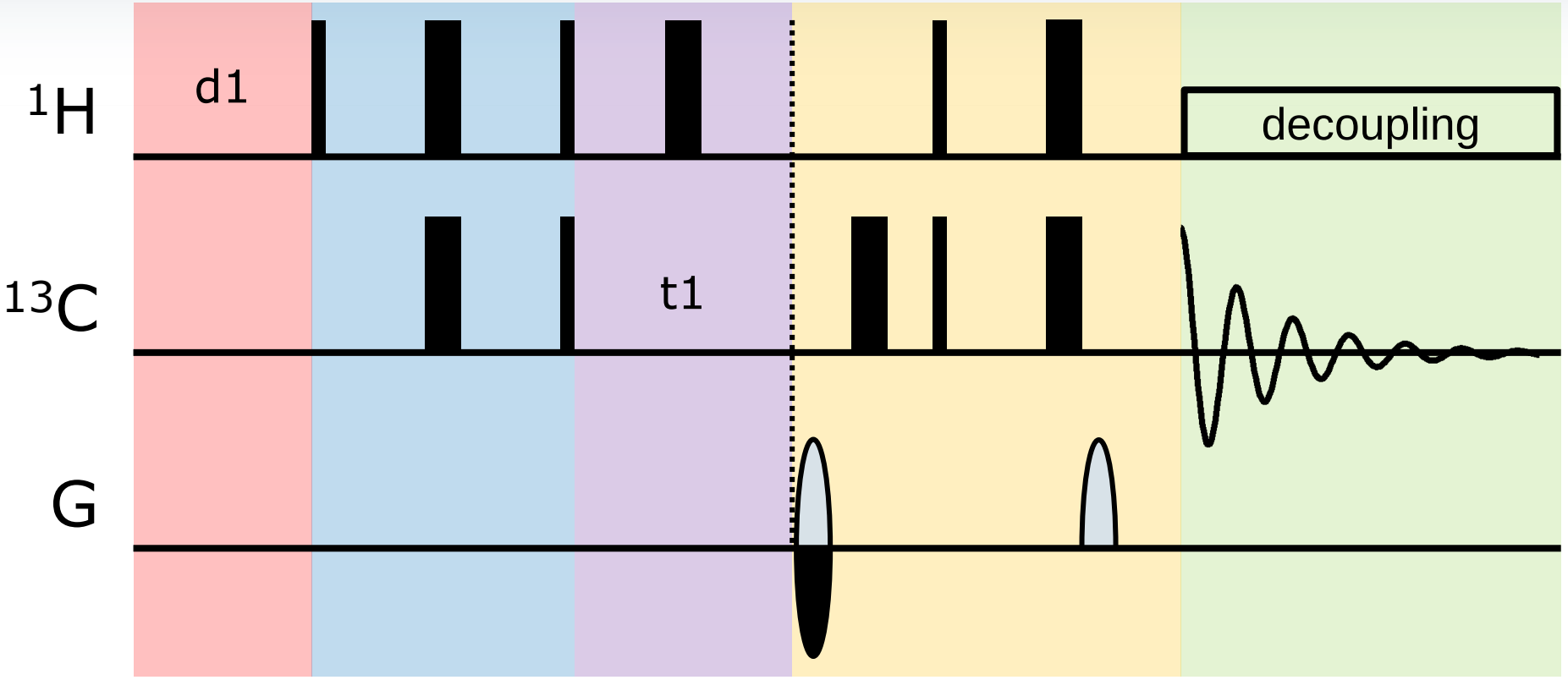
- **Relaxation delay**: time needed for relaxation
- **Preparation**: spins are excited by one or more pulses
- **Evolution**: evolution of the spins during a time t1
- **Mixing**: one or more pulses to select desired correlations
- **Acquisition**: Signal is detected as a function of time t2

2D Experiments



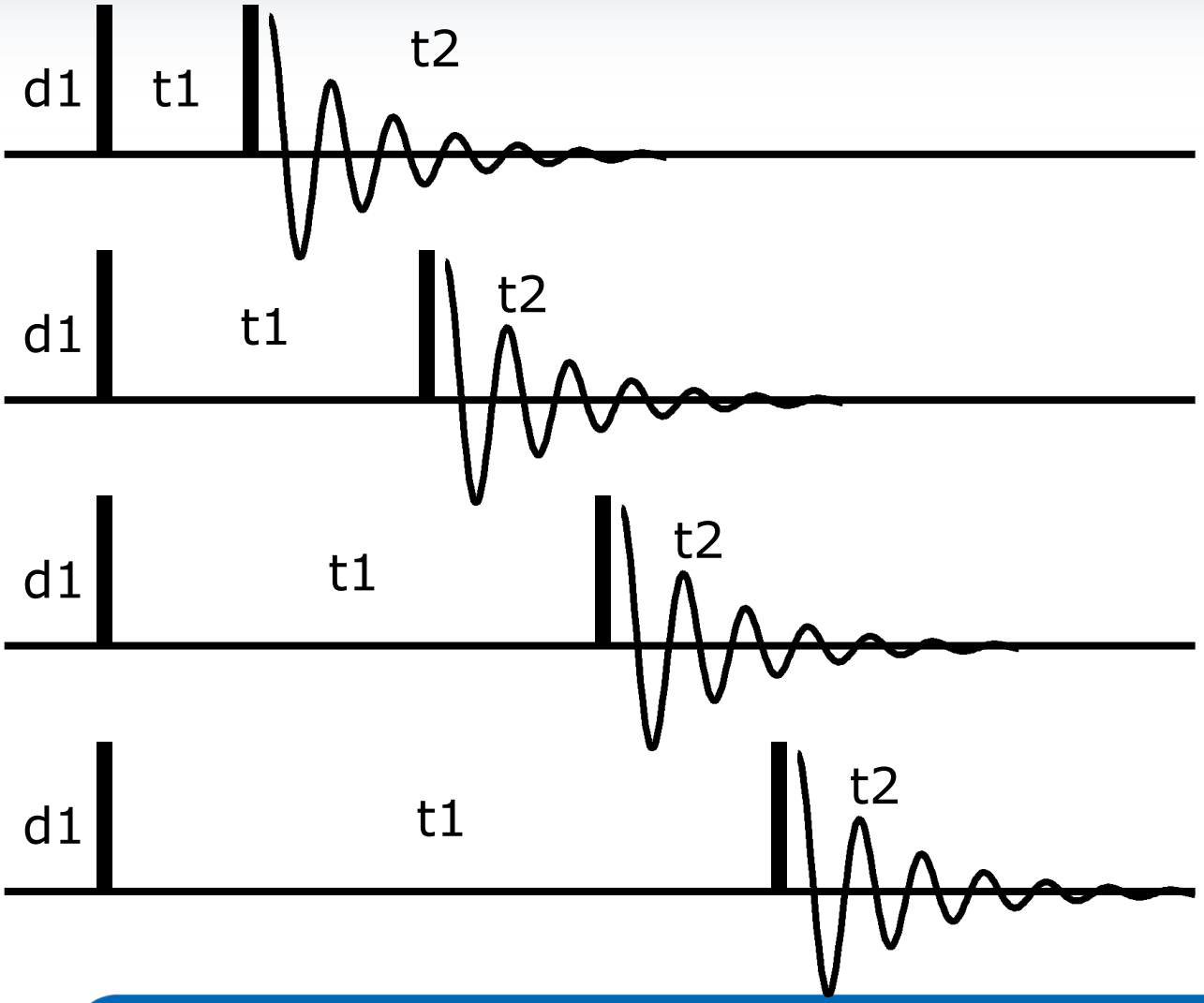
Relaxation delay	Preparation	Evolution	Mixing	Acquisition

2D Experiments



Relaxation delay	Preparation	Evolution	Mixing	Acquisition

2D Experiments



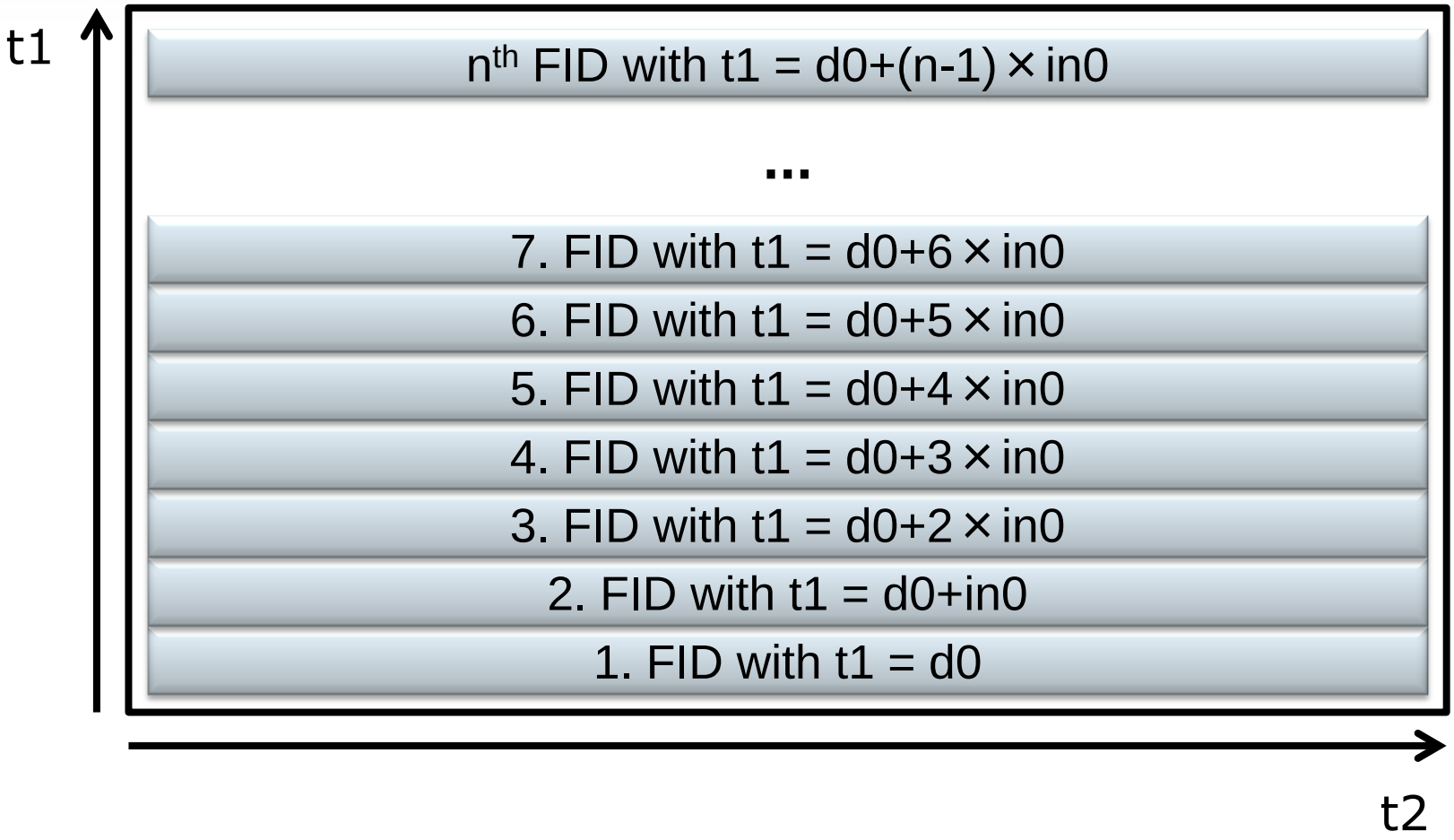
t1=d0

t1=d0+in0

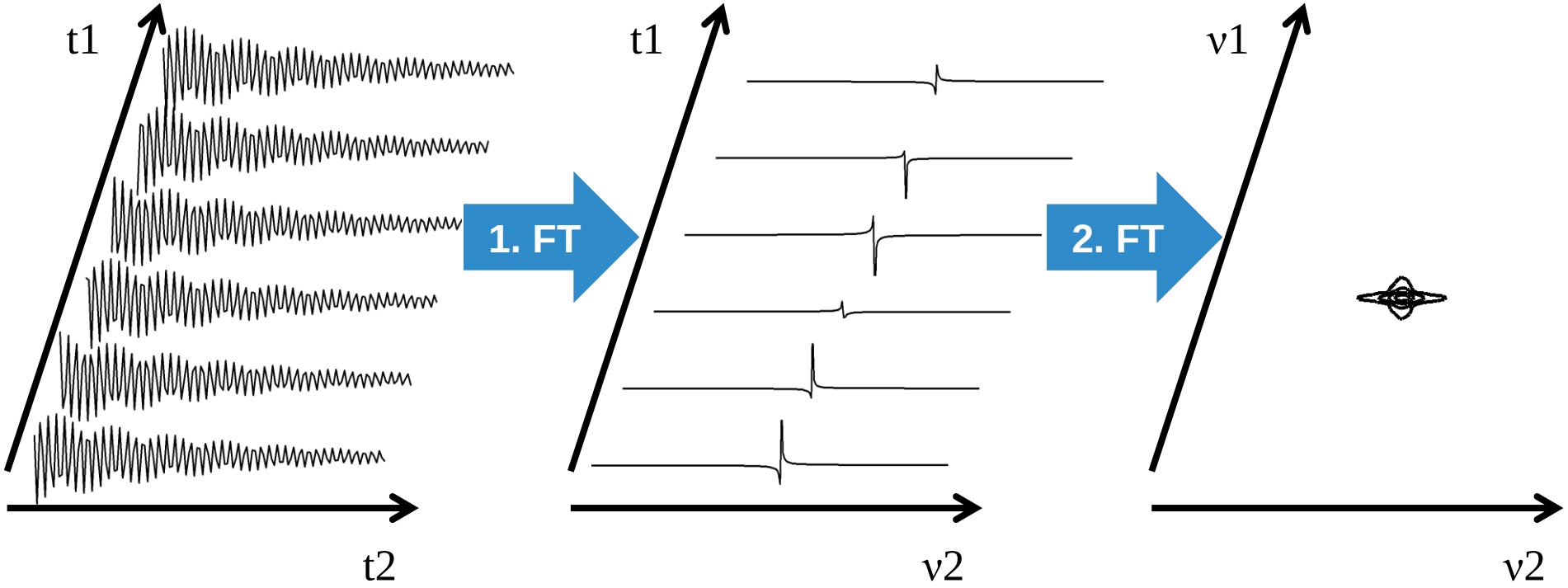
t1=d0+2×in0

t1=d0+3×in0

2D Experiments



Fourier Transformation



Parameters

- Parameters are :

  time domain TD
  spectral width SW/SWH
  dwell time DW
  incremented delay IN0
  acquisition time AQ

- **Some parameters are needed twice!**
  - TD (TD(F2), TD(F1))
  - SW/SWH (SW(F2), SW(F1))

- IN0 is the equivalent to DW for the second dimension.

Spectral width

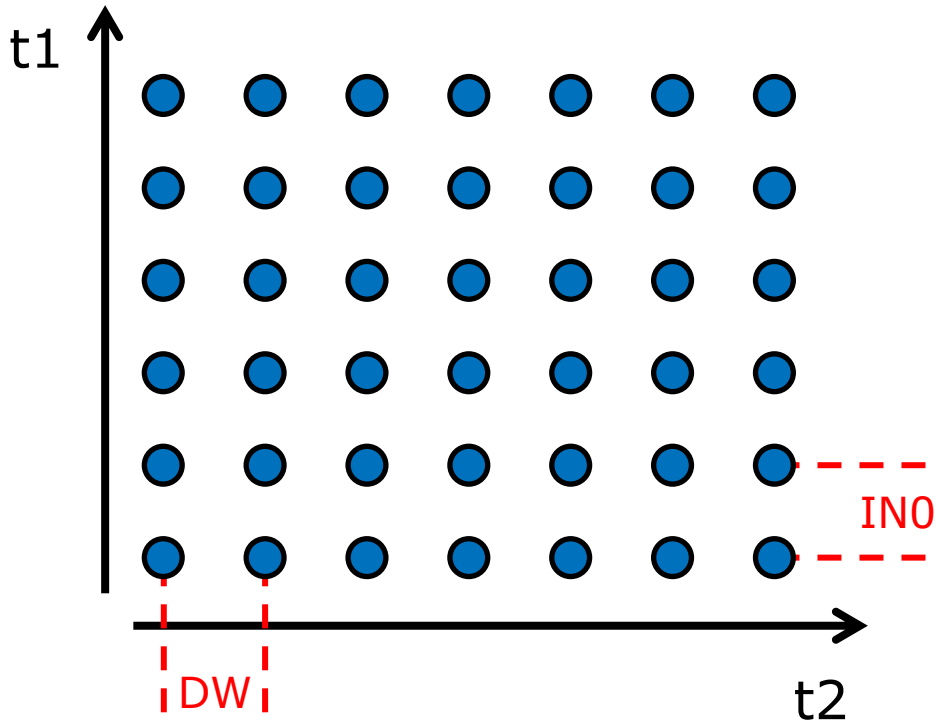
- Spectral width in 1D (now F2) was:

$$SWH(F2) = \frac{1}{2 \cdot DW}$$

- Corresponding to DW in F1 is IN0

- Spectral width in F1:

$$SWH(F1) = \frac{1}{2 \cdot IN0}$$



Resolution

- Resolution in 1D (now F2) was:

$$HzpPt(F2) = \frac{1}{AQ(F2)} = \frac{1}{DW \cdot TD(F2)}$$

To get a good resolution, many points need to be acquired.

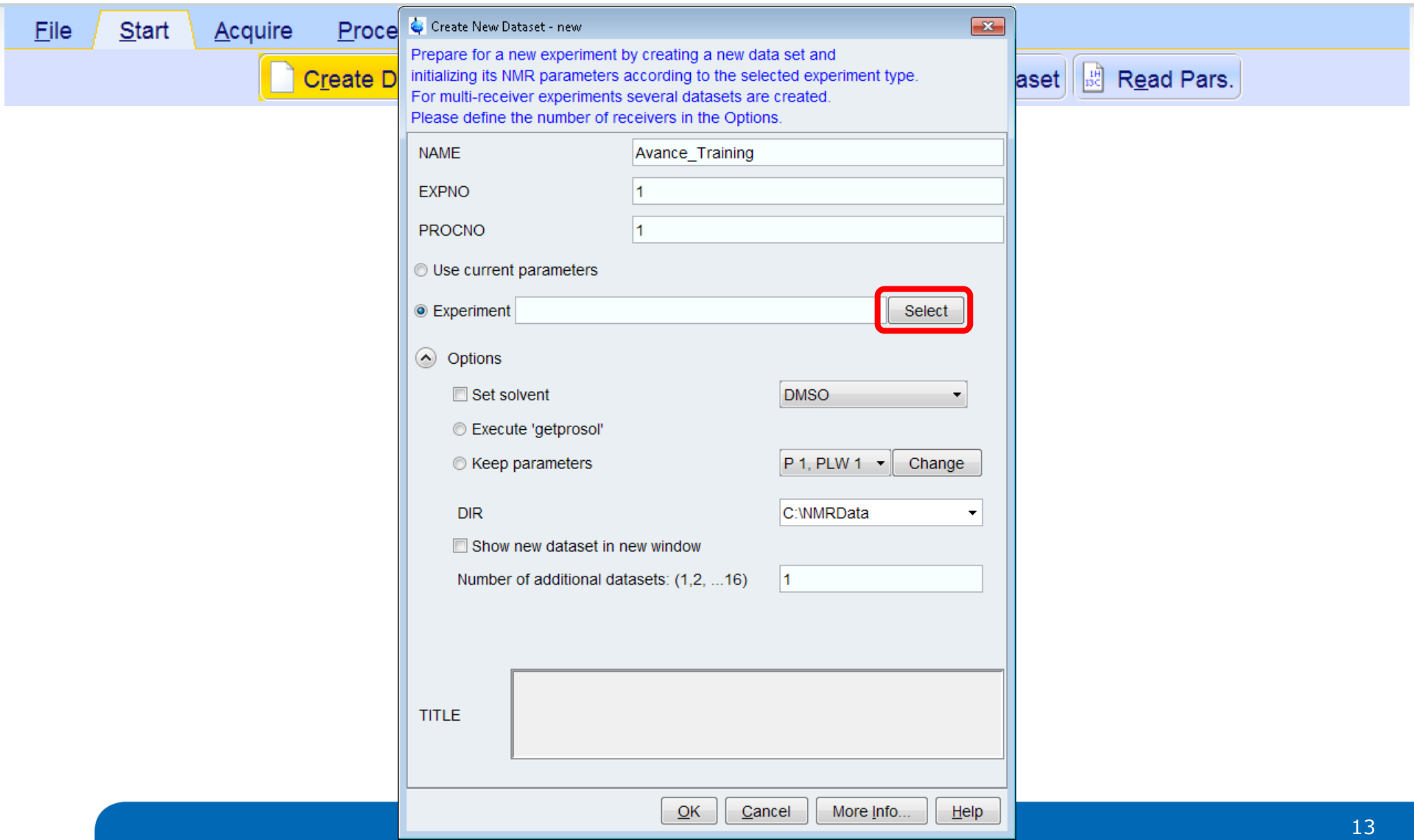
- Now for F1:

$$HzpPt(F1) = \frac{1}{AQ(F1)} = \frac{1}{IN0 \cdot TD(F1)}$$

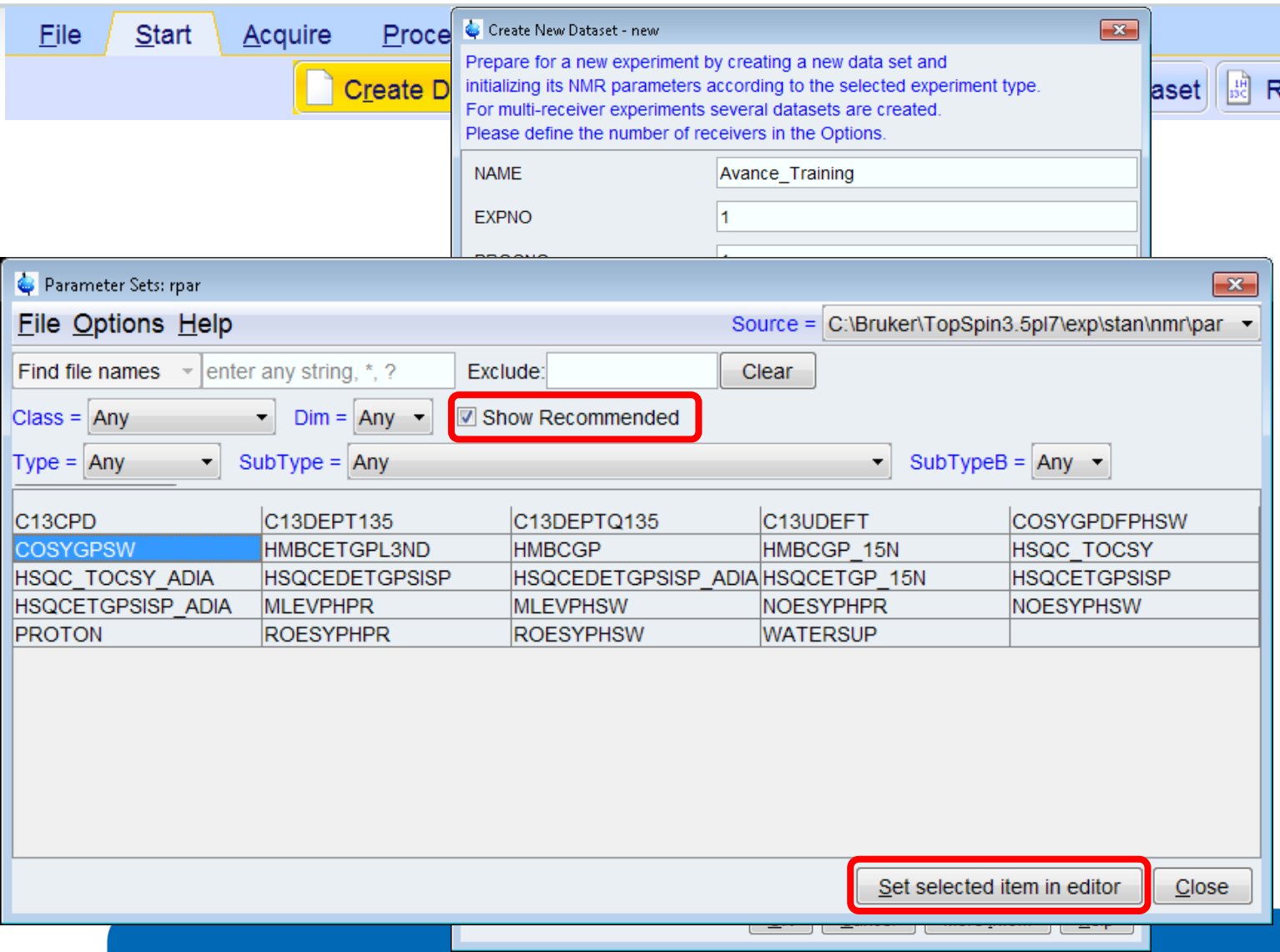
If a good resolution in F1 is needed, experiment time will get very long!

- **How to setup a 2D data set?**

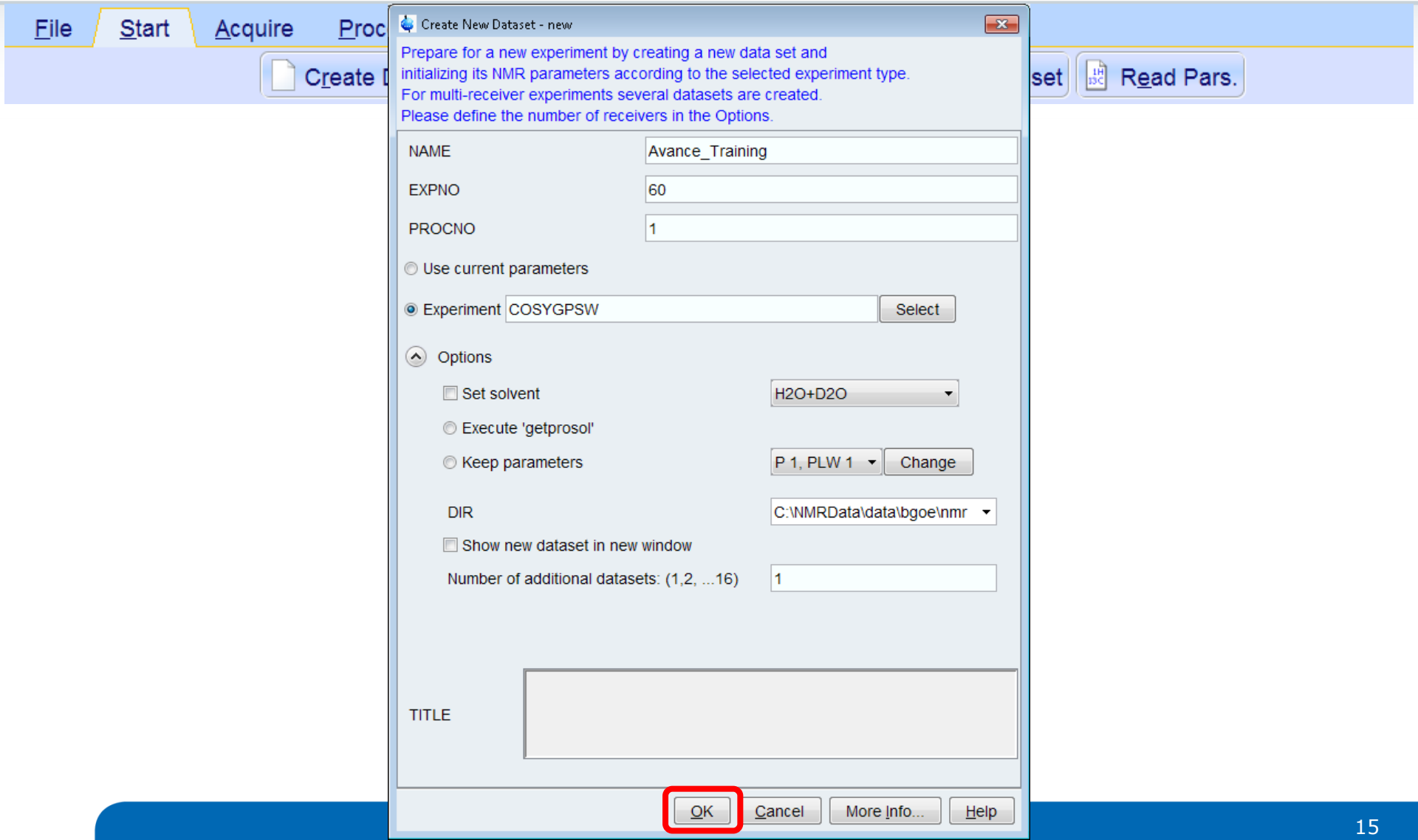
Create new experiment [**new**/**edc**]



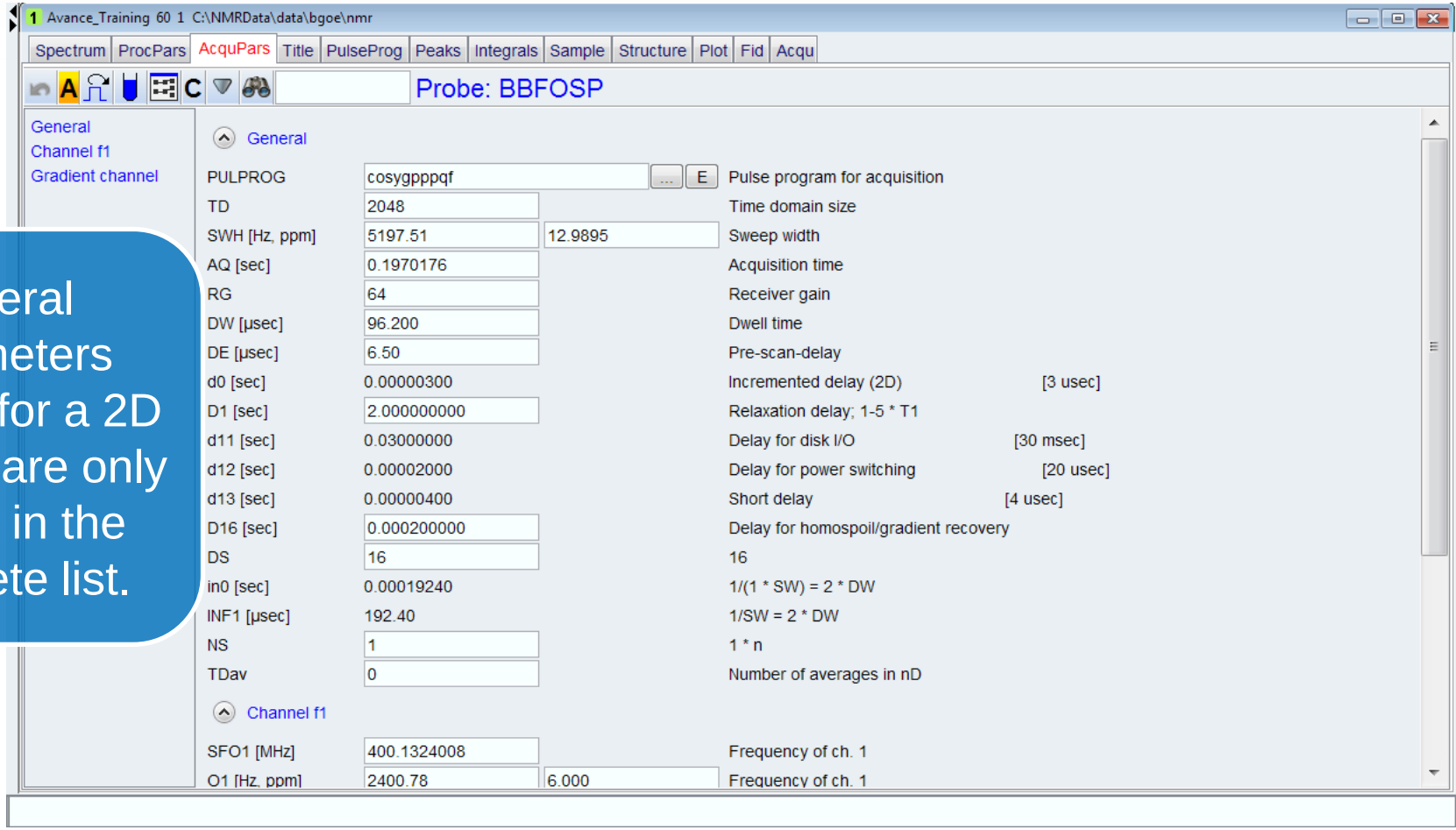
Create new experiment [**new**/**edc**]



Create new experiment [**new**/**edc**]

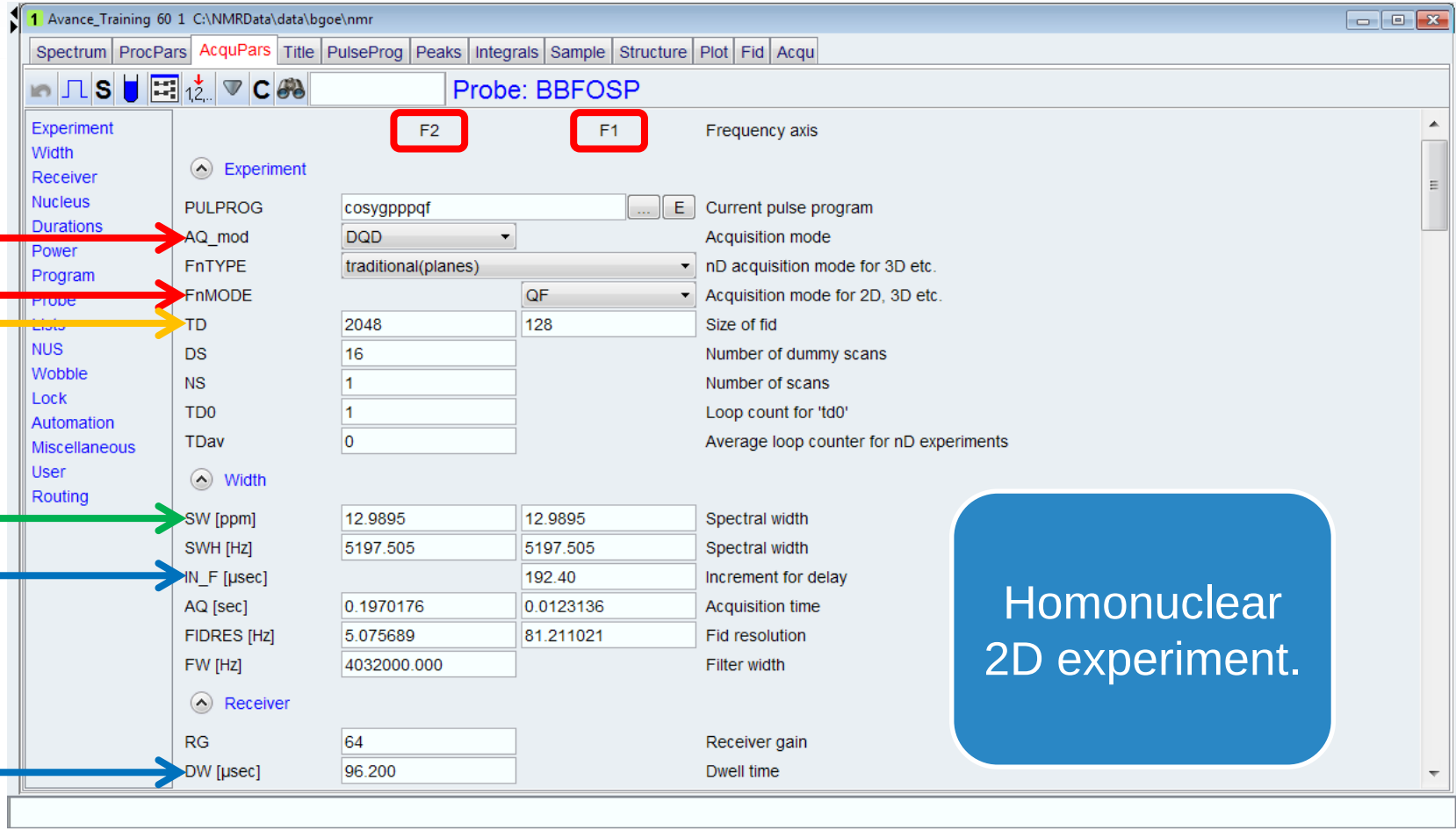


Acquisition parameters [**ased**]

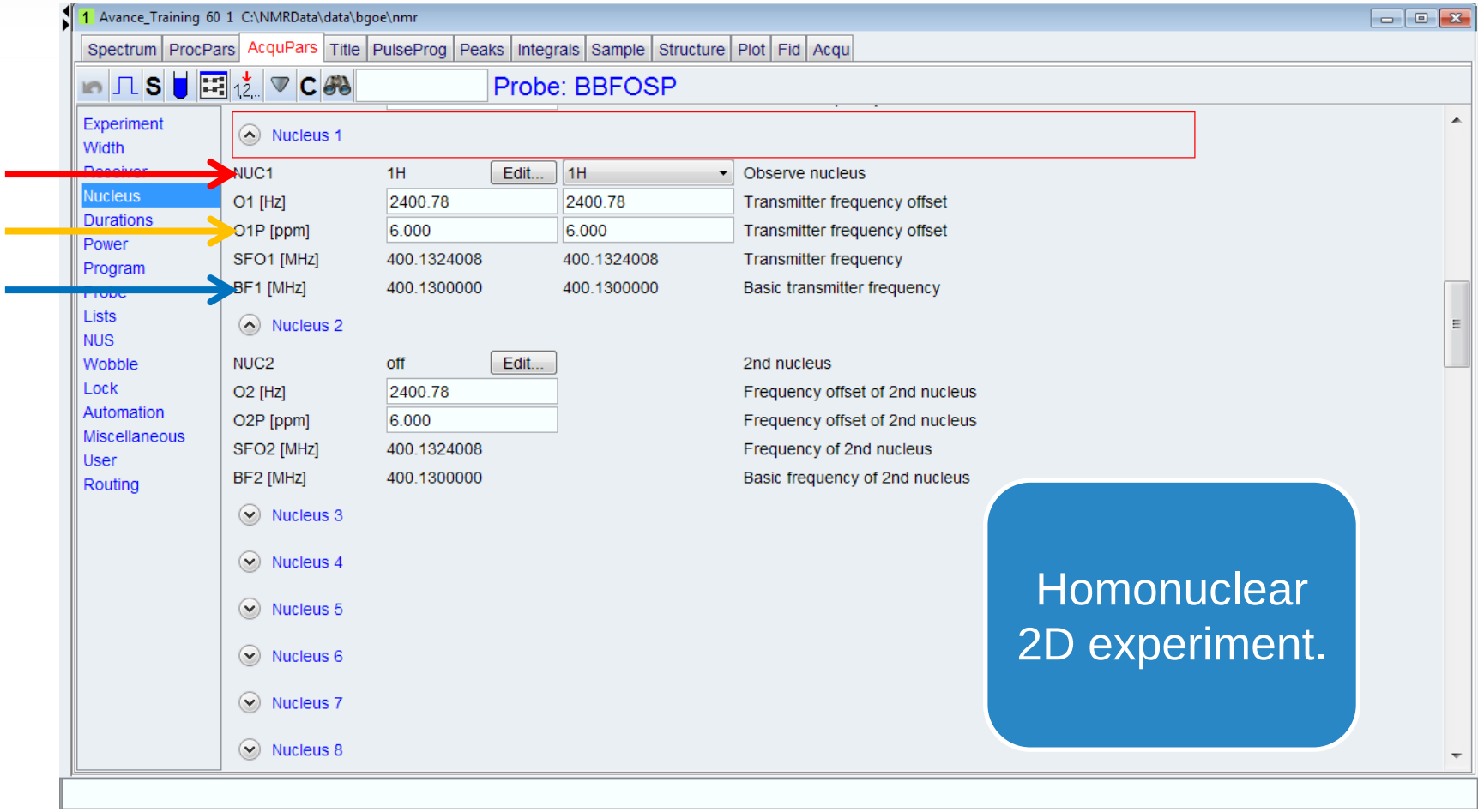


Several parameters needed for a 2D data set are only shown in the complete list.

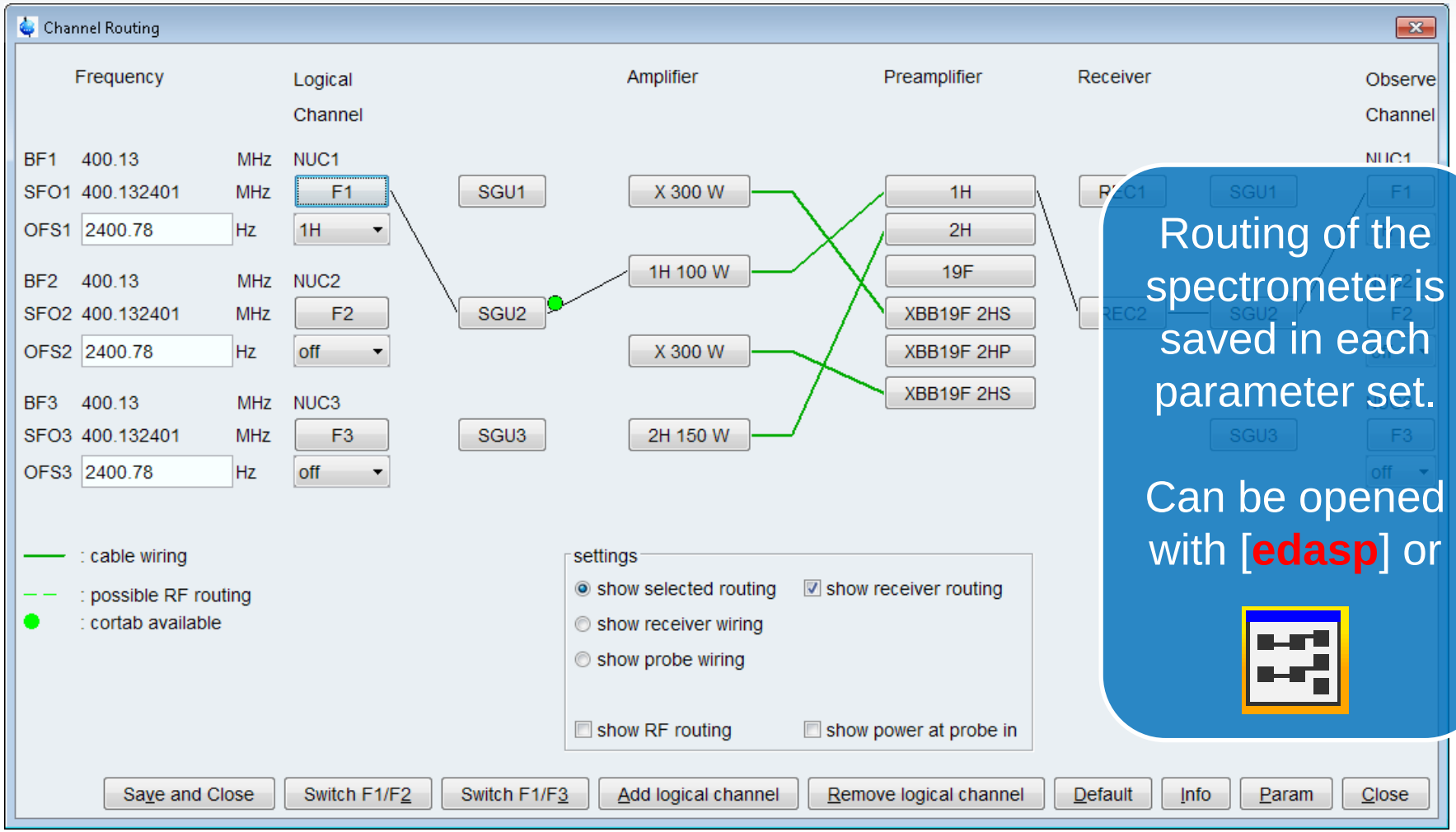
Acquisition parameters [eda]



Acquisition parameters [**eda**]



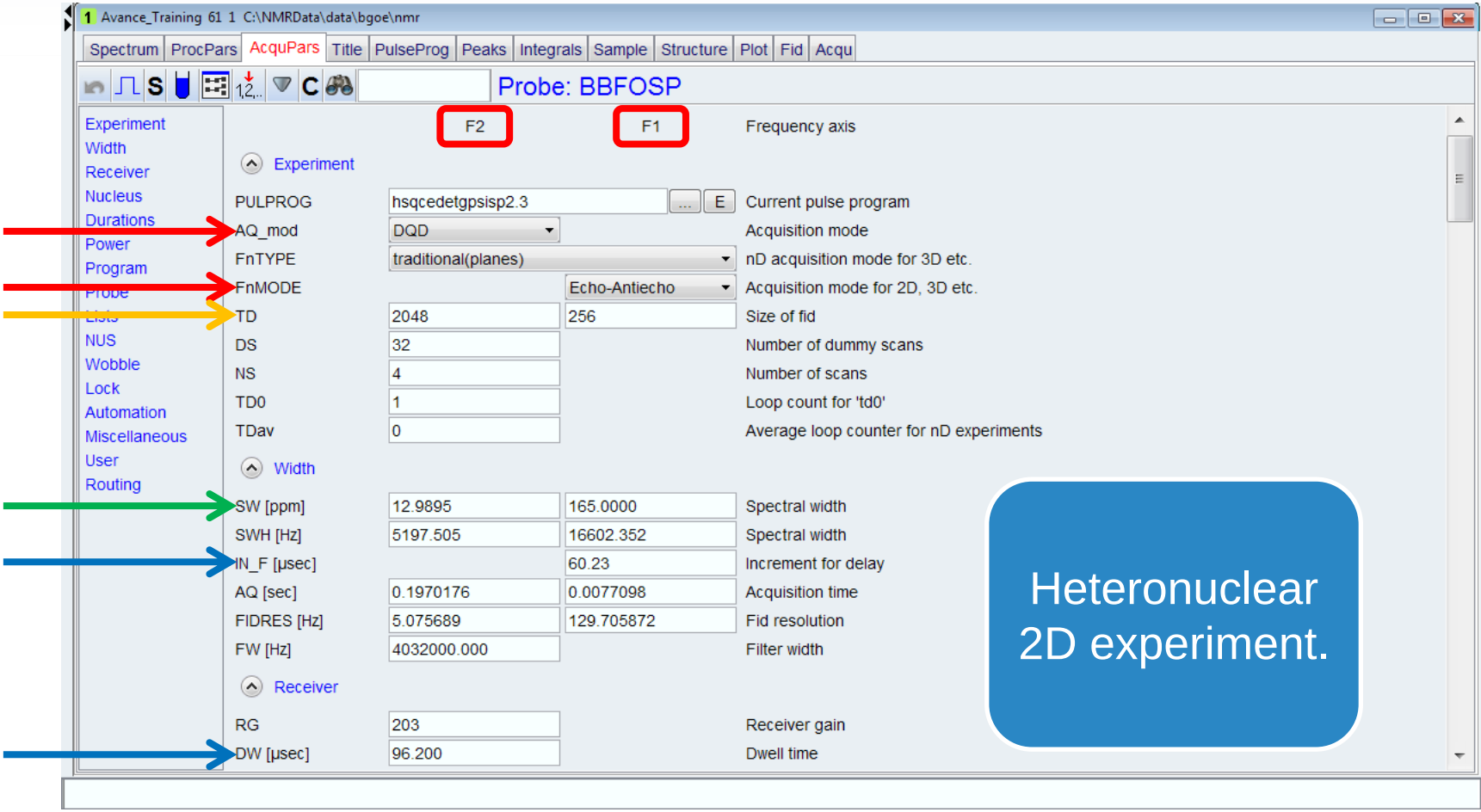
Channel Routing [edasp]



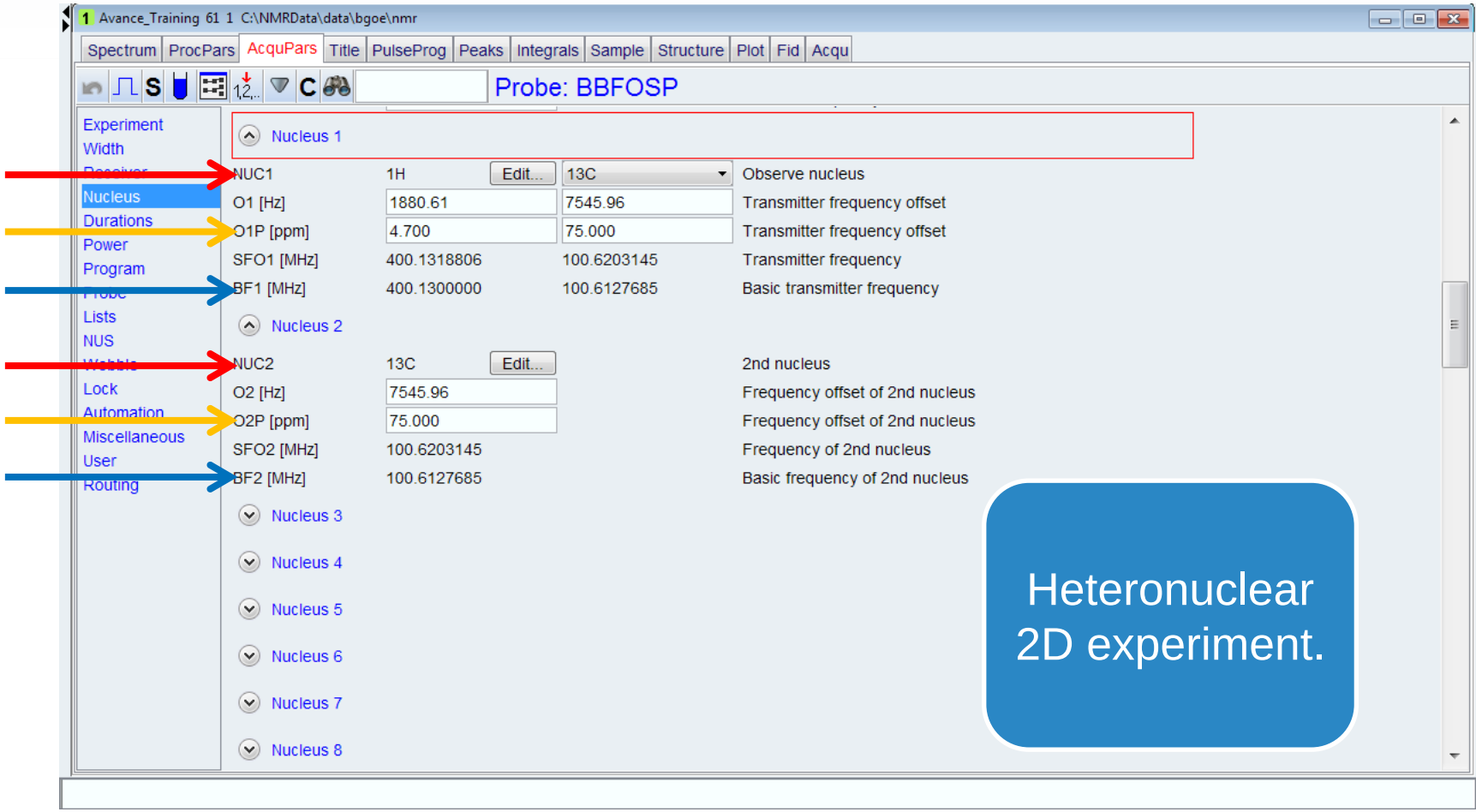
Routing of the spectrometer is saved in each parameter set.

Can be opened with [edasp] or

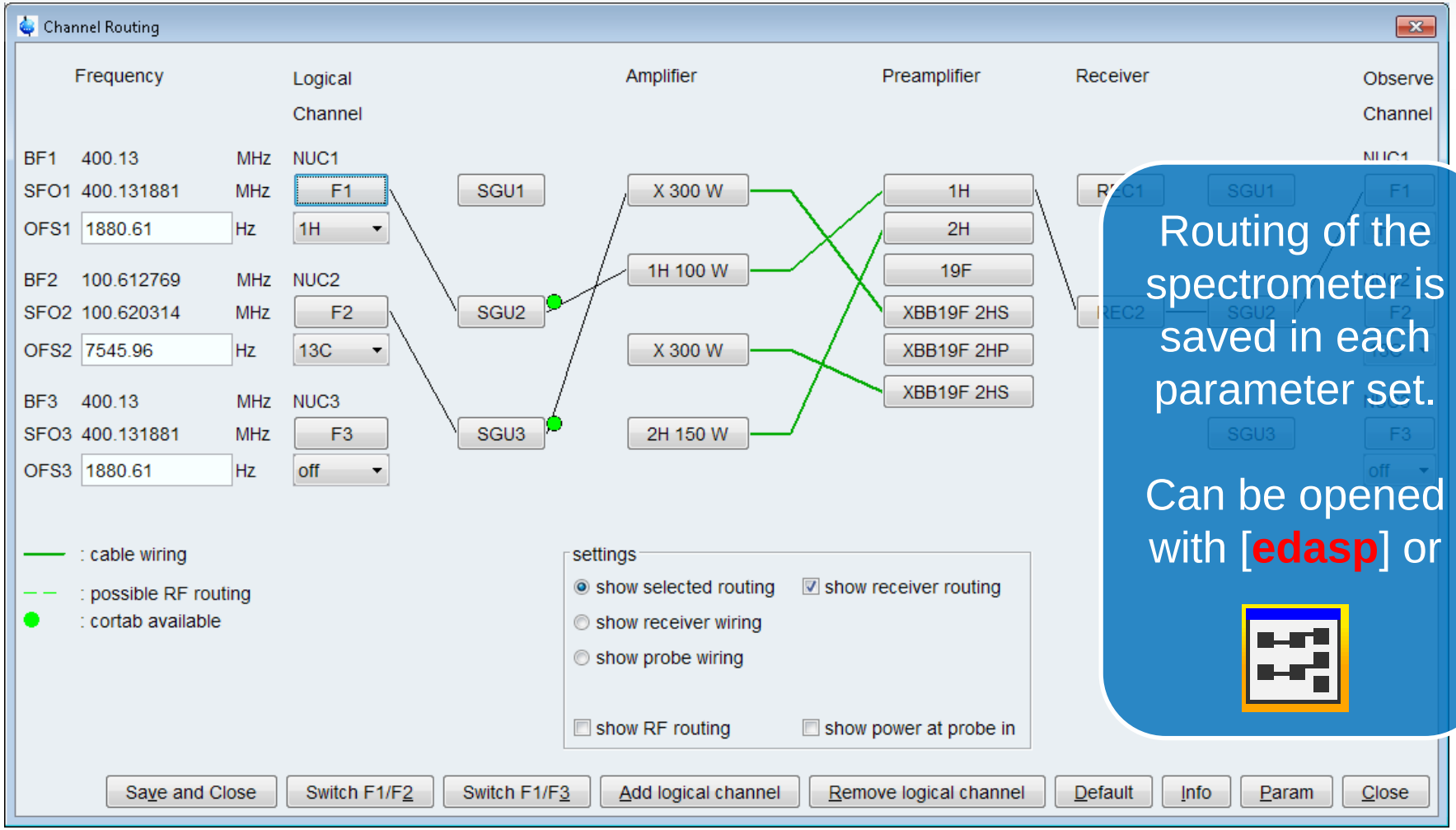
Acquisition parameters [eda]



Acquisition parameters [**eda**]



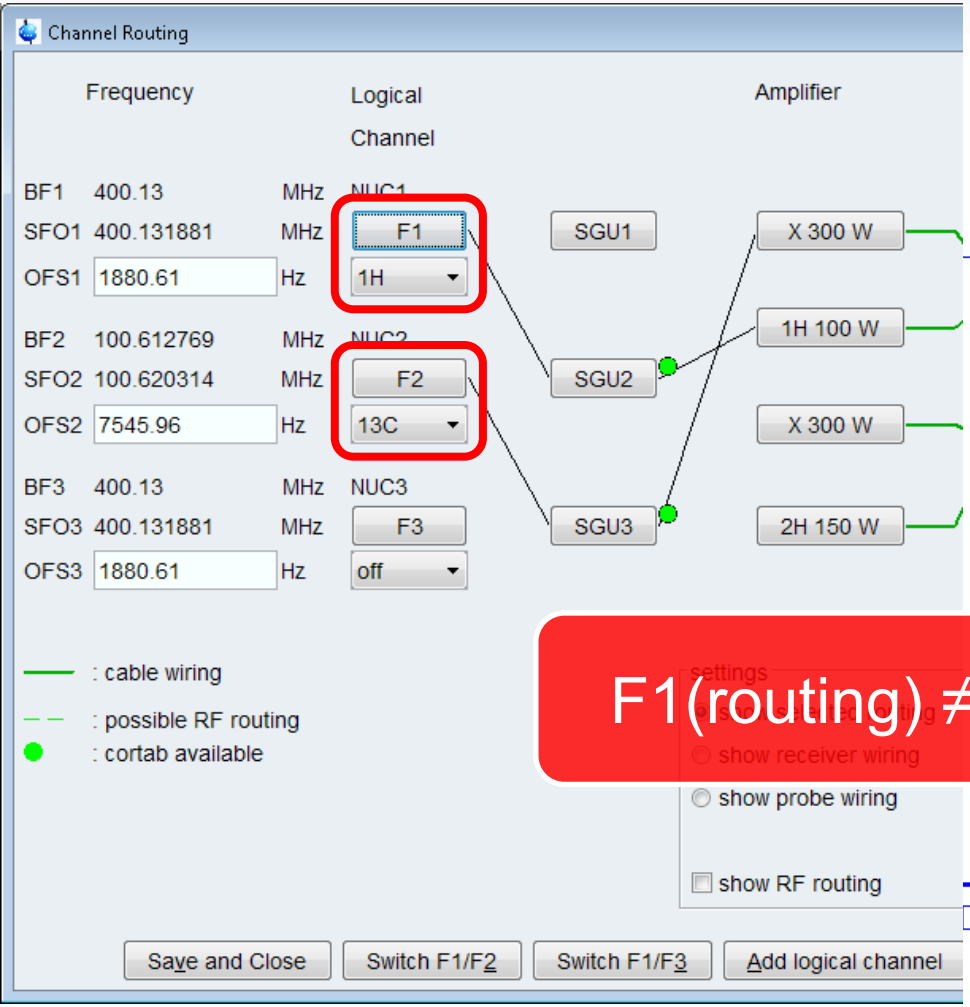
Channel Routing [edasp]



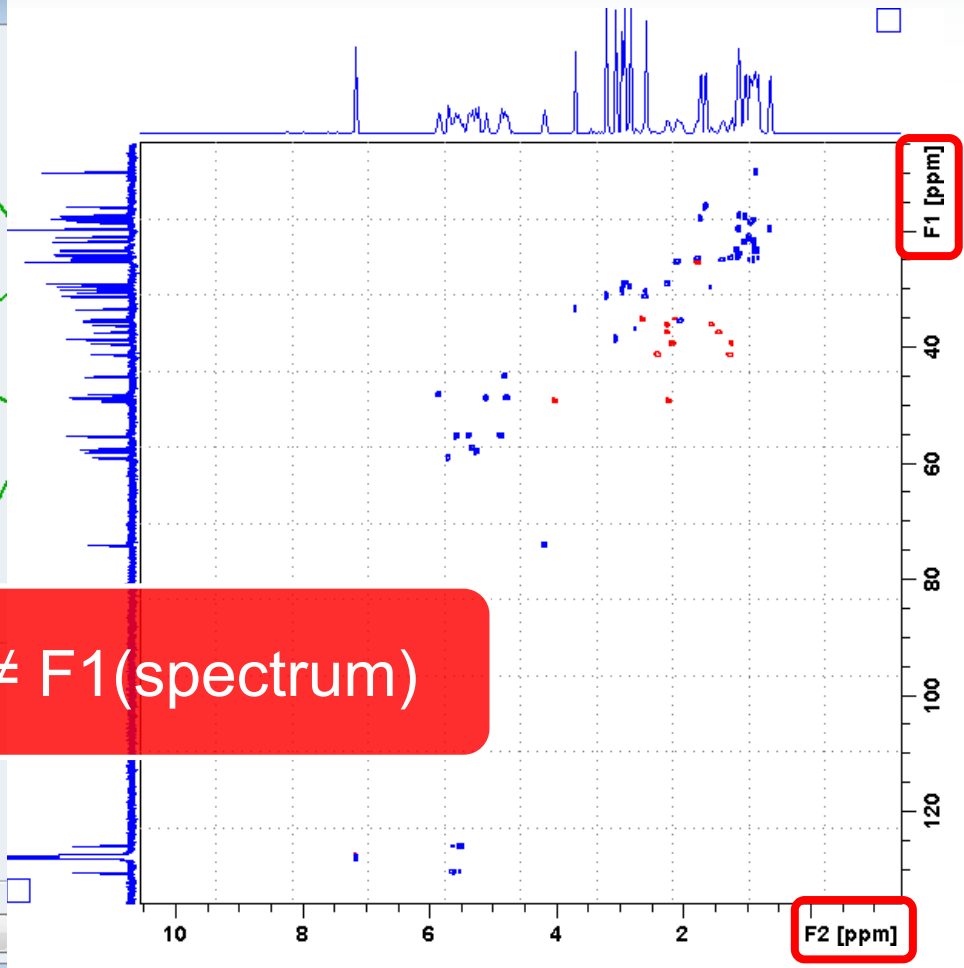
Routing of the spectrometer is saved in each parameter set.

Can be opened with [edasp] or

Channels and spectral axis



F1(routing) ≠ F1(spectrum)

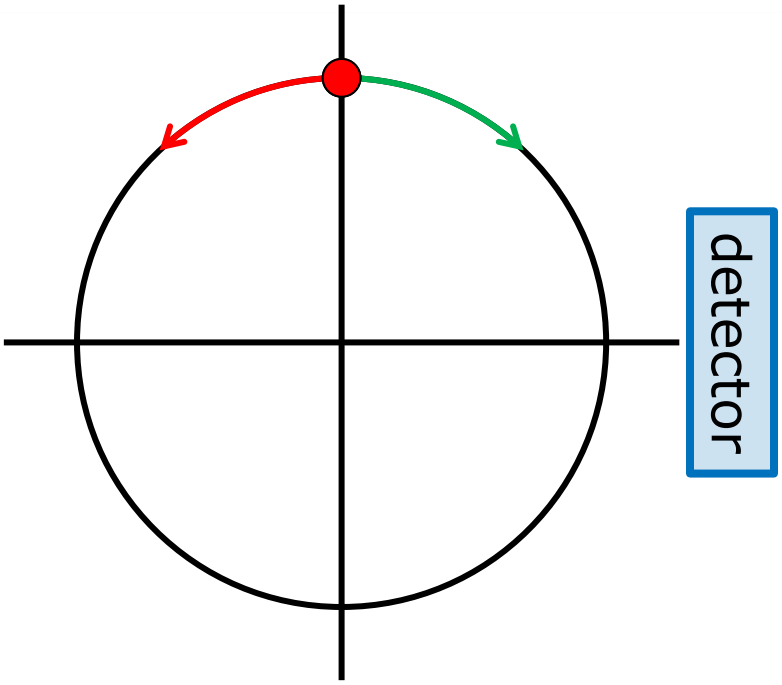


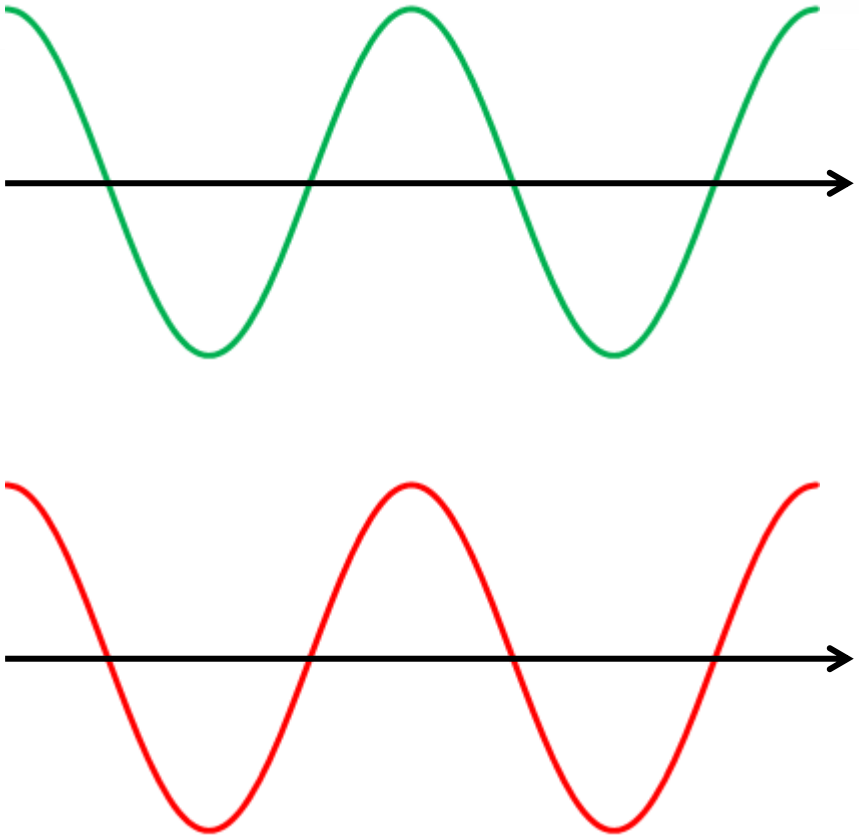
- **Acquisition Mode**

Acquisition mode

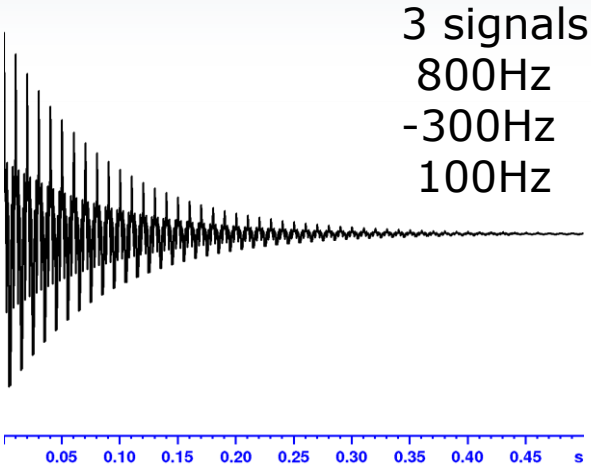
- Defines how the data is acquired.
- Acquisition mode for F1 and F2 are defined by FnMODE and AQ_mod, respectively.
- Necessary to get correct signal position and phase.
- AQ_mod is always DQD (digital quadrature detection)

Quadrature detection



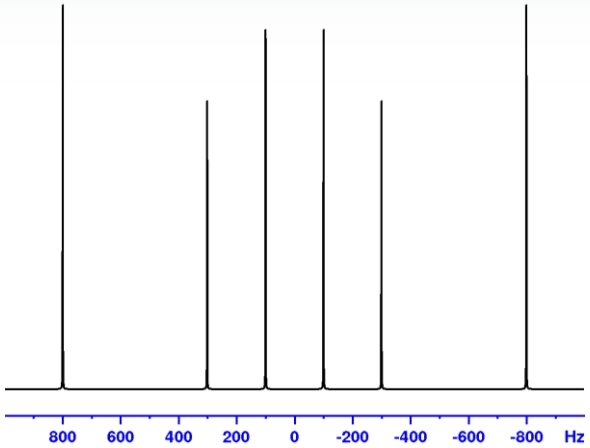


Quadrature detection

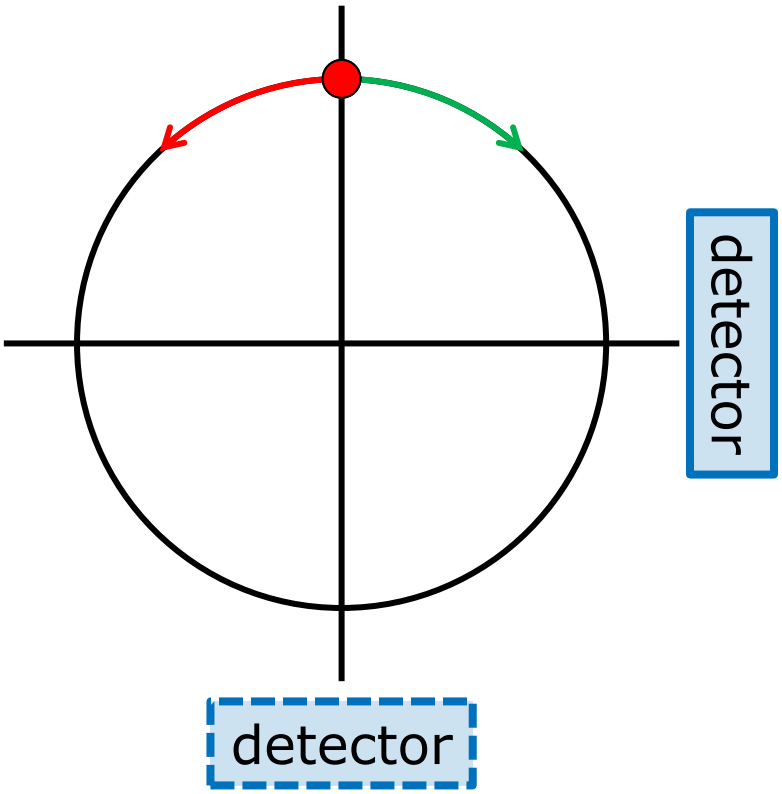


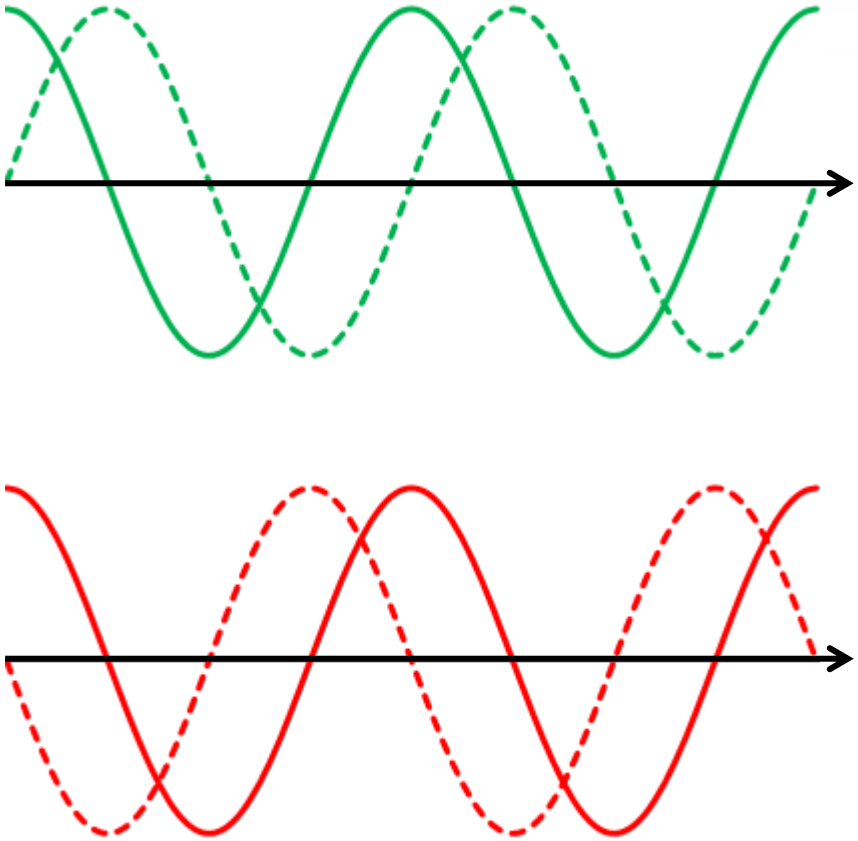
FT

only cos

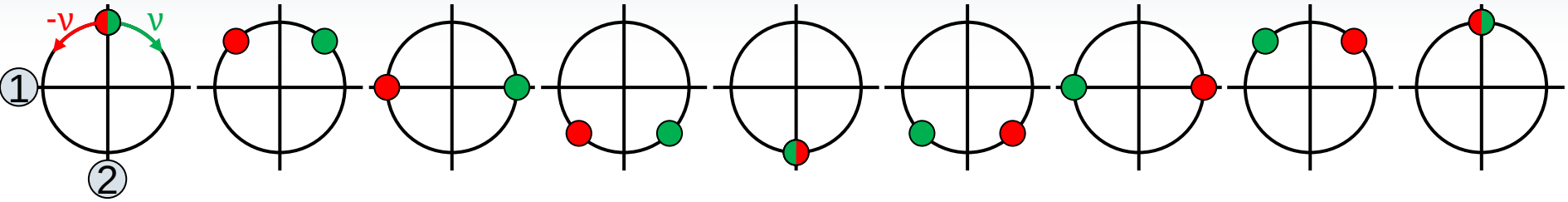


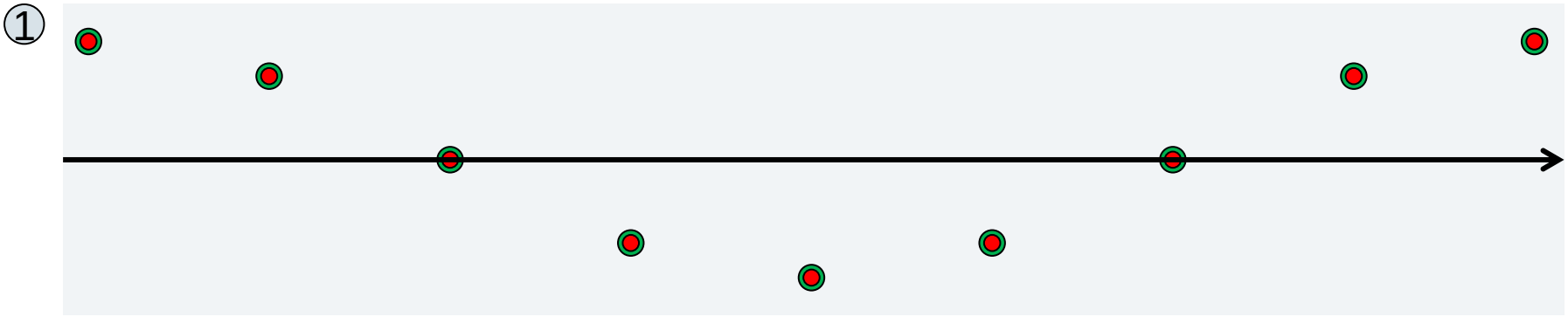
Quadrature detection

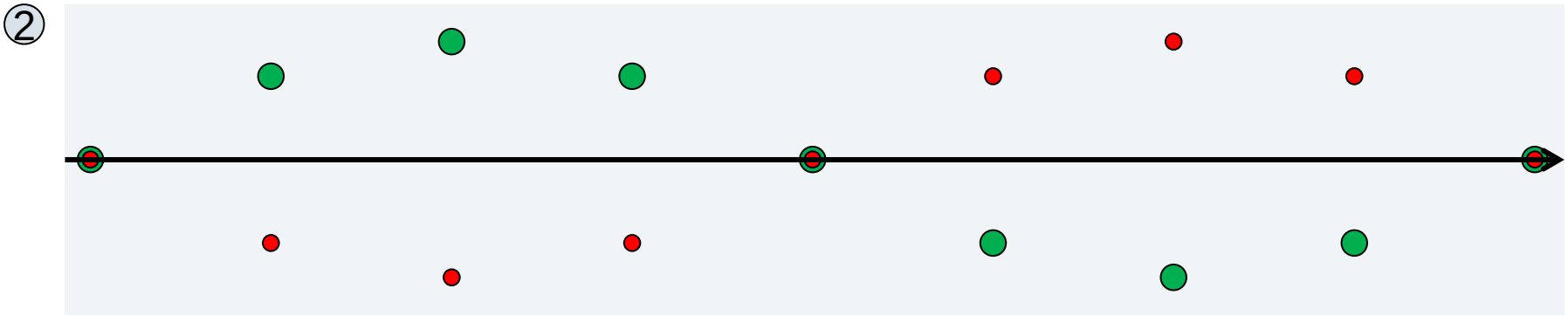




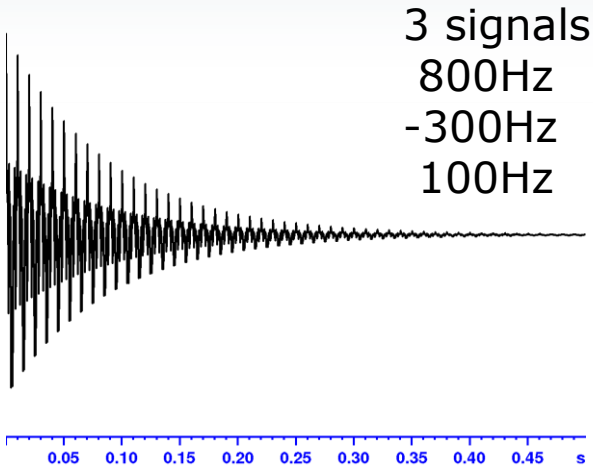
Quadrature detection





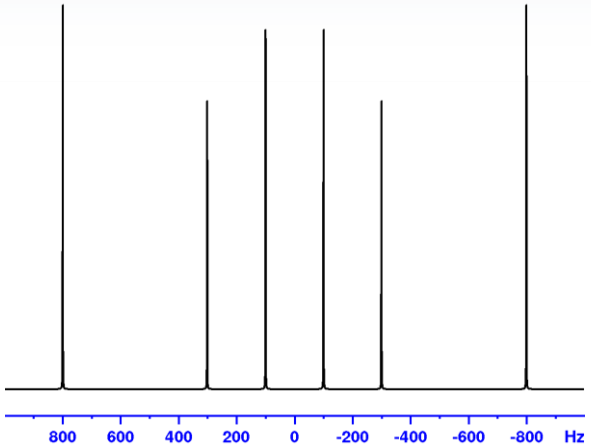


Quadrature detection



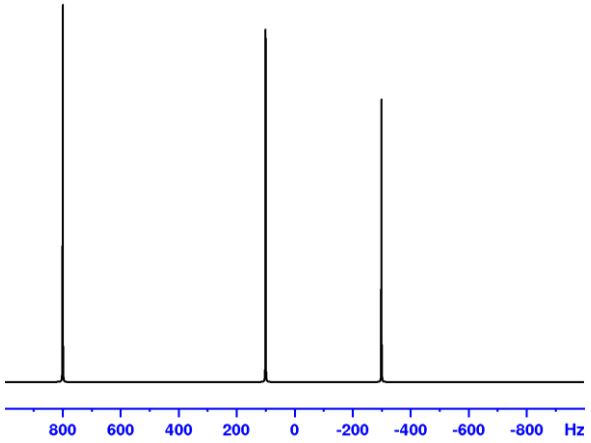
FT

only cos



FT

sin & cos



Phase sensitive detection in 2D

- Quadrature detection in the F1 Dimension in 2D experiments is not possible!

- Frequency discrimination is done by phase cycling/gradient selection

- **not phase sensitive experiments**:
  storage of the cosine and sine component not separately
- **phase sensitive experiments**:
  storage of the cosine and sine component separately

Acquisition modes

FnMODE	**QF**	**cosygpppqf**
not phase sensitive		**hmbcgplpndqf**
FnMODE	**TPPI**	**cosygpphpp**
phase sensitive	**States**	**mlevphpp**
	States-TPPI	**noesygpphpp**
FnMODE	**Echo-Antiecho**	**hsqcetgpsisp2.2**
phase sensitive		**hmbcetgpl3nd**

Recommended 2D Experiments

H-H

COSY

cosygpmfphpp, cosygpppqf

TOCSY

mlevphpp, mlevphpr.2

NOESY

noesygpphpp, noesygpphpr

ROESY

roesyphpp.2, roesyphpr.2

H-X

^{13}C-HSQC

hsqcdietgpsisp.2, hsqcedetgpsisp2.3, hsqcetgpsisp2.2

^{13}C-HMBC

hmbcetgpl3nd, hmbcgplpndqf

^{15}N-HSQC/HMBC

hsqcetgpsi2/hmbcgpndqf

JRES

jresqf, jresprqf

Recommended 2D Parameter Sets

H-H

COSY

COSYGPDFPHSW, COSYGPSW

TOCSY

MLEVPHSW, MLEVPHPR

NOESY

NOESYPHSW, NOESYPHPR

ROESY

ROESYPHSW, ROESYPHPR

H-X

^{13}C-HSQC

HSQC_TOCSY
HSQCEDETGPSISP, HSQCETGPSISP

^{13}C-HMBC

HMBCETGPL3ND, HMBCGP

^{15}N-HSQC/HMBC

HSQCETGP_15N/HMBCGP_15N

JRES

PROF_JRES (presat)

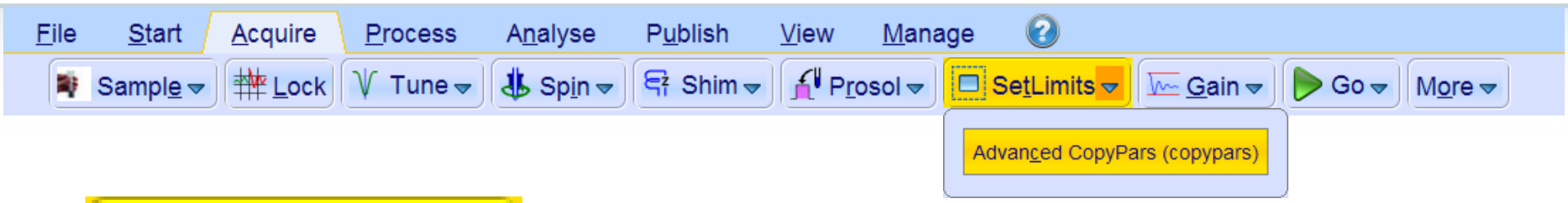
- **How to acquire a spectrum?**

Acquire Toolbar

Workflow

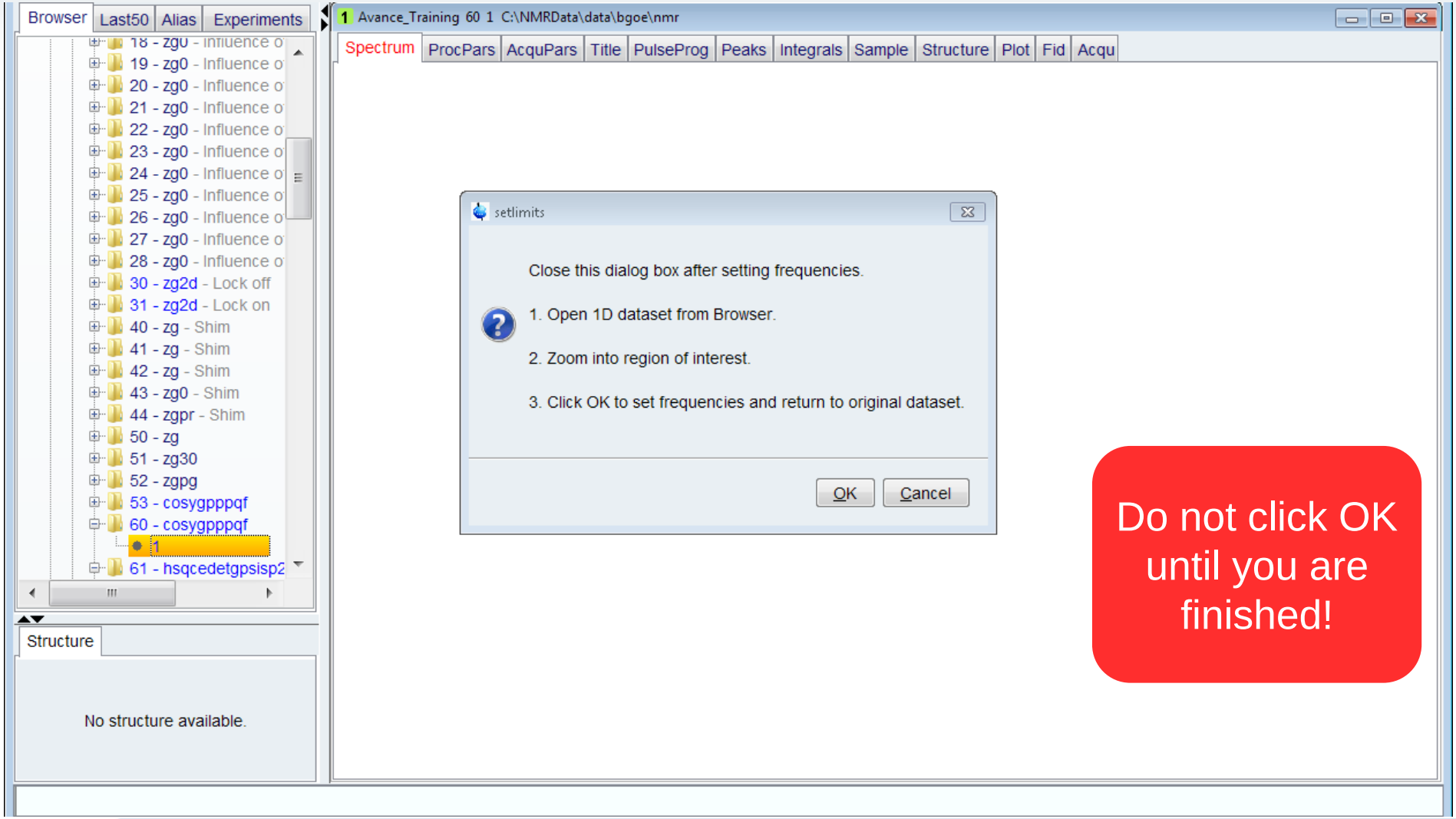
Very similar to 1D acquisition.

Acquire Toolbar - SetLimits

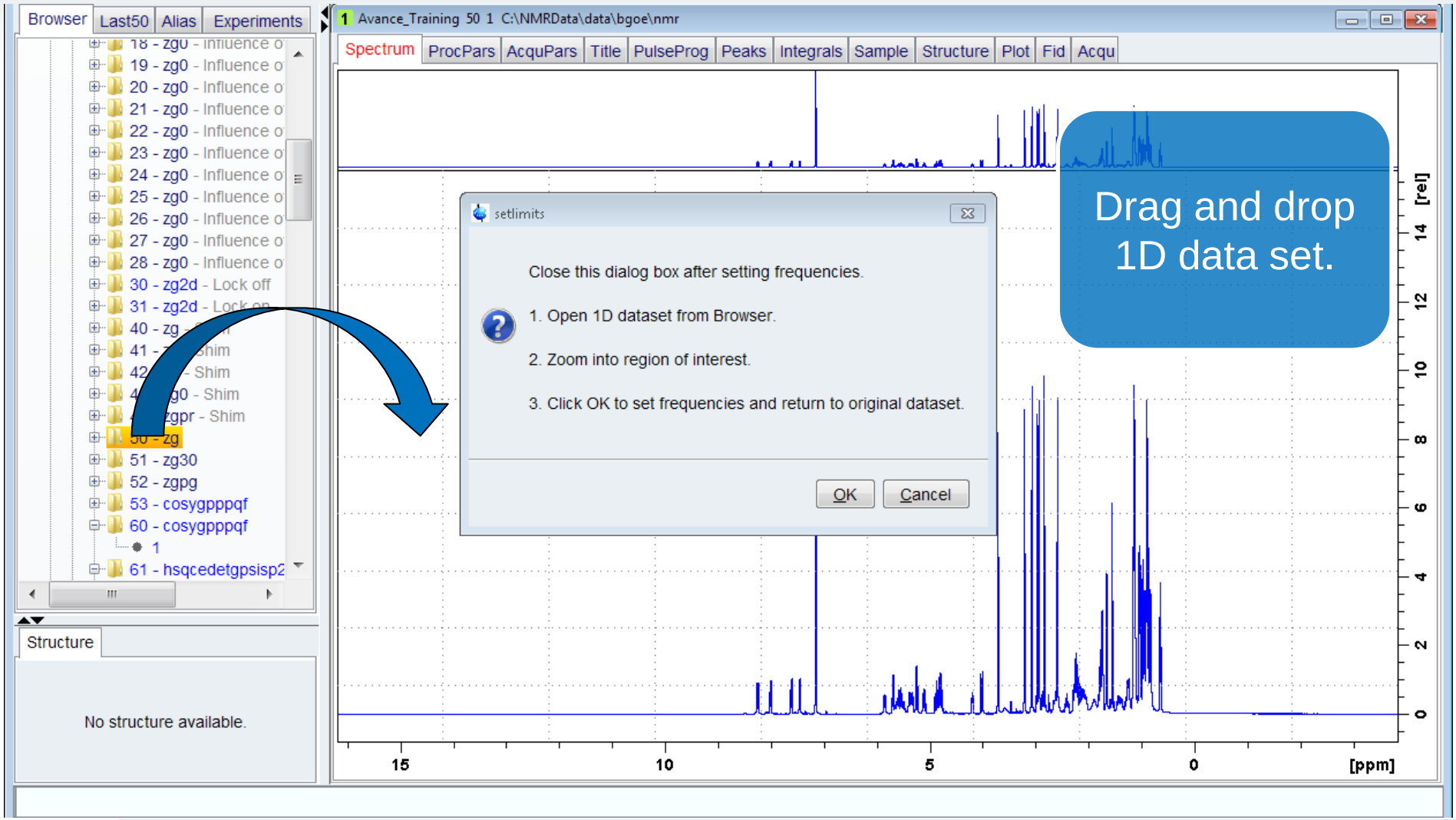


- Helps to set limits for 2D spectra from 1D data sets
- Interactive mode to define spectral width (SW) and transmitter frequency (O1P/O2P)
- [**copypars**] will be discussed in Tips & Tricks session

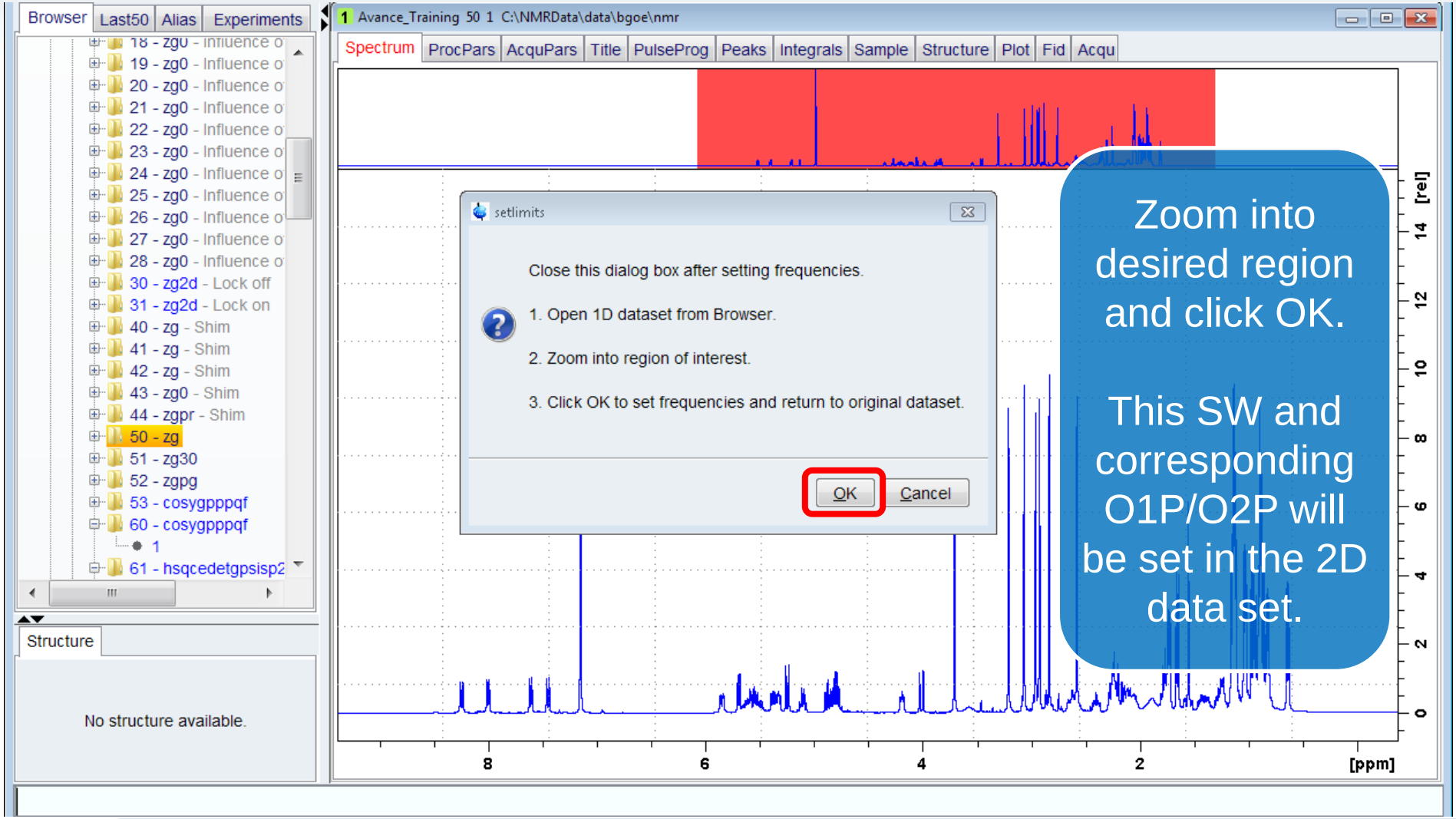
Acquire Toolbar - SetLimits



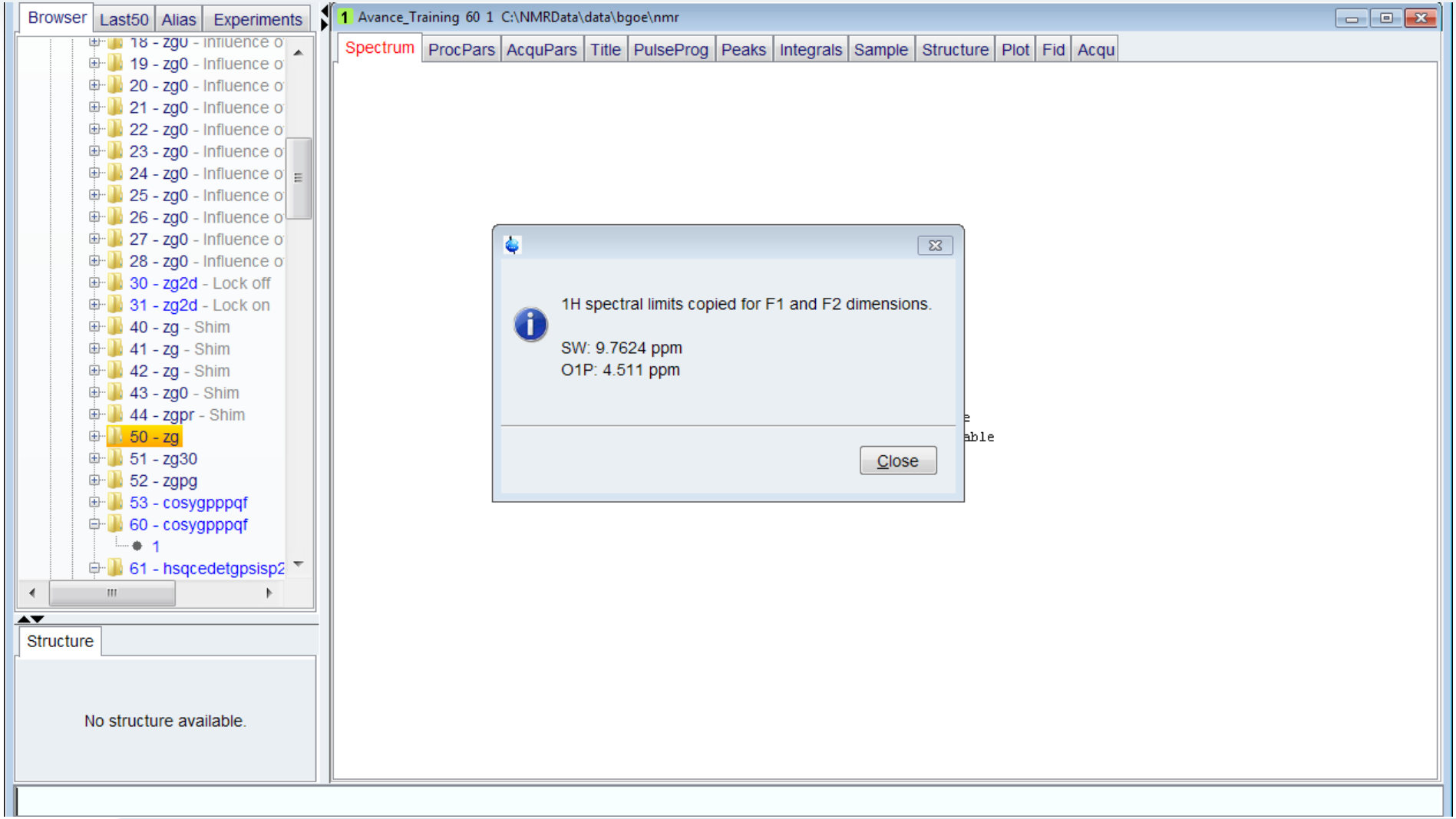
Acquire Toolbar - SetLimits



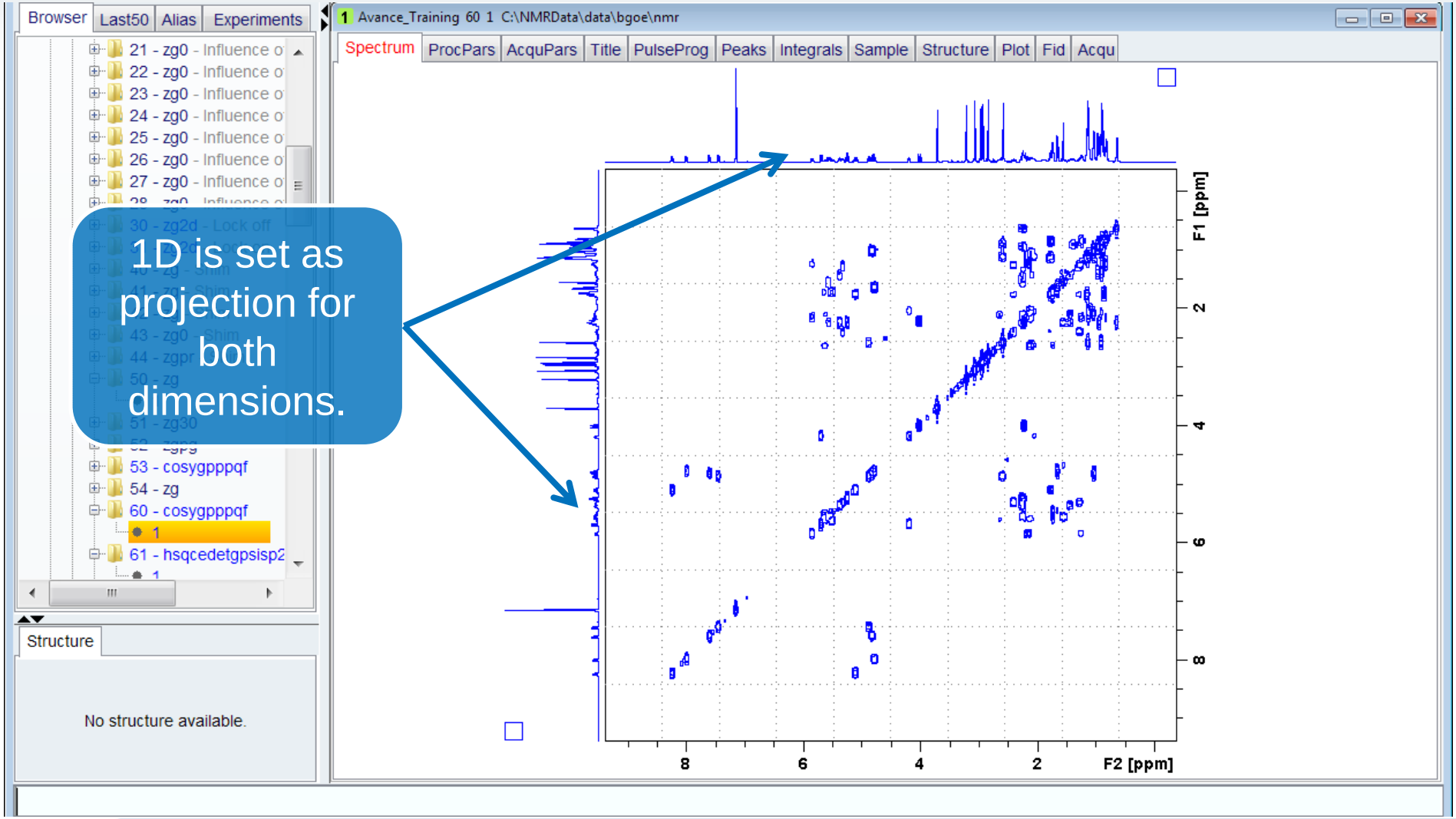
Acquire Toolbar - SetLimits



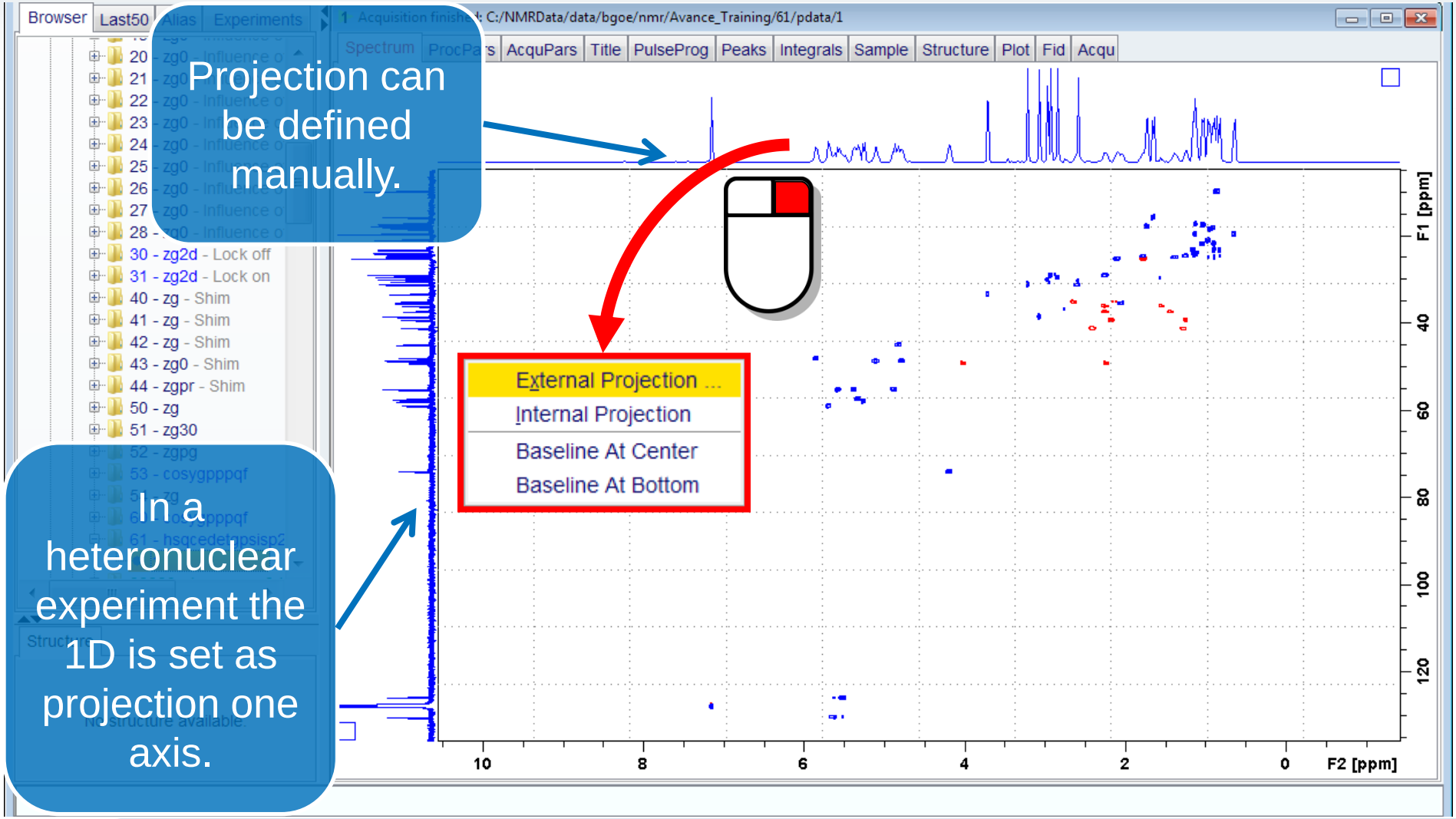
Acquire Toolbar - SetLimits



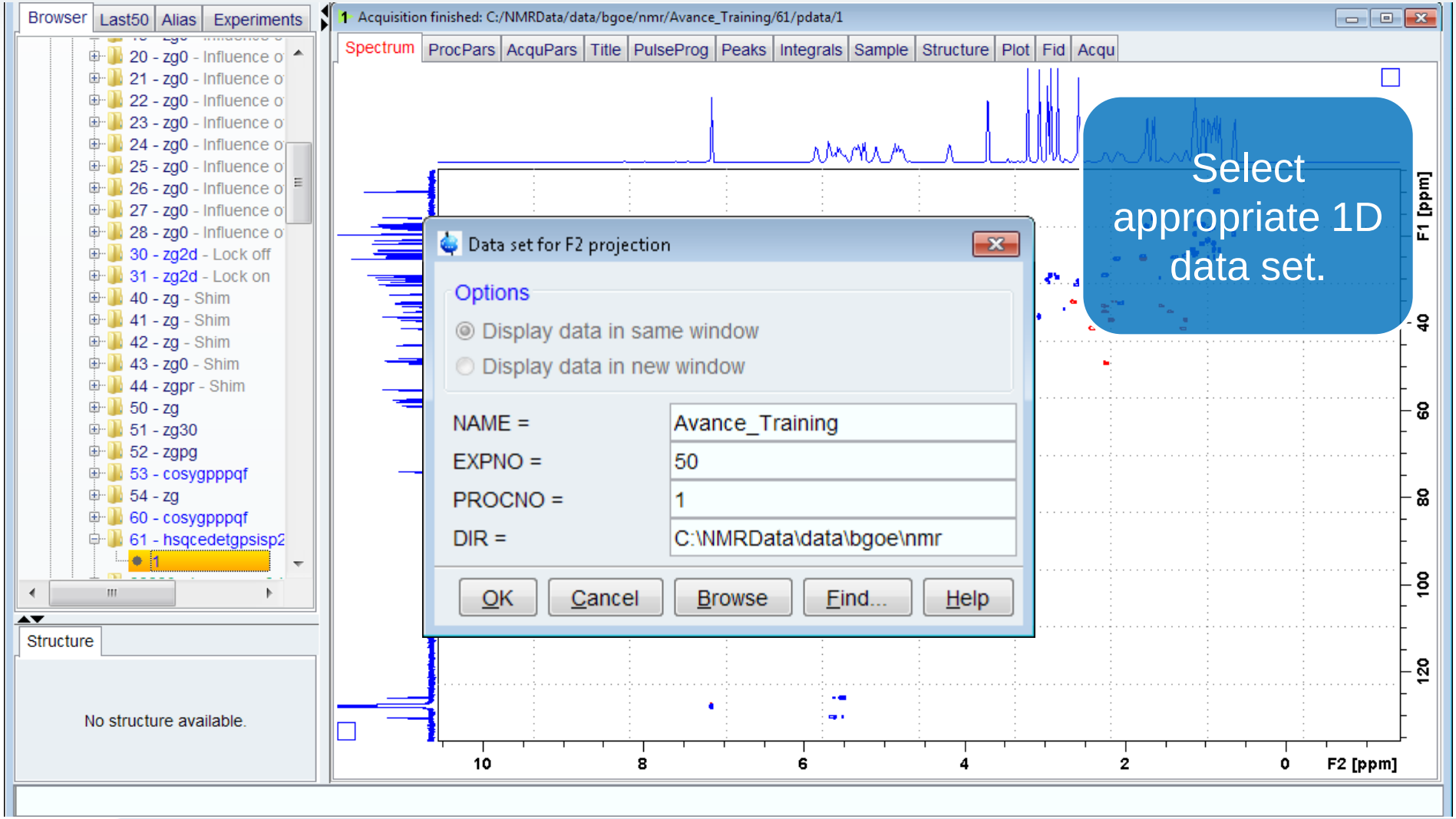
Projections



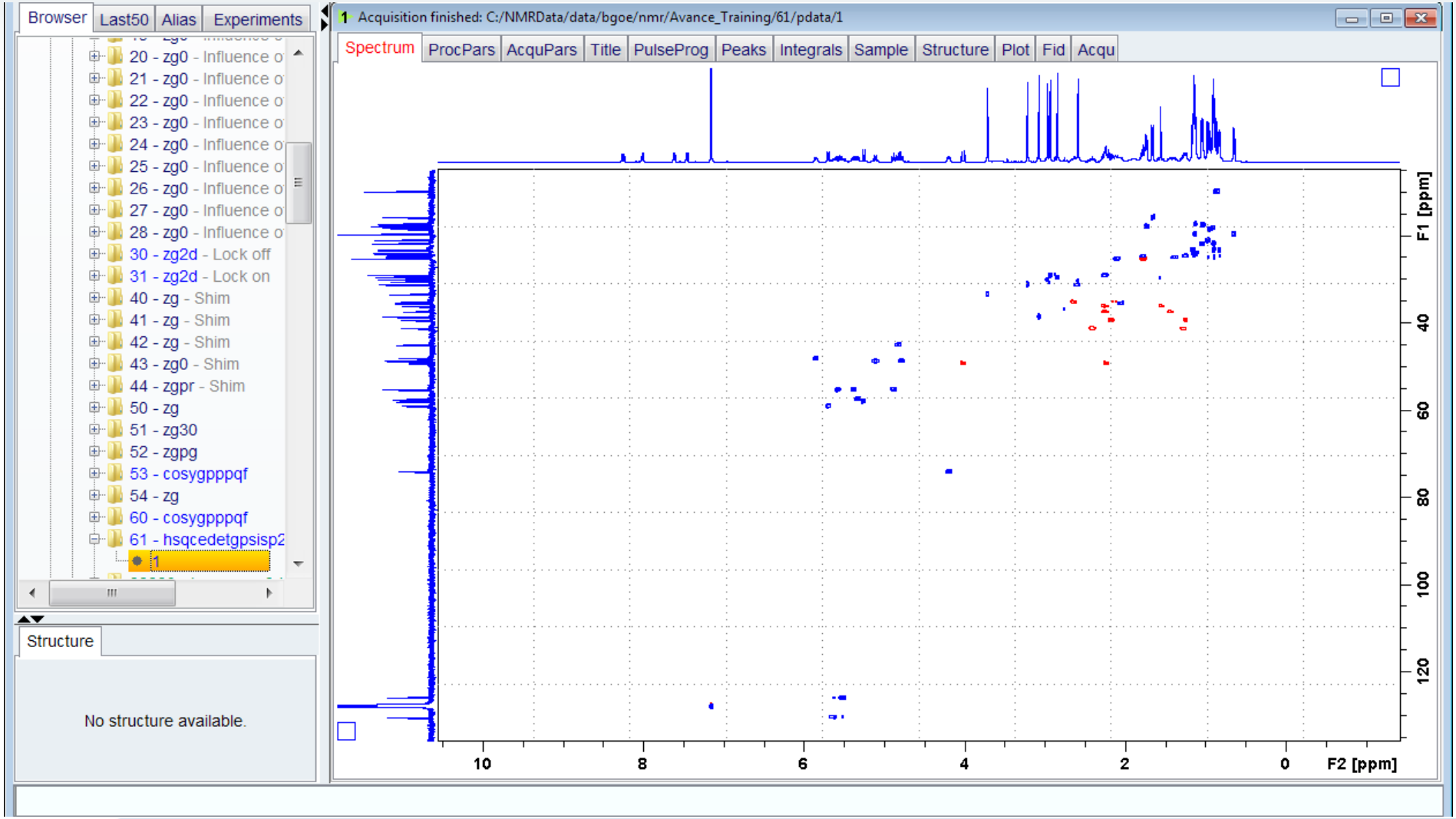
Projections



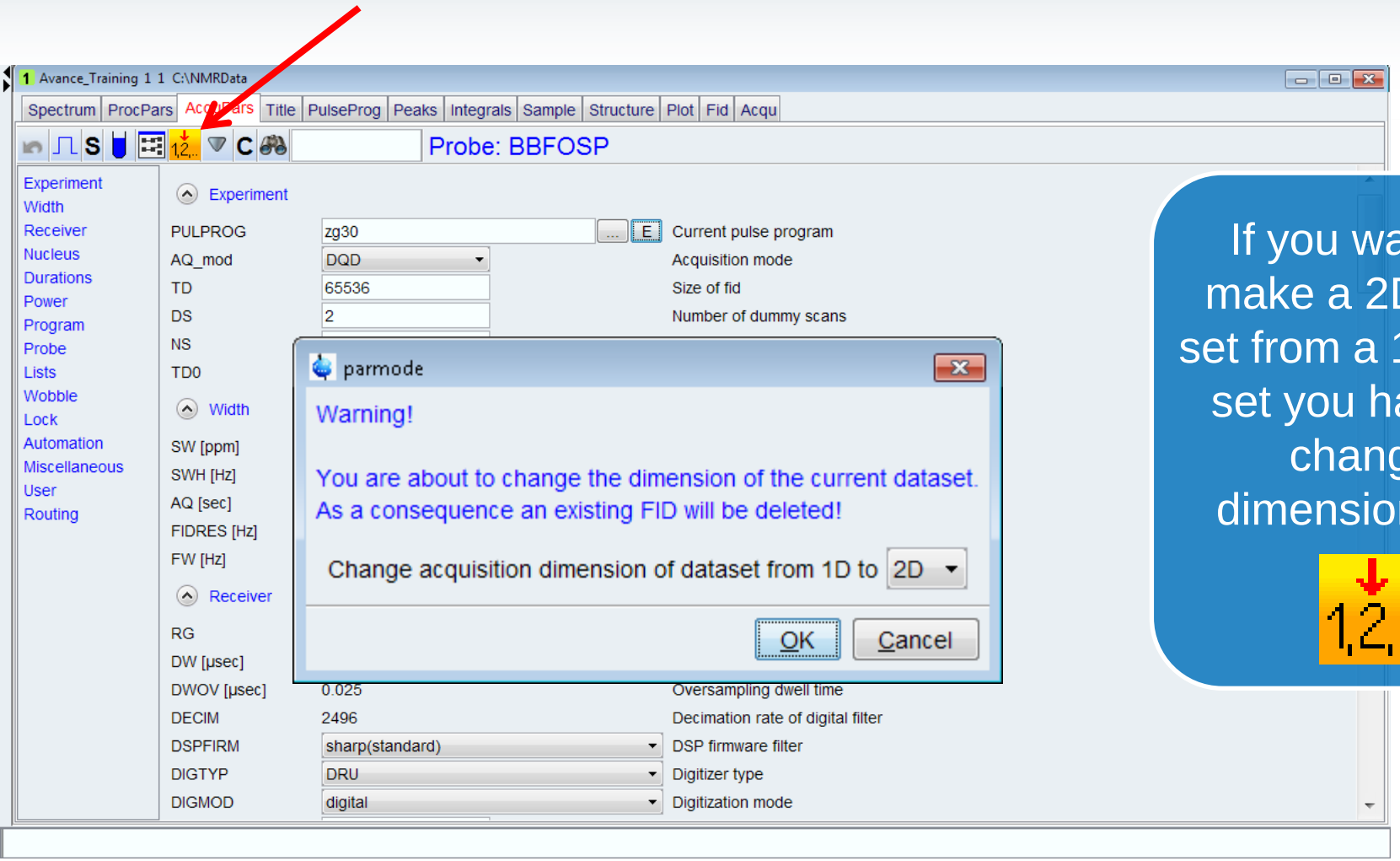
Projections



Projections



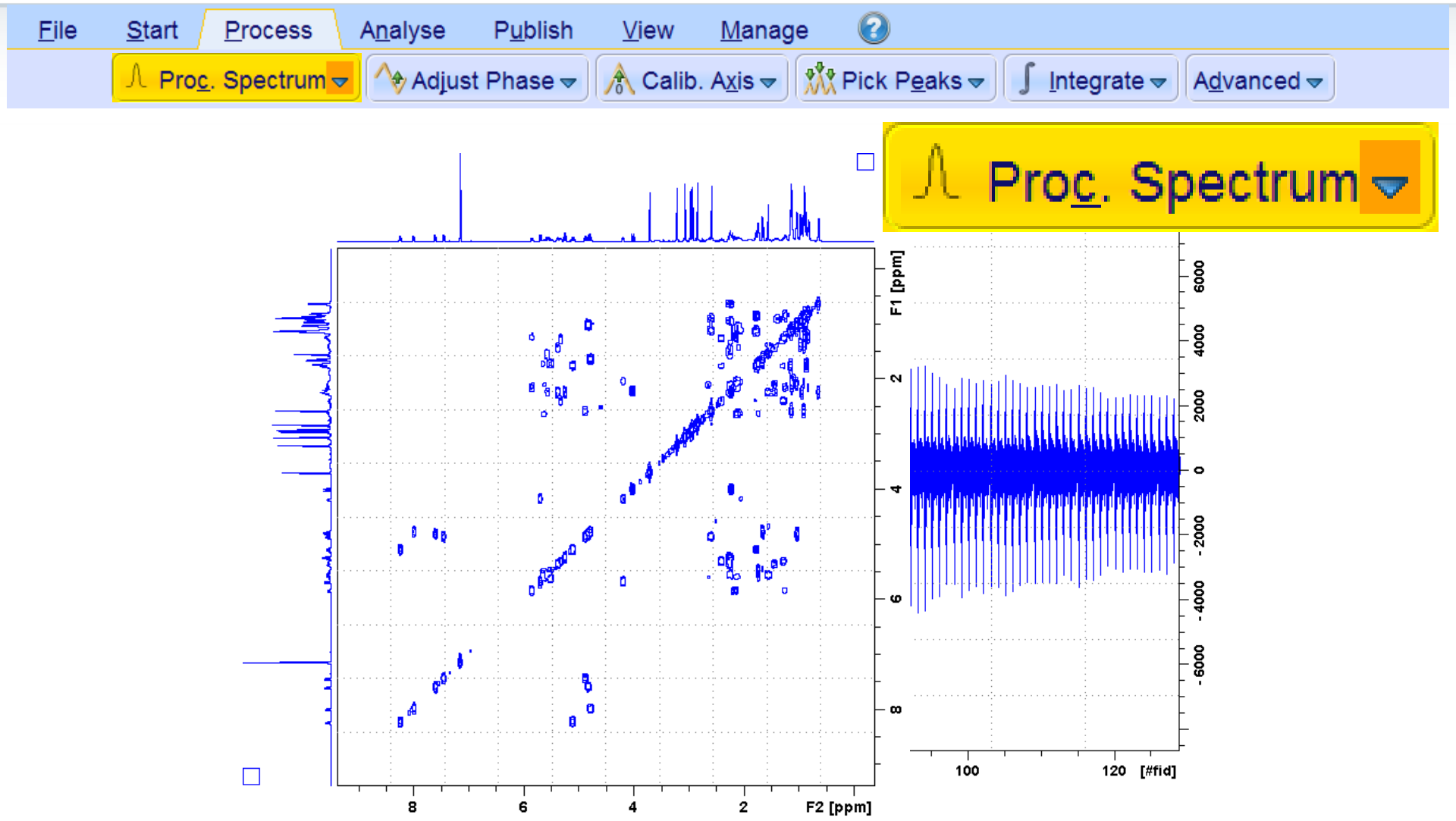
From 1D to 2D



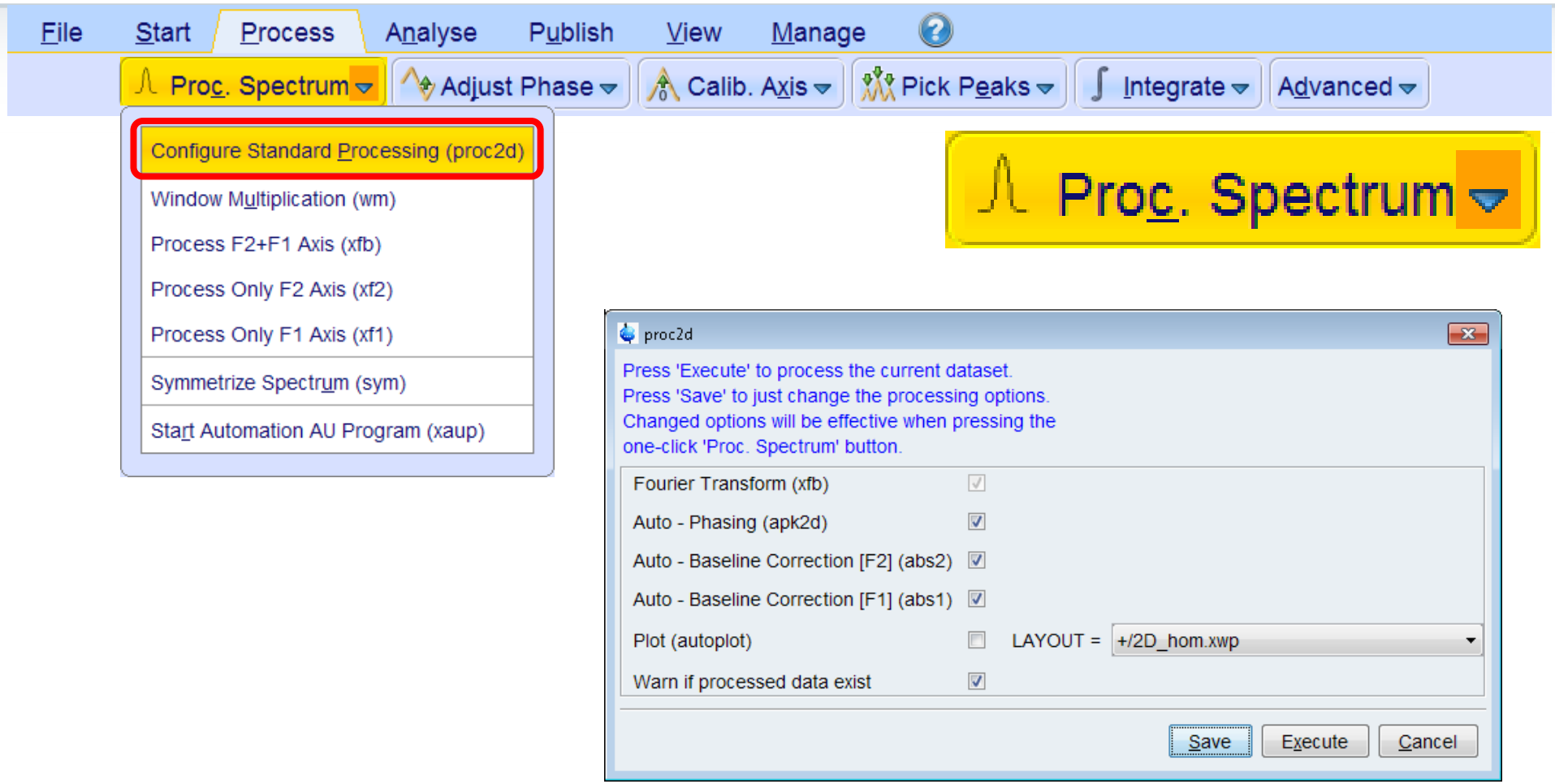
If you want to make a 2D data set from a 1D data set you have to change dimensionality.

- **2D Processing**

1-Click processing



1-Click processing options



Processing commands

- [**xfb**] processes data in both dimensions with the following steps:
  - Baseline correction
  - Linear prediction
  - Window multiplication
  - Fourier transformation
  - Phase correction
- [**xf2**] processes data only in F2
- [**xf1**] processes data only in F1

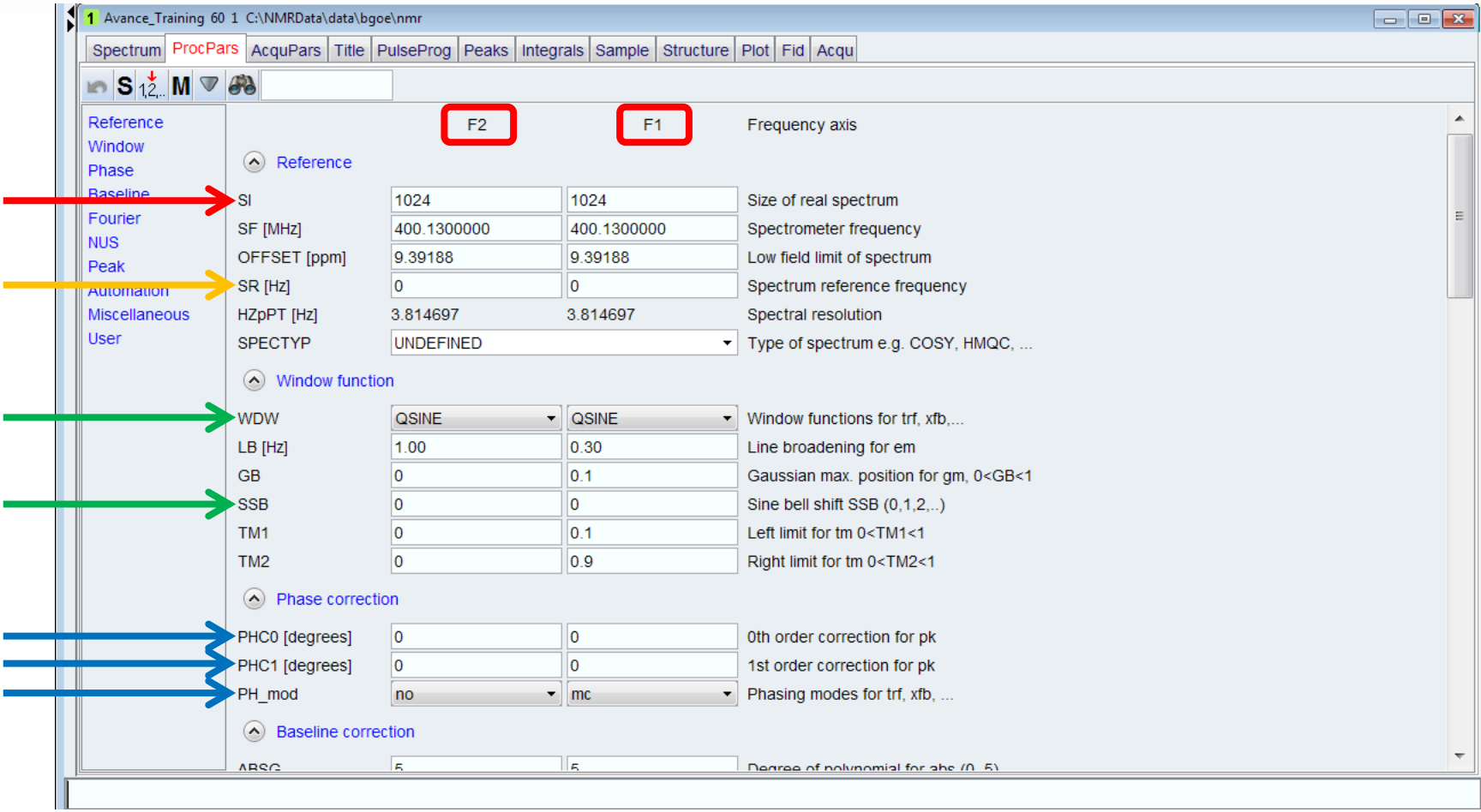
Parameters

- Parameters are :

  size SI
  spectrum reference frequency SR
  spectral resolution HzpPt
  window function WDW
  sine bell shift SSB
  phase mode PH_mod
  0^{th} order correction PHC0
  1^{st} order correction PHC1

- **All of these processing parameters are needed twice!**

Processing parameters [edp]



Typical processing parameters

	F2 dimension	F1 dimension
SI	TD(F2)	4(8)×TD(F1)
WDW	QSIN	QSIN
SSB	0	0
PH_mod	no	mc
PHC0	0	-
PHC1	0	-

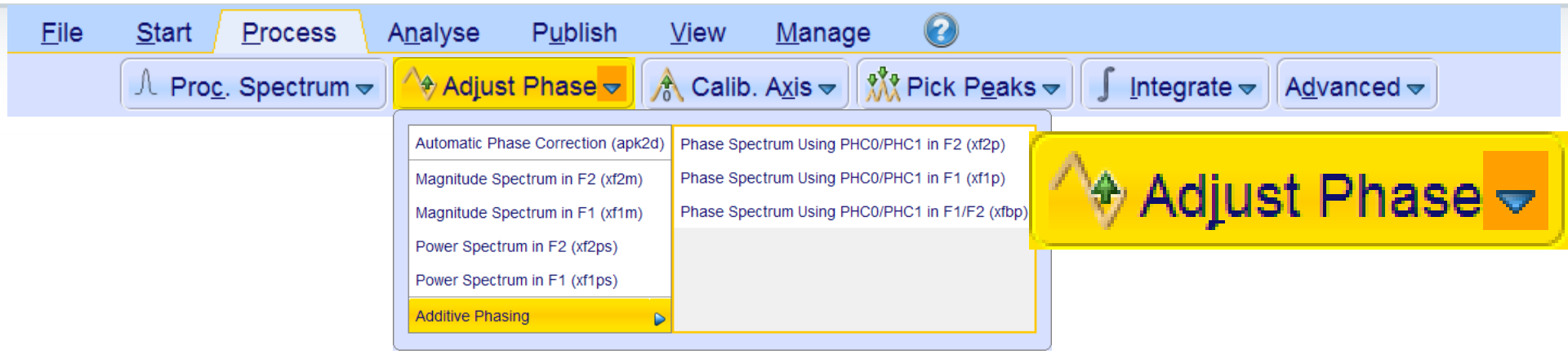
For FnMODE: QF

Typical processing parameters

	F2 dimension	F1 dimension
SI	**TD(F2)**	**4(8)×TD(F1)**
WDW	**QSIN**	**QSIN**
SSB	**2**	**2**
PH_mod	**pk**	**pk**
PHC0	**0**	Depends on
PHC1	**0**	experiment

For FnMODE: States, TPPI, States-TPPI, Echo-Antiecho

Adjust Phase



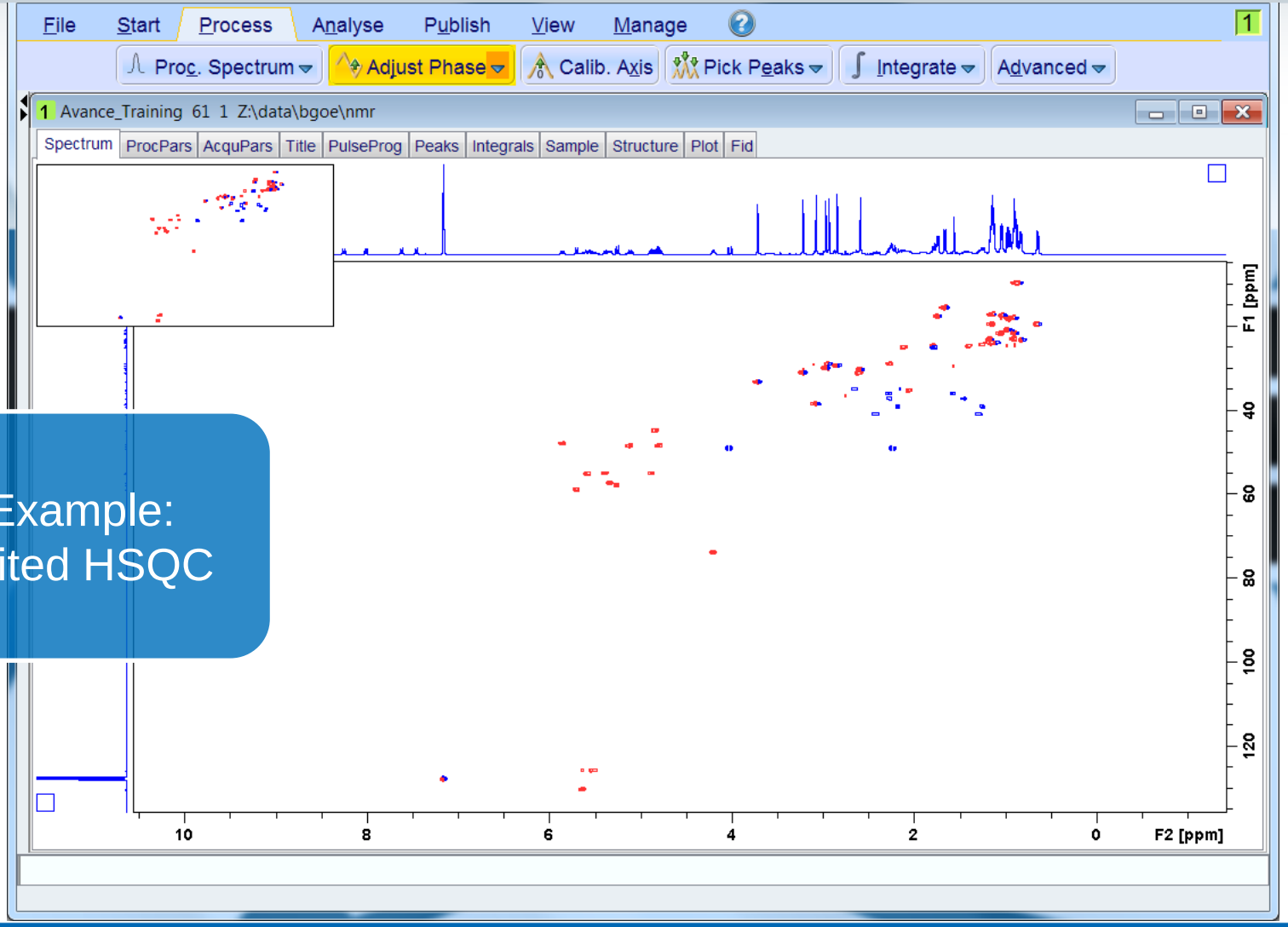
- Manual phase correction with [**.ph**]
- Automatic phase correction with [**apk2d**]
- [**xfb**] uses values of PHC0(F1/F2) and PHC1(F1/F2)

Phase correction of 2D experiments

No phase correction is necessary for not phase sensitive experiments!

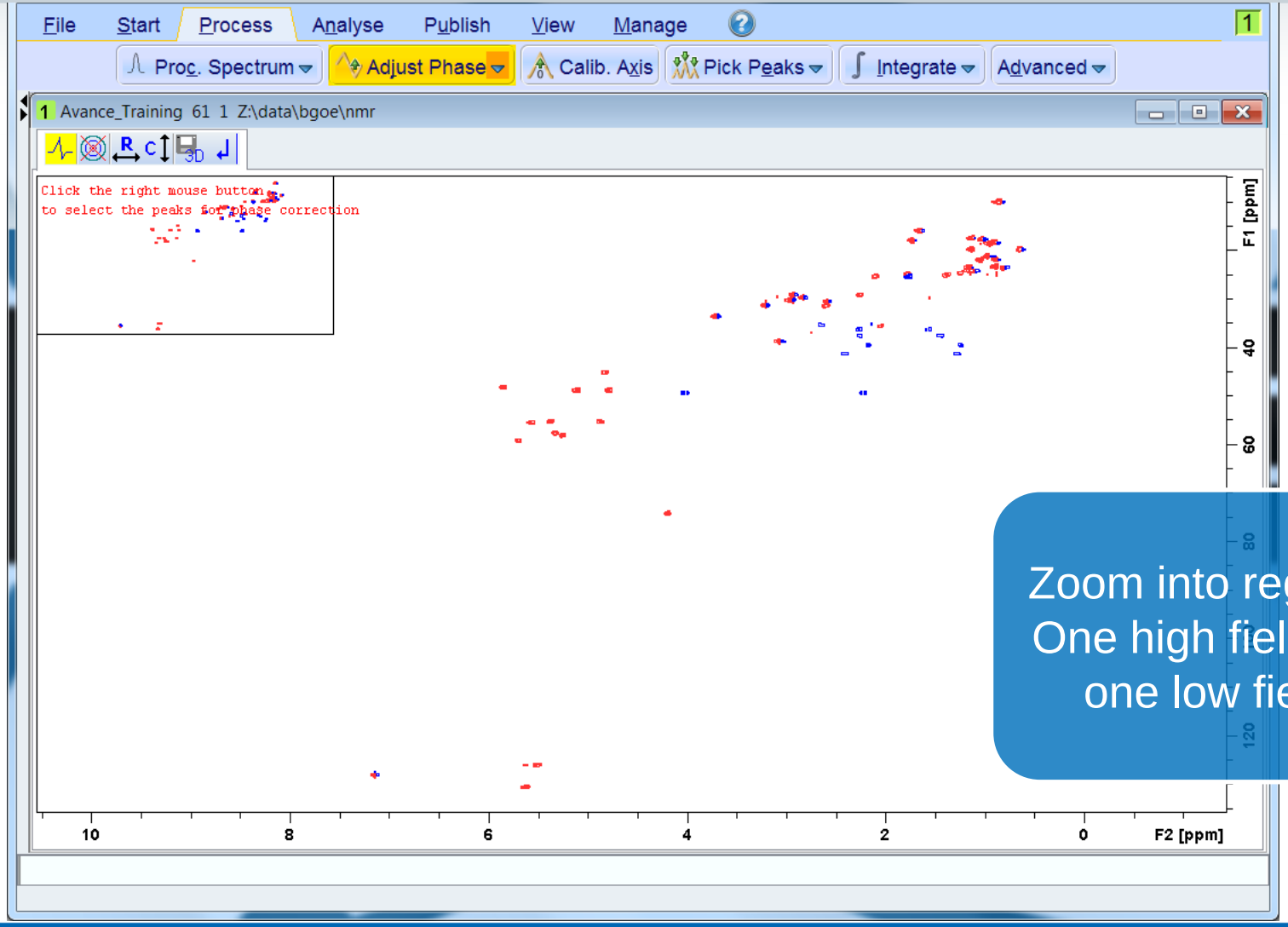
- Phase correction for phase sensitive experiments are typically only necessary for the F2-dimension.
- The values for homonuclear experiments for F1 are mentioned in the pulse program.
- Heteronuclear experiments are automatically corrected in F1.

Phasing



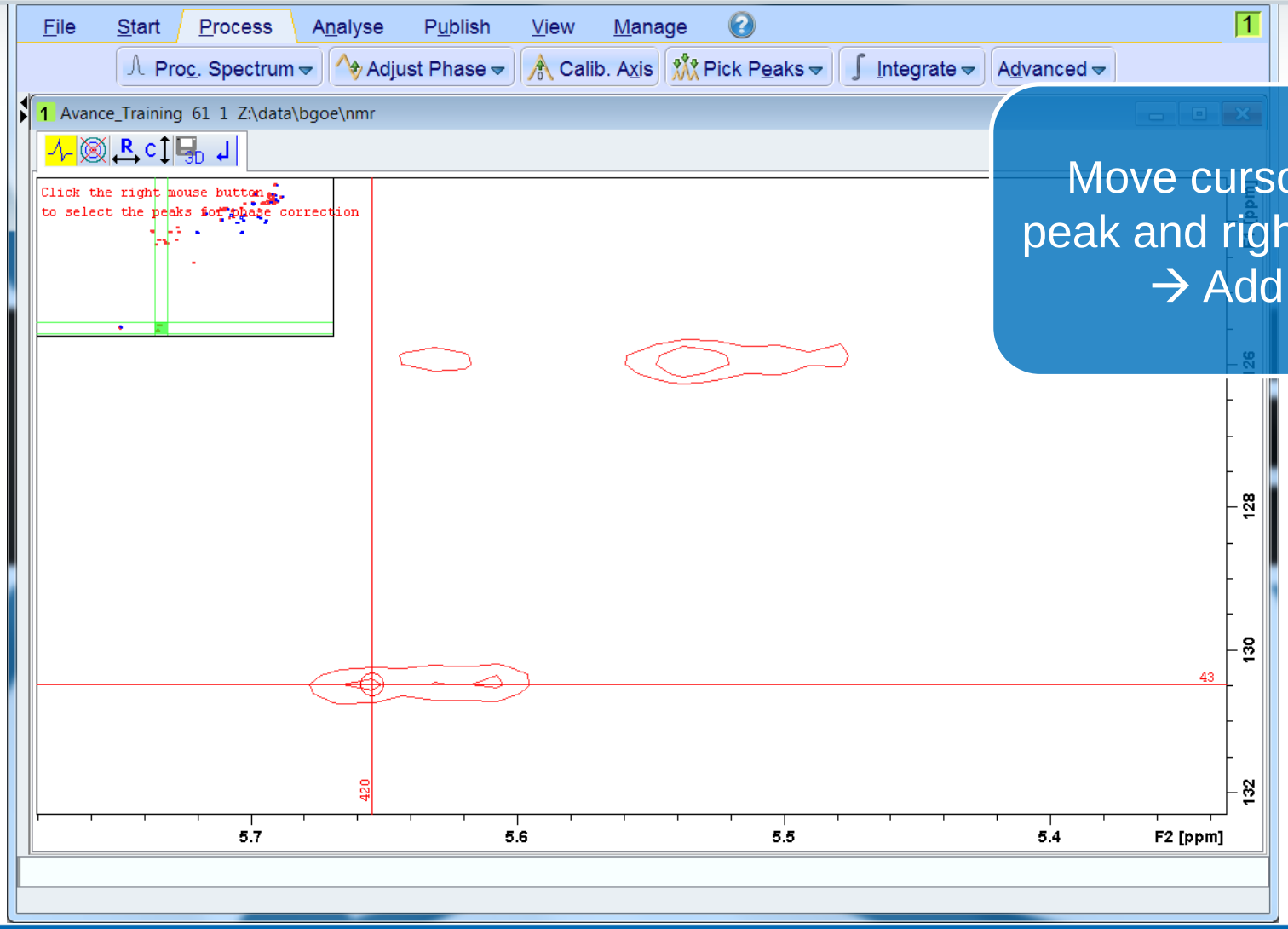
Example:
edited HSQC

Phasing

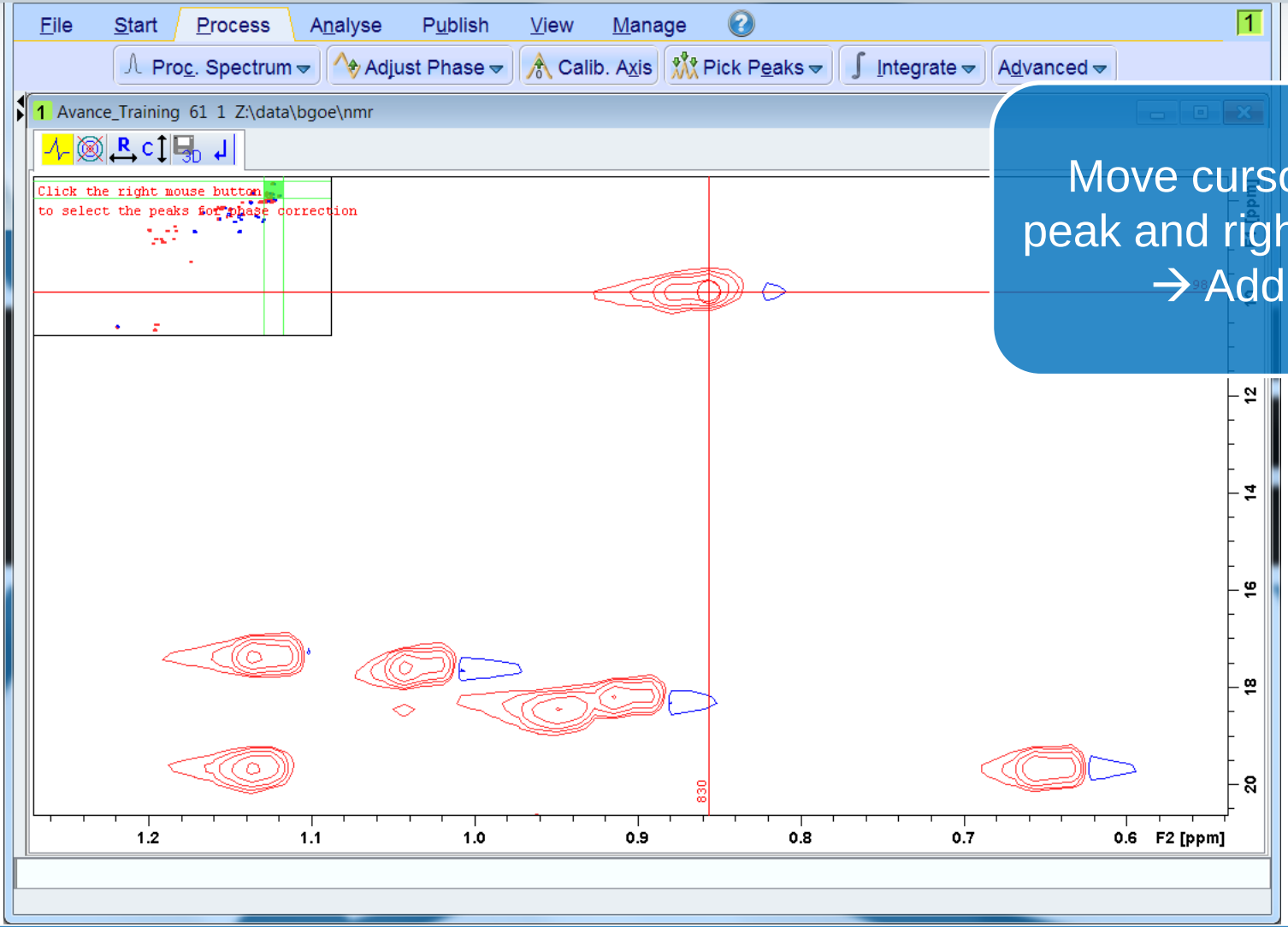


Zoom into regions. One high field and one low field.

Phasing

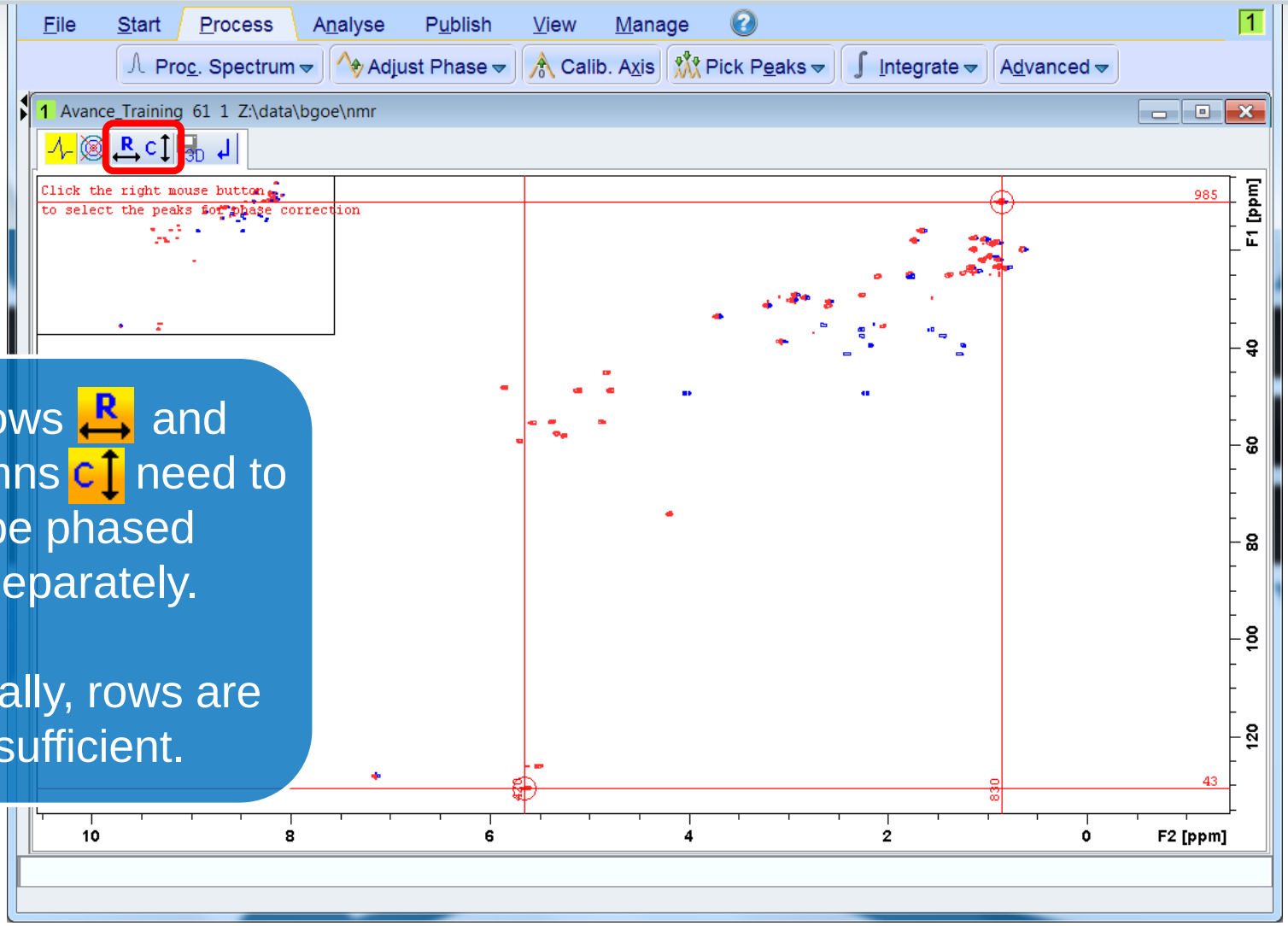

File
Start
Process
Analyse
Publish
View
Manage
Proc. Spectrum
Adjust Phase
Calib. Axis
Pick Peaks
Integrate
Advanced
1 Avance_Training 61 1 Z:\data\bgoe\nmr
Click the right mouse button
to select the peaks for phase correction
Move cursor to peak and right click → Add
5.7
5.6
5.5
5.4
F2 [ppm]
128
130
132
420
43

Phasing



Move cursor to peak and right click → Add

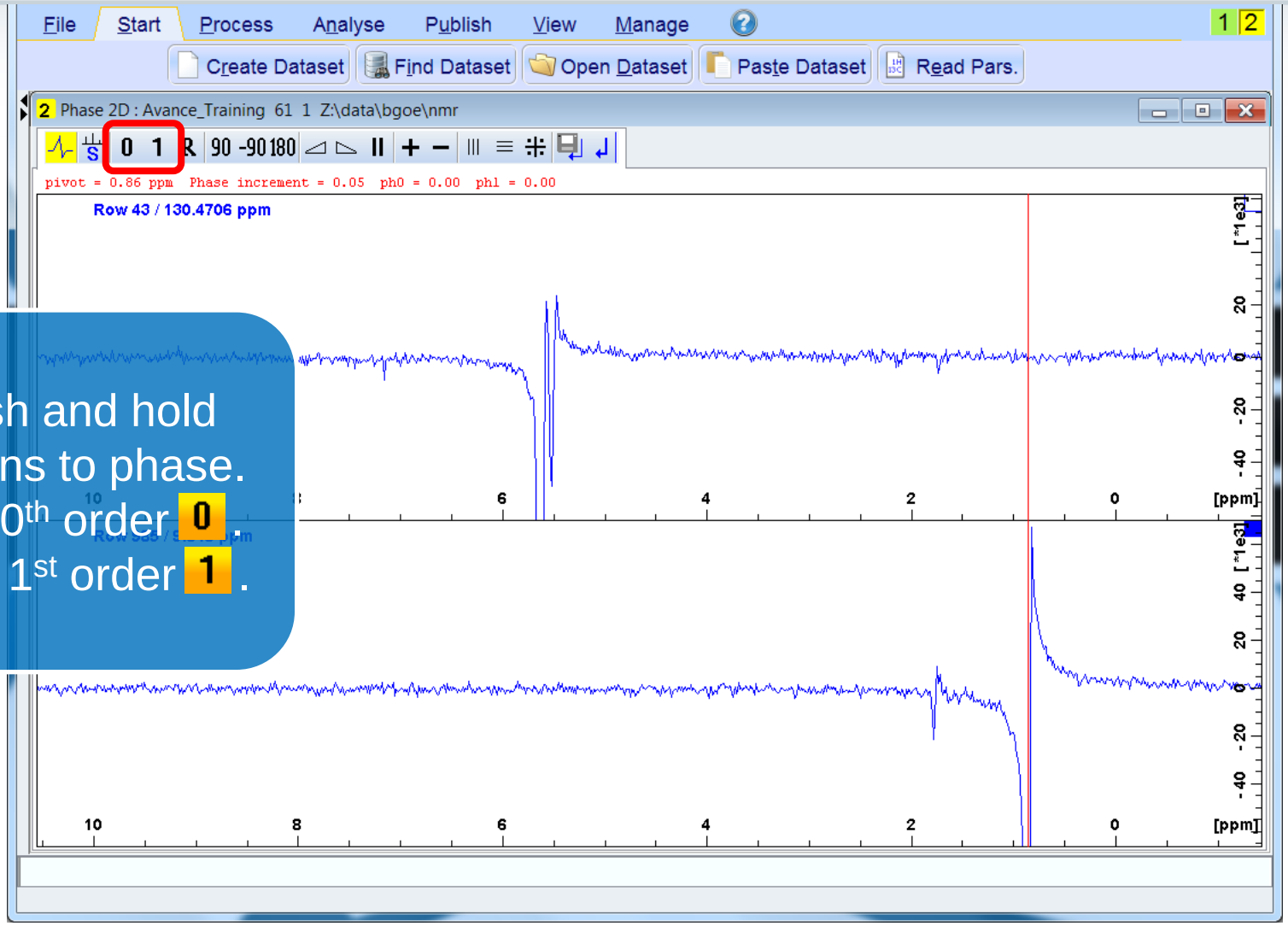
Phasing



Rows R and columns C need to be phased separately.

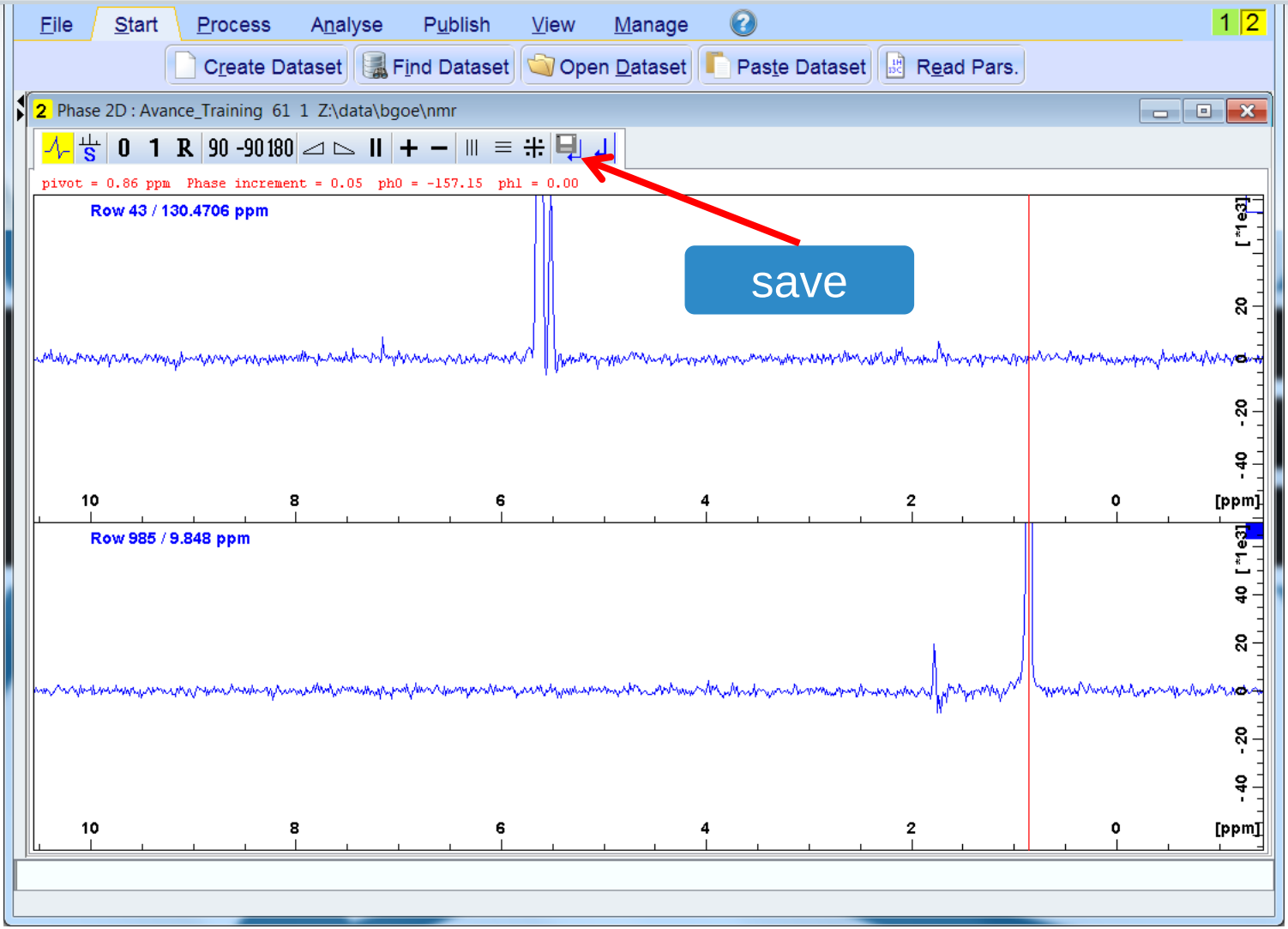
Usually, rows are sufficient.

Phasing

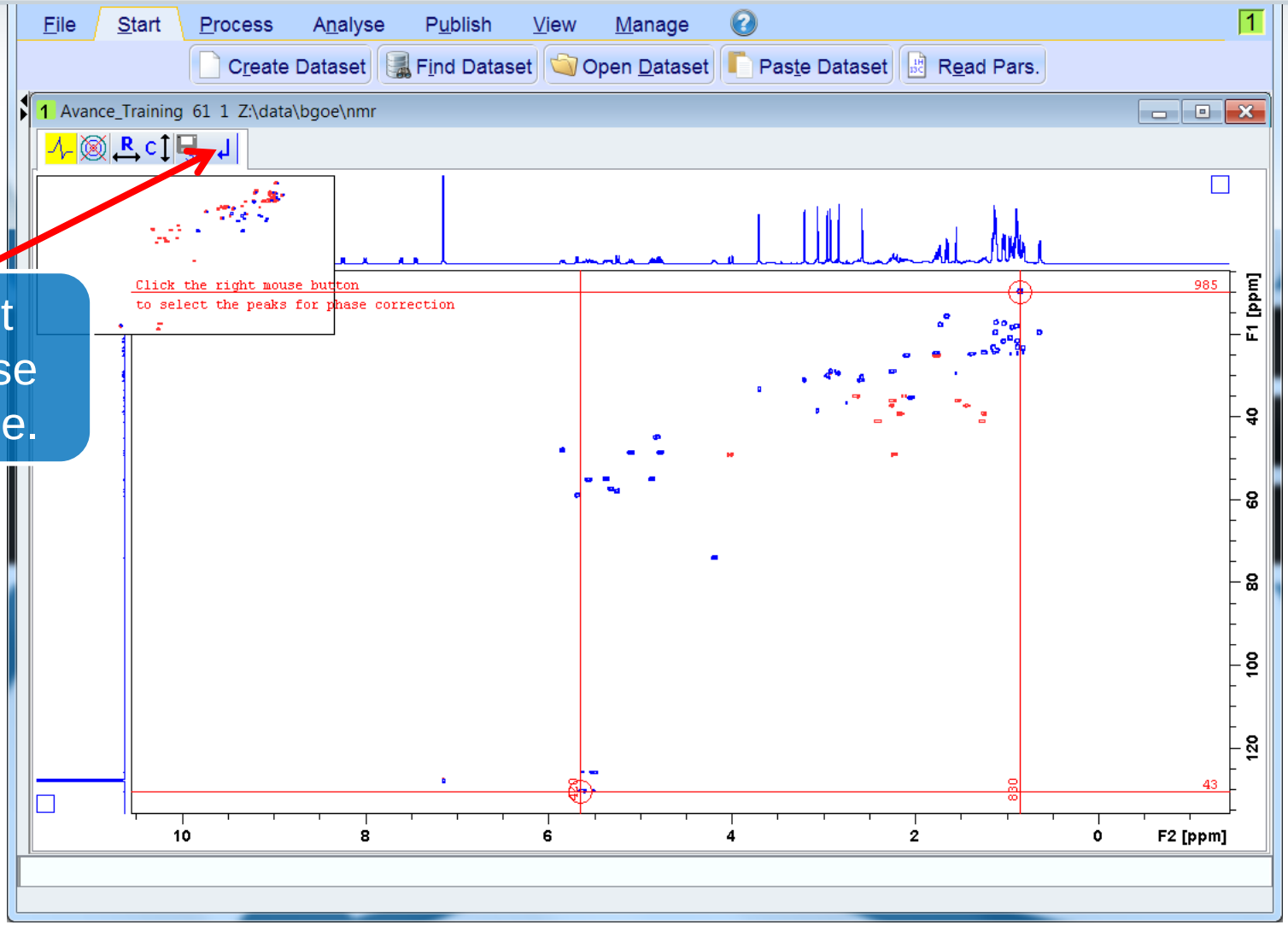


Push and hold buttons to phase.
First 0th order **0**.
Then 1st order **1**.

Phasing

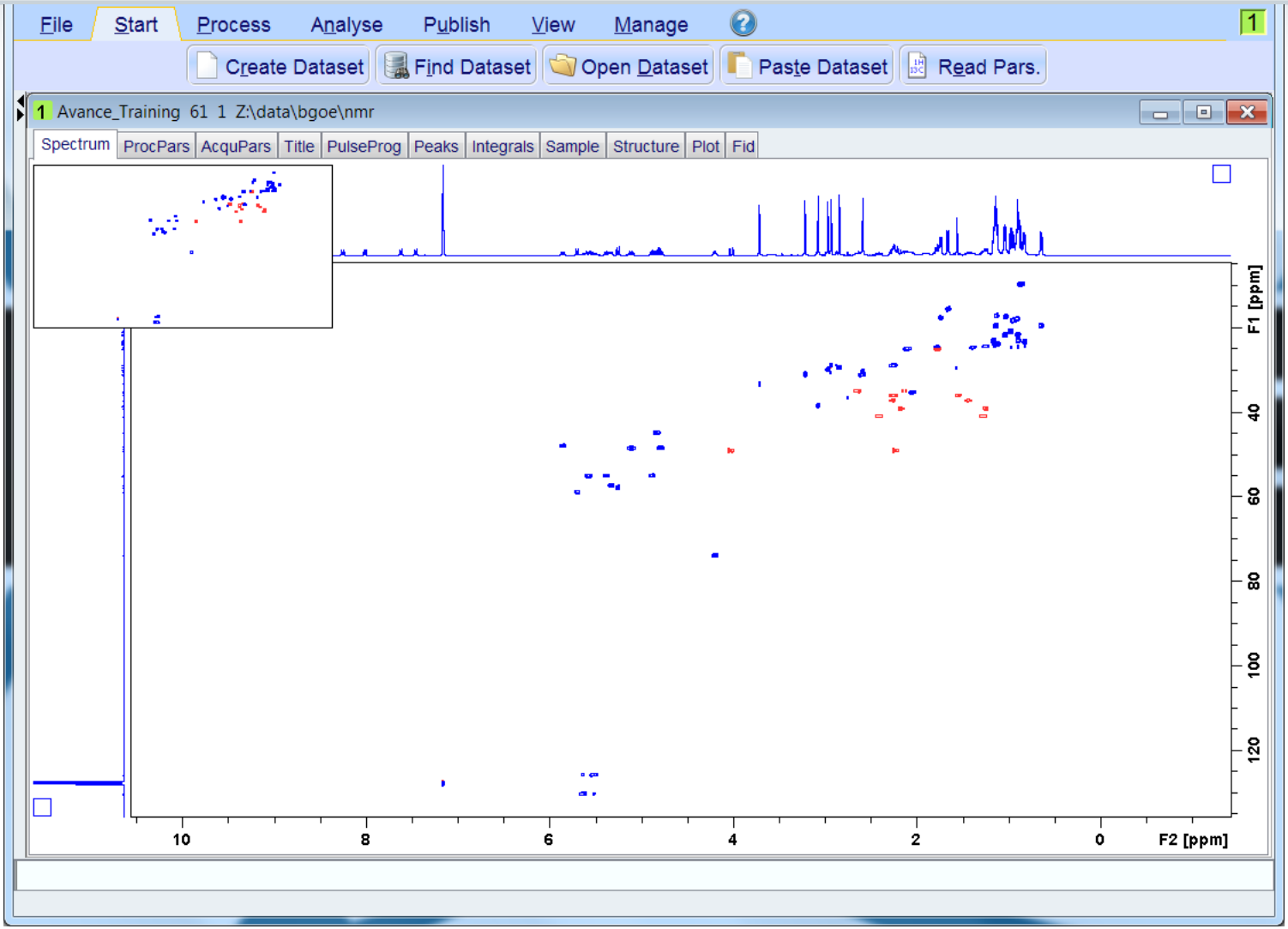

File
Start
Process
Analyse
Publish
View
Manage
Create Dataset
Find Dataset
Open Dataset
Paste Dataset
Read Pars.
2 Phase 2D : Avance_Training 61 1 Z:\data\bgoe\nmr
pivot = 0.86 ppm Phase increment = 0.05 ph0 = -157.15 ph1 = 0.00
Row 43 / 130.4706 ppm
save
Row 985 / 9.848 ppm
10
8
6
4
2
0
[ppm]

Phasing

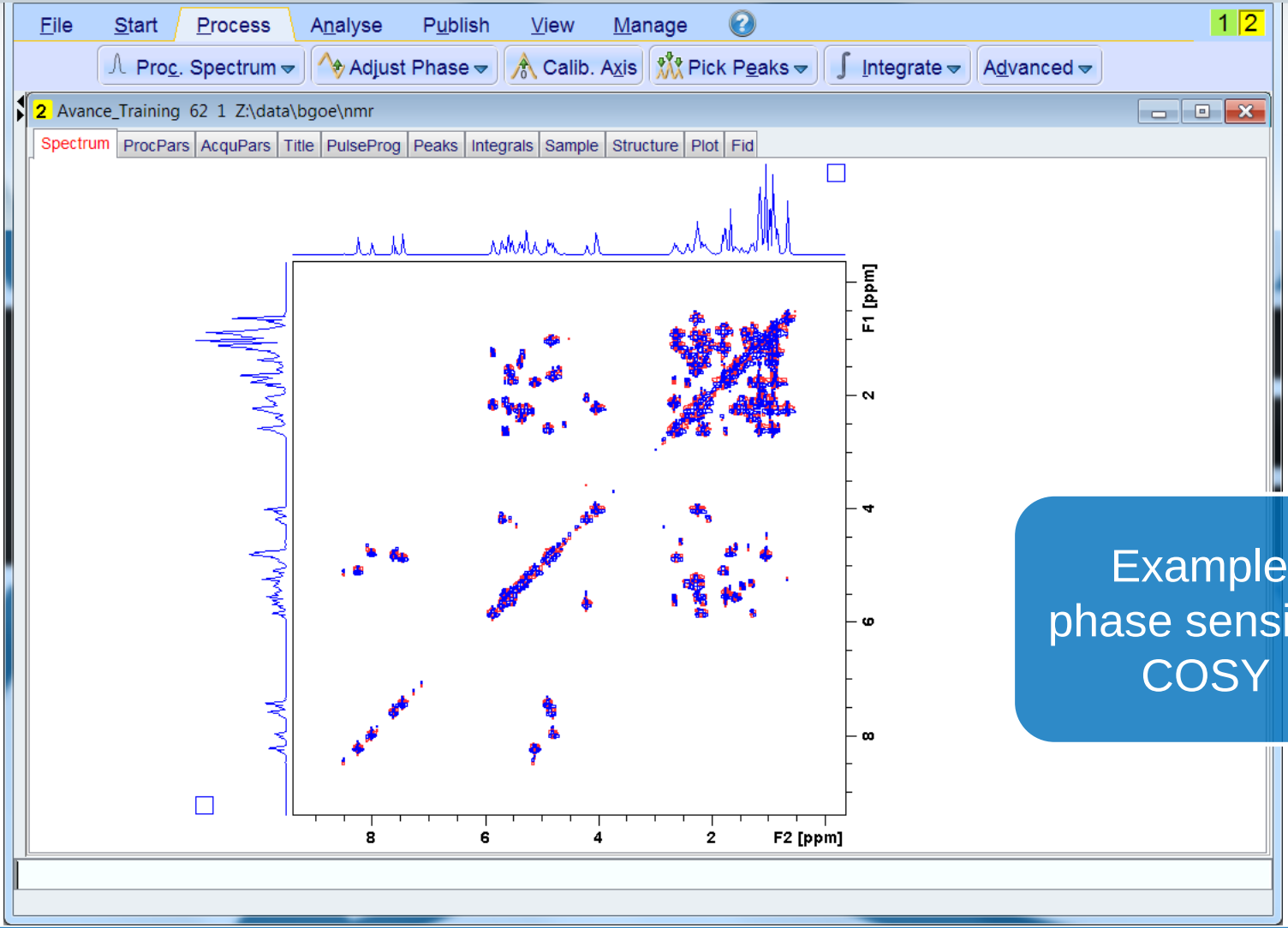


Exit phase mode.

Phasing

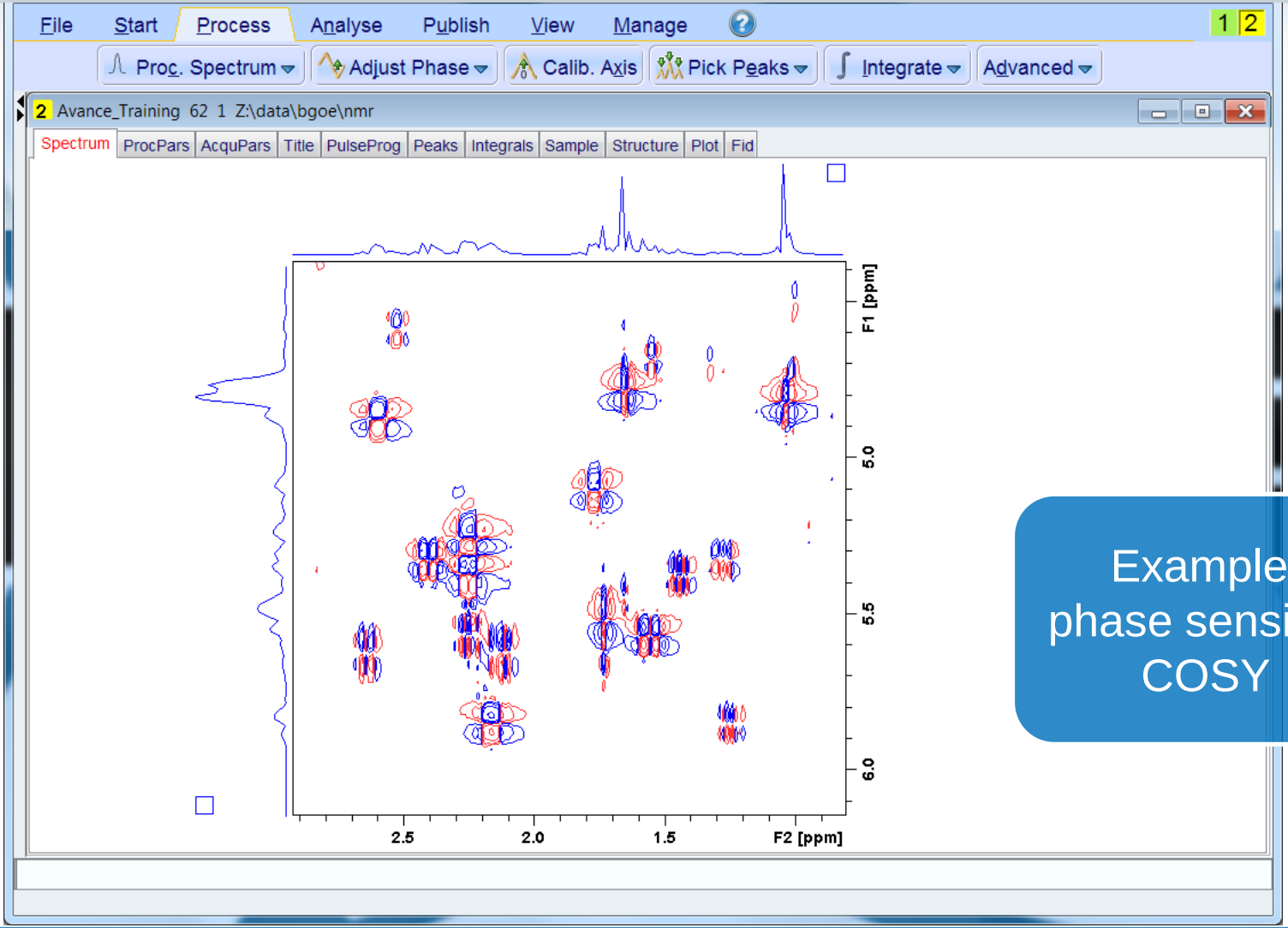

File
Start
Process
Analyse
Publish
View
Manage
Create Dataset
Find Dataset
Open Dataset
Paste Dataset
Read Pars.
1 Avance_Training 61 1 Z:\data\bgoe\nmr
Spectrum
ProcPars
AcquPars
Title
PulseProg
Peaks
Integrals
Sample
Structure
Plot
Fid
F1 [ppm]
F2 [ppm]
10
8
6
4
2
0
40
60
80
100
120

Phasing
DQF-COSY



Example:
phase sensitive
COSY

Phasing
DQF-COSY



Example:
phase sensitive
COSY

Phasing DQF-COSY

Phase correction for F1 is mentioned in pulse program.

cosygpmfphpp (C:\Bruker\TopSpin3.5pl7\exp\stan\nmr\lists\pp)

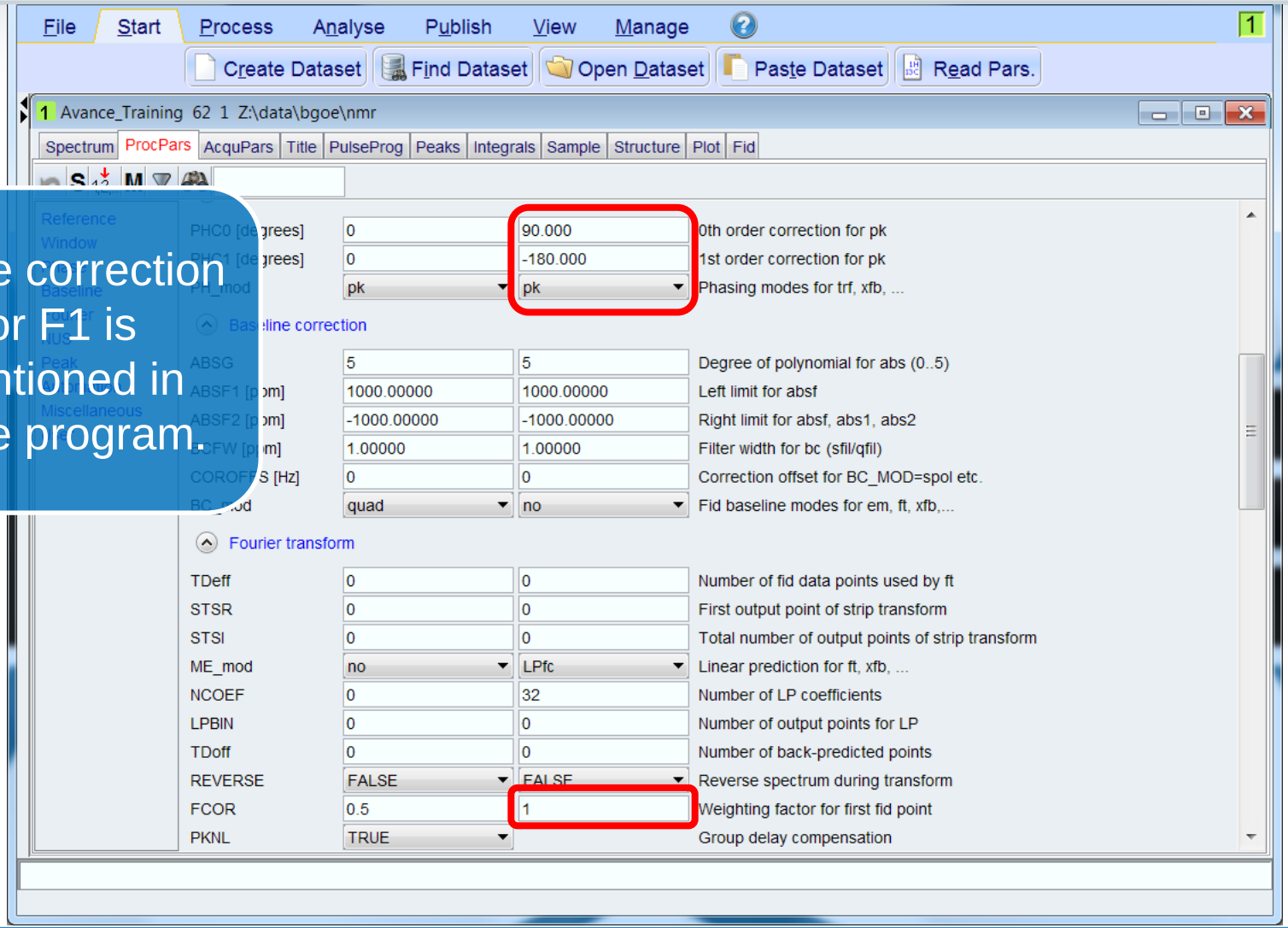
File Edit Search

Graphical Edit | Set PULPROG

```
;pl10: f1 channel - power level for TOCSY-spinlock
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse
;p17: f1 channel - trim pulse                        [2.5 msec]
;d0 : incremented delay (2D)
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O                             [30 msec]
;d12: delay for power switching                      [20 usec]
;d16: delay for homospoil/gradient recovery
;inf1: 1/SW = 2 * DW
;in0: 1/(1 * SW) = 2 * DW
;nd0: 1
;ns: 1 * n
;ds: 16
;td1: number of experiments
;FnMODE: States-TPPI, TPPI, States or QSEQ

;use gradient ratio:  gp 1 : gp 2
;       10 :   20   for double quantum filter
;       10 :   30   for triple quantum filter

;for z-only gradients:
;gpz1: 10%
;gpz2: 20% for DQF, 30% for TQF

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100


;Processing

;PHC0(F1): 90
;PHC1(F1): -180
;FCOR(F1): 1


;$Id: cosygpmfphpp,v 1.3 2012/01/31 17:49:22 ber Exp $
```

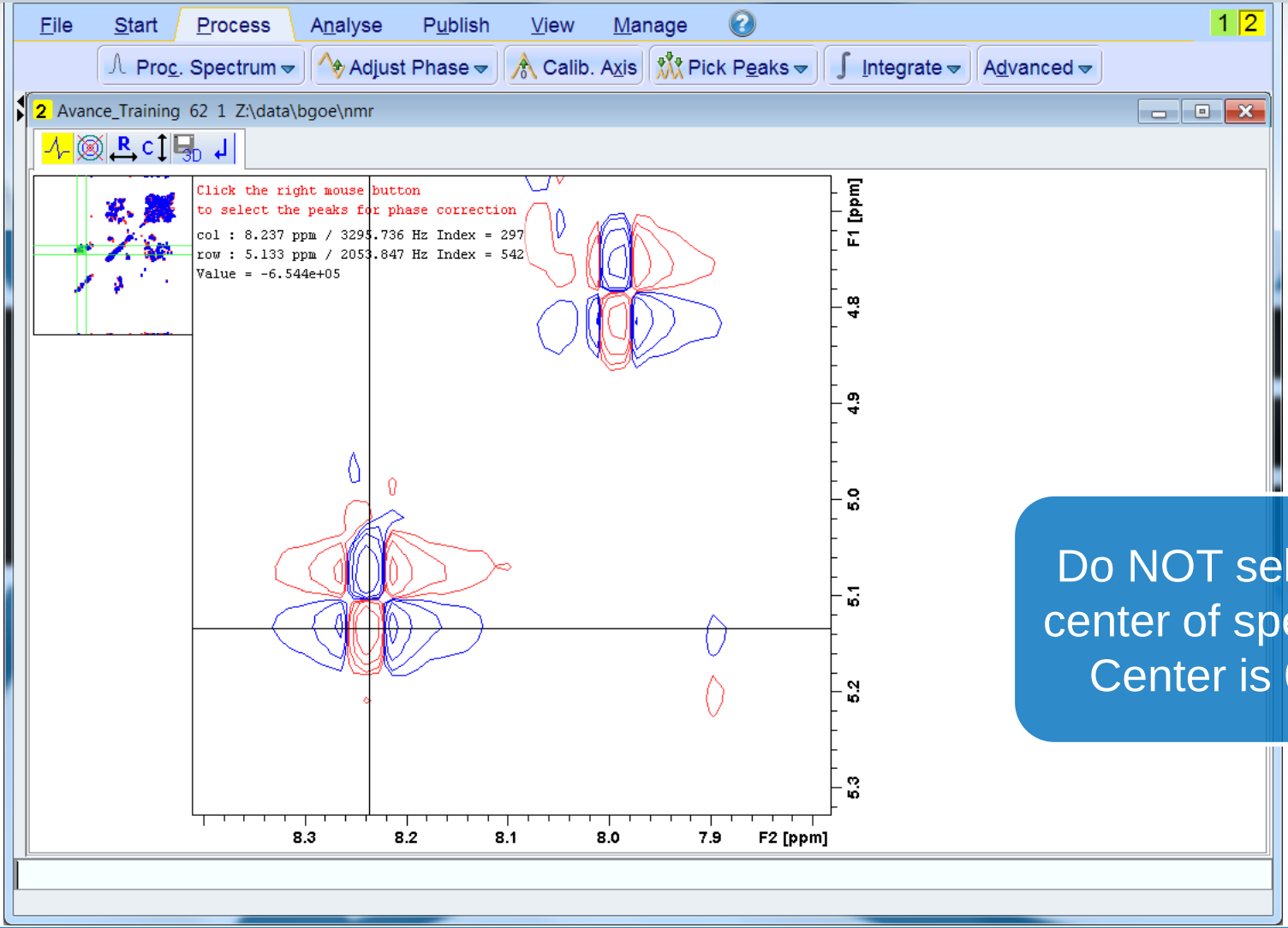
1 : 1

Phasing
DQF-COSY



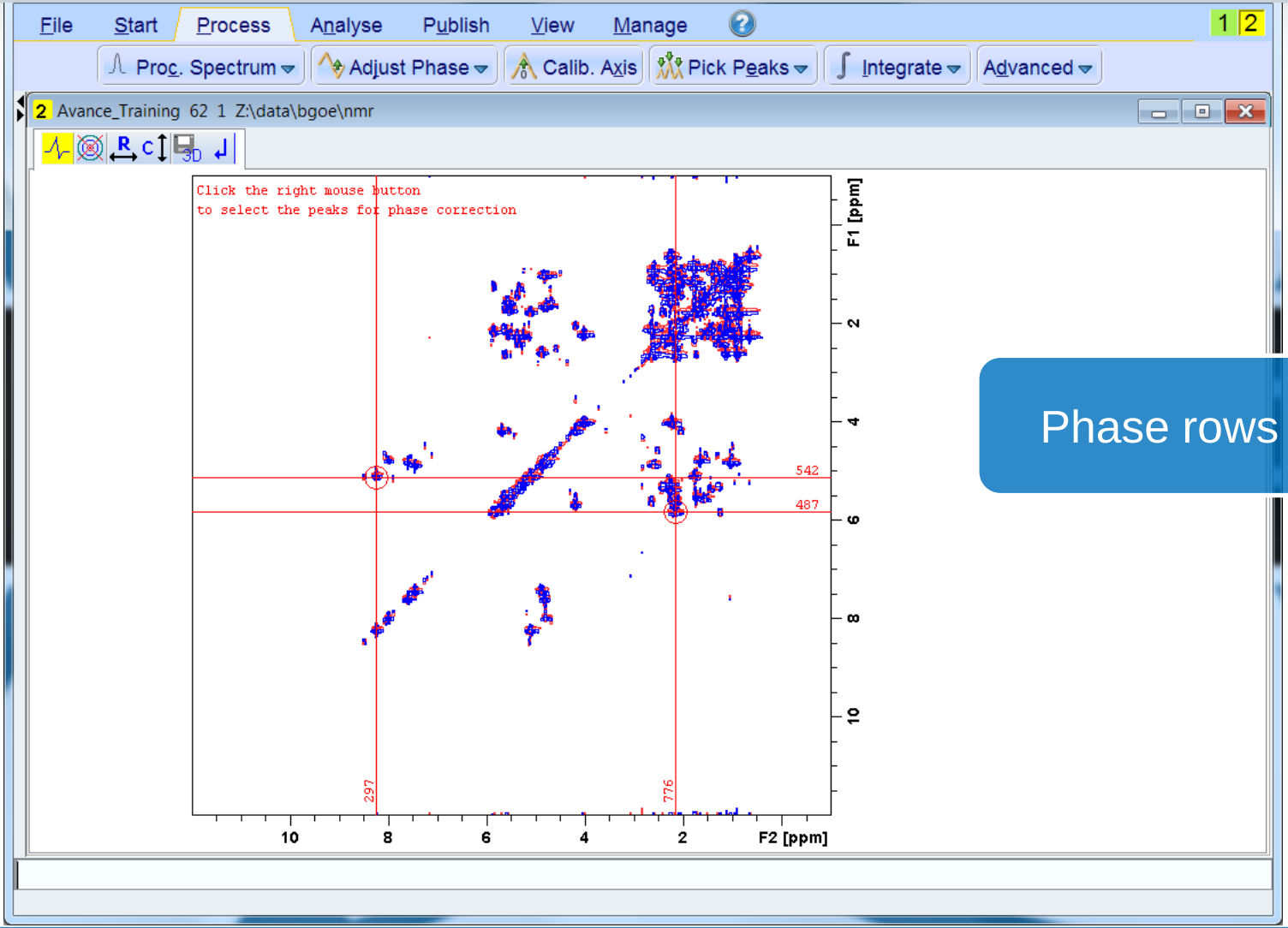
Phase correction for F1 is mentioned in pulse program.

Phasing
DQF-COSY



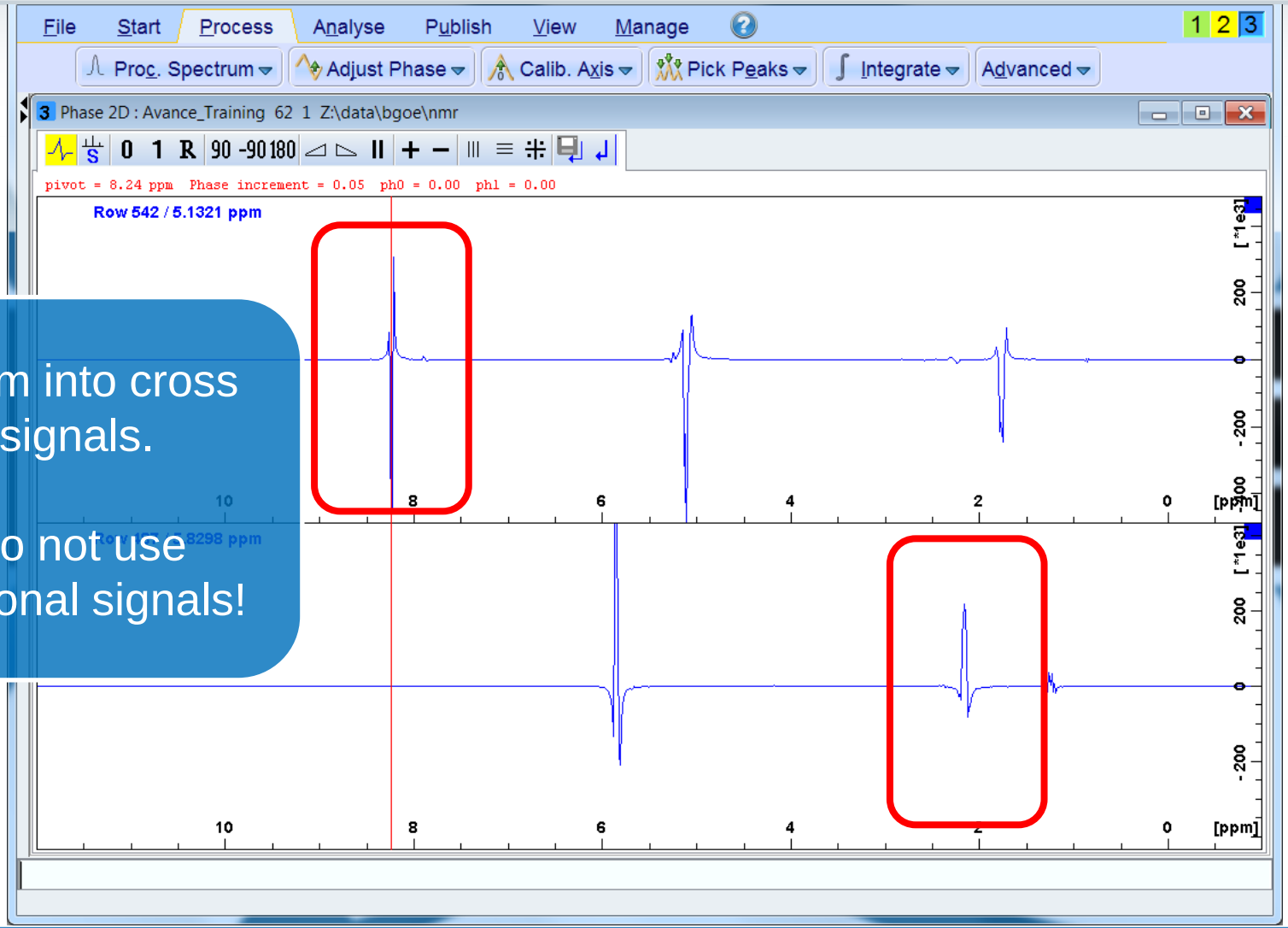
Do NOT select center of speak. Center is 0.

Phasing
DQF-COSY



Phase rows R.

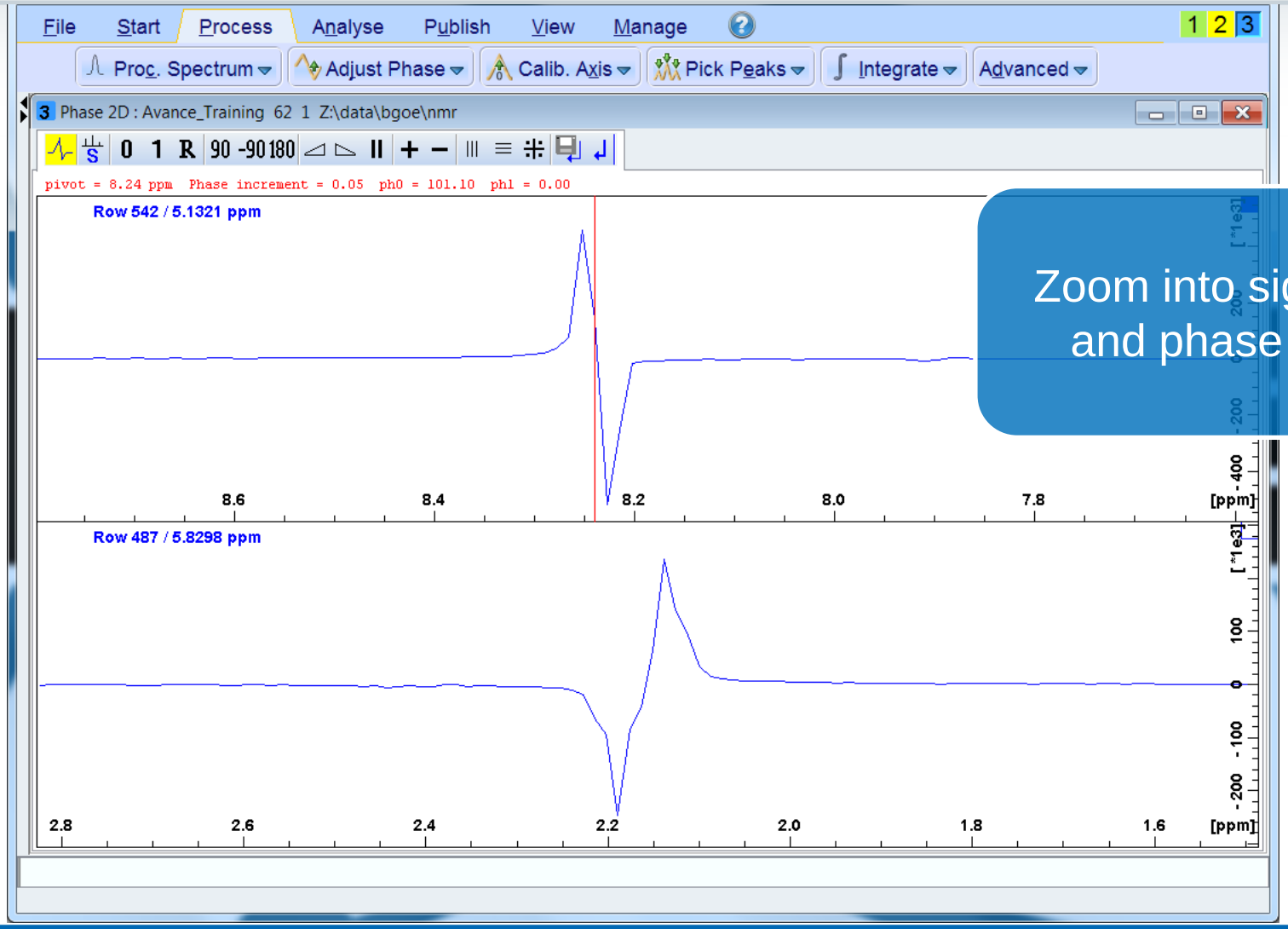
Phasing
DQF-COSY



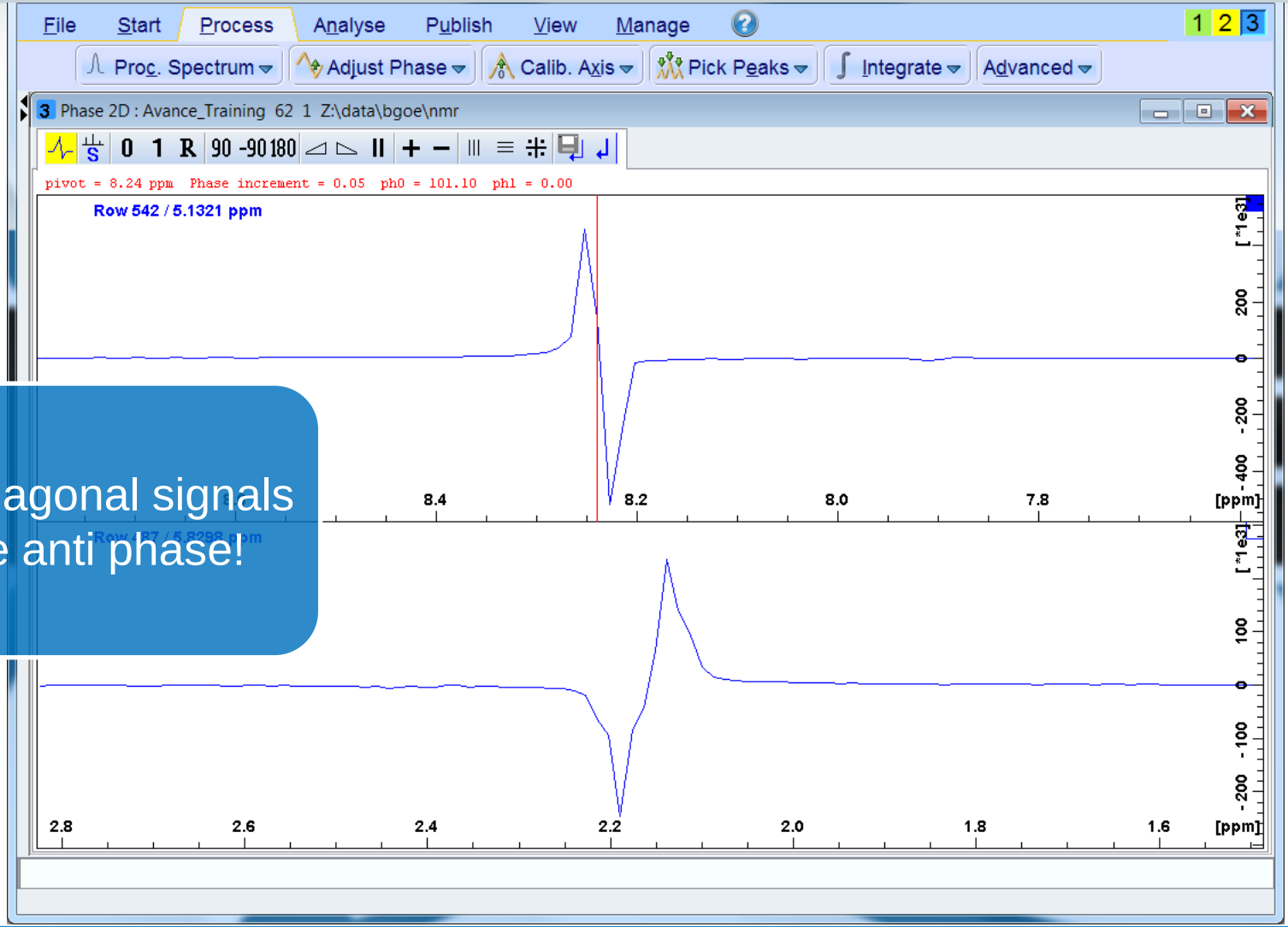
Zoom into cross signals.

Do not use diagonal signals!

Phasing
DQF-COSY

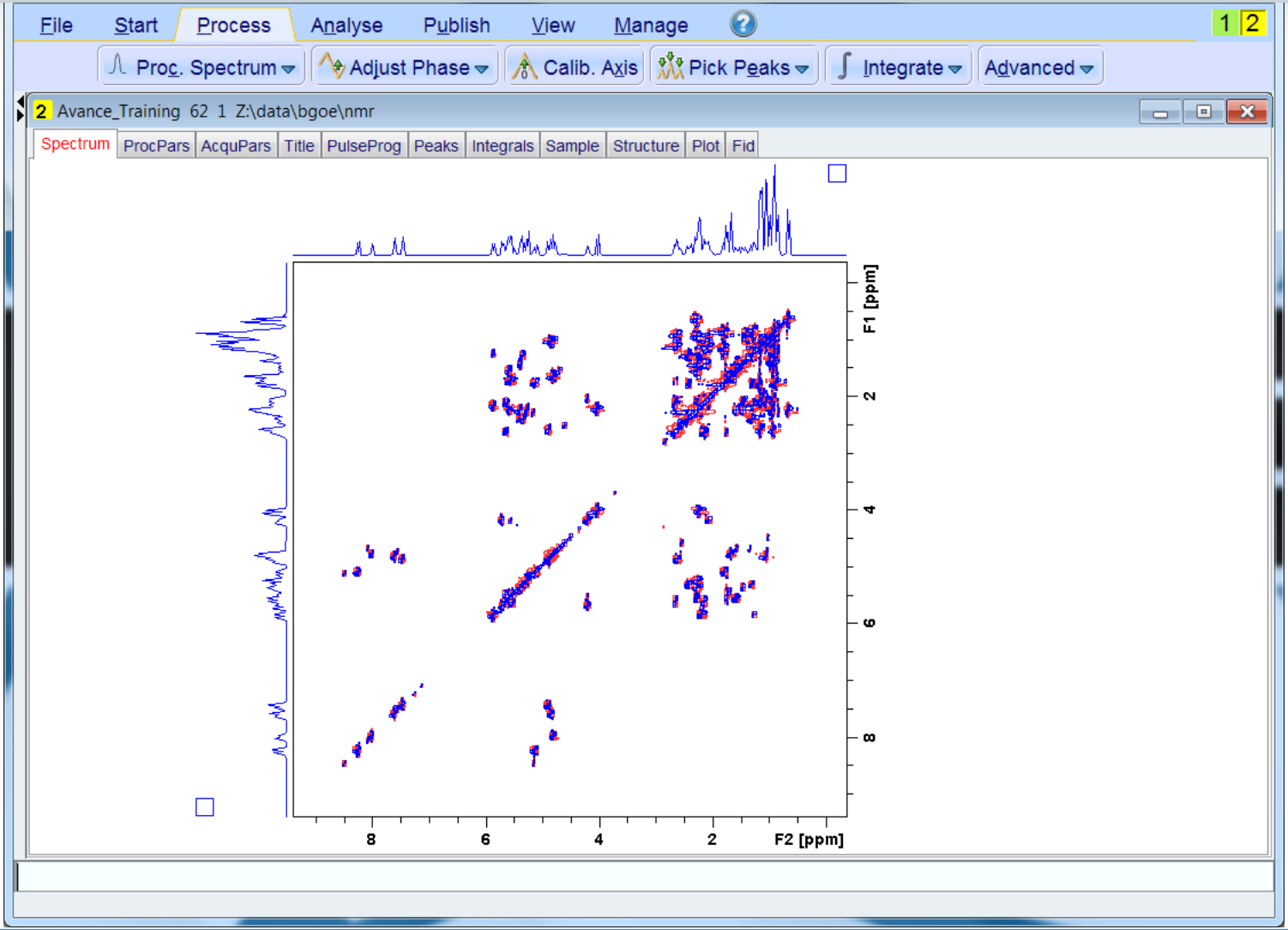


Phasing
DQF-COSY

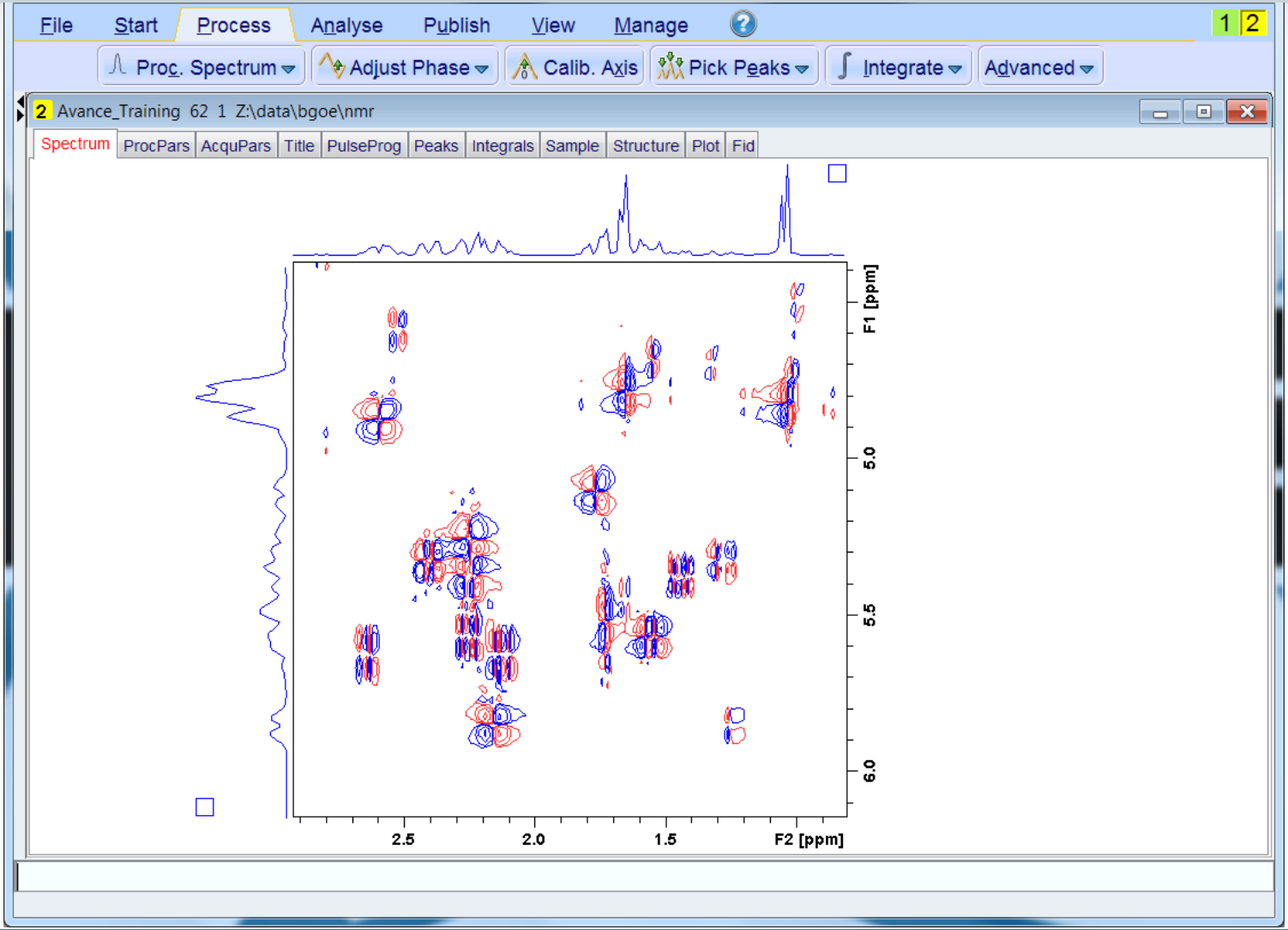


Off diagonal signals are anti phase!

Phasing
DQF-COSY



Phasing
DQF-COSY

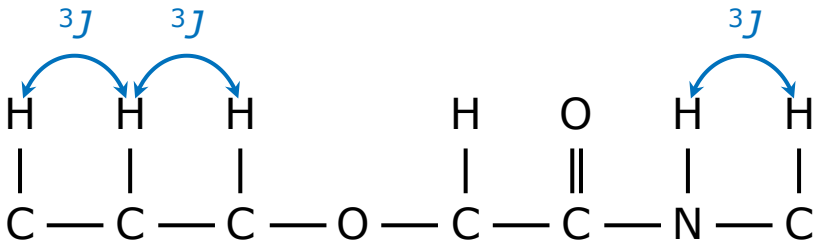


- **Basic 2D experiments**

COSY
COrrelation **S**pectroscop**Y**

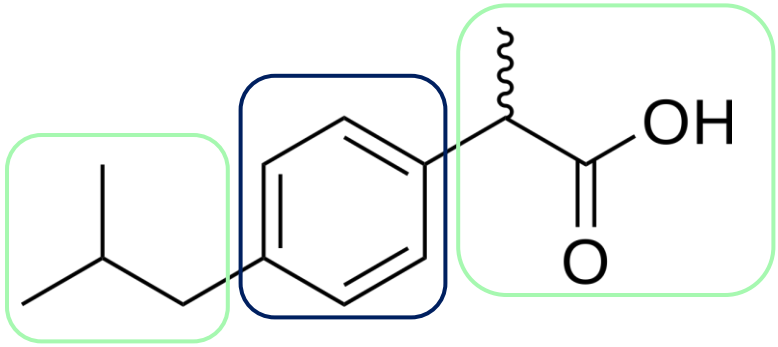
- Correlation between directly coupled protons

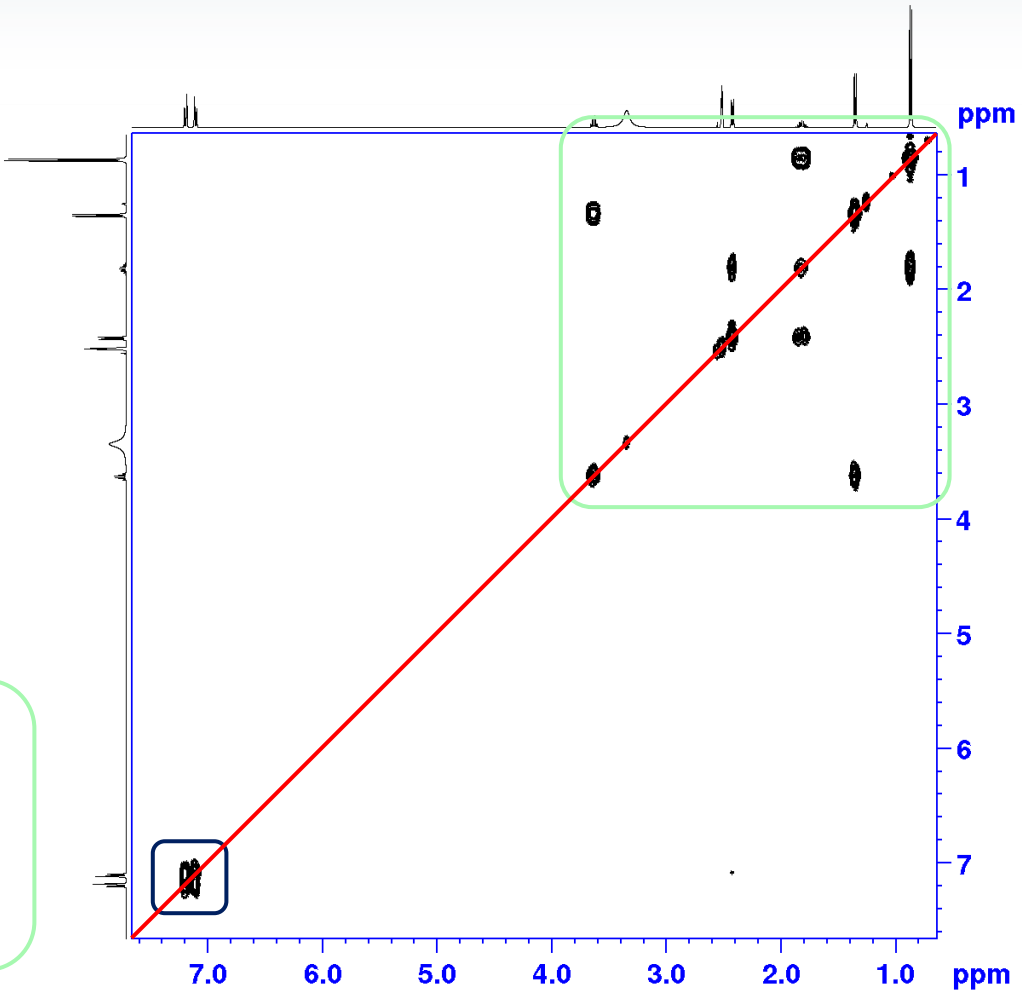
- Via scalar couplings



COSY
COrrelation **S**pectroscop**Y**

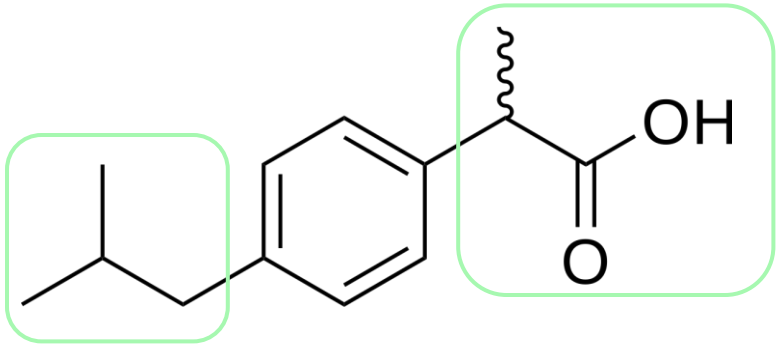
- Correlation between directly coupled protons
- Diagonal ≙ 1D spectrum
- No new information

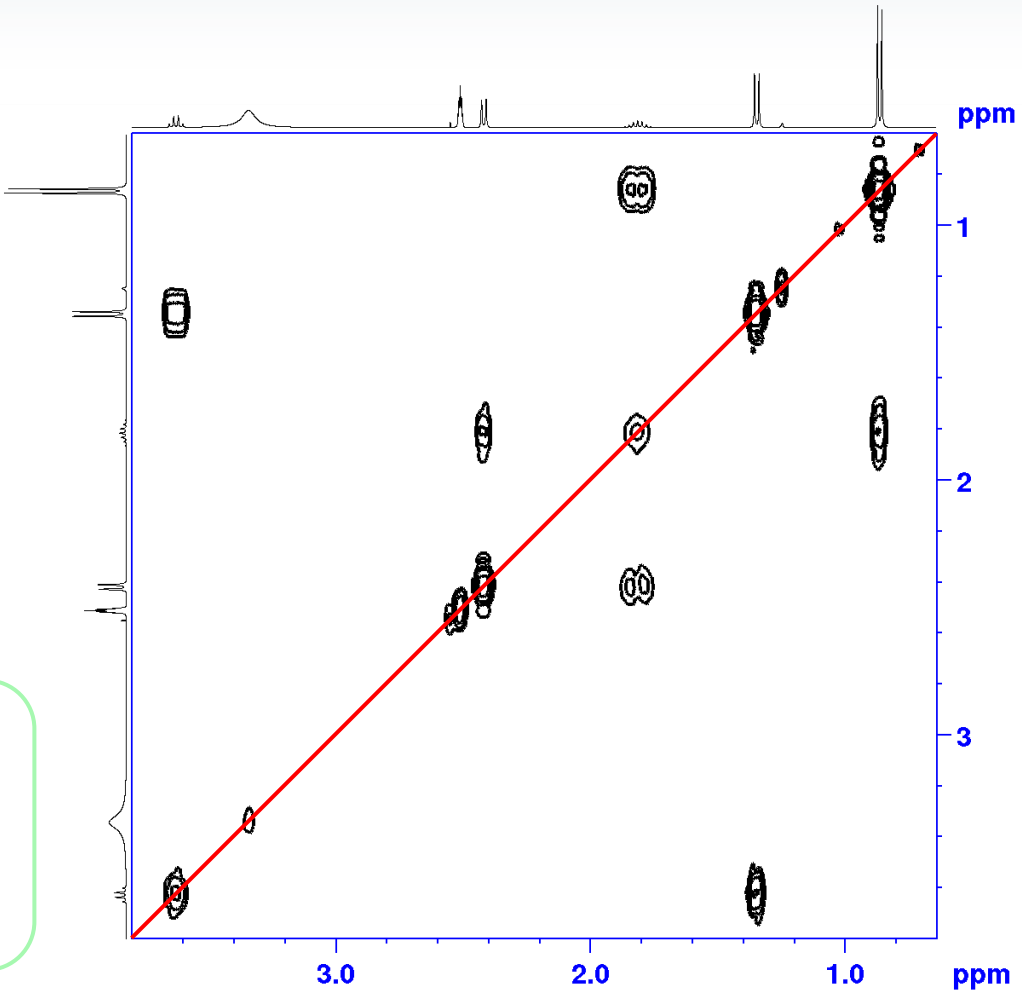




COSY
COrrelation **S**pectroscop**Y**

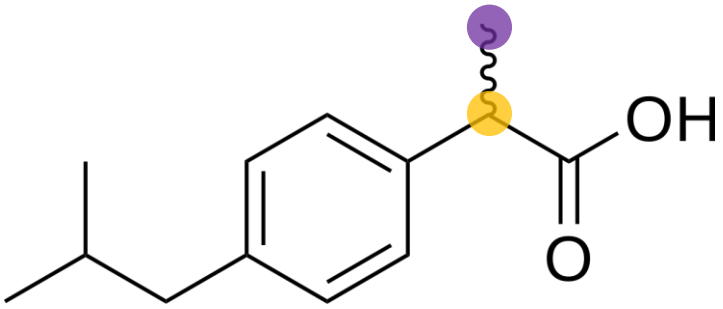
- Correlation between directly coupled protons
- Diagonal ≙ 1D spectrum
- No new information

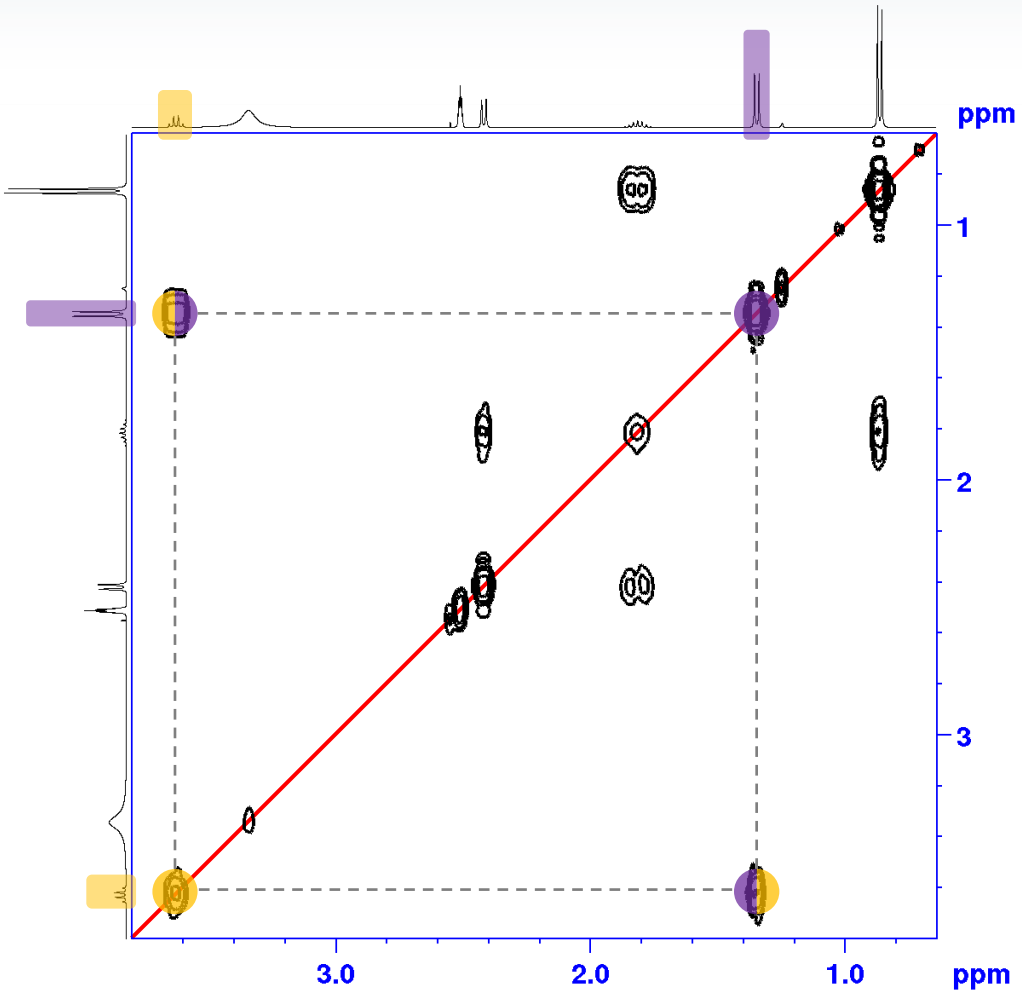




COSY
COrrelation **S**pectroscop**Y**

- Off diagonal peaks from coupled spins
- Typically ^{2}J and ^{3}J couplings





COSY
COrrelation **S**pectroscop**Y**

- Off diagonal peaks from coupled spins
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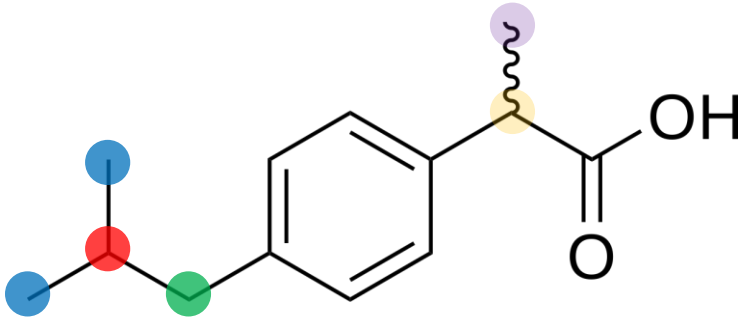

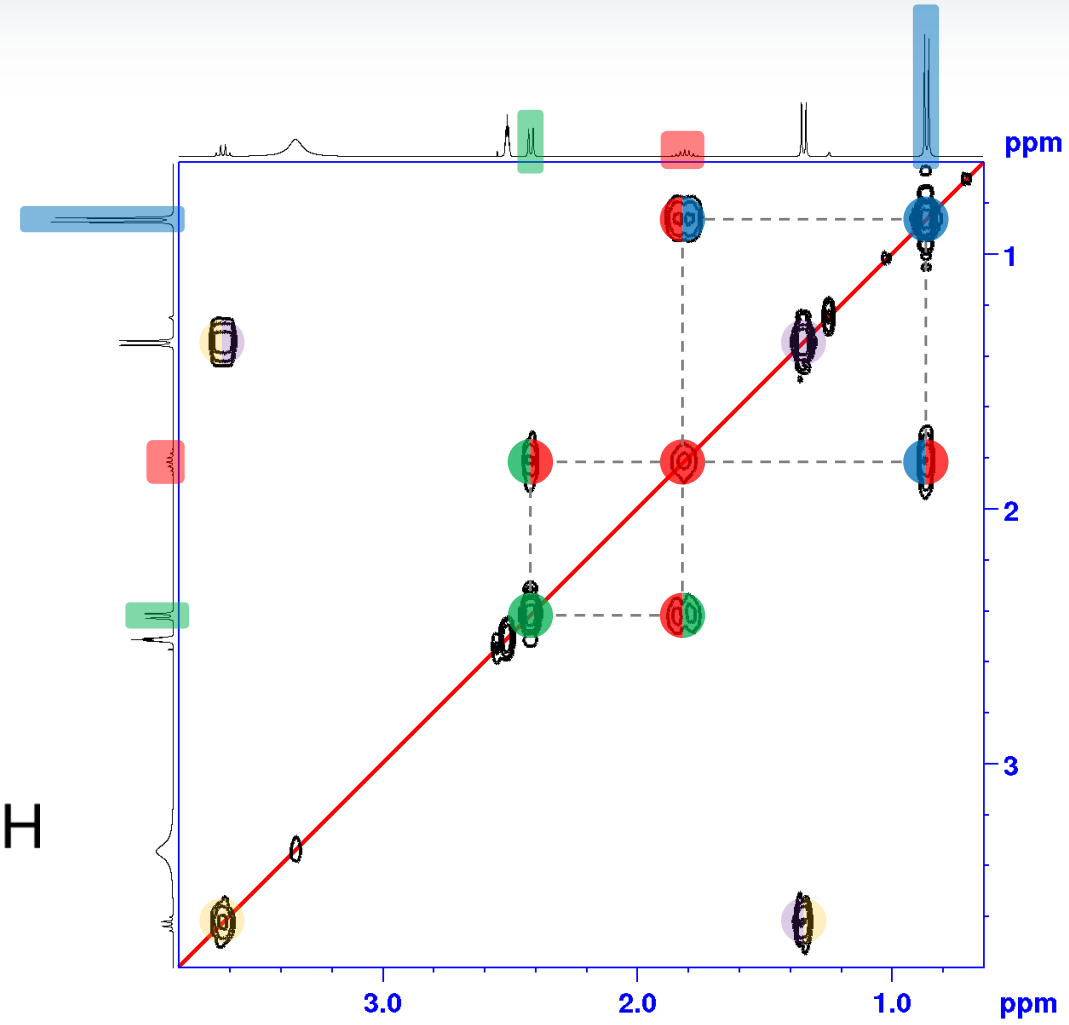

COSY
COrrelation **S**pectroscop**Y**

- Off diagonal peaks from coupled spins
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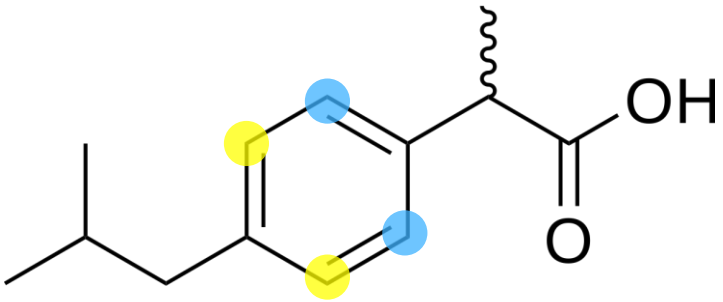
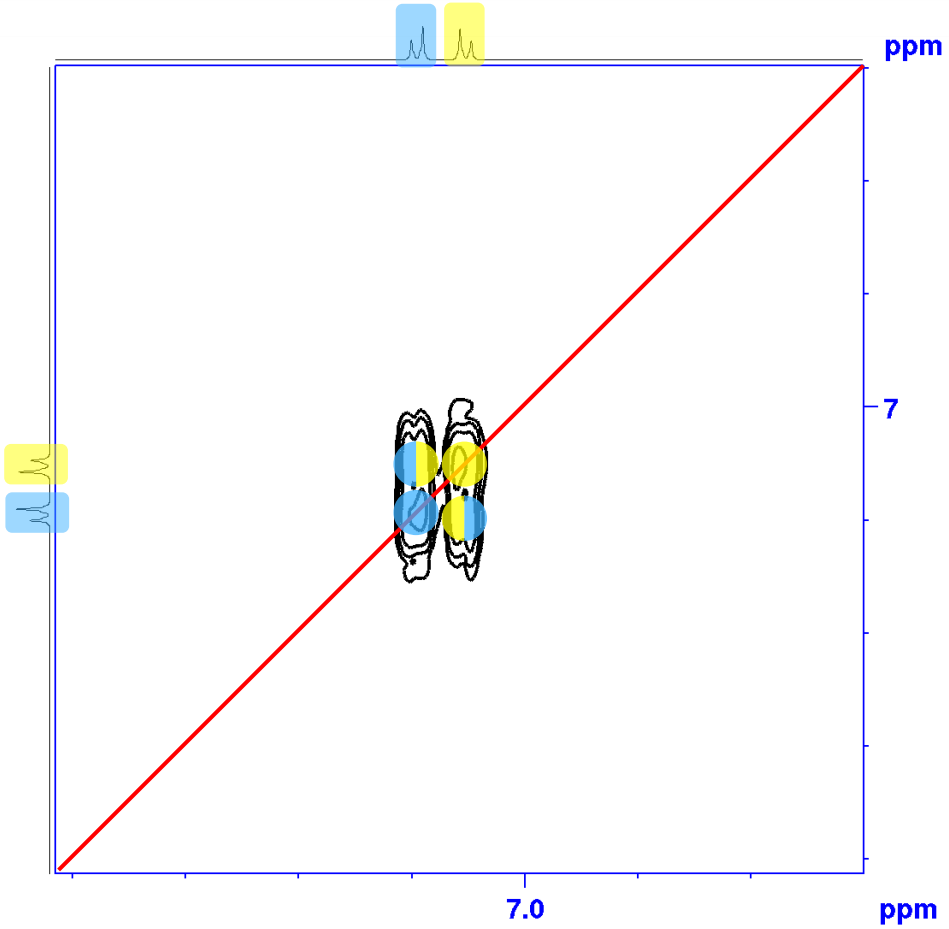
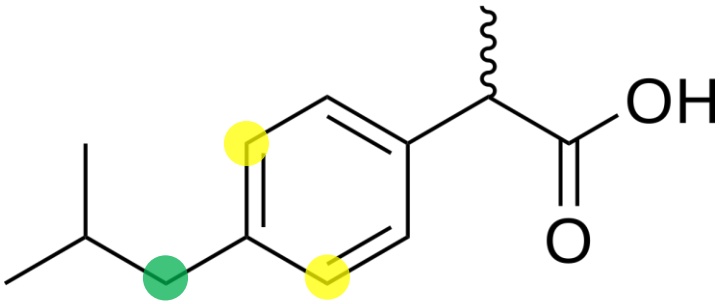
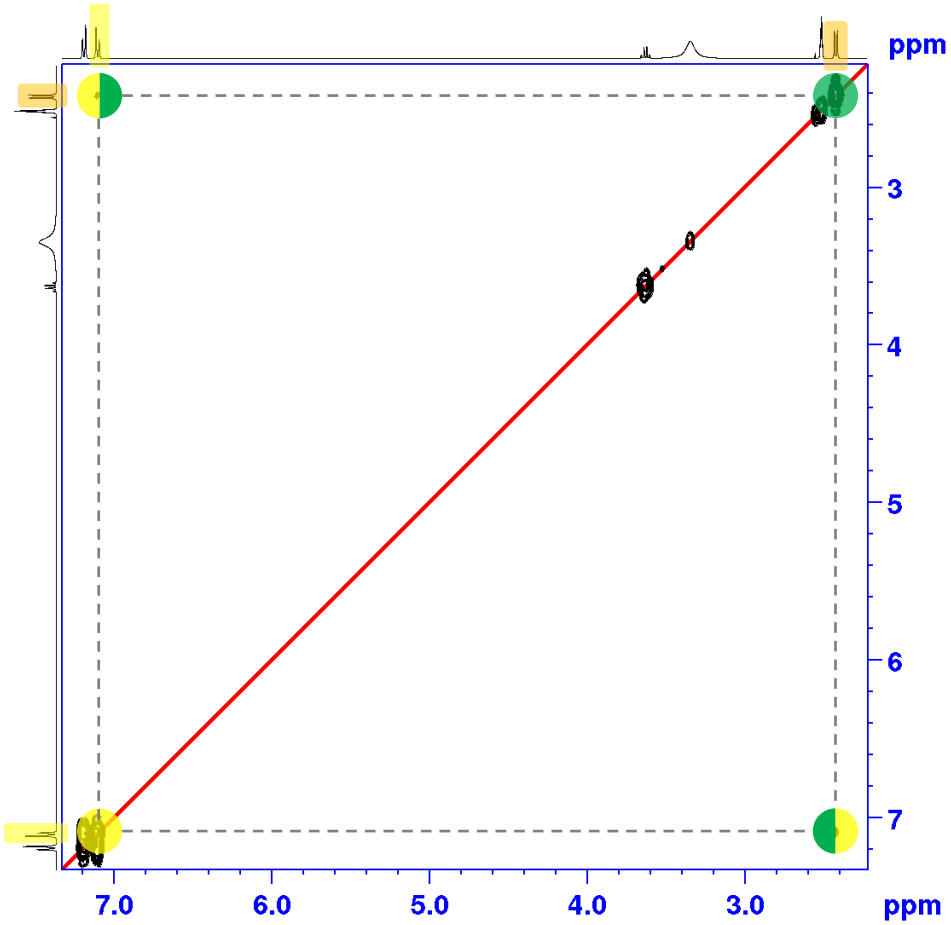




COSY
COrrelation **S**pectroscop**Y**

- Off diagonal peaks from coupled spins
- Typically ^{2}J and ^{3}J couplings
- ^{4}J can be visible as well

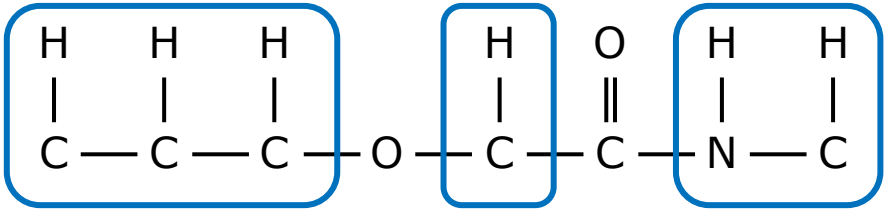




TOCSY
Total **CO**rrelation **S**pectroscop**Y**

- Correlation between all spins within one spin system

- Spin system: all spins that are connected via scalar couplings



TOCSY
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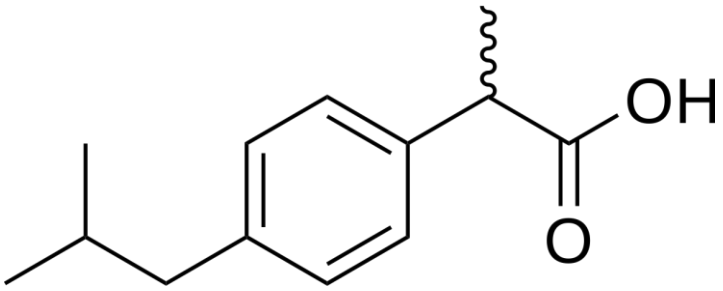

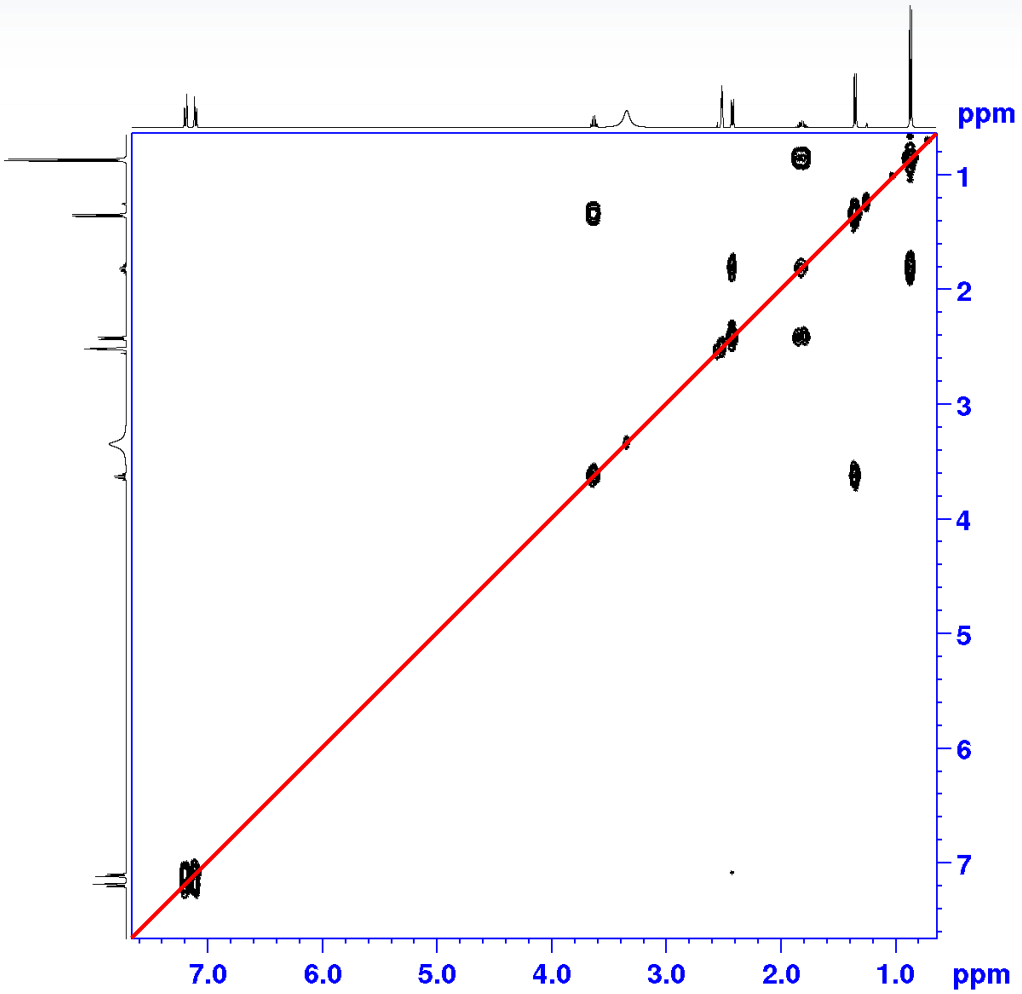

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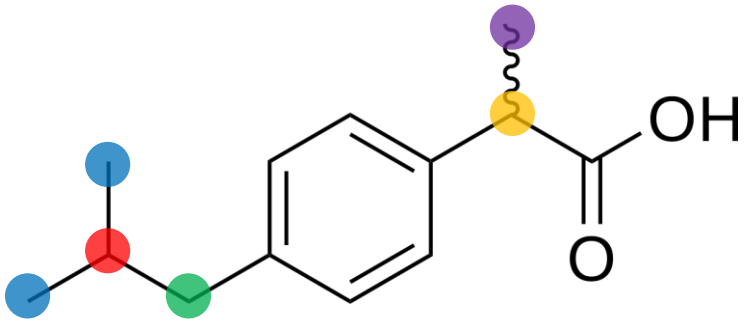
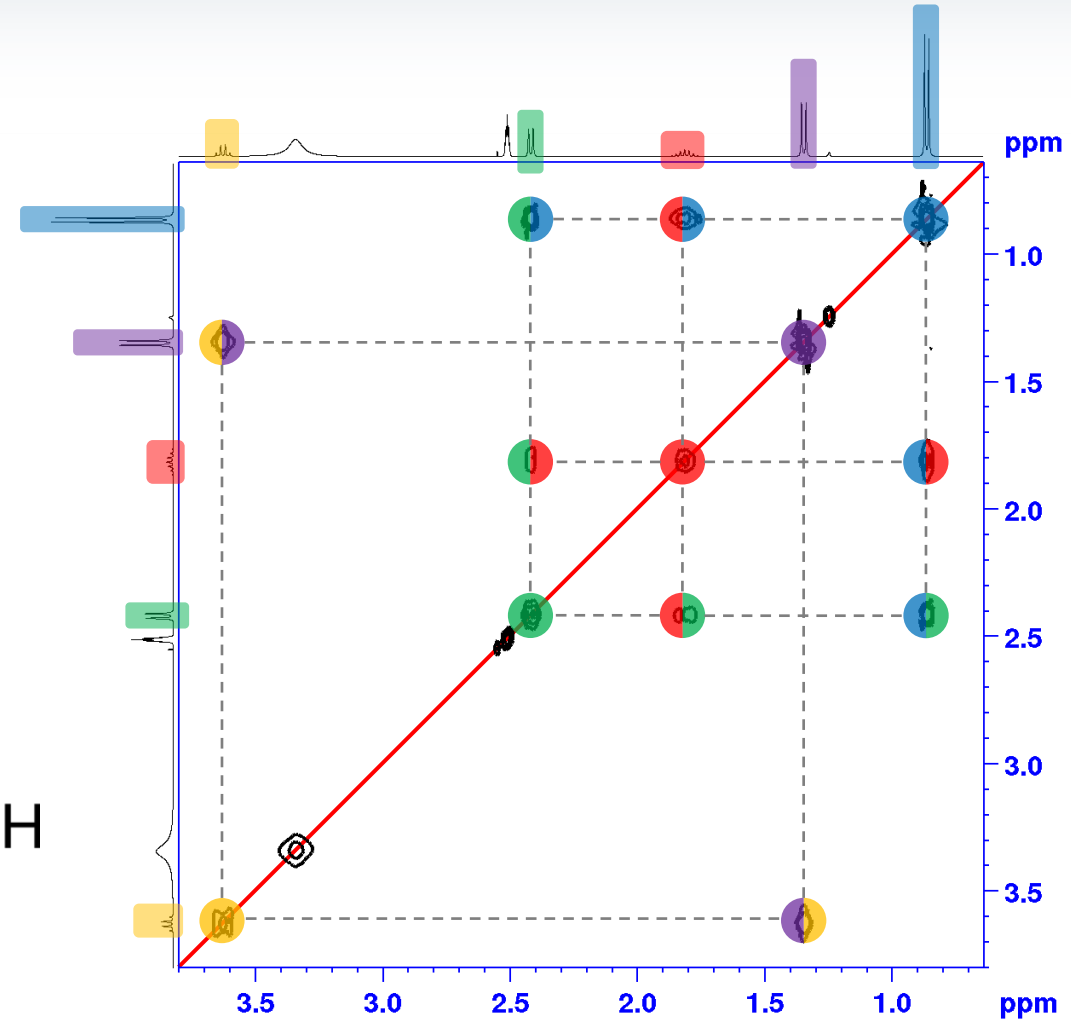
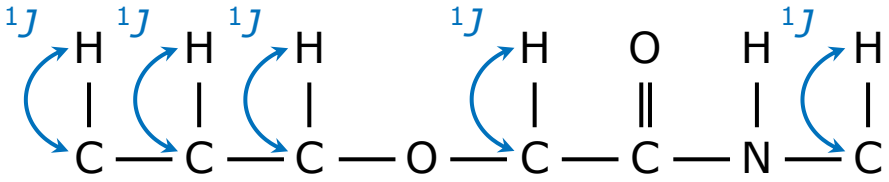




HSQC
Heteronuclear **S**ingle **Q**uantum **C**oherence spectroscopy

- Correlation between protons and directly coupled carbons

- Only carbons with attached protons are visible



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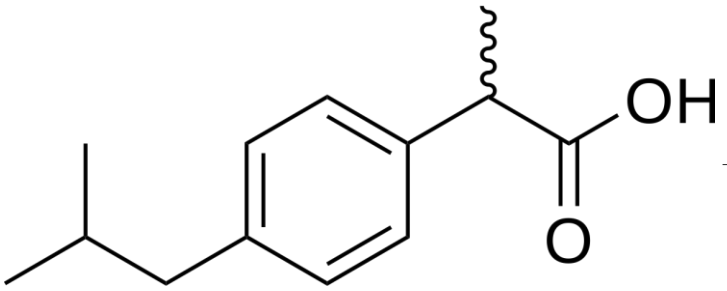

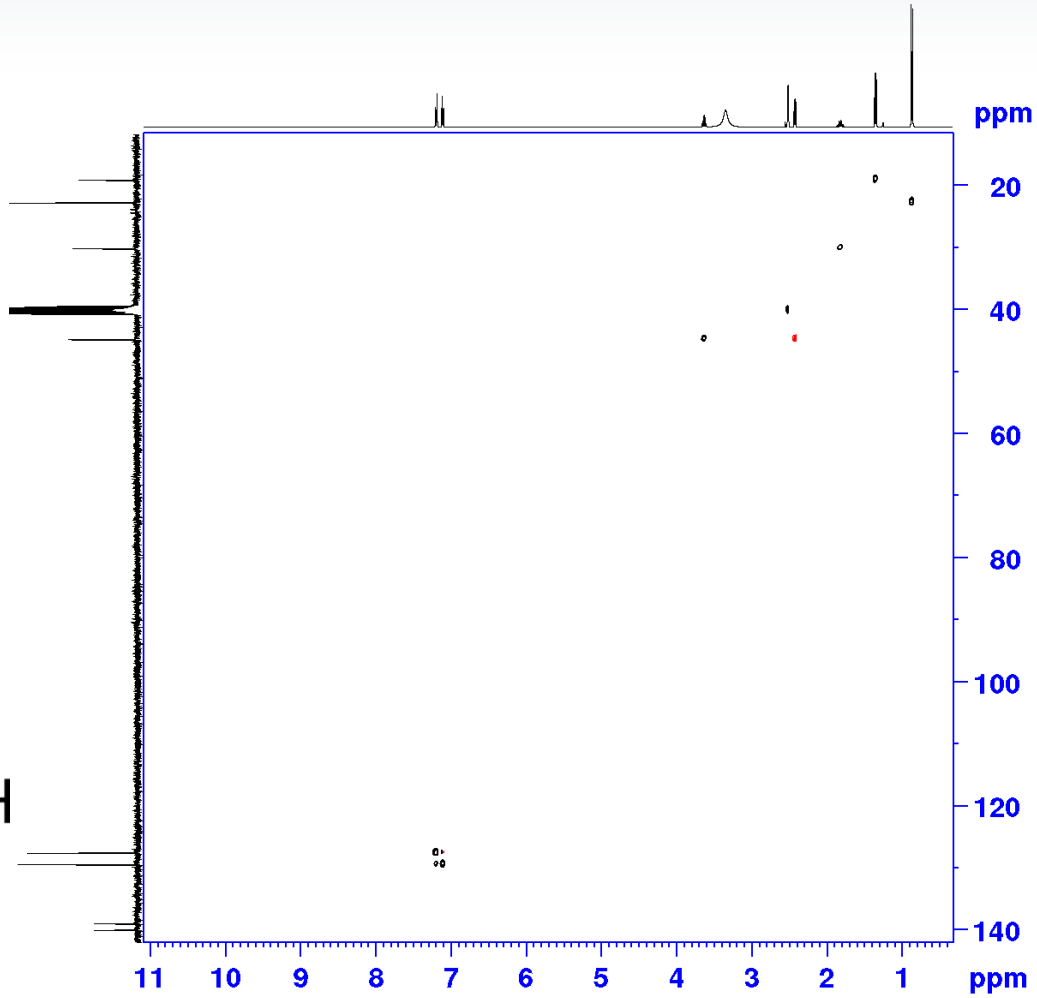

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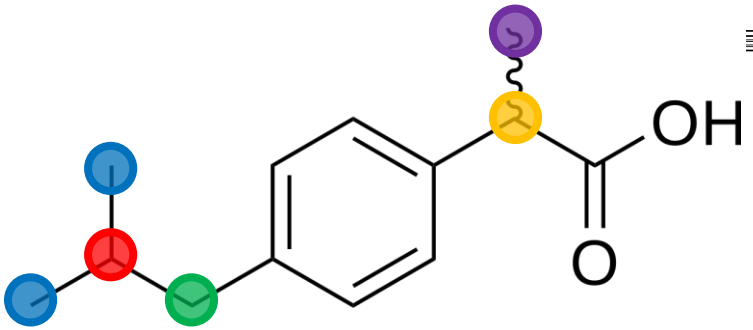

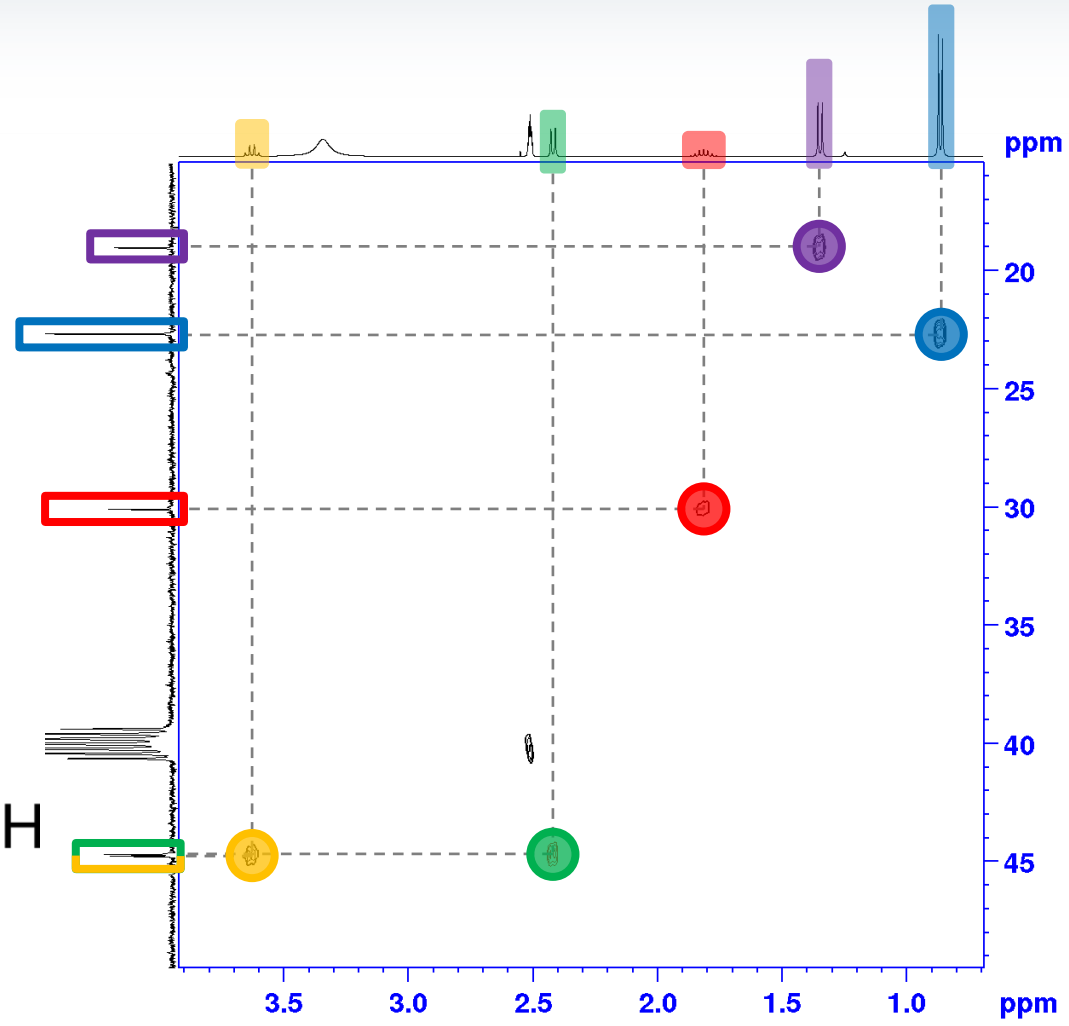

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Heteronuclear **S**ingle **Q**uantum **C**oherence spectroscopy

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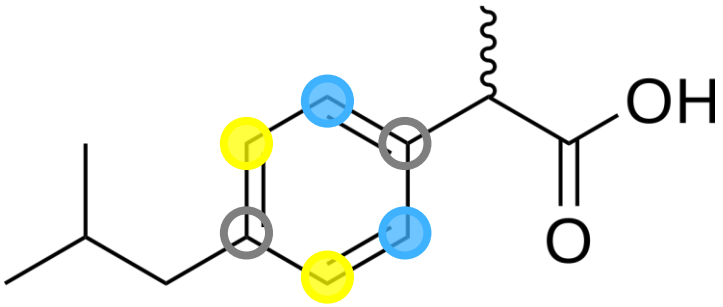
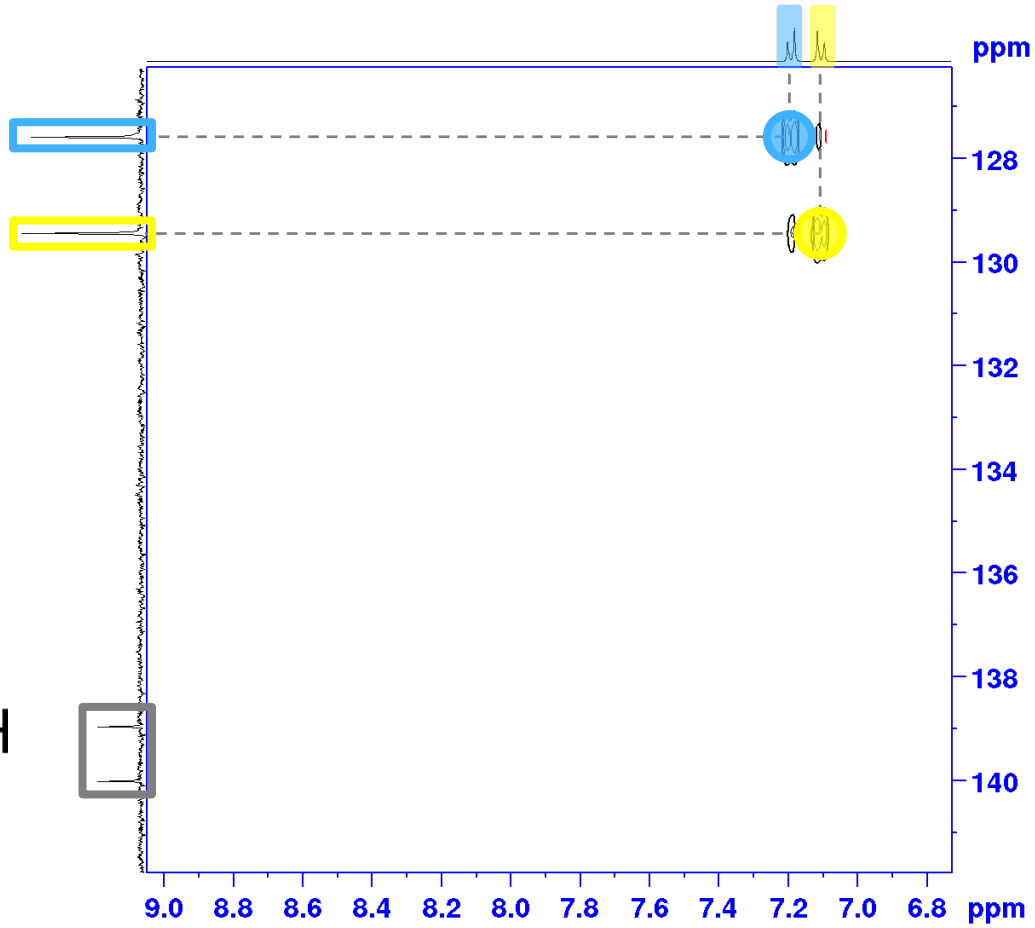
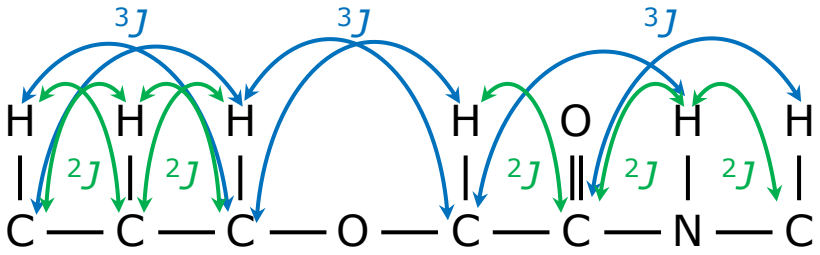




HMBC
Heteronuclear **M**ultiple **B**ond **C**orrelation spectroscopy

- Correlation between protons and carbons that are two or three bonds away

- Coupling to quaternary carbons possible



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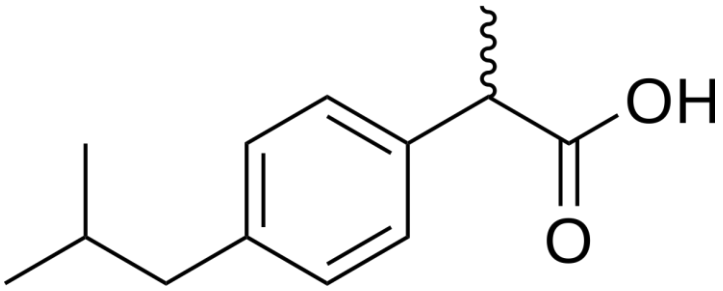
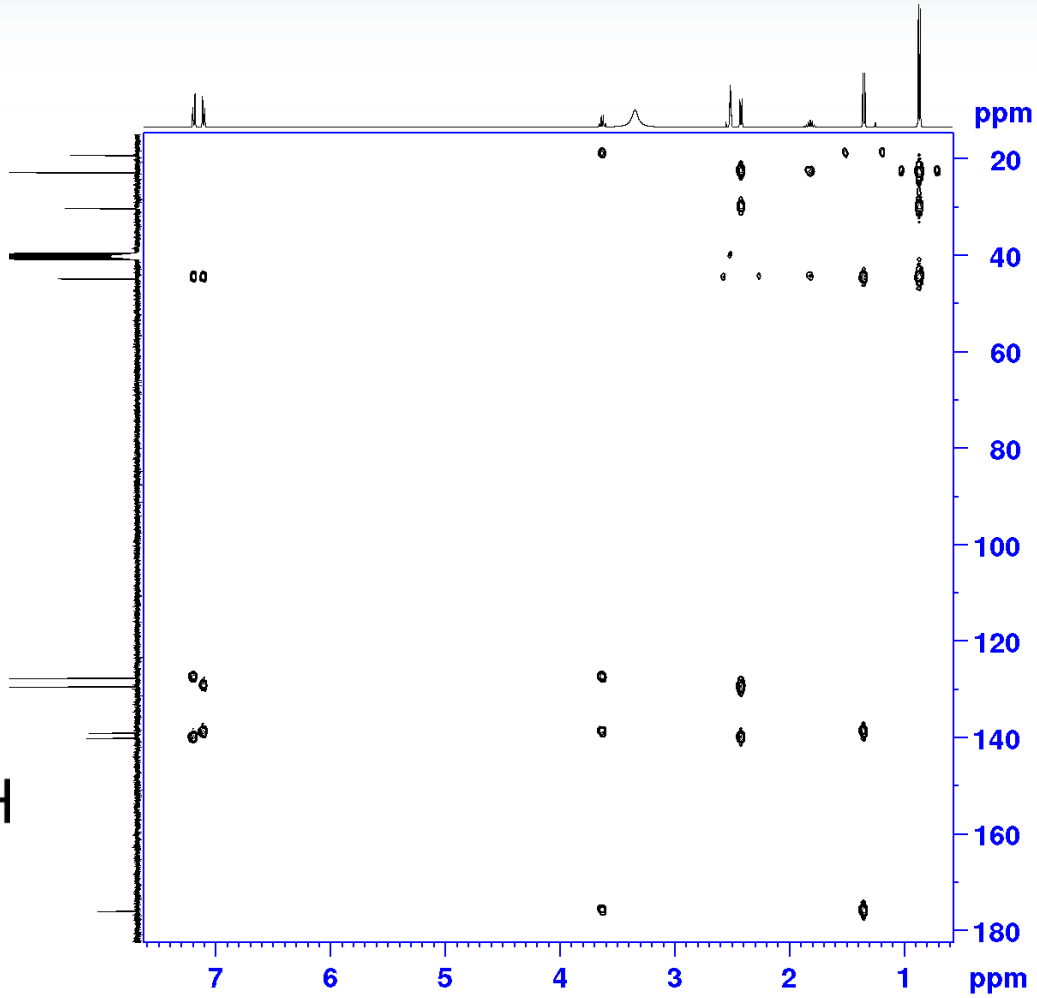
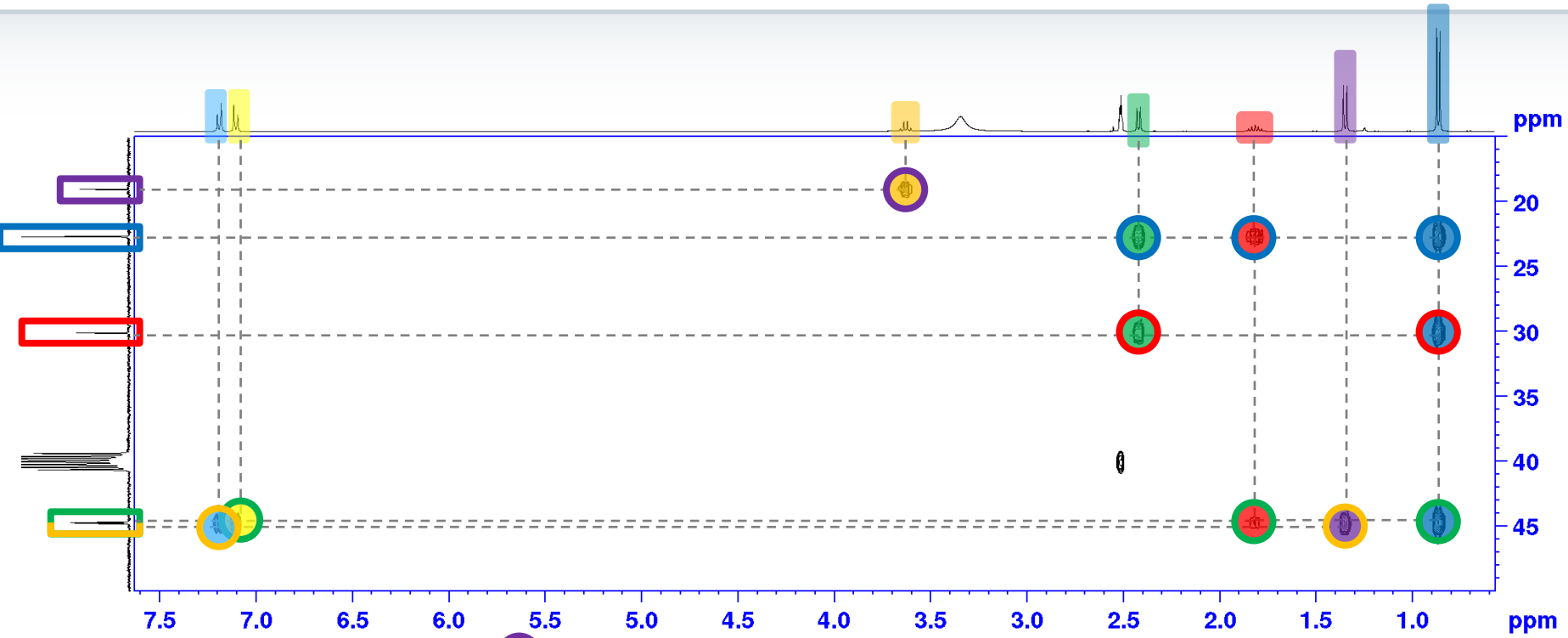
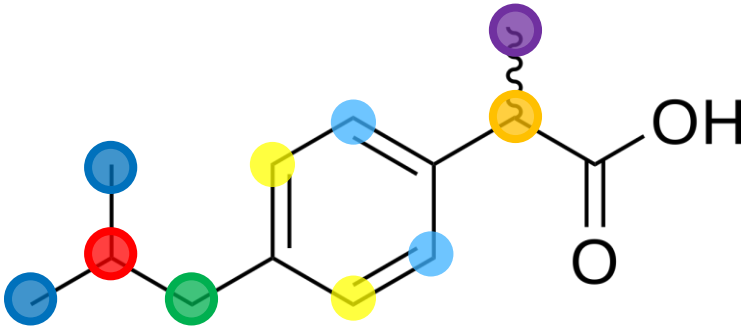


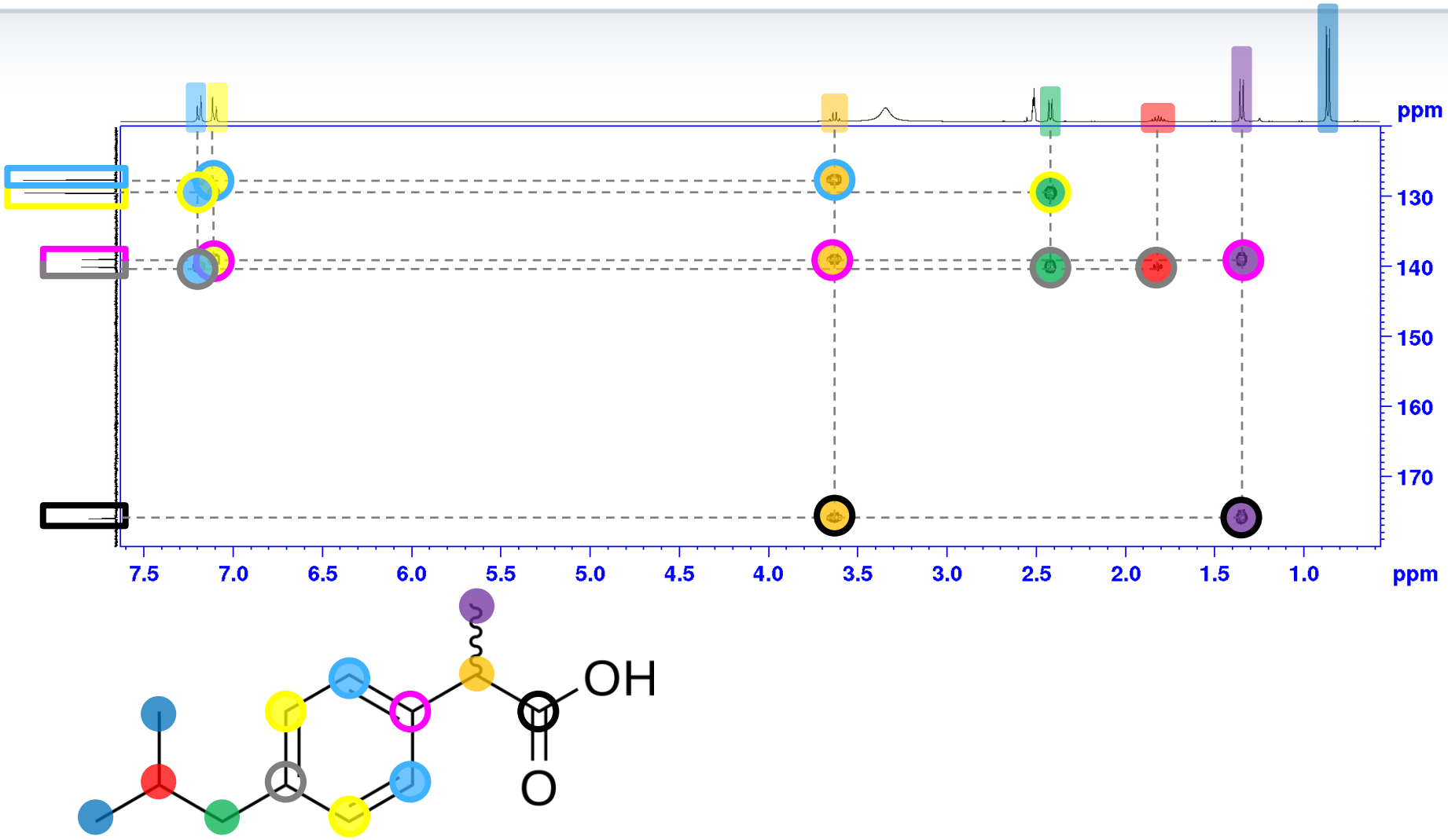


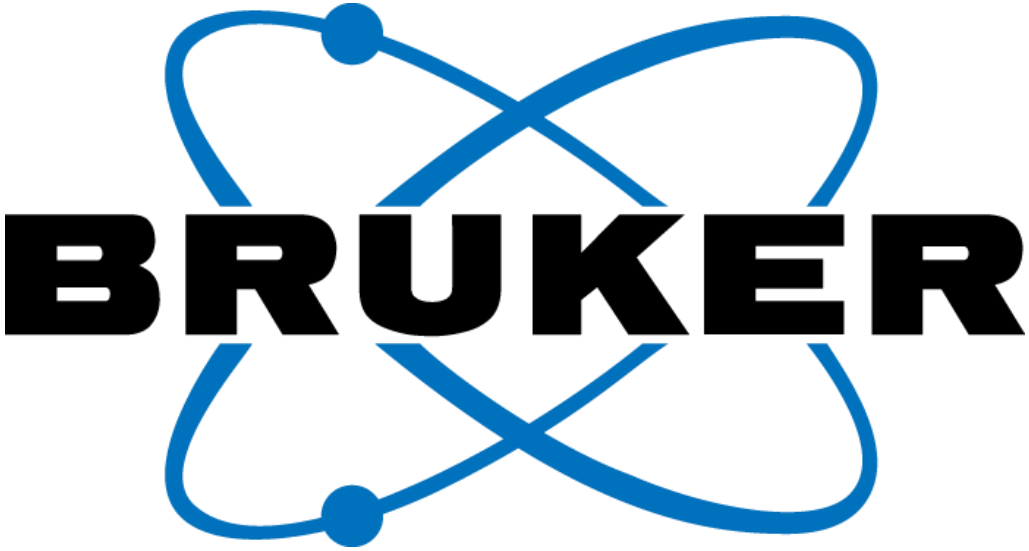
HMBC
Heteronuclear **M**ultiple **B**ond **C**orrelation spectroscopy





HMBC
Heteronuclear **M**ultiple **B**ond **C**orrelation spectroscopy





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