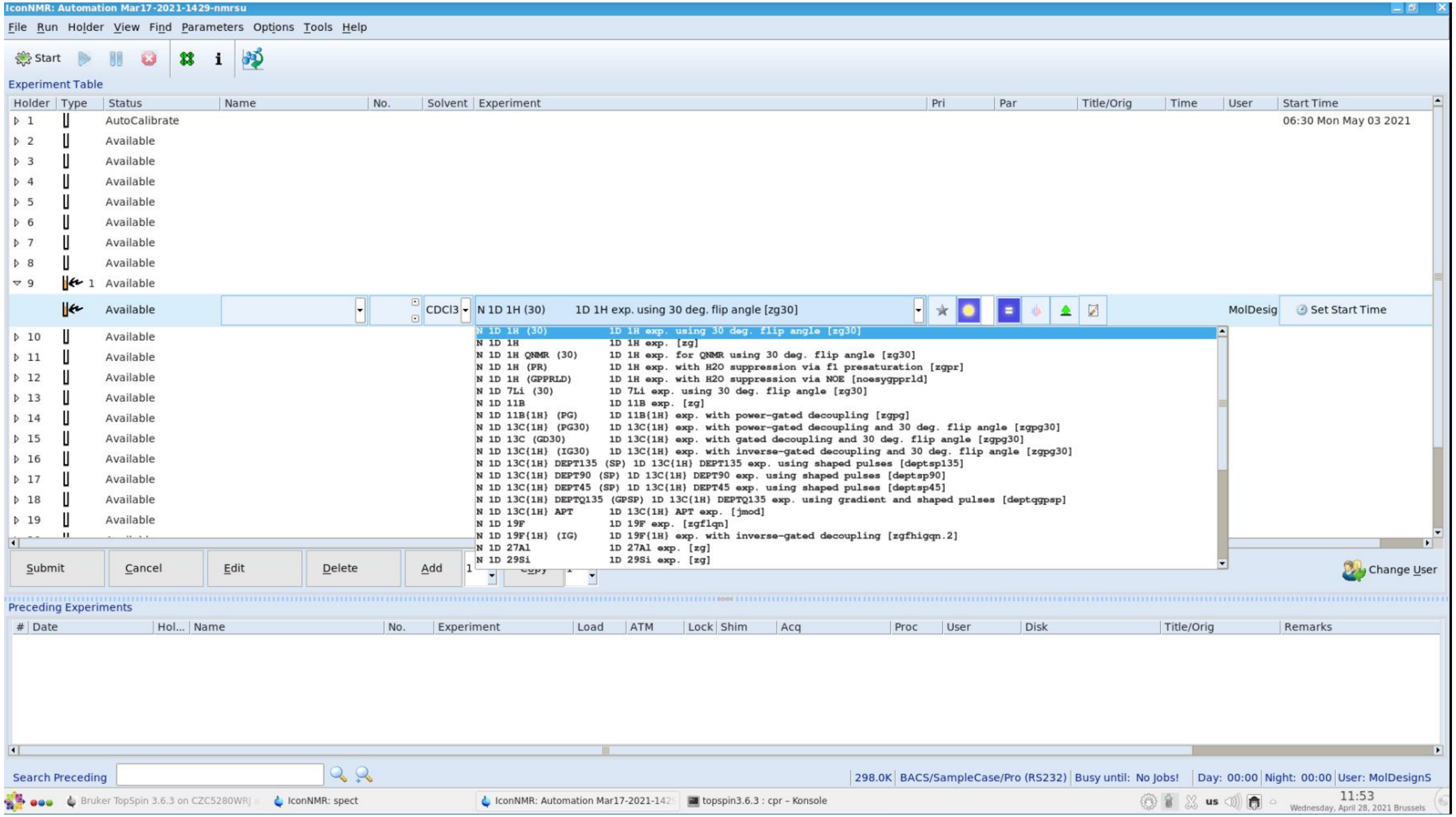
Choosing the proper NMR experiment

A walkthrough of the available experiments at the Leuven Chem&Tech Liquid NMR Core Facilities.

Gert Steurs

10 May 2021

Choosing an experiment



https://nmrfacilities.chem.kuleuven.be/experiments_in_automation

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Icon-NMR Codes

Icon-NMR Code	Meaning	Icon-NMR Code	Meaning
30	Using a 30 degree flip angle	PH	Acquisition for phase-sensitive spectra
PR	With presaturation	LR	Optimized for long-range coupling
GP	Using gradient pulses	ED	With multiplicity-editing
LD	Using low power CPD decoupling	ET	Acquisition for phase-sensitive spectra using echo/antiecho-TPPI method
PG	Using power-gated decoupling	SI	With sensitivity-improvement
GD	Using gated-decoupling	ADIA	Using adiabatic pulses
IG	Using inverse-gated decoupling	LP	With low-pass J-filter
SP	Using shaped pulses	L3	With three-fold low-pass *J*-filter
PP	Using purge pulses	ND	With no decoupling during acquisition
QF	Acquisition for magnitude mode (qf) spectra	LOCK	Using ^{2}H lock channel (for ^{2}H acquisition)
DF	Using a double-quantum filter	X{Y}	X-observed experiment with Y-decoupling during acquisition

https://nmrfacilities.chem.kuleuven.be/bruker_abbreviations

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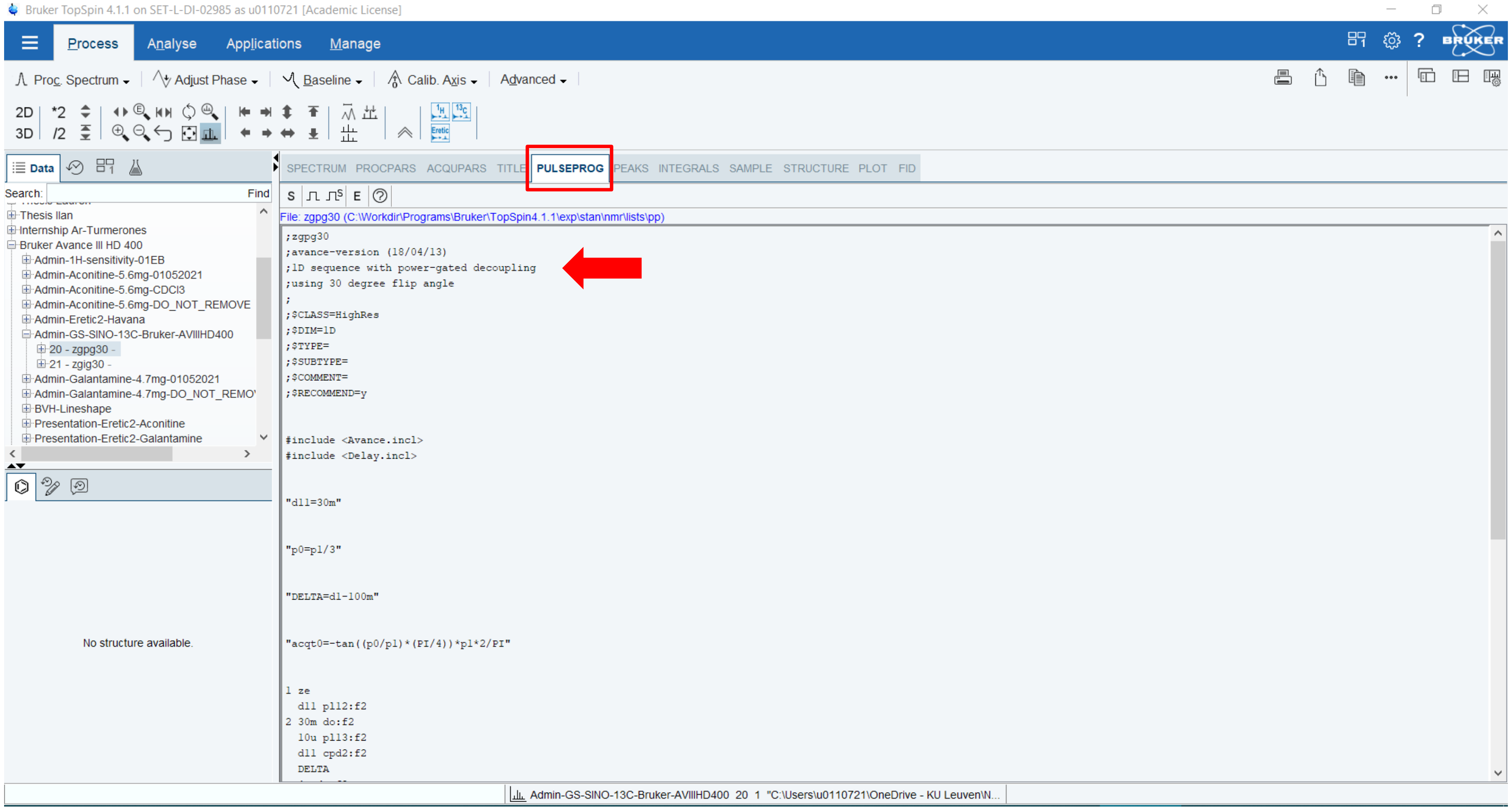
Icon-NMR Codes – examples

Icon-NMR Name	**Meaning**
1D 1H (30)	1D ^{1}H exp. with a 30 degree flip angle [zg30]
1D 1H (GPPRLD)	1D ^{1}H exp. with gradient pulses, presaturation and low-power CPD decoupling [noesygpprld]
1D 13C{1H} (PG30)	1D ^{1}H-decoupled ^{13}C exp. with power-gated decoupling and a 30 degree flip angle [zgpg30]
1D 19F{1H} (IG)	1D ^{1}H-decoupled ^{19}F exp. with inverse-gated decoupling [zgfhigqn.2]
2D 1H-1H COSY (GPPPQF)	2D ^{1}H-^{1}H COSY exp. with gradient pulses and purge pulses, which acquires data for magnitude mode (qf) spectra [cosygpppqf]
2D 1H-13C HSQC-DEPT (EDETGPSISP2.2ADIA)	2D ^{1}H-^{13}C HSQC-DEPT exp. with multiplicity-editing, phase-sensitive acquisition using echo/antiecho-TPPI method, gradient pulses, sensitivity improvement, shaped pulses and adiabatic pulses [hsqcedetgpsisp2.2]

Icon-NMR Codes



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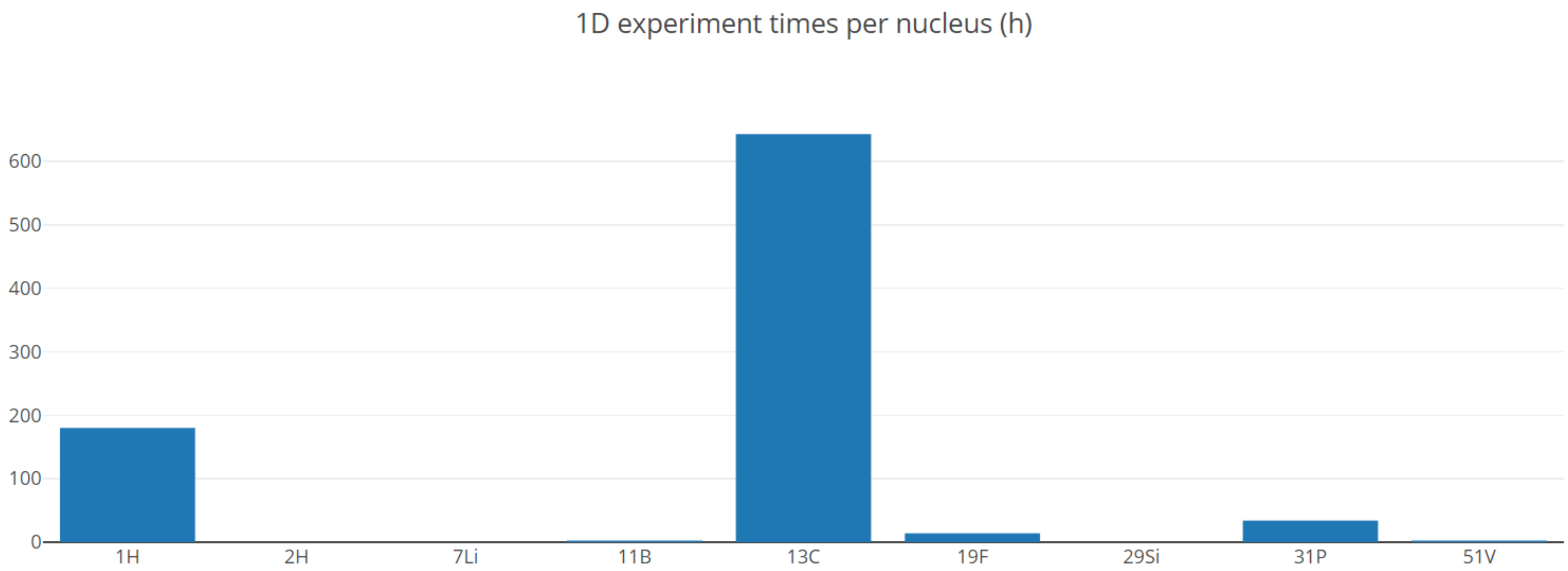
1D experiments

1D experiments



On the Bruker Avance III HD 400 over the past 75 days.

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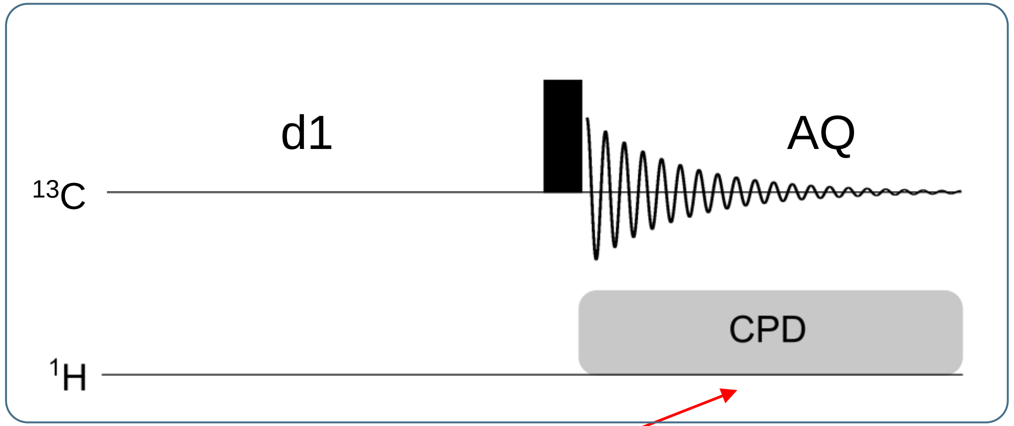
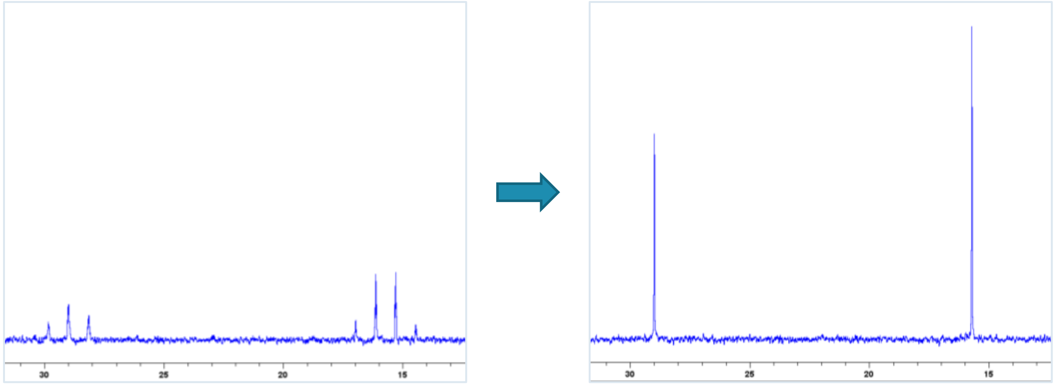
^{13}C experiments

- Available 1D ^{13}C-detected experiments
  - Regular experiments
    - 1D 13C{1H} (PG30) [zgpg30]
    - 1D 13C (GD30) [zggd30]
    - 1D 13C{1H} (IG30) [zgig30]
  - Multiplicity-edited experiments due to 180° phase shifts ("peaks are up or down")
    - 1D 13C{1H} DEPT135 (SP) [deptsp135]
    - 1D 13C{1H} DEPT90 (SP) [deptsp90]
    - 1D 13C{1H} DEPT45 (SP) [deptsp45]
    - 1D 13C{1H} DEPTQ135 (GPSP) [deptqgpsp]
    - 1D 13C{1H} APT [jmod]

^{13}C experiments – Decoupling



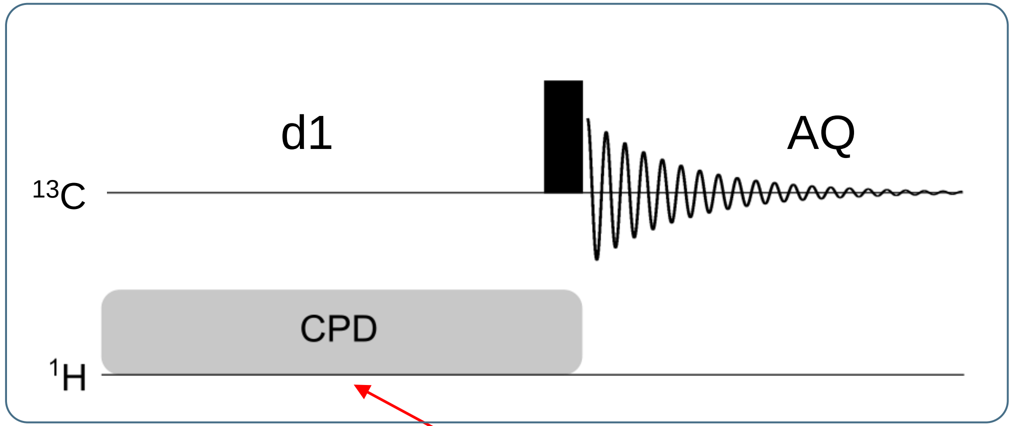


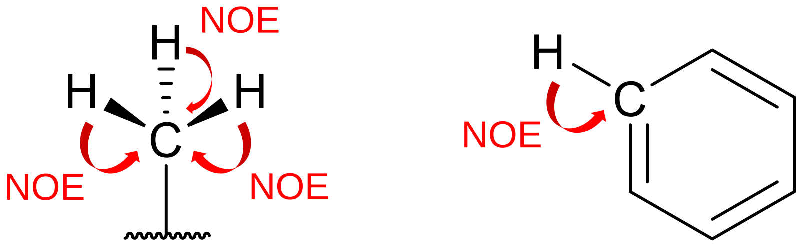
- Saturating ^{1}H during acquisition time (AQ) of ^{13}C experiment causes C-H multiplets to collapse into singlets

⇒ Spectrum becomes simplified

⇒ Higher SINO, as singlet area should remain equal to multiplet area

^{13}C experiments – Decoupling





(a) ^{13}C is boosted by 3 ^{1}H's. (b) ^{13}C is boosted by 1 ^{1}H.

- Saturating ^{1}H during relaxation delay (d1) of ^{13}C experiment causes NOE to build up. This NOE will enhance ^{13}C signals according to number of ^{1}H nuclei close to (attached to) the ^{13}C's.

⇒ ^{13}C intensity is boosted according to number of ^{1}H's close to (~ attached to) the ^{13}C atom

⇒ NOE-enhanced spectra must NOT be integrated (⇒ no quantification!!)

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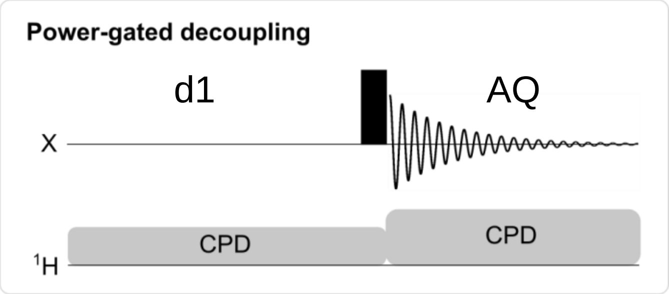
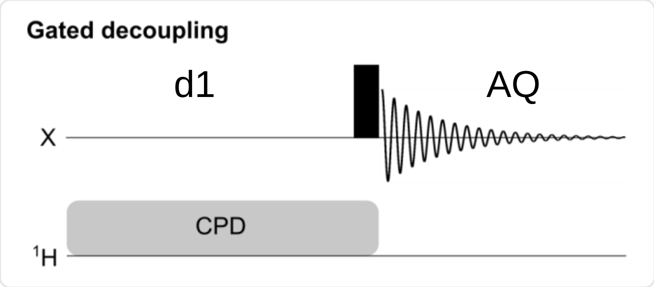
^{13}C experiments – PG, GD and IG



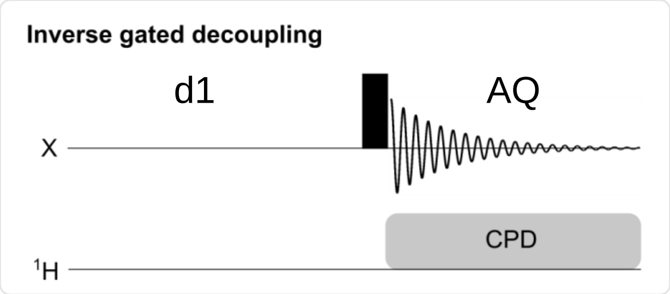
Power-Gated decoupling (1D 13C{1H} (PG30))

- Singlets (decoupling during AQ)
- ✓ Highest SINO (NOE enhancement + decoupling during AQ)
- × Not quantifiable (NOE enhancement)
- ✓ Fast



Gated Decoupling (1D 13C (GD30))

- Multiplets (no decoupling during AQ)
- × Lowest SINO (only NOE enhancement)
- × Not quantifiable (NOE enhancement)
- × Slow



Inverse-Gated decoupling (1D 13C{1H} (IG30))

- Singlets
- × Lower SINO (only decoupling during AQ)
- ✓ Quantifiable (no NOE enhancement)
- Intermediate time

^{13}C experiments

- Available 1D ^{13}C-detected experiments
  - Regular experiments
    - 1D 13C{1H} (PG30) [zgpg30]
    - 1D 13C (GD30) [zggd30]
    - 1D 13C{1H} (IG30) [zgig30]
  - Multiplicity-edited experiments due to 180° phase shifts (“peaks are up or down”)
    - 1D 13C{1H} DEPT135 (SP) [deptsp135]
    - 1D 13C{1H} DEPT90 (SP) [deptsp90]
    - 1D 13C{1H} DEPT45 (SP) [deptsp45]
    - 1D 13C{1H} DEPTQ135 (GPSP) [deptqgpsp]
    - 1D 13C{1H} APT [jmod]

^{13}C experiments – DEPT

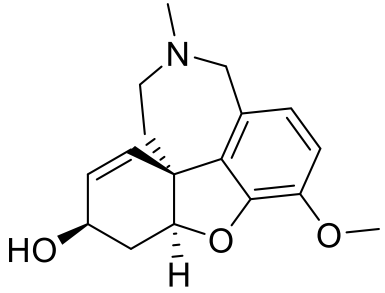
- DEPT experiments
  - More sensitive than regular $^{13}C\{^1H\}$ (zgpg30) experiment for protonated carbons
  - Automation
    - 1D 13C{1H} DEPT135 (SP) [deptsp135]
      - CH & CH_3 positive, CH_2 negative, no C_q
    - 1D 13C{1H} DEPT90 (SP) [deptsp90]
      - CH only
    - 1D 13C{1H} DEPT45 (SP) [deptsp45]
      - CH, CH_2 & CH_3 positive, no C_q

^{13}C experiments – DEPTQ and APT

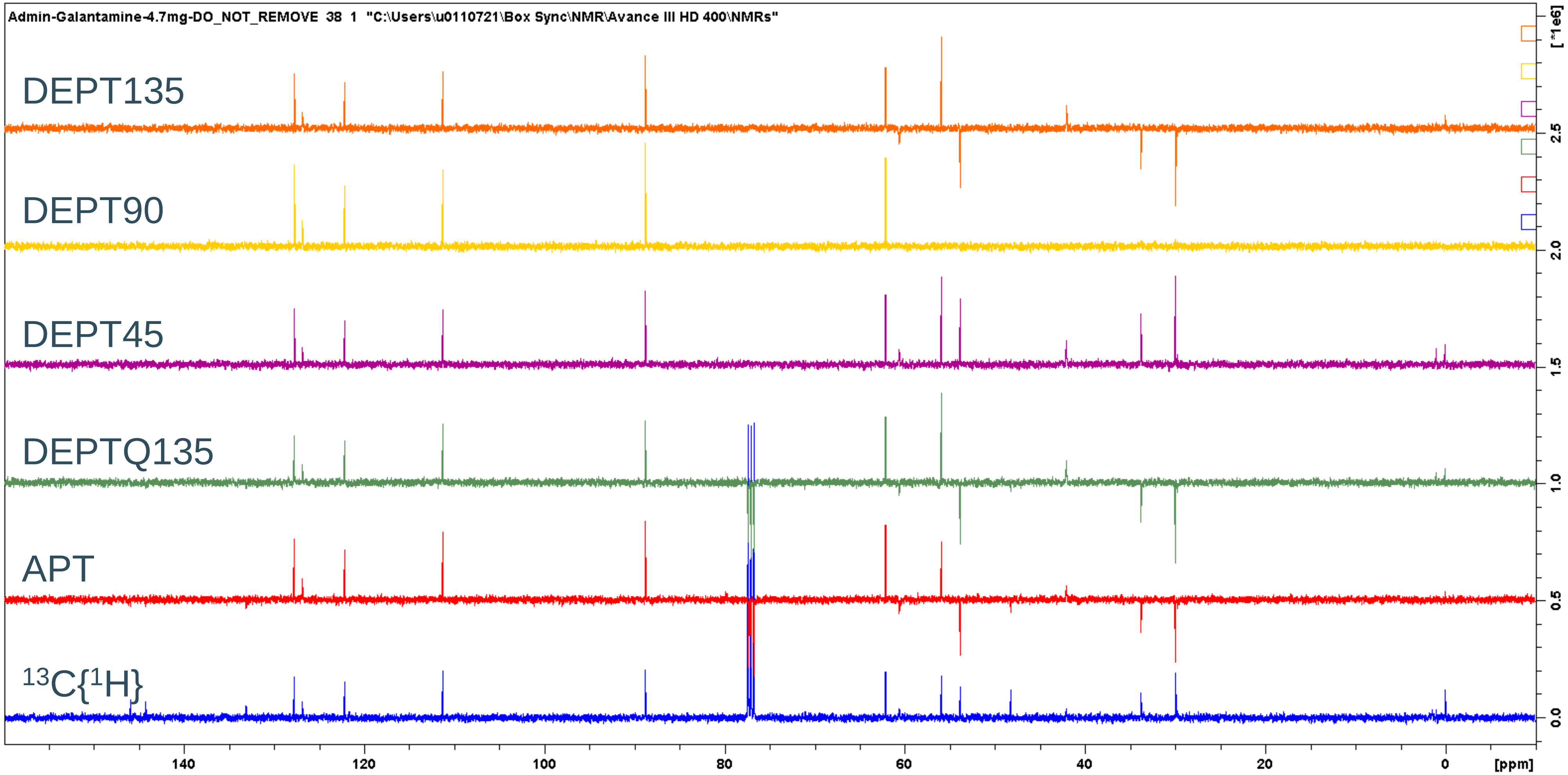
- DEPTQ
  - More / less sensitive than regular $^{13}C\{^1H\}$ (zgpg30) experiment for protonated / quaternary carbons, resp.
  - Automation
    - 1D 13C{1H} DEPTQ135 (GPSP) [deptqgpsp]
      - CH & CH_3 positive, CH_2 & C_q negative
- APT
  - Less sensitive than regular $^{13}C\{^1H\}$ (zgpg30) experiment
  - Automation
    - 1D 13C{1H} APT [jmod]
      - CH & CH_3 positive, CH_2 & C_q negative

^{13}C experiments

Sample: 4.7 mg galanthamine in $CDCl_3$ (33 mM)



galanthamine



All spectra

- NS 256
- Expt ~ 15 min

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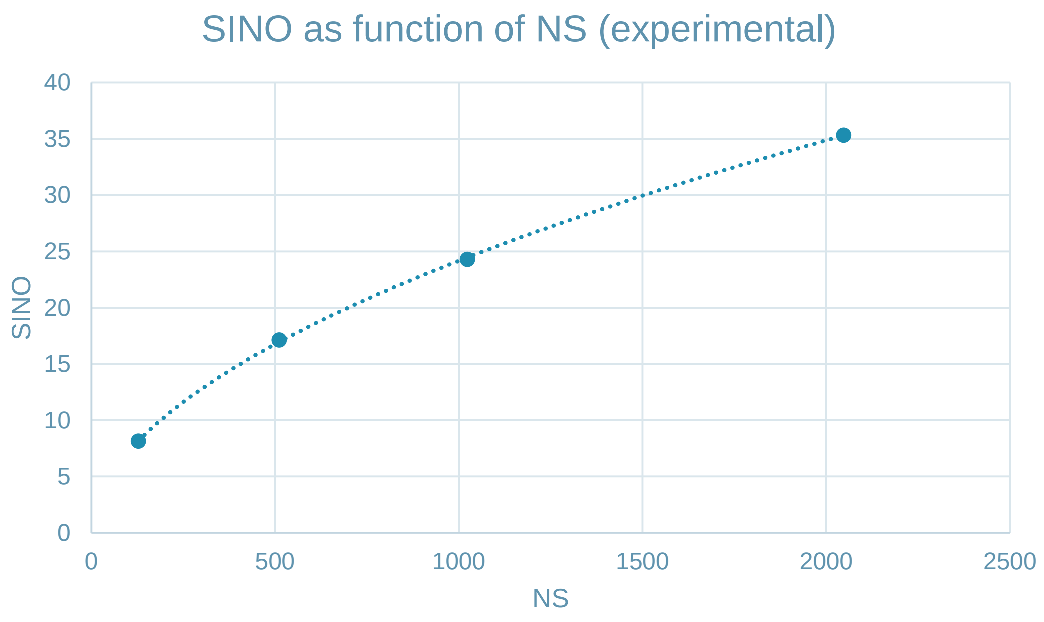
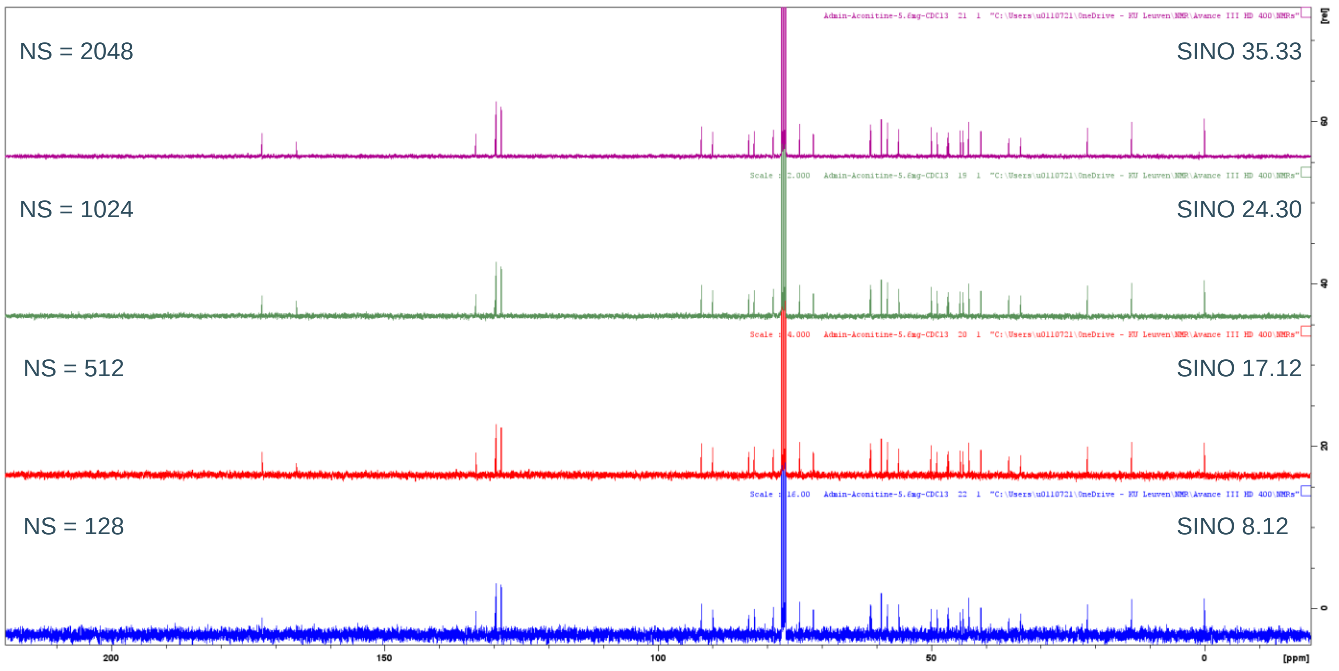
Reminder

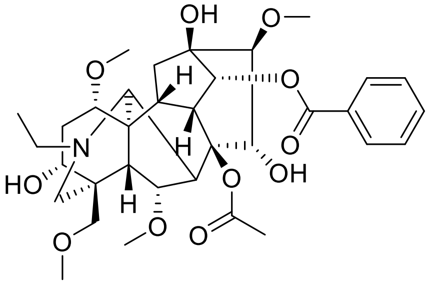
- SINO increases with √NS!
- To double the SINO, four times more scans need to be recorded





Reminder

Sample: 5.6 mg aconitine in $CDCl_3$ (17 mM)



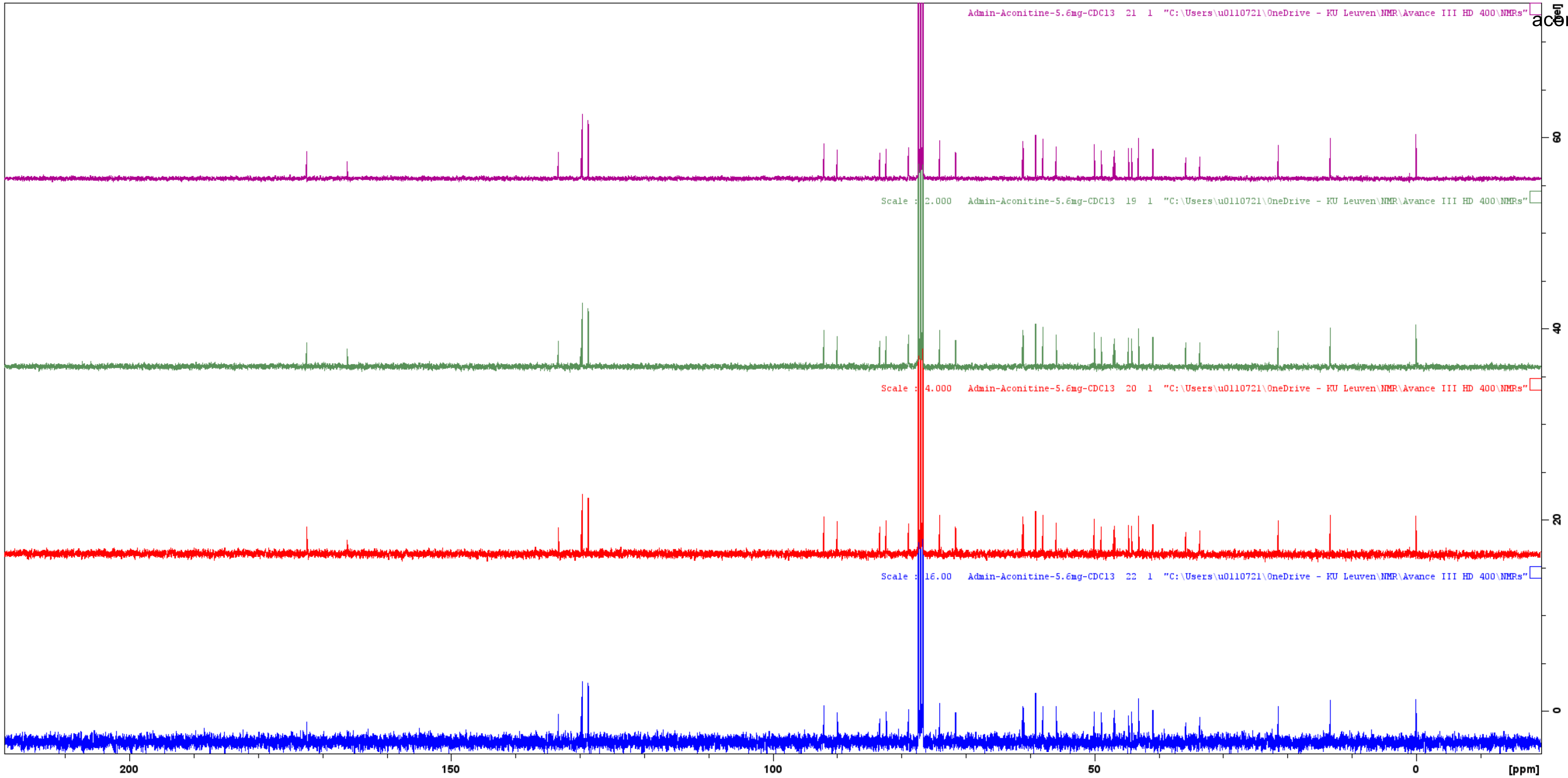
aconitine

NS = 2048
EXPT ≈ 2 h
SINO = 35.33

NS = 1024
EXPT ≈ 1 h
SINO = 24.30

NS = 512
EXPT ≈ 30 min
SINO = 17.12

NS = 128
EXPT ≈ 8 min
SINO = 8.12



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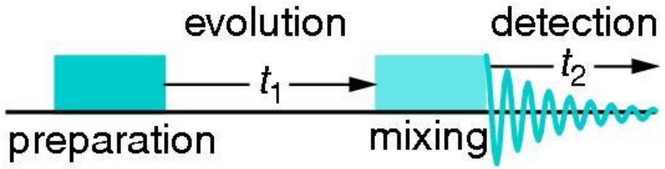
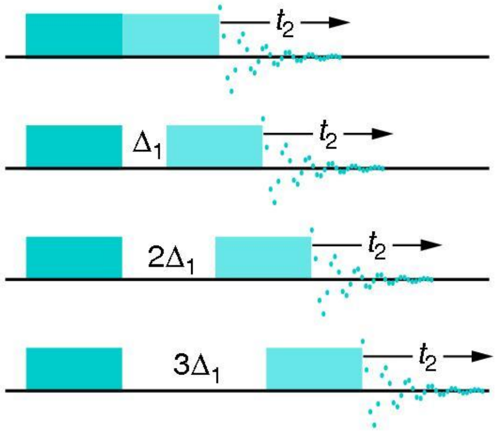
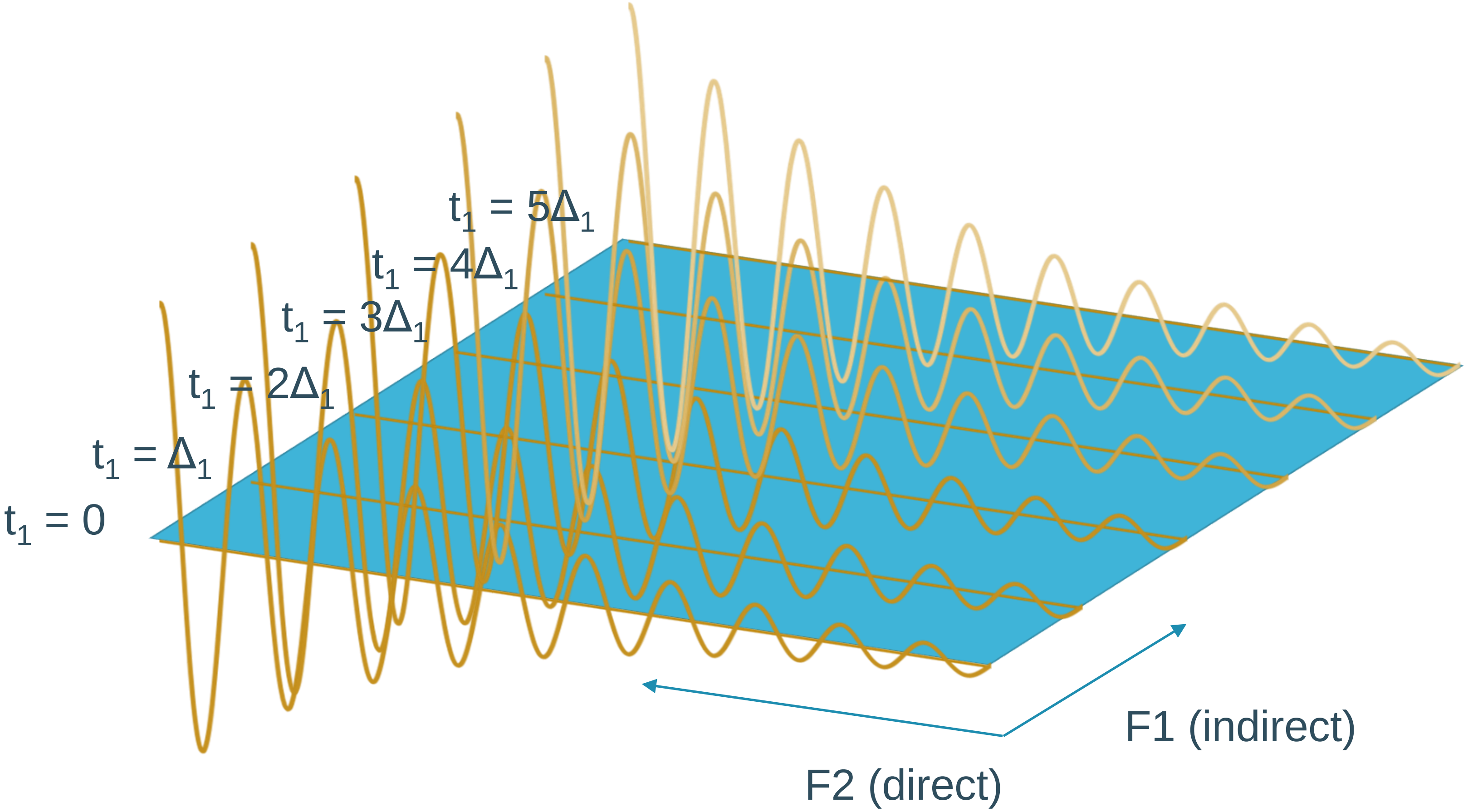
2D experiments

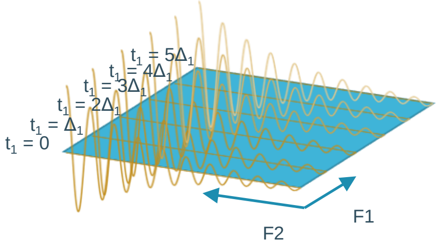
2D acquisition





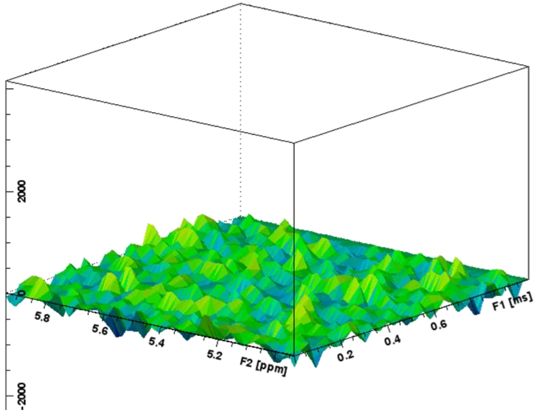


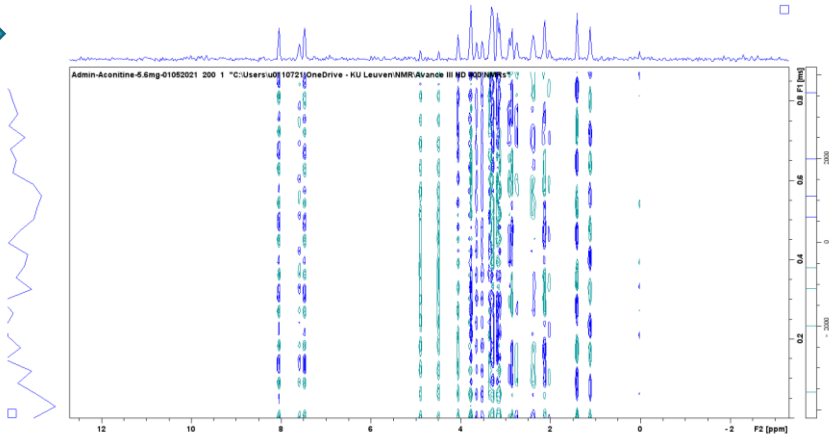
2D acquisition



FFT

in F2



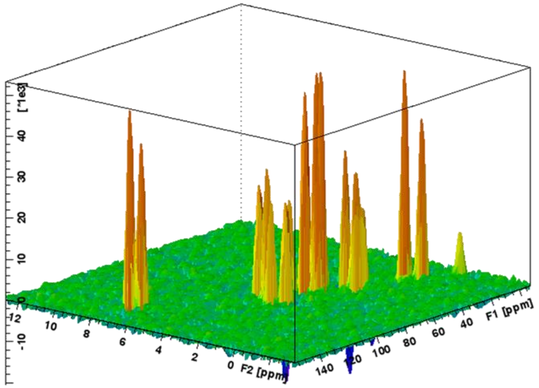


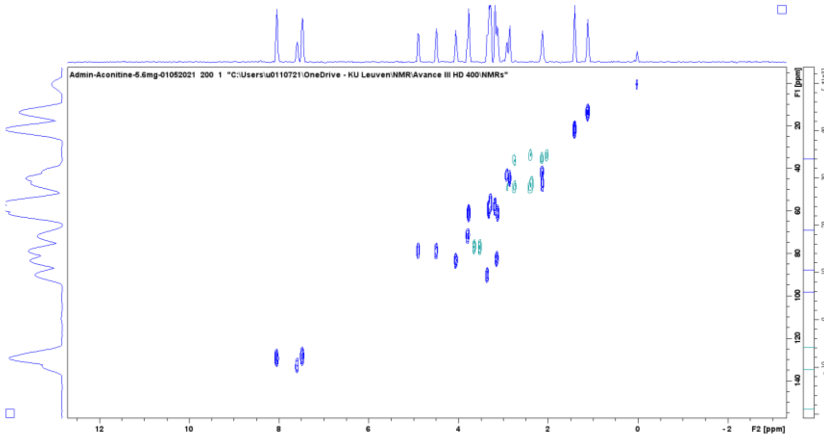
F1 (indirect)

F2 (direct)

FFT

in F1





F1 (indirect)

F2 (direct)

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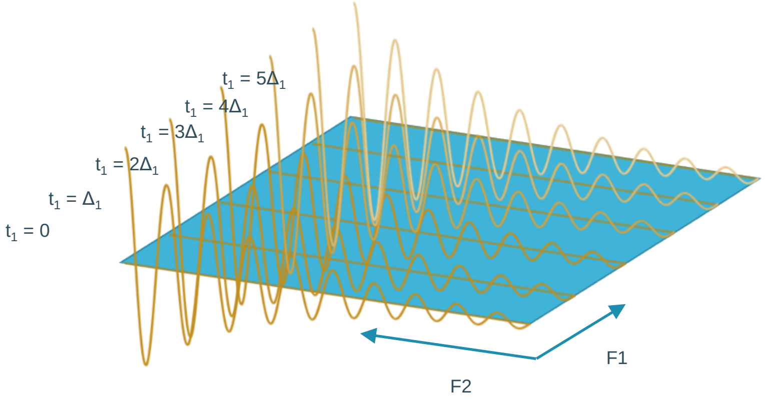
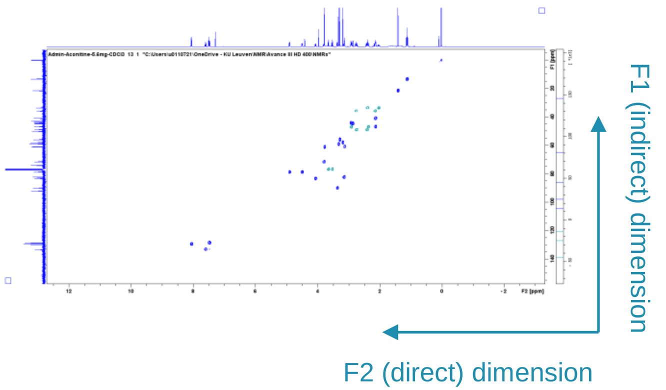
Parameters in 2D



- Most important parameters in 2D experiments include
  - NS: the number of scans per slice
  - TD: number of data points recorded (in both dimensions)
  - AQ: acquisition time (in both dimensions, sec)
  - SW: spectral width (in both dimensions, ppm)
  - O1P: carrier frequency = middle of the spectrum (in both dimensions, ppm)
- As for 1D spectra,
  - Resolution is determined by TD (or AQ)
  - SINO is determined by NS*



* And also by the resolution, see further.

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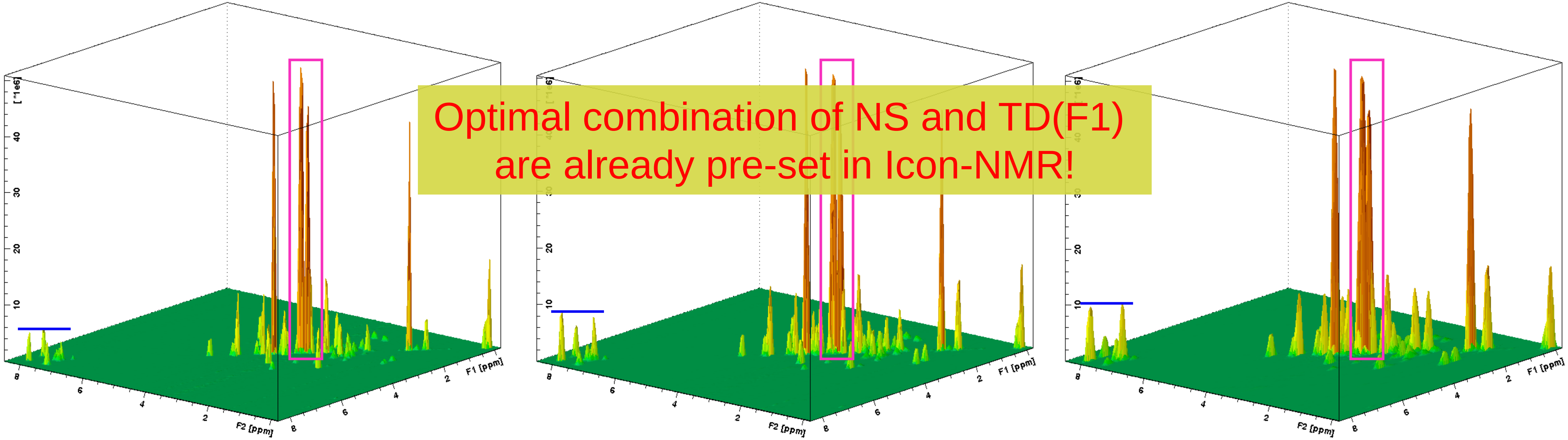
Obtaining better 2D data – more NS?

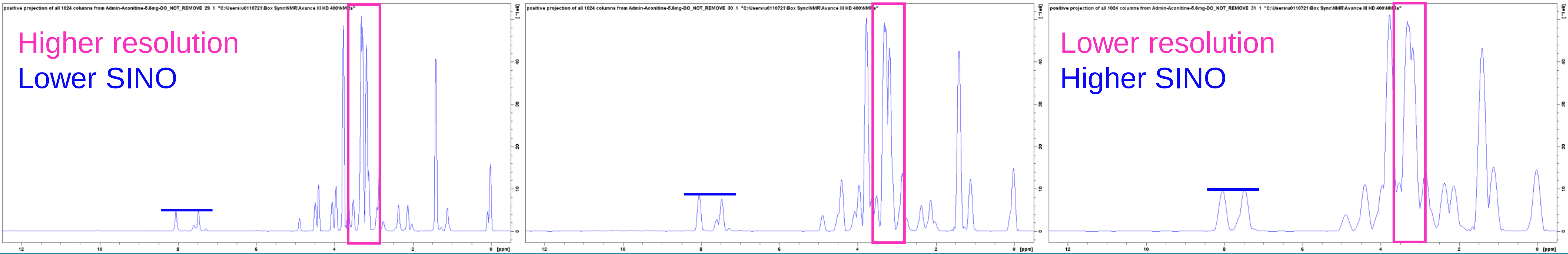
- Need for high NS in 1D (for indirect nucleus) does not presuppose need for high NS in 2D!
- As for 1D, recording NS more scans, increases SINO with $\sqrt{NS}$
- Increasing number of slices in 2D (= TD(F1) = 1TD) increases resolution in F1 (vertical) dimension, but also
  - More resolution = narrower lines = higher signal (narrower signal with same integration area = higher signal) ⇒ higher SINO*
  - Amount of overall signal in 2D matrix increases ⇒ higher SINO
- Optimal values for most applications are already set in Icon-NMR for each experiment! Don't just start adjusting parameters at random!

* Providing that still enough signal is present at higher evolution times. If not, increasing TD(F1) will add more noise = decrease SINO!

NS	2	4	8
TD(F1)	256	128	64
Expt	19:55	19:49	19:45



Optimal combination of NS and TD(F1) are already pre-set in Icon-NMR!



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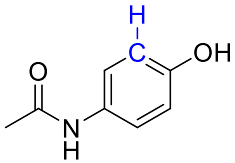
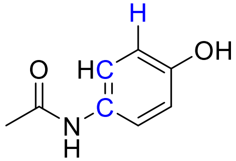
Finding your way in the 2D space

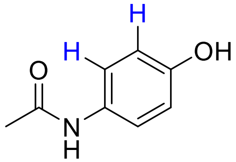
- Most common 2D experiments
  - HSQC
    - ^{1}H-^{13}C $^{1}J_{CH}$ heteronuclear bond correlations
    - "^{1}H and ^{13}C that are directly attached"



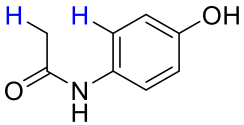
  - HMBC
    - ^{1}H-^{13}C $^{2\text{-}3}J_{CH}$ heteronuclear bond correlations
    - "^{1}H and ^{13}C that are 2 to 3 bonds separated"



  - COSY
    - ^{1}H-^{1}H homonuclear bond correlations
    - "Two (or more) ^{1}H's that couple with each other"



  - NOESY
    - ^{1}H-^{1}H correlations through space (< 5 Å) and ^{1}H-^{1}H exchange (chemical or rotational)
    - "^{1}H's that are close in space or exchange with one another"



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HSQC

2D 1H-13C HSQC-DEPT (EDETGPSISP2.2ADIA)

- Very sensitive experiment (here even sensitivity-improved!!)
- Typical NS = 1-4 (1*n)
- Typical TD(F1) = 128
- Multiplicity-edited = also DEPT information in cross peaks (positive/negative)
- ^{13}C-decoupled (no multiplicities visible in cross peaks)
- Use this for routine experiments

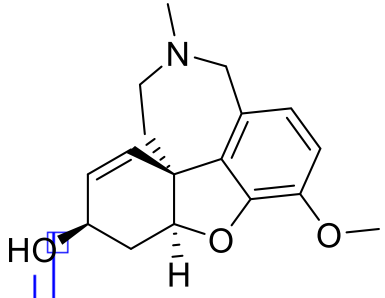
2D 1H-13C HSQC (ETGPSP.3)*

- Less sensitive
- Typical NS = 2-8 (1*n)
- Typical TD(F1) = 128
- Not multiplicity-edited
- ^{13}C-decoupled (no multiplicities visible in cross peaks)
- For ^{13}C-labeled molecules!
- Do not use this for routine experiments

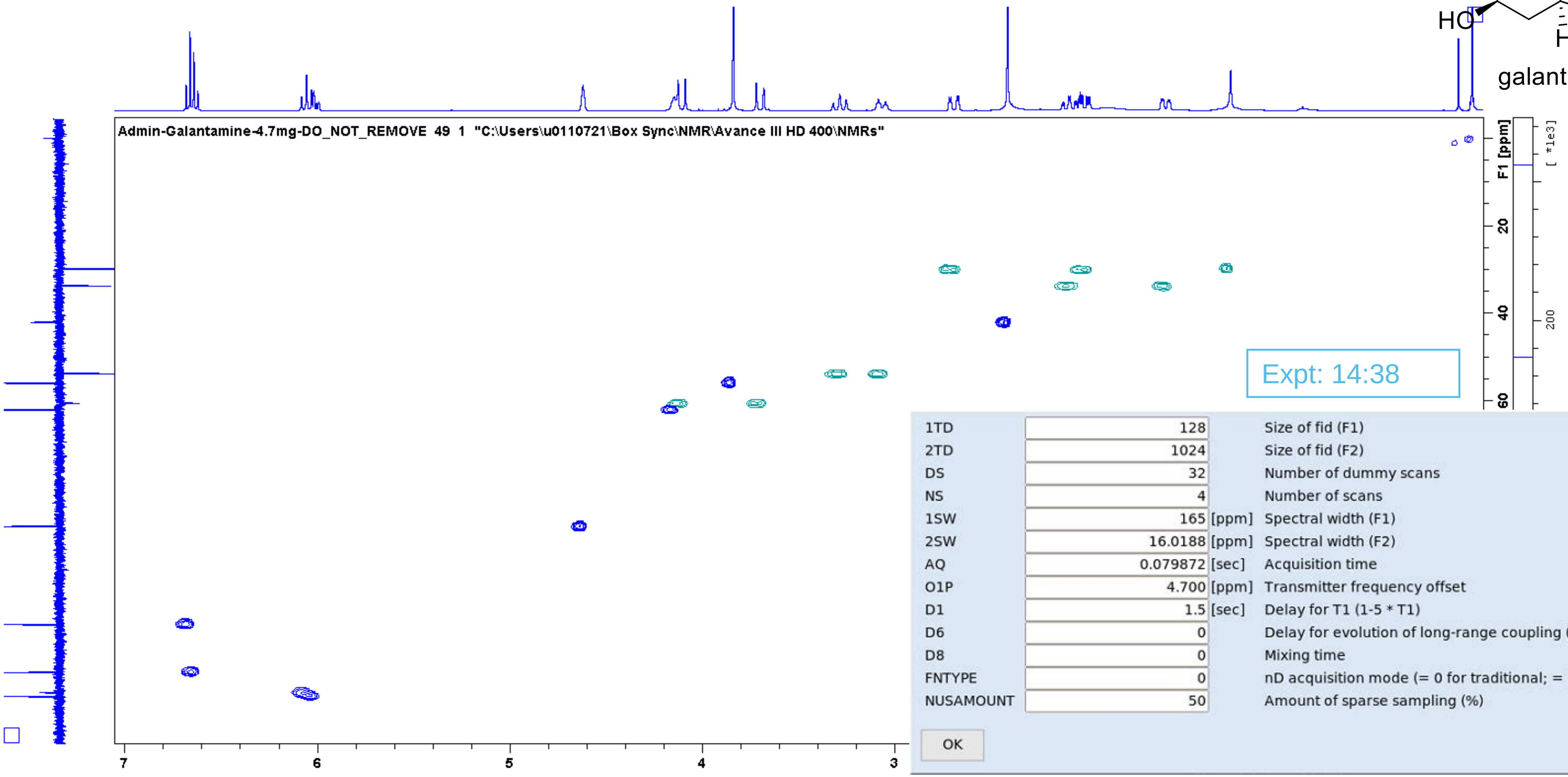
*This experiment will not be considered further.

HSQC

Sample: 4.7 mg galanthamine in $CDCl_3$ (33 mM)



galanthamine



Expt: 14:38

1TD	128		Size of fid (F1)
2TD	1024		Size of fid (F2)
DS	32		Number of dummy scans
NS	4		Number of scans
1SW	165	[ppm]	Spectral width (F1)
2SW	16.0188	[ppm]	Spectral width (F2)
AQ	0.079872	[sec]	Acquisition time
O1P	4.700	[ppm]	Transmitter frequency offset
D1	1.5	[sec]	Delay for T1 (1-5 * T1)
D6	0		Delay for evolution of long-range coupling (LR-COSY, 1/(2J))
D8	0		Mixing time
FNTYPE	0		nD acquisition mode (= 0 for traditional; = 2 for NUS)
NUSAMOUNT	50		Amount of sparse sampling (%)

OK

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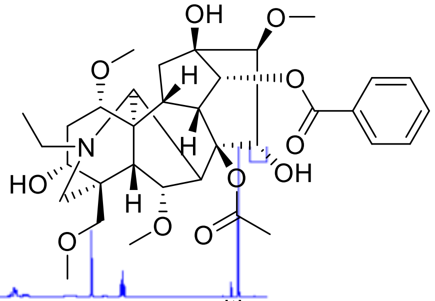
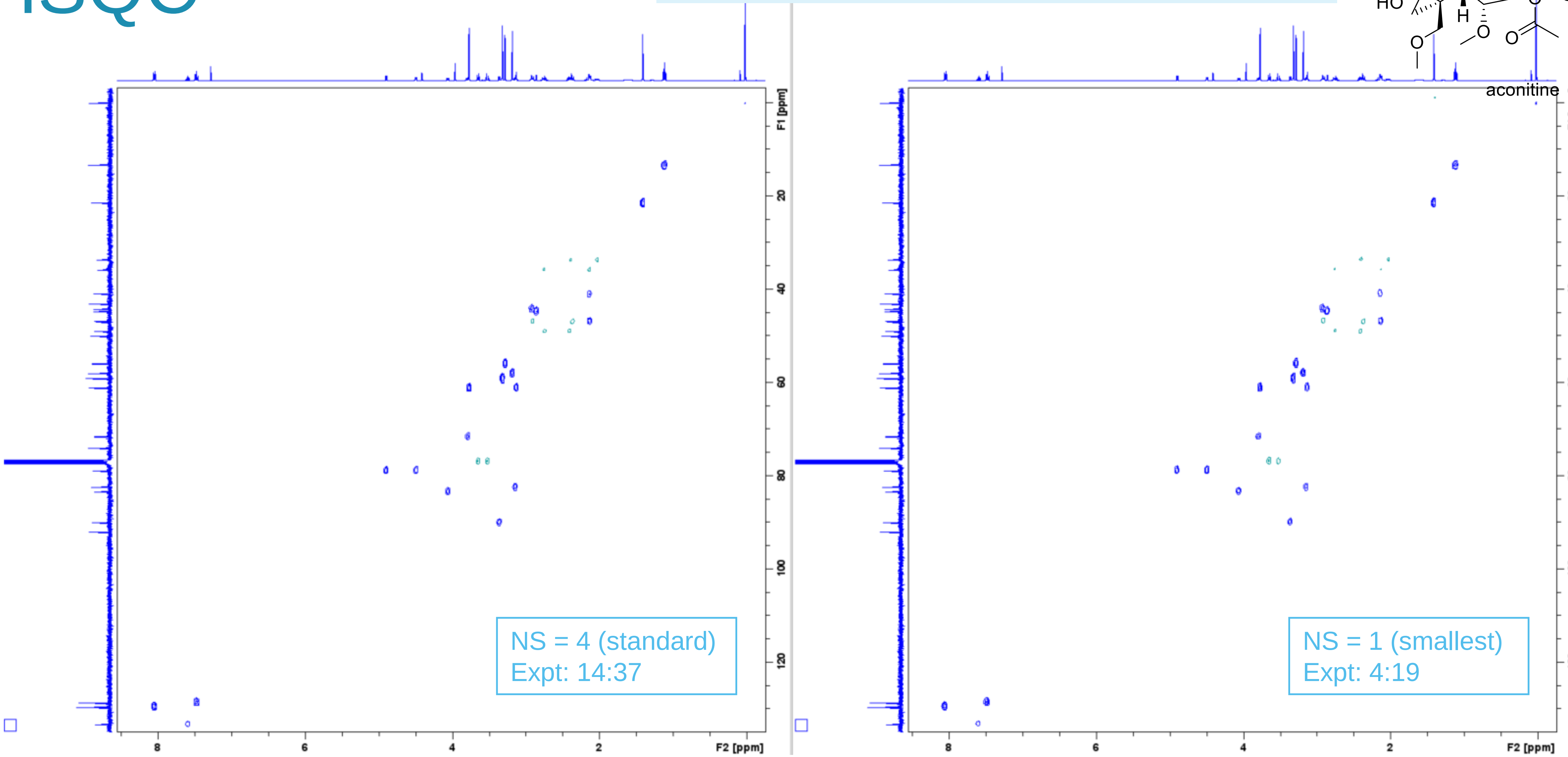
HSQC

Sample: 5.6 mg aconitine in $CDCl_3$ (17 mM)



aconitine



Experiment: HSQC (EDETGPSISP2.2ADIA).

HSQC

- Important!
  - The acquisition time in the direct dimension (AQ) must **NEVER** be higher than 0.2 sec, so be very careful when changing AQ (or TD)!!
  - Due to ^{13}C-decoupling during the acquisition time (AQ), the probe would overheat and be destroyed after a few scans!
  - HSQC experiments with AQ > 0.2 sec will automatically be aborted

1TD	128		Size of fid (F1)
2TD	1024		Size of fid (F2)
DS	32		Number of dummy scans
NS	4		Number of scans
1SW	165	[ppm]	Spectral width (F1)
2SW	16.0188	[ppm]	Spectral width (F2)
AQ	0.079872	[sec]	Acquisition time
O1P	4.700	[ppm]	Transmitter frequency offset
D1	1.5	[sec]	Delay for T1 (1-5 * T1)
D6	0		Delay for evolution of long-range coupling (LR-COSY, 1/(2J))
D8	0		Mixing time
FNTYPE	0		nD acquisition mode (= 0 for traditional; = 2 for NUS)
NUSAMOUNT	50		Amount of sparse sampling (%)

OK

HMBC

2D 1H-13C HMBC (GPLPNDQF)

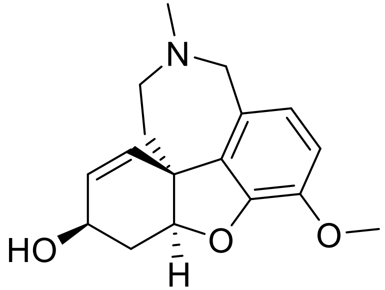
- More sensitive
- Magnitude mode
- Typical NS = 2-4 (2*n)
- Typical TD(F1) = 128
- Not decoupled
- 1-fold *J*-filter: more HSQC residuals
- Meant to obtain high SINO spectra

2D 1H-13C HMBC (ETGPL3ND)

- Somewhat less sensitive
- Phase sensitive
- Typical NS = 2-8 (2*n)
- Typical TD(F1) = 128-256
- Not decoupled
- 3-fold *J*-filter: less to no HSQC residuals
- Meant to obtain high-res spectra

HMBC

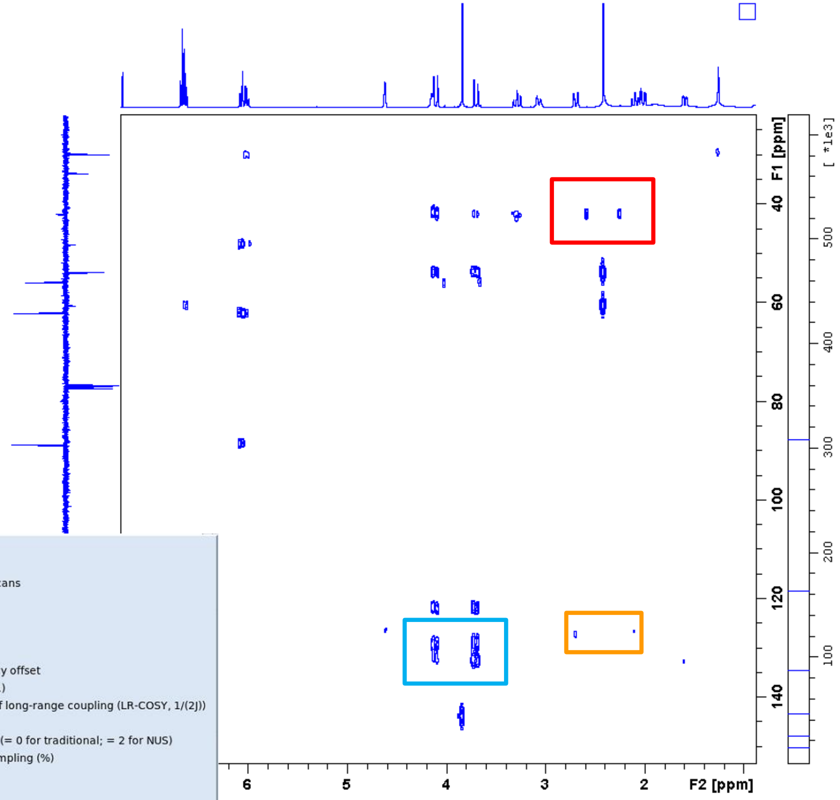
Sample: 4.7 mg galanthamine in $CDCl_3$ (33 mM)



galanthamine

2D 1H-13C HMBC (GPLPNDQF)

- ✓ Higher sensitivity



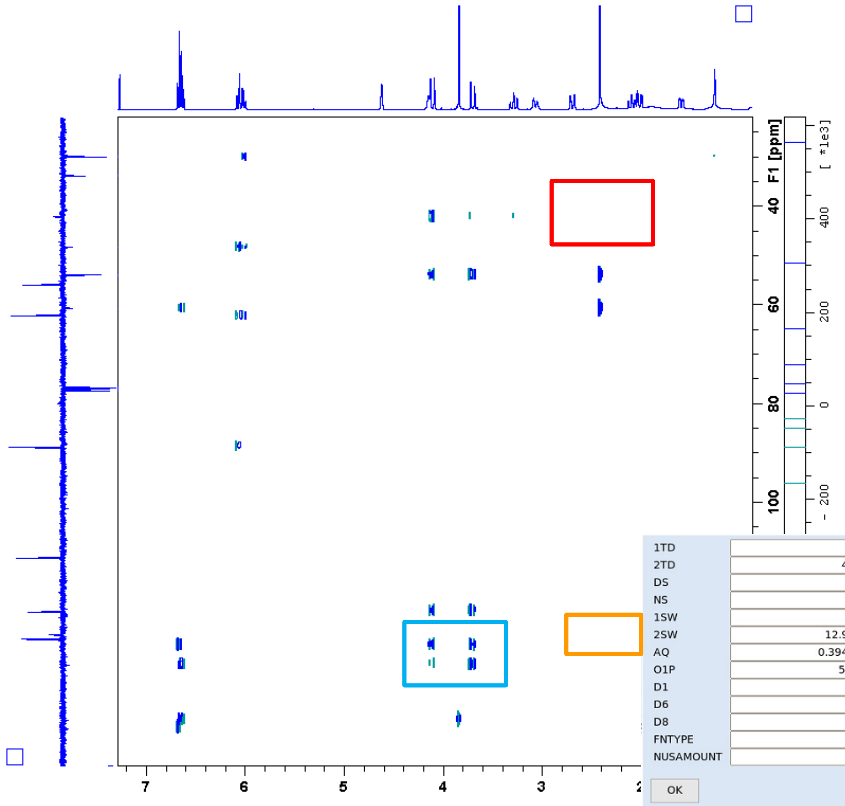
Expt: 15:30

1TD	128		Size of fid (F1)
2TD	2048		Size of fid (F2)
DS	16		Number of dummy scans
NS	4		Number of scans
1SW	220	[ppm]	Spectral width (F1)
2SW	12.9882	[ppm]	Spectral width (F2)
AQ	0.197018	[sec]	Acquisition time
O1P	6.300	[ppm]	Transmitter frequency offset
D1	1.5	[sec]	Delay for T1 (1-5 * T1)
D6	0		Delay for evolution of long-range coupling (LR-COSY, 1/(2J))
D8	0		Mixing time
FNTYPE	0		nD acquisition mode (= 0 for traditional; = 2 for NUS)
NUSAMOUNT	50		Amount of sparse sampling (%)

OK

2D 1H-13C HMBC (ETGPL3ND)

- ✓ No HSQC residuals
- ✓ Higher resolution



Expt: 21:50

1TD	128		Size of fid (F1)
2TD	4096		Size of fid (F2)
DS	16		Number of dummy scans
NS	4		Number of scans
1SW	220	[ppm]	Spectral width (F1)
2SW	12.9882	[ppm]	Spectral width (F2)
AQ	0.394035	[sec]	Acquisition time
O1P	5.500	[ppm]	Transmitter frequency offset
D1	2	[sec]	Delay for T1 (1-5 * T1)
D6	0		Delay for evolution of long-range coupling (LR-COSY, 1/(2J))
D8	0		Mixing time
FNTYPE	0		nD acquisition mode (= 0 for traditional; = 2 for NUS)
NUSAMOUNT	50		Amount of sparse sampling (%)

OK

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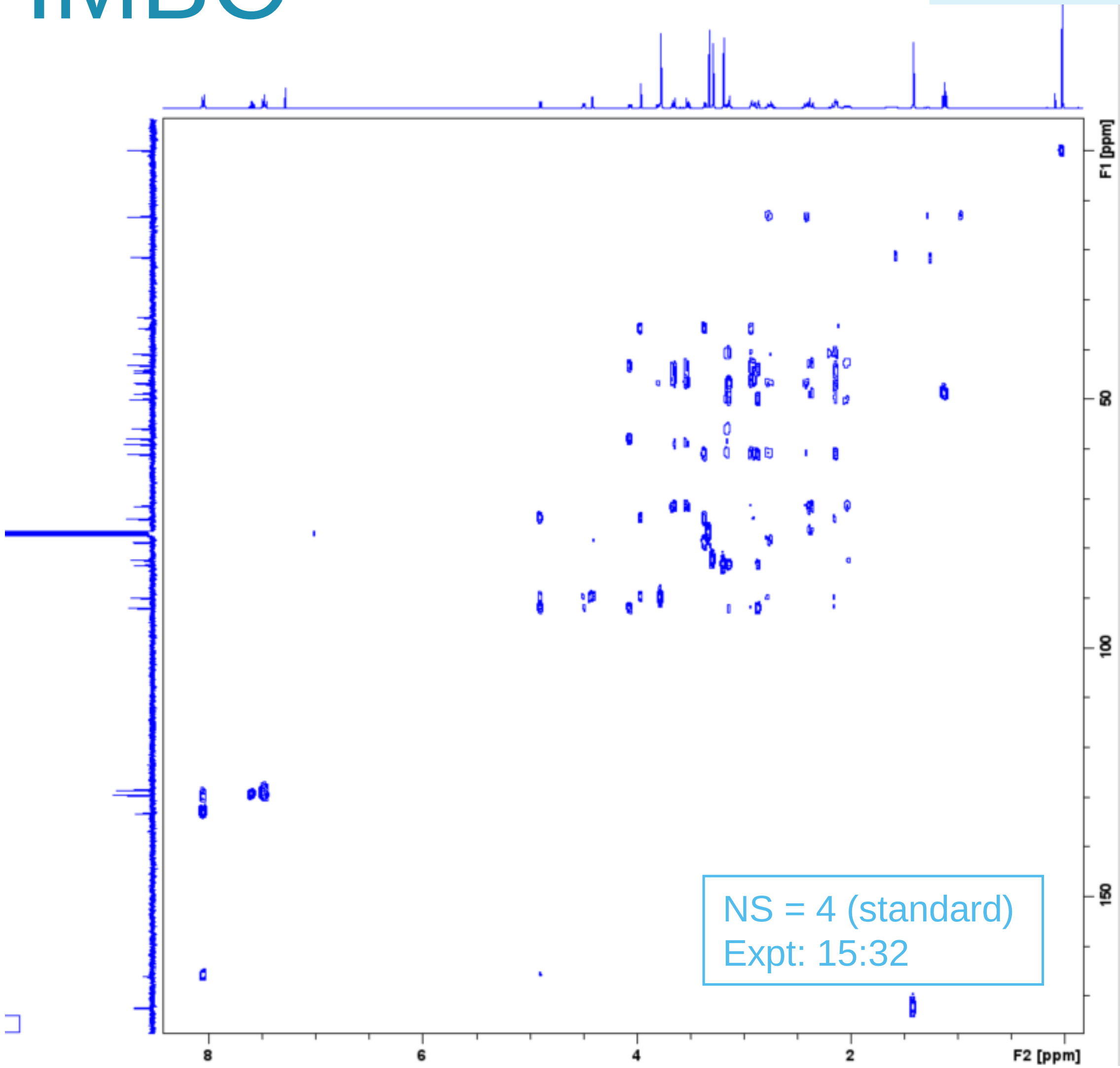
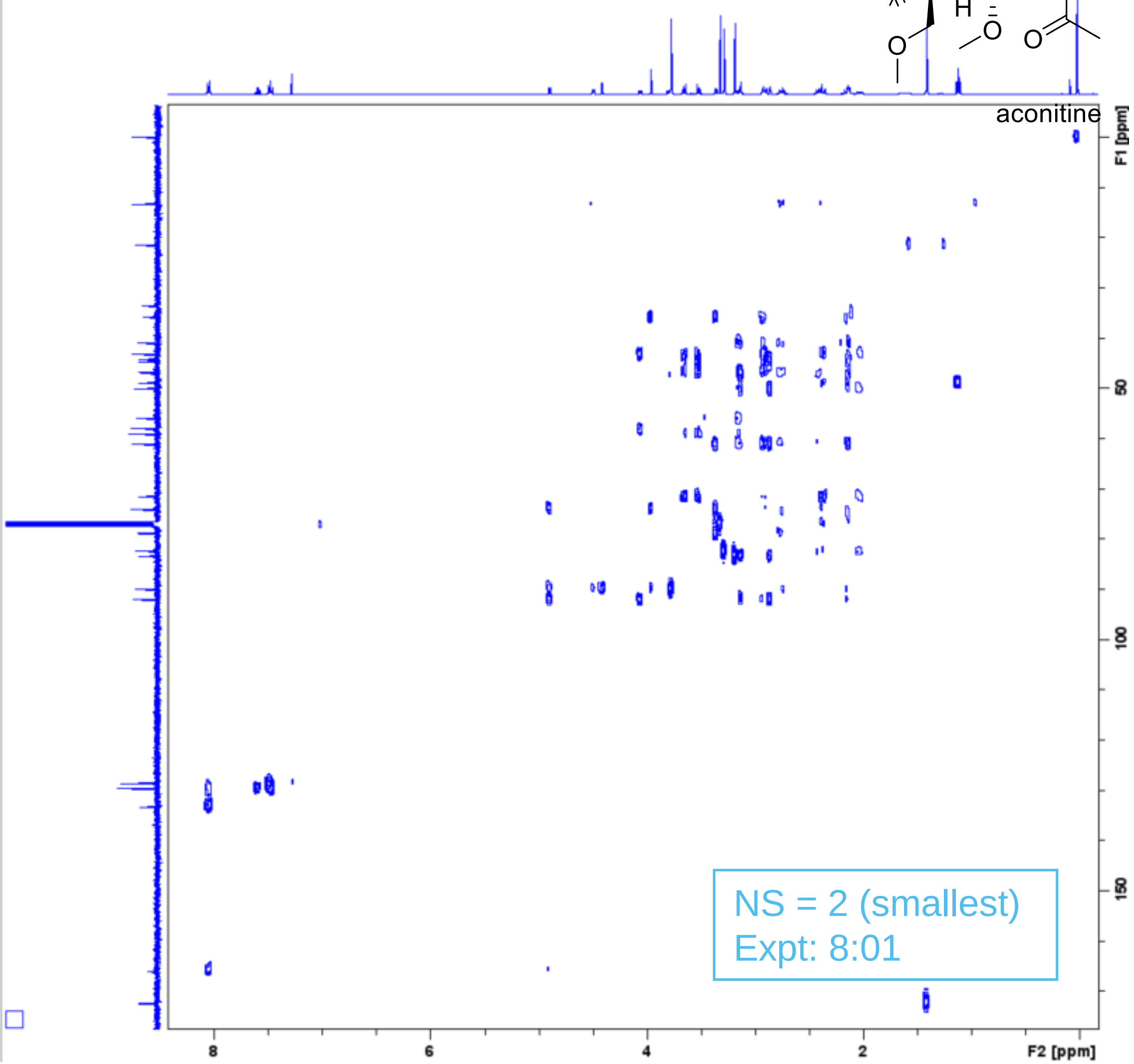
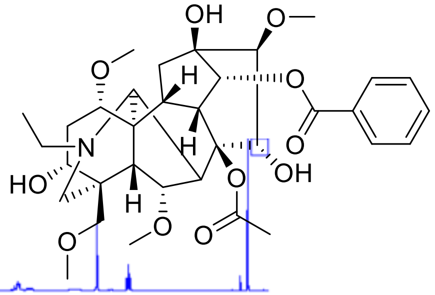
HMBC

Sample: 5.6 mg aconitine in $CDCl_3$ (17 mM)







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Experiment: HMBC (GPLPNDQF).

COSY

2D 1H-1H COSY (GPPPQF)

- Regular COSY
- Magnitude mode
- Highly sensitive
- Typical NS = 1-2 (1*n)
- Typical TD(F1) = 128
- Lot of overlap with diagonal
- Meant to obtain high SINO spectra

2D 1H-1H DQF-COSY (GPDFPHPP)

- DQF-COSY
- Phase-sensitive
- Somewhat less sensitive
- Typical NS = 2-4 (1*n)
- Typical TD(F1) = 128-256
- Less overlap with diagonal
- Meant to obtain high-res spectra

2D 1H-1H LR-COSY (GPLRPPQF)

- LR-COSY
- Magnitude mode
- Less sensitive
- Typical NS = 2-8 (1*n)
- Typical TD(F1) = 128
- Meant to visualize long-range ^1H-^1H couplings *via* $d6 = \frac{1}{2J}$ (0.1-0.4 sec)

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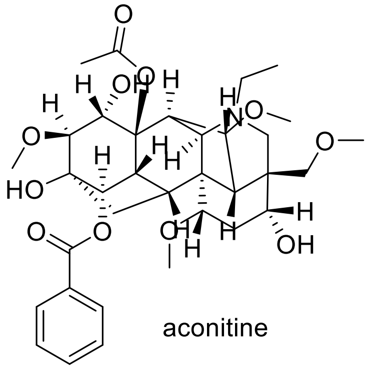
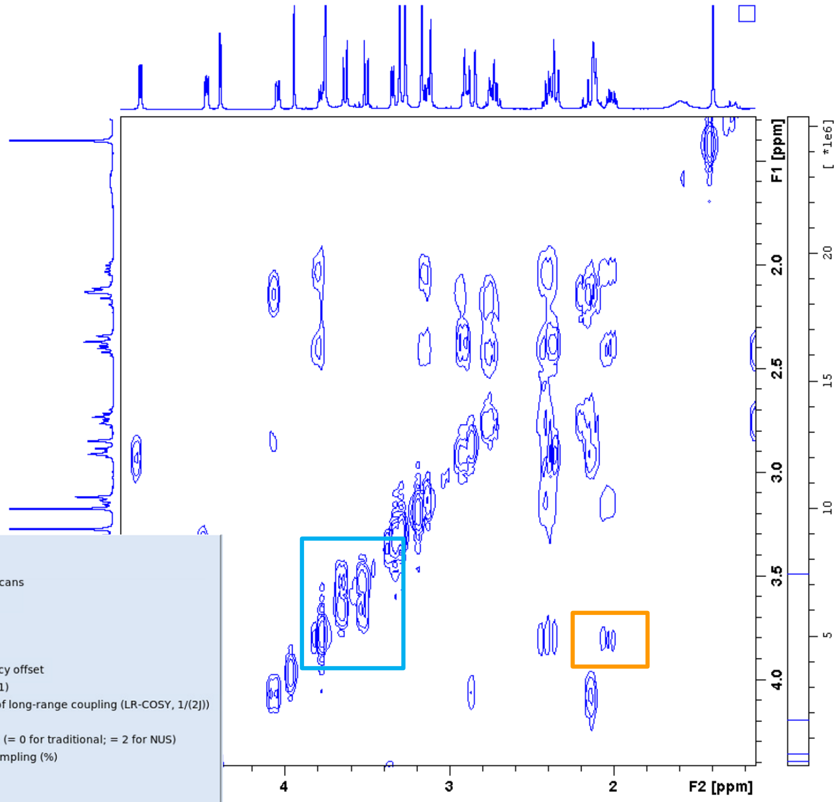
COSY

Sample: 5.6 mg aconitine in $CDCl_3$ (17 mM)



2D 1H-1H COSY (GPPPQF)

- ✓ Higher sensitivity
- ✓ Faster



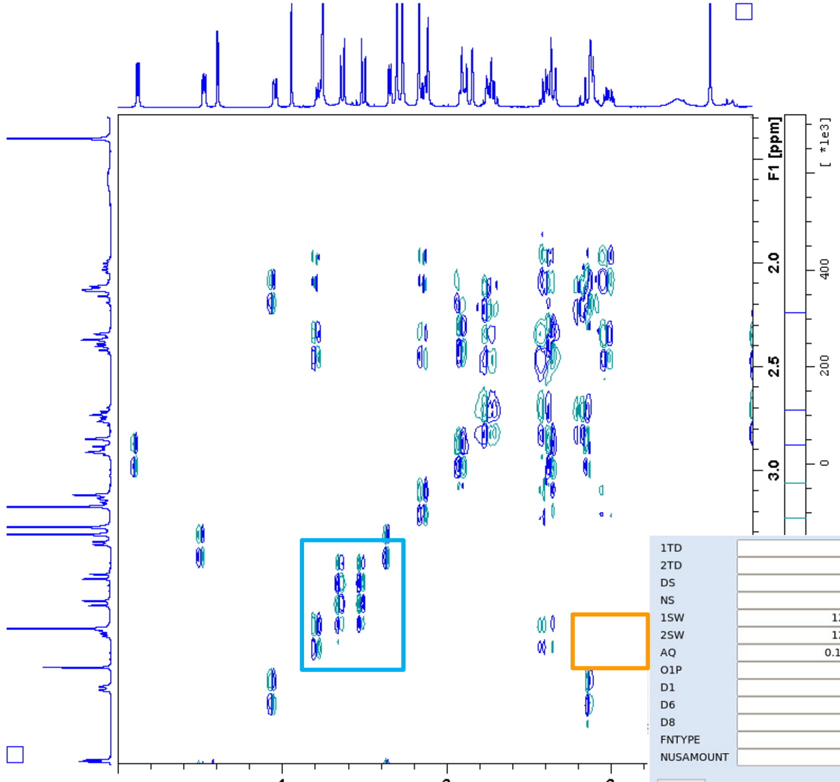
Expt: 10:12

1TD	128	Size of fid (F1)
2TD	2048	Size of fid (F2)
DS	16	Number of dummy scans
NS	2	Number of scans
1SW	12.9882 [ppm]	Spectral width (F1)
2SW	12.9882 [ppm]	Spectral width (F2)
AQ	0.197018 [sec]	Acquisition time
O1P	6.000 [ppm]	Transmitter frequency offset
D1	2 [sec]	Delay for T1 (1-5 * T1)
D6	0	Delay for evolution of long-range coupling (LR-COSY, 1/(2J))
D8	0	Mixing time
FNTYPE	0	nD acquisition mode (= 0 for traditional; = 2 for NUS)
NUSAMOUNT	50	Amount of sparse sampling (%)

OK

2D 1H-1H DQF-COSY (GPDFPHPP)

- ✓ Higher resolution



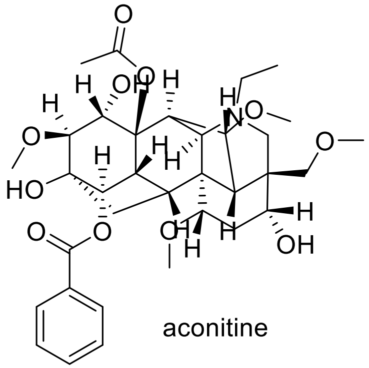
Expt: 19:47

1TD	128	Size of fid (F1)
2TD	2048	Size of fid (F2)
DS	16	Number of dummy scans
NS	4	Number of scans
1SW	12.9882 [ppm]	Spectral width (F1)
2SW	12.9882 [ppm]	Spectral width (F2)
AQ	0.197018 [sec]	Acquisition time
O1P	5.500 [ppm]	Transmitter frequency offset
D1	2 [sec]	Delay for T1 (1-5 * T1)
D6	0	Delay for evolution of long-range coupling (LR-COSY, 1/(2J))
D8	0	Mixing time
FNTYPE	0	nD acquisition mode (= 0 for traditional; = 2 for NUS)
NUSAMOUNT	50	Amount of sparse sampling (%)

OK

COSY

Sample: 5.6 mg aconitine in $CDCl_3$ (17 mM)



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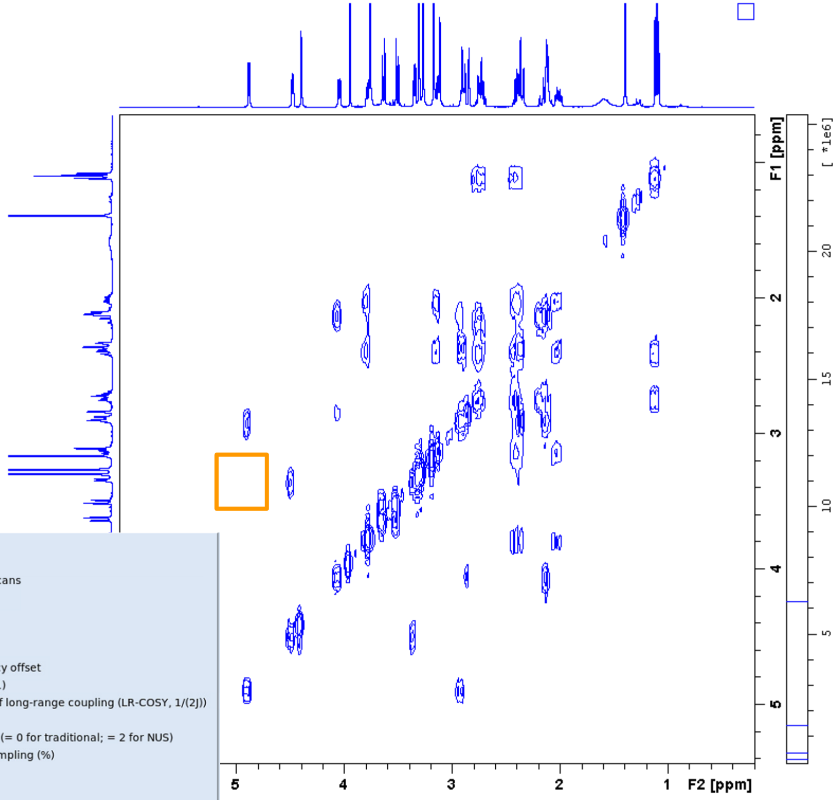
2D 1H-1H COSY (GPPPQF)

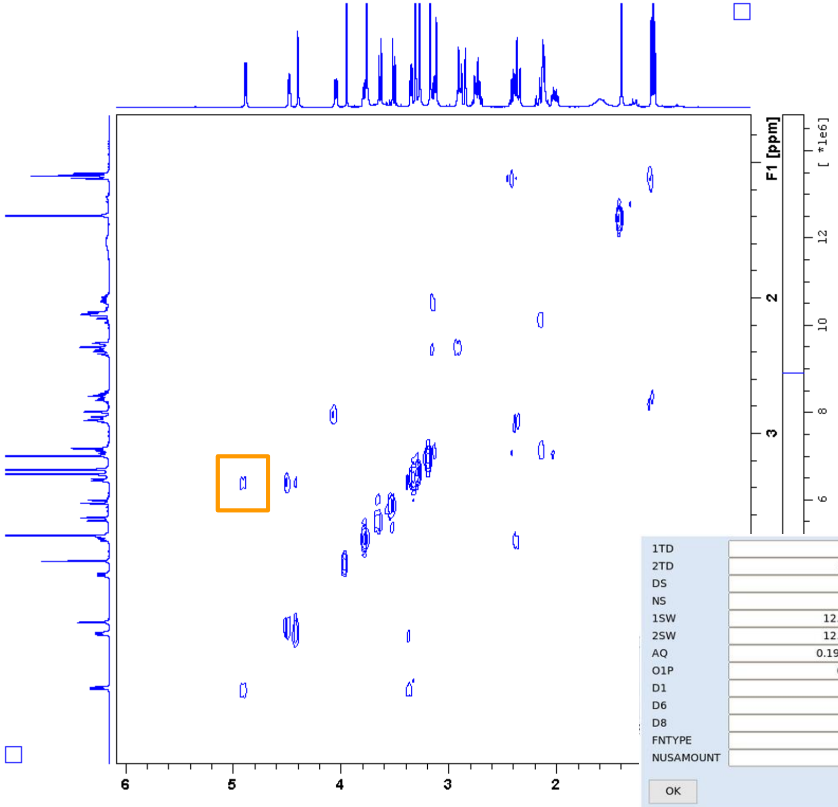


Expt: 10:12

1TD	128		Size of fid (F1)
2TD	2048		Size of fid (F2)
DS	16		Number of dummy scans
NS	2		Number of scans
1SW	12.9882	[ppm]	Spectral width (F1)
2SW	12.9882	[ppm]	Spectral width (F2)
AQ	0.197018	[sec]	Acquisition time
O1P	6.000	[ppm]	Transmitter frequency offset
D1	2	[sec]	Delay for T1 (1-5 * T1)
D6	0		Delay for evolution of long-range coupling (LR-COSY, 1/(2J))
D8	0		Mixing time
FNTYPE	0		nD acquisition mode (= 0 for traditional; = 2 for NUS)
NUSAMOUNT	50		Amount of sparse sampling (%)

OK

2D 1H-1H LR-COSY (GPLRPPQF)



✓ Long-range couplings

Expt: 12:01

1TD	128		Size of fid (F1)
2TD	2048		Size of fid (F2)
DS	16		Number of dummy scans
NS	2		Number of scans
1SW	12.9882	[ppm]	Spectral width (F1)
2SW	12.9882	[ppm]	Spectral width (F2)
AQ	0.197018	[sec]	Acquisition time
O1P	6.000	[ppm]	Transmitter frequency offset
D1	2	[sec]	Delay for T1 (1-5 * T1)
D6	0.2		Delay for evolution of long-range coupling (LR-COSY, 1/(2J))
D8	0		Mixing time
FNTYPE	0		nD acquisition mode (= 0 for traditional; = 2 for NUS)
NUSAMOUNT	50		Amount of sparse sampling (%)

OK

LR-COSY is less sensitive than a ‘regular COSY’, but this is – of course – very logical, as we are looking for very small couplings!

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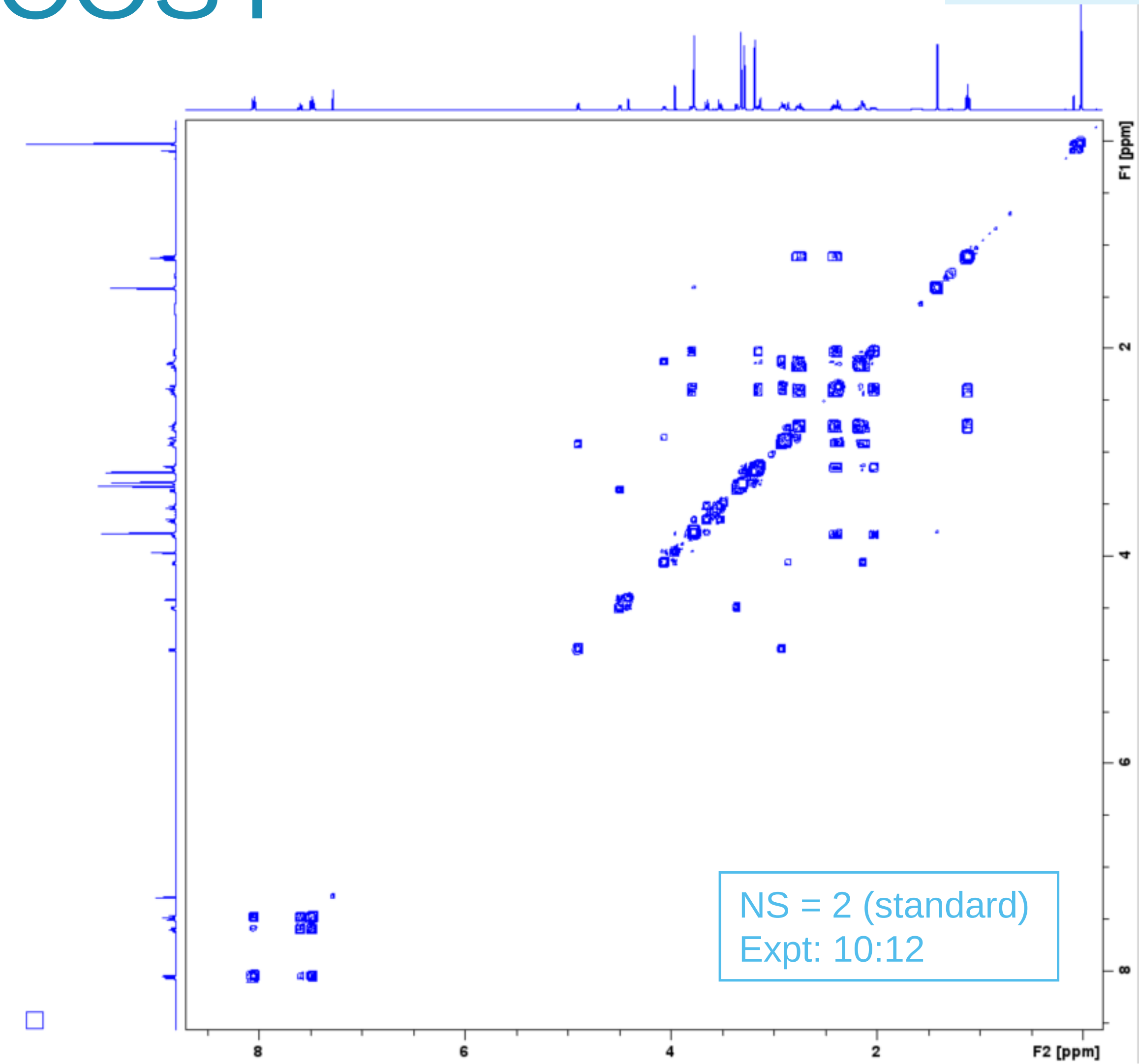
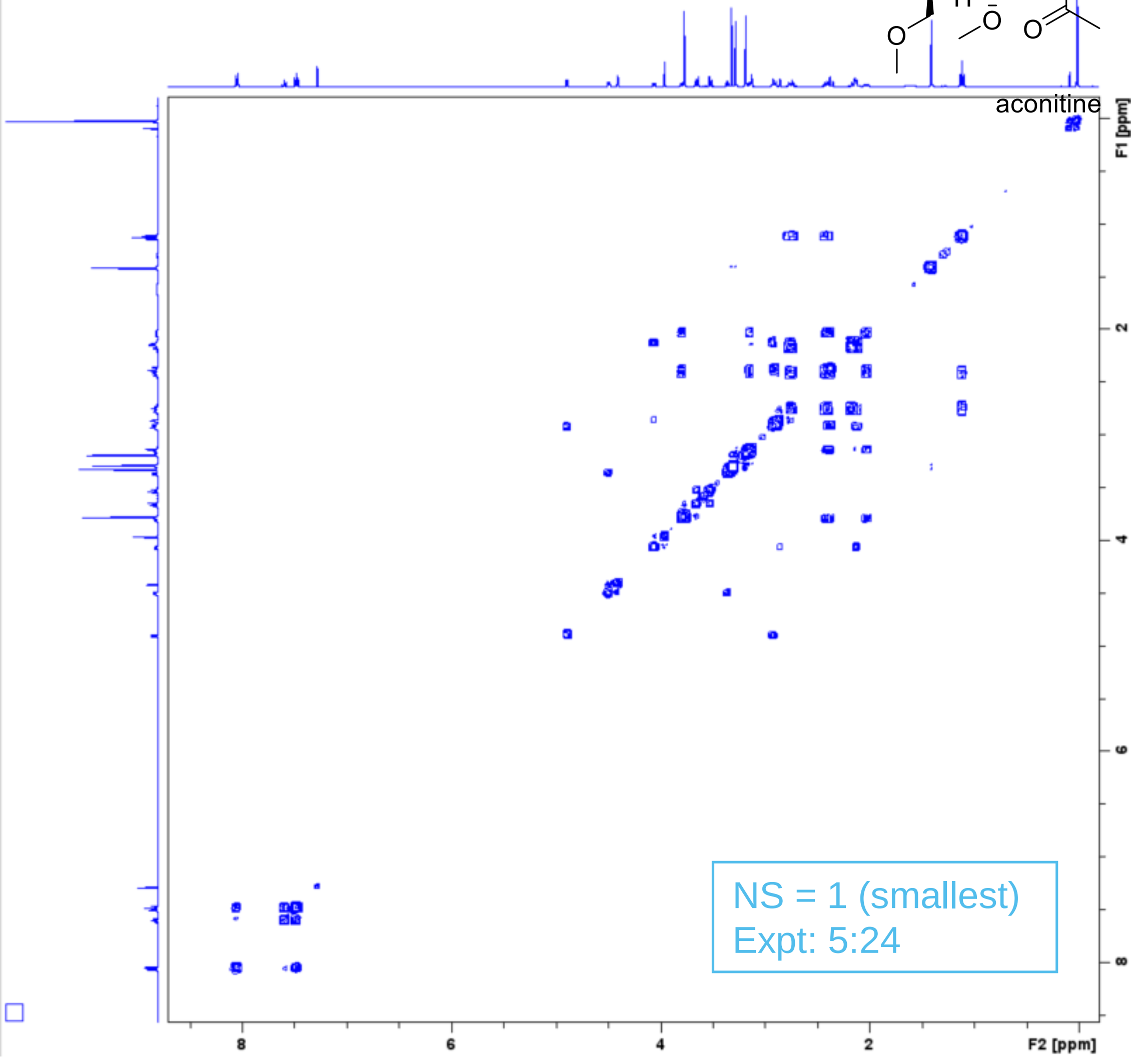
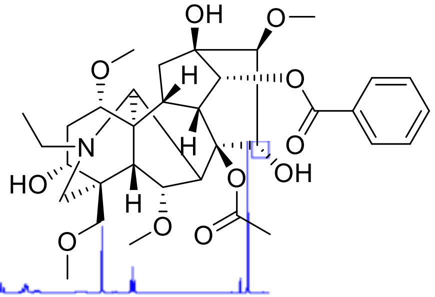
COSY

Sample: 5.6 mg aconitine in $CDCl_3$ (17 mM)







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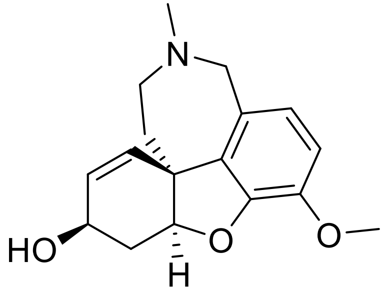
NOESY

- **2D 1H-1H NOESY (GPPHPP)**
- Phase-sensitive
- Somewhat less sensitive
- Typical NS = 2-8 (2*n)
- Typical TD(F1) = 128
- Two types of information in one spectrum*
  - Cross peaks with opposite phase (= color) of diagonal peaks
    - ^{1}H’s that are close in space (< 5 Å)
  - Cross peaks with same phase (= color) as diagonal peaks
    - Chemical exchange
    - Rotational exchange

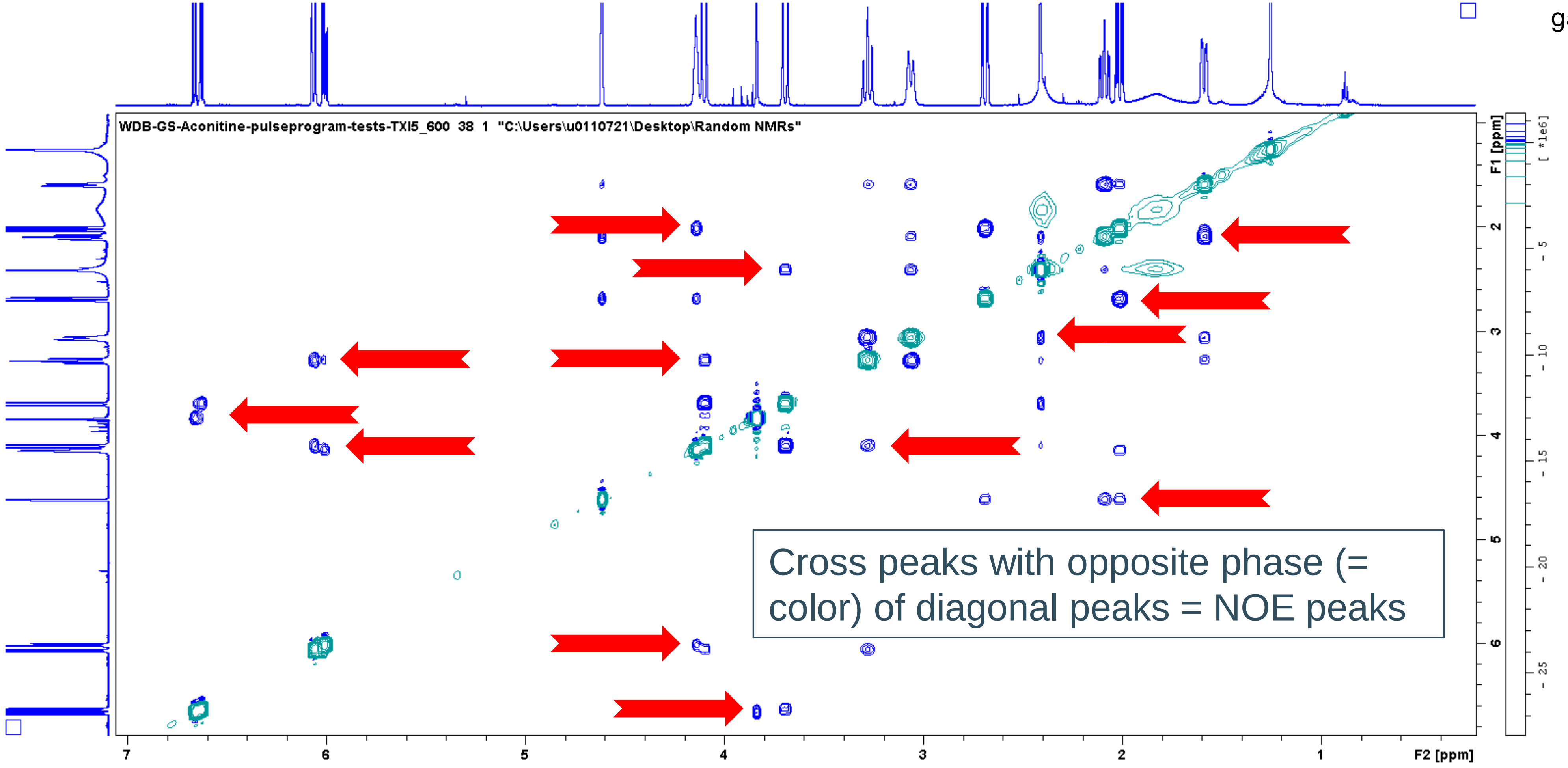
* For molecules with small correlation times, i.e. small molecules in non-viscous solvents.

NOESY

Sample: 4.7 mg galanthamine in $CDCl_3$ (33 mM)



galanthamine



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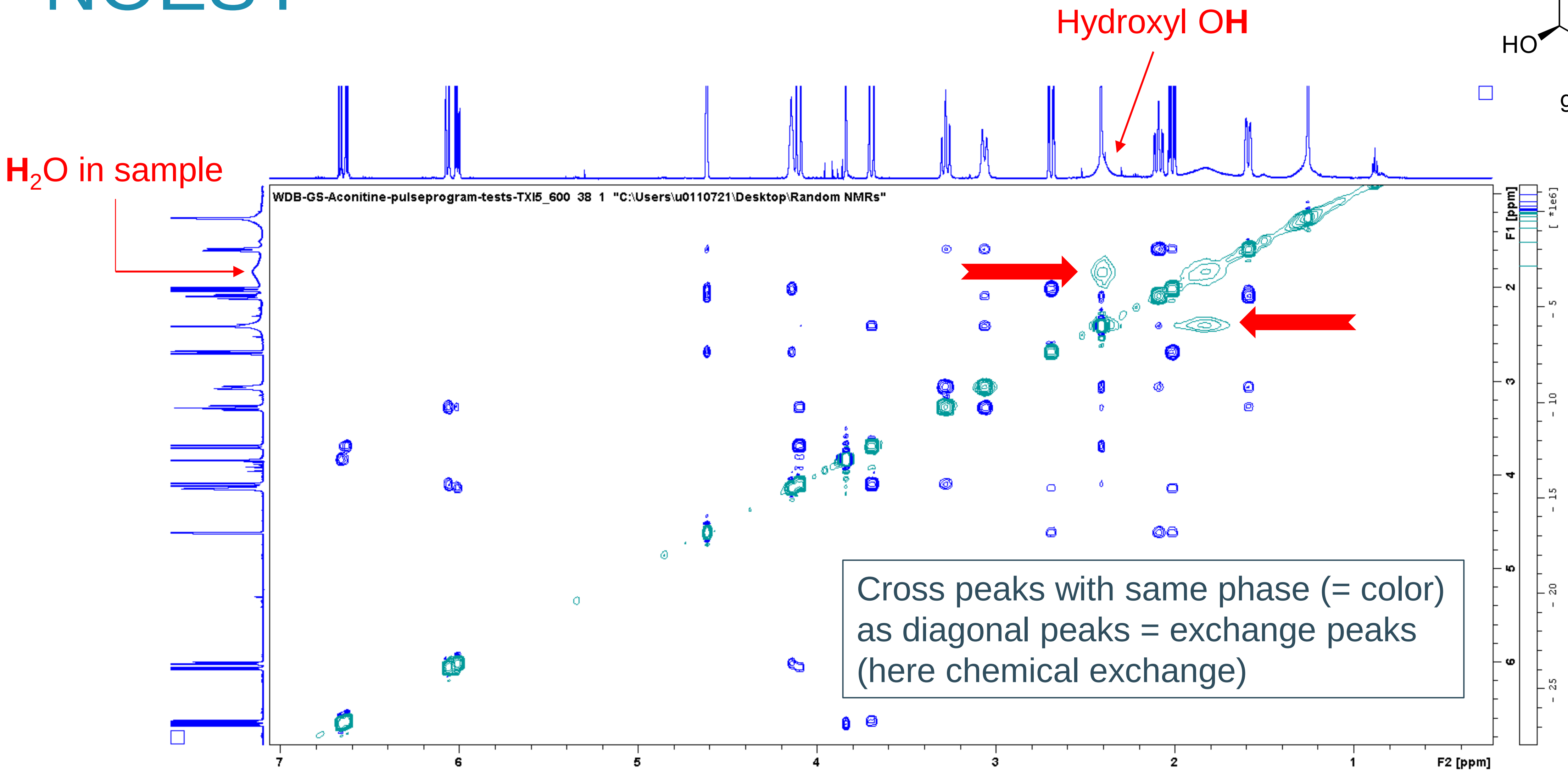
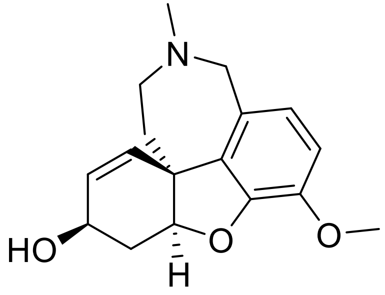
NOESY

Sample: 4.7 mg galanthamine in $CDCl_3$ (33 mM)





galanthamine

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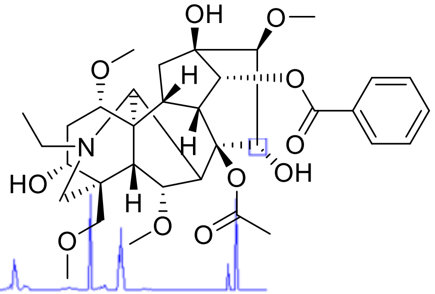
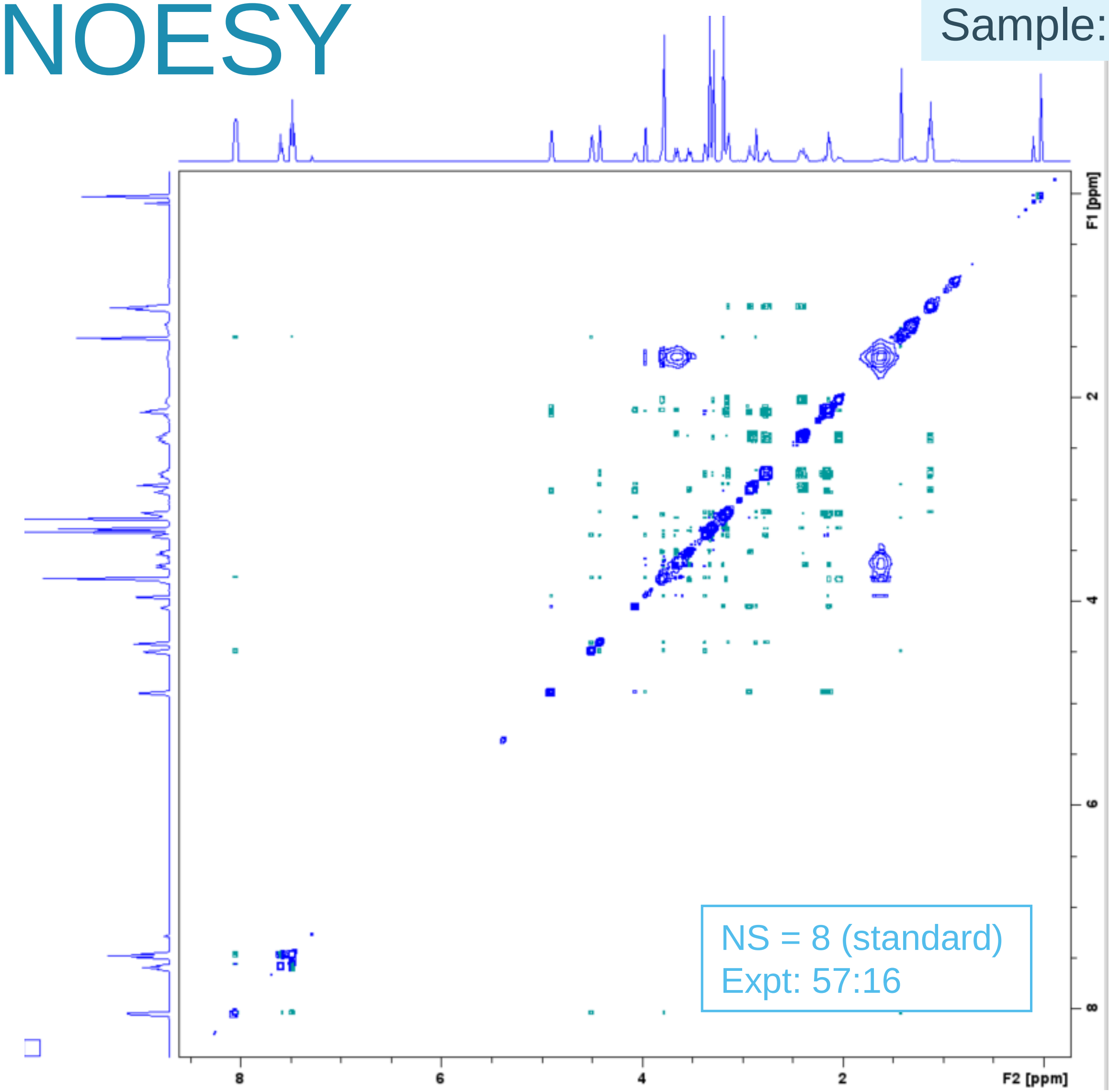
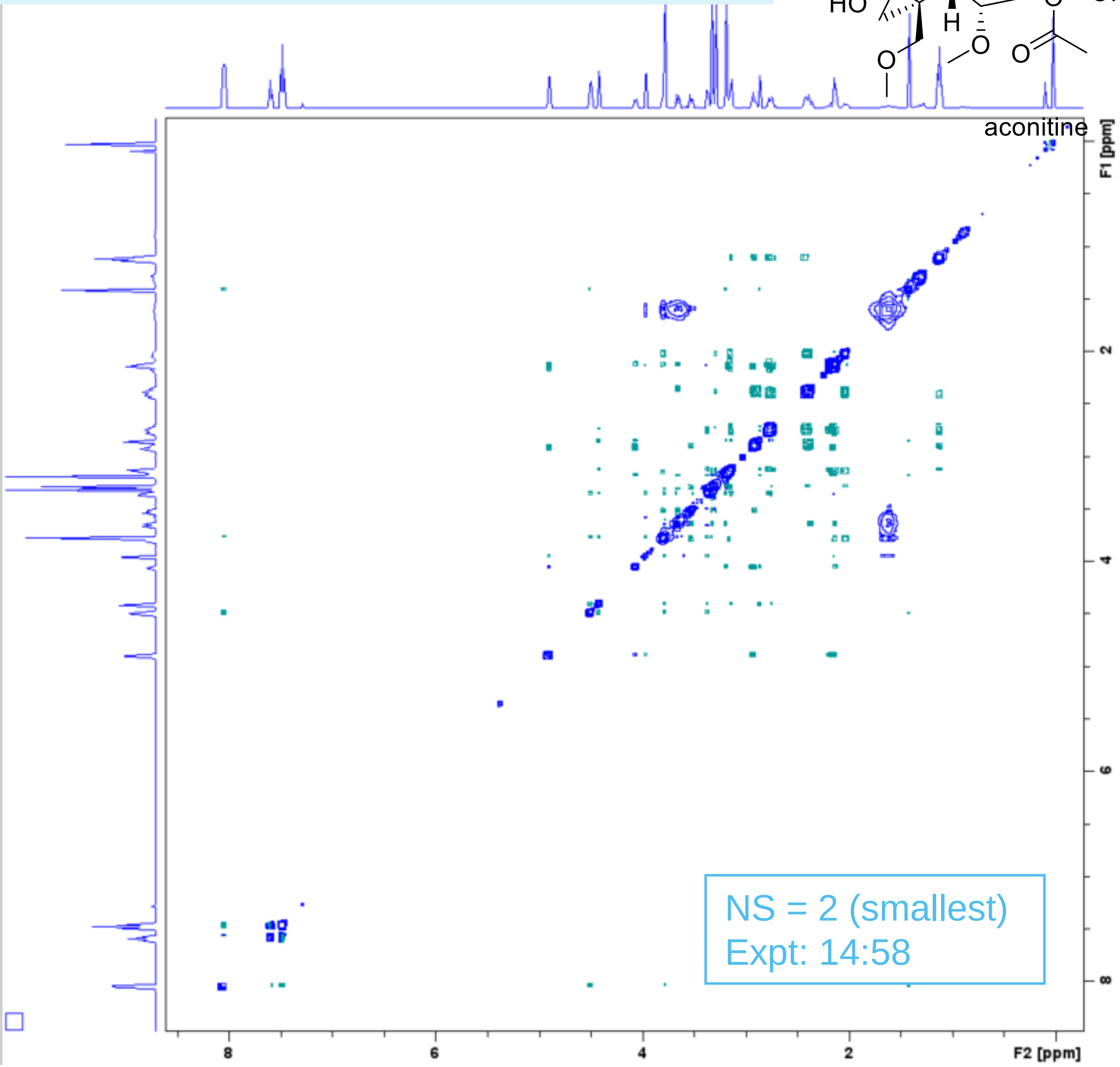
NOESY

Sample: 5.6 mg aconitine in $CDCl_3$ (17 mM)



aconitine

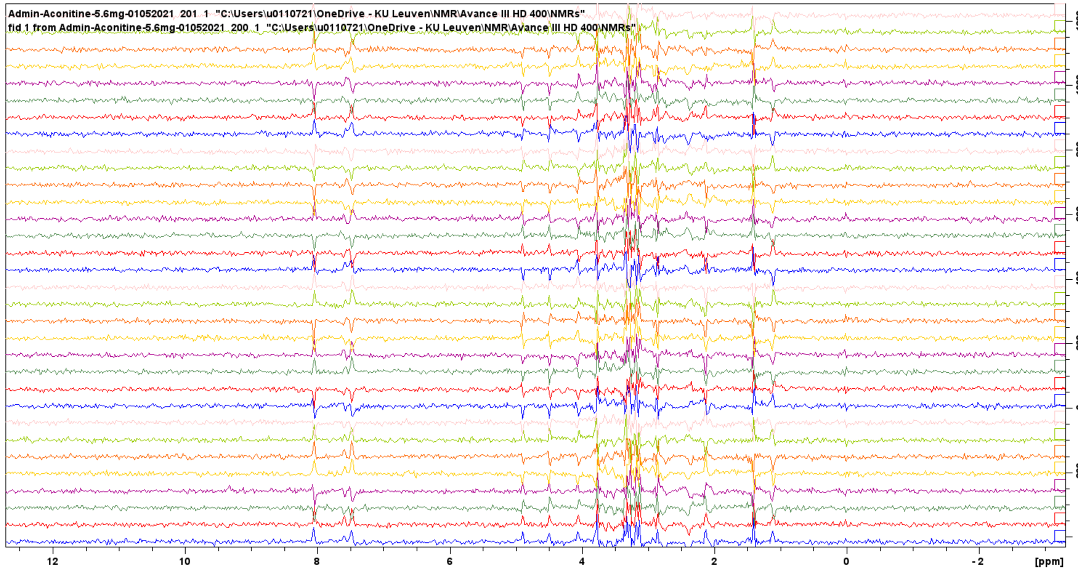




Experiment: NOESY (GPPHPP).

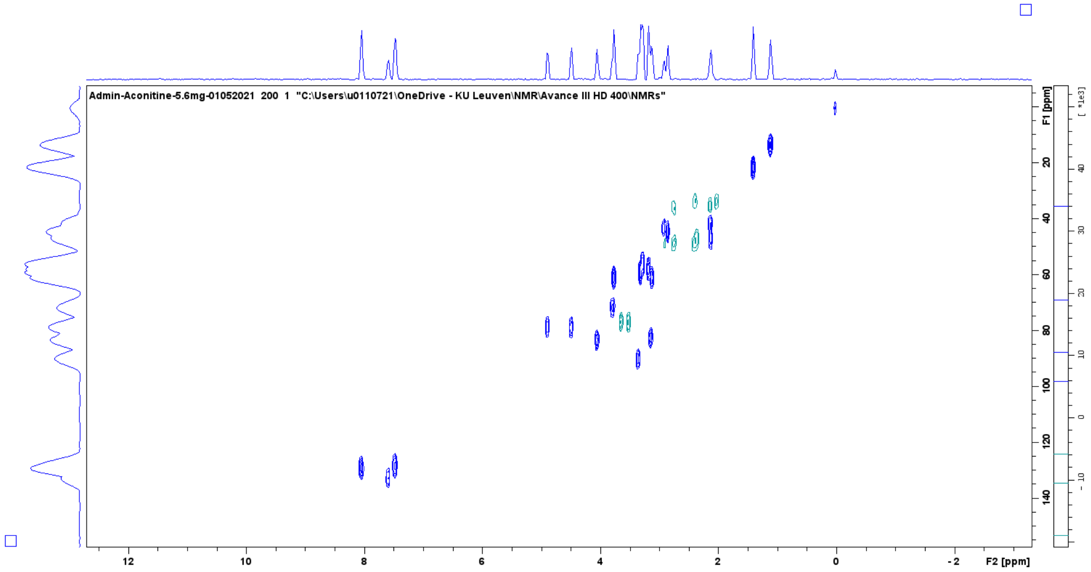
Time-optimization in 2D: NUS

- Traditional acquisition (uniform sampling): collect all TD(F1) slices in indirect dimension, FT in both dimensions



Raw data with 32 slices recorded.

FFT

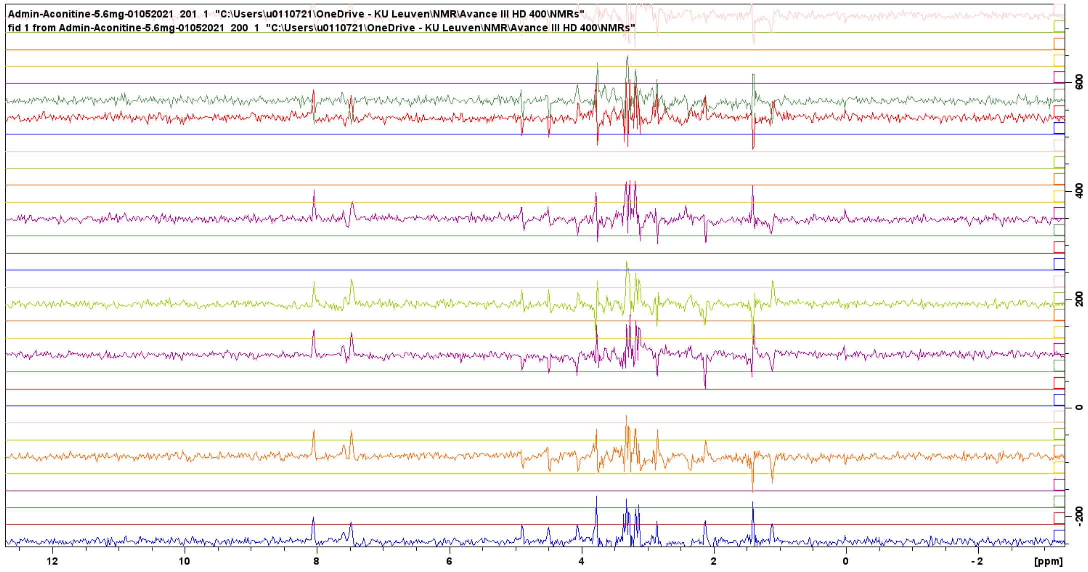


Obtained 2D spectrum using 32 slices.

In this case, TD(F1) = 32.

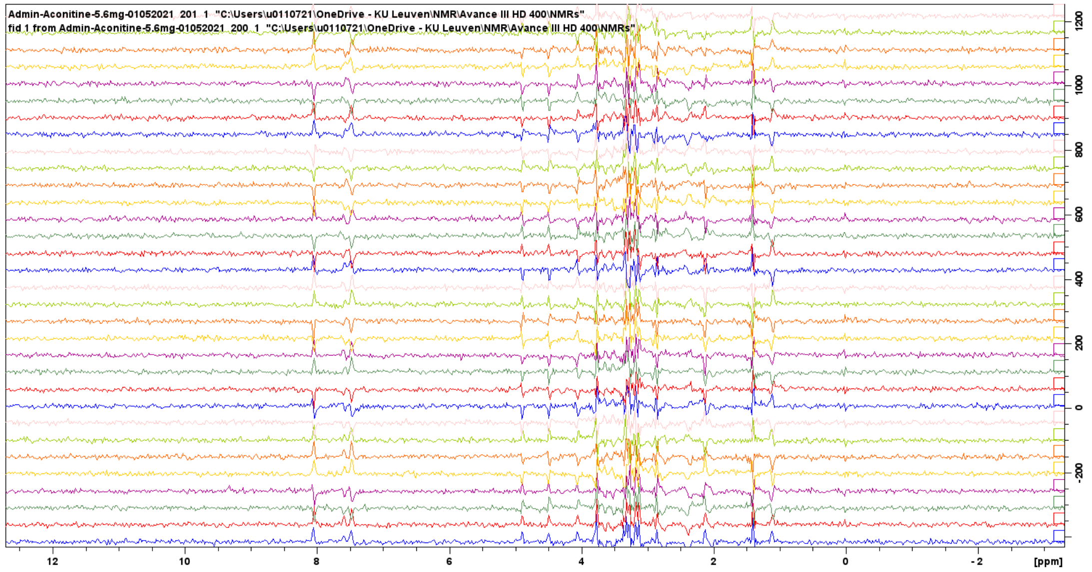
Time-optimization in 2D: NUS

- NUS = **N**on-**U**niform **S**ampling acquisition: collect random fraction of TD(F1) slices in indirect dimension, reconstruct the missing slices and FT in both dimensions



Raw data with 8 slices recorded.

1. Reconstruction



Reconstructed raw data with 32 slices (8 recorded, 24 calculated).

In this case, TD(F1) = 32, NUS = 25%.

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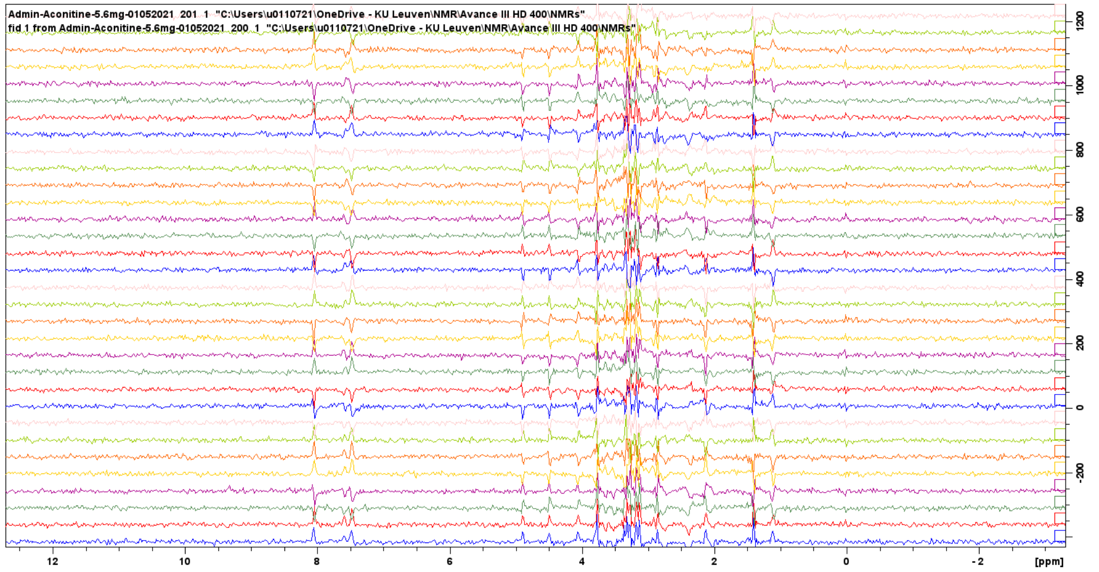
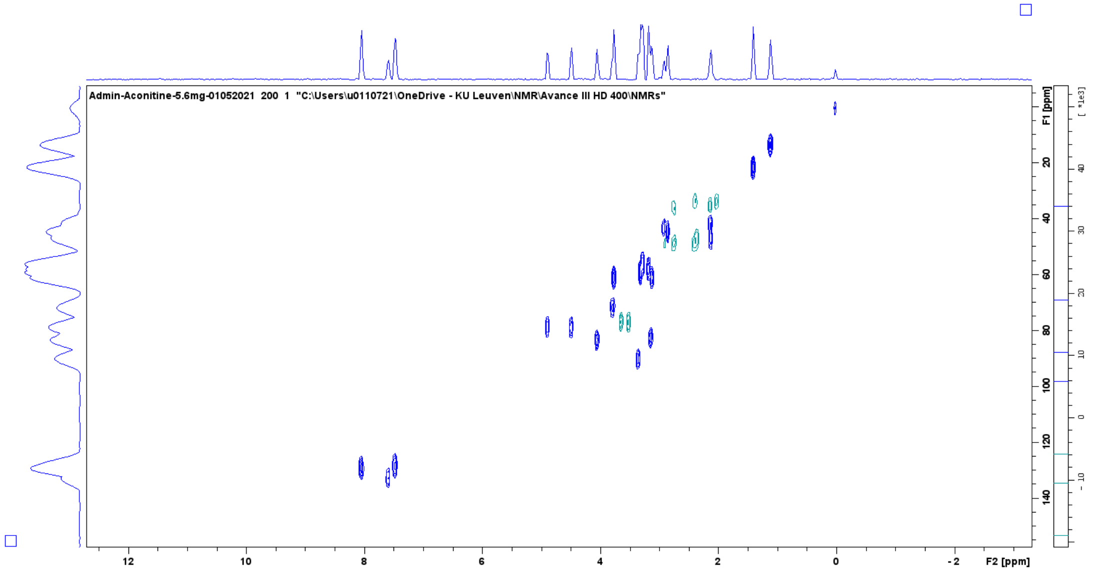
Time-optimization in 2D: NUS

- NUS = **N**on-**U**niform **S**ampling acquisition: collect random fraction of TD(F1) slices in indirect dimension, reconstruct the missing slices and FT in both dimensions



Reconstructed raw data with 32 slices (8 recorded, 24 calculated).

2. FFT



Obtained 2D spectrum using 32 slices.

In this case, TD(F1) = 32, NUS = 25%.

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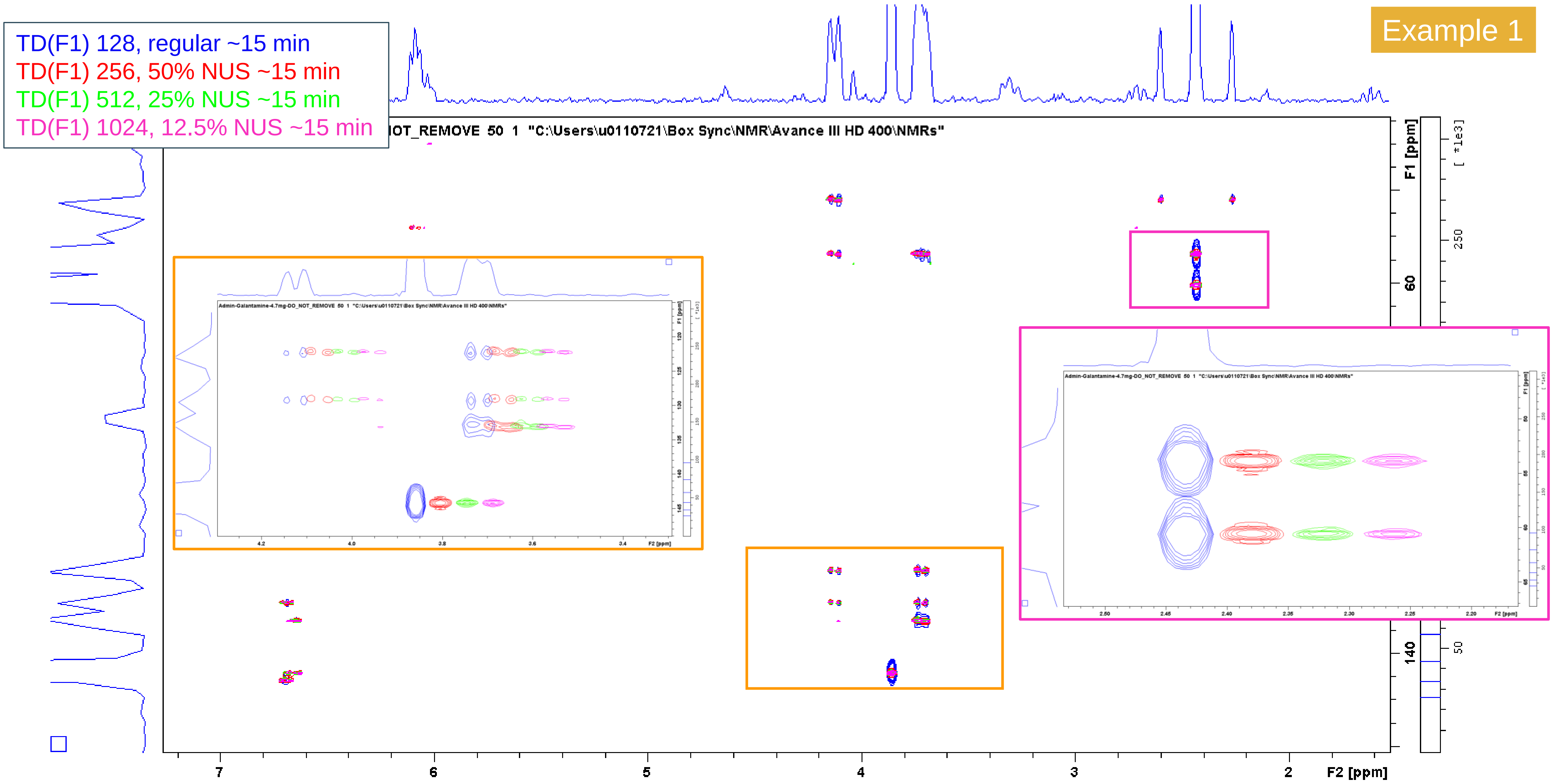
Time-optimization in 2D: NUS

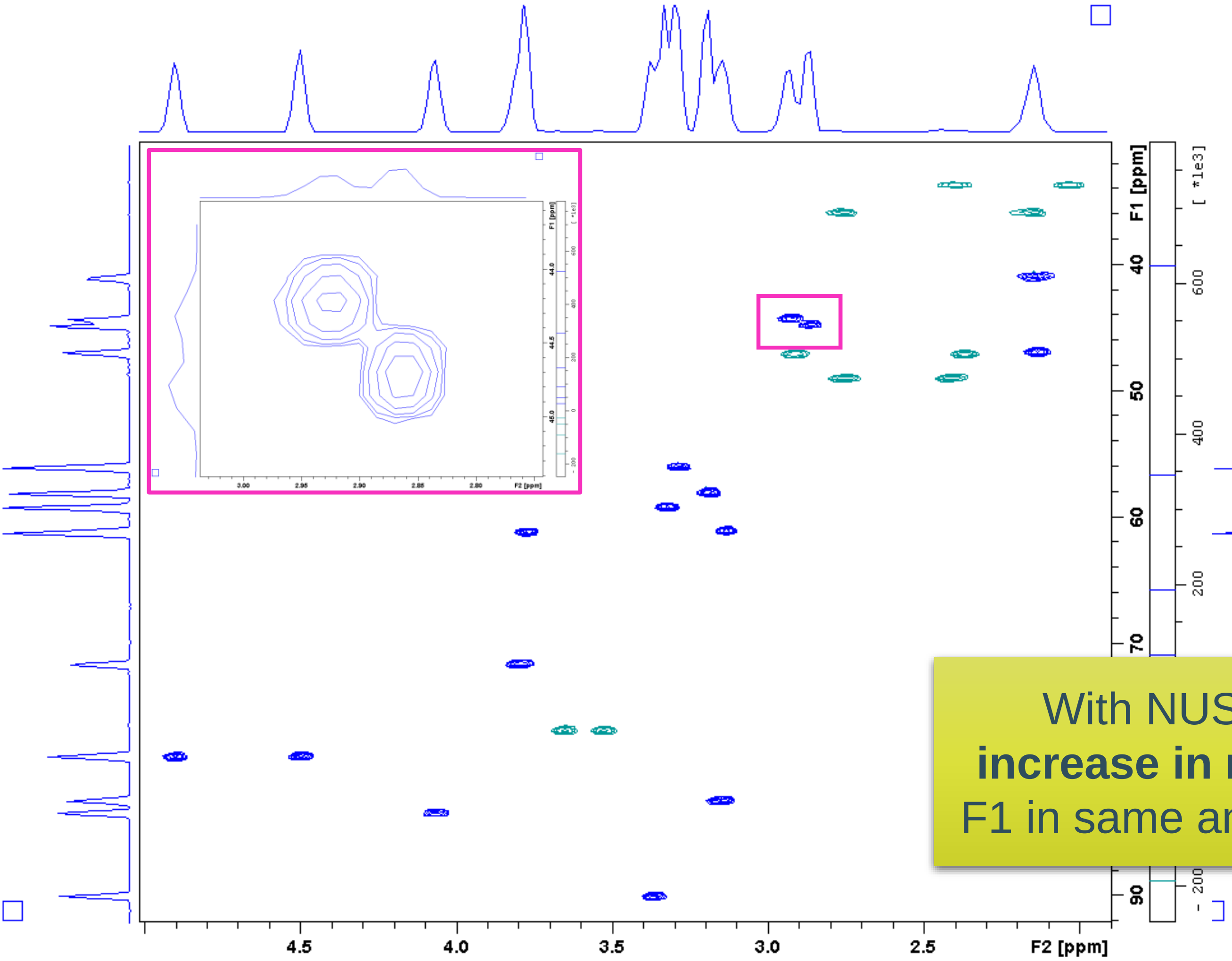
- Advantages of NUS
  - Higher resolution in F1 in same amount of time, *or*
  - Same resolution in F1 in much less time

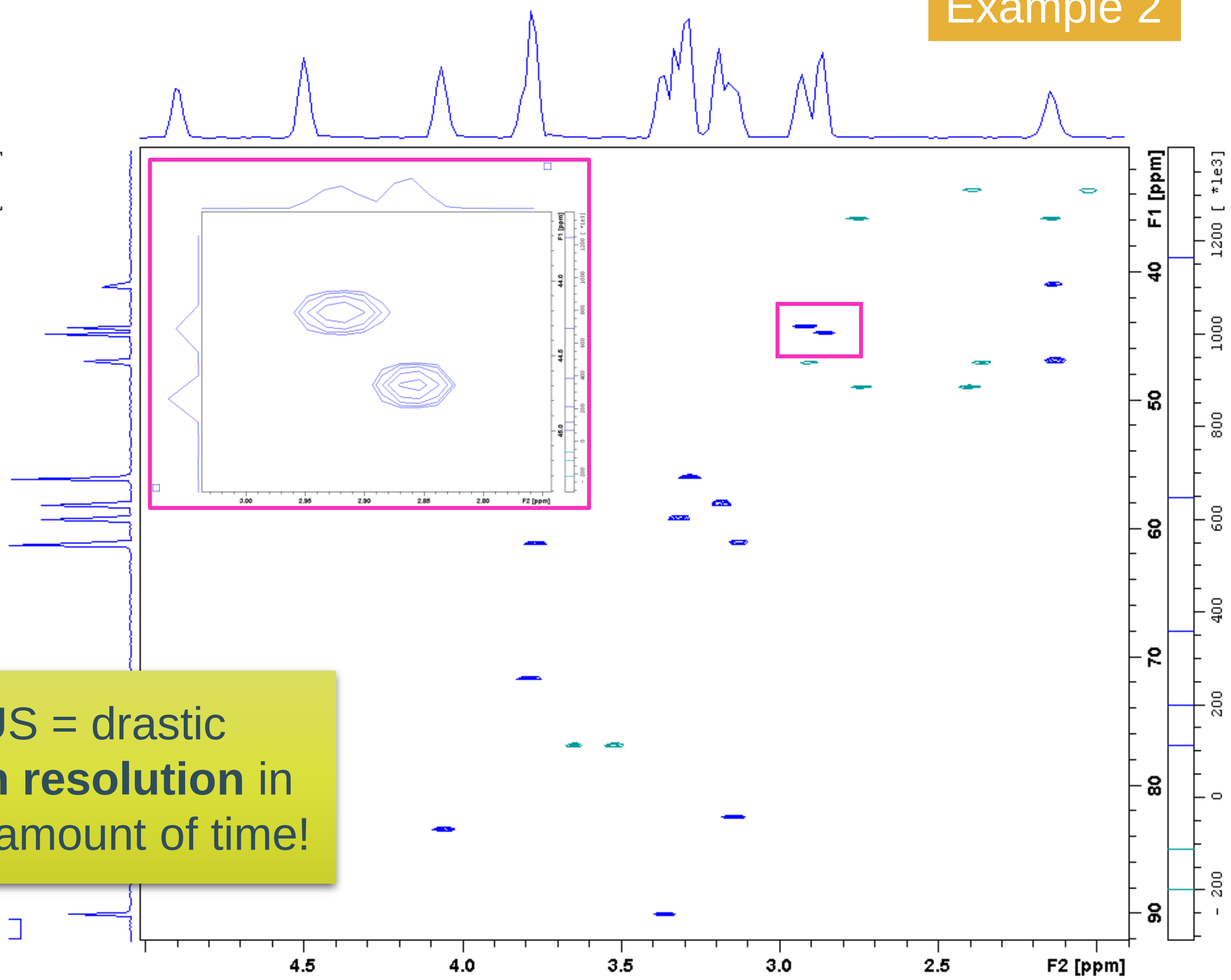
- Example
  - TD(F1) = 1024 with 25 % NUS: only 256 slices (25 %) will really be recorded and 768 additional slices will be calculated during reconstruction. The reconstructed raw data contain 1024 data points.



HMBC (hmbcgplpndqf) of 4.7 mg galanthamine in $CDCl_3$ (33 mM)

Example 2





With NUS = drastic **increase in resolution** in F1 in same amount of time!

Regular HSQC
TD(F1) 512 ~ 56 min

HSQC with NUS
TD(F1) 4k, 12.5% NUS ~ 57 min

HSQC (hsqcedetgpsisp2.2) of 5.6 mg aconitine in $CDCl_3$ (17 mM).

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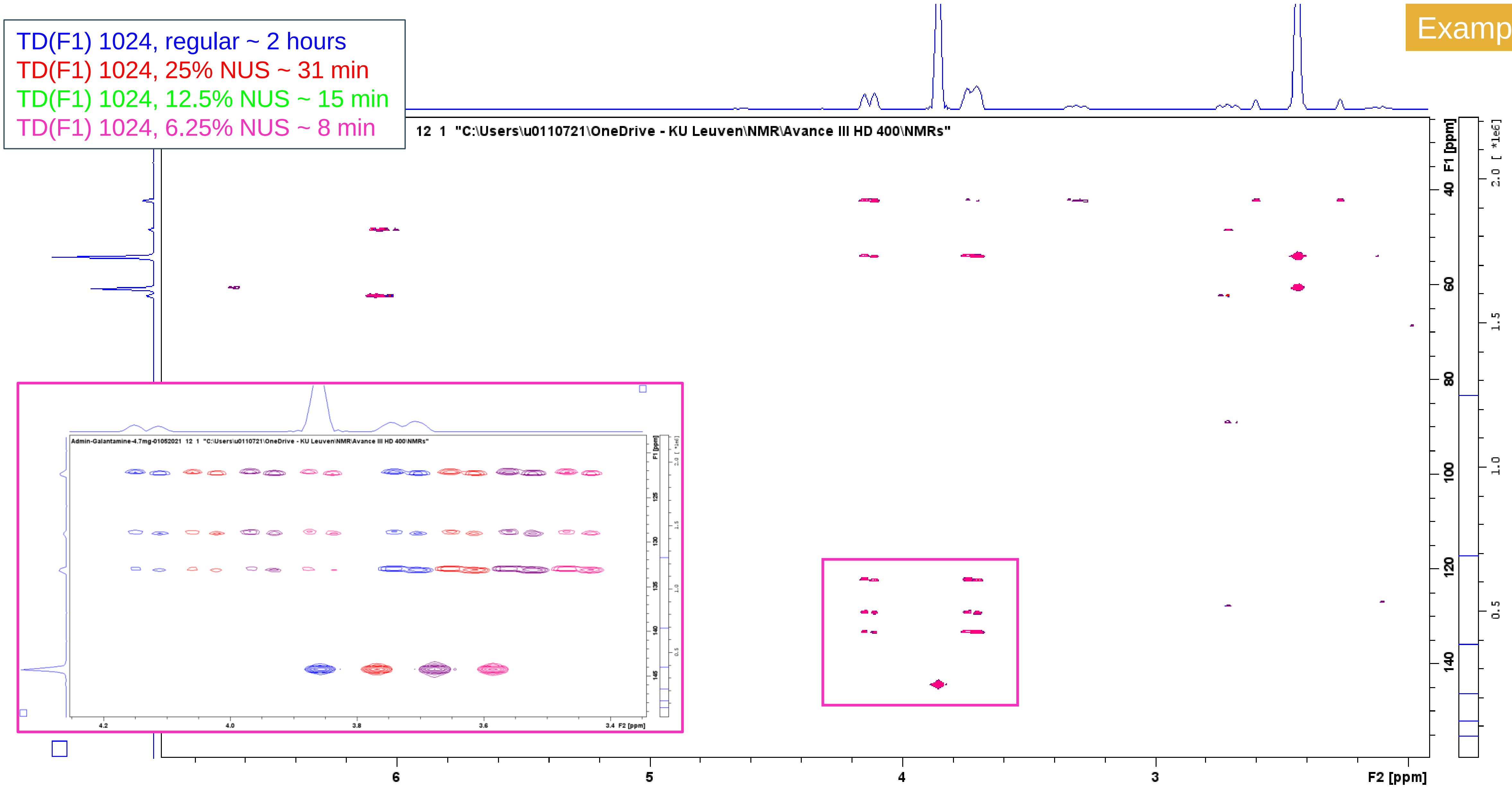
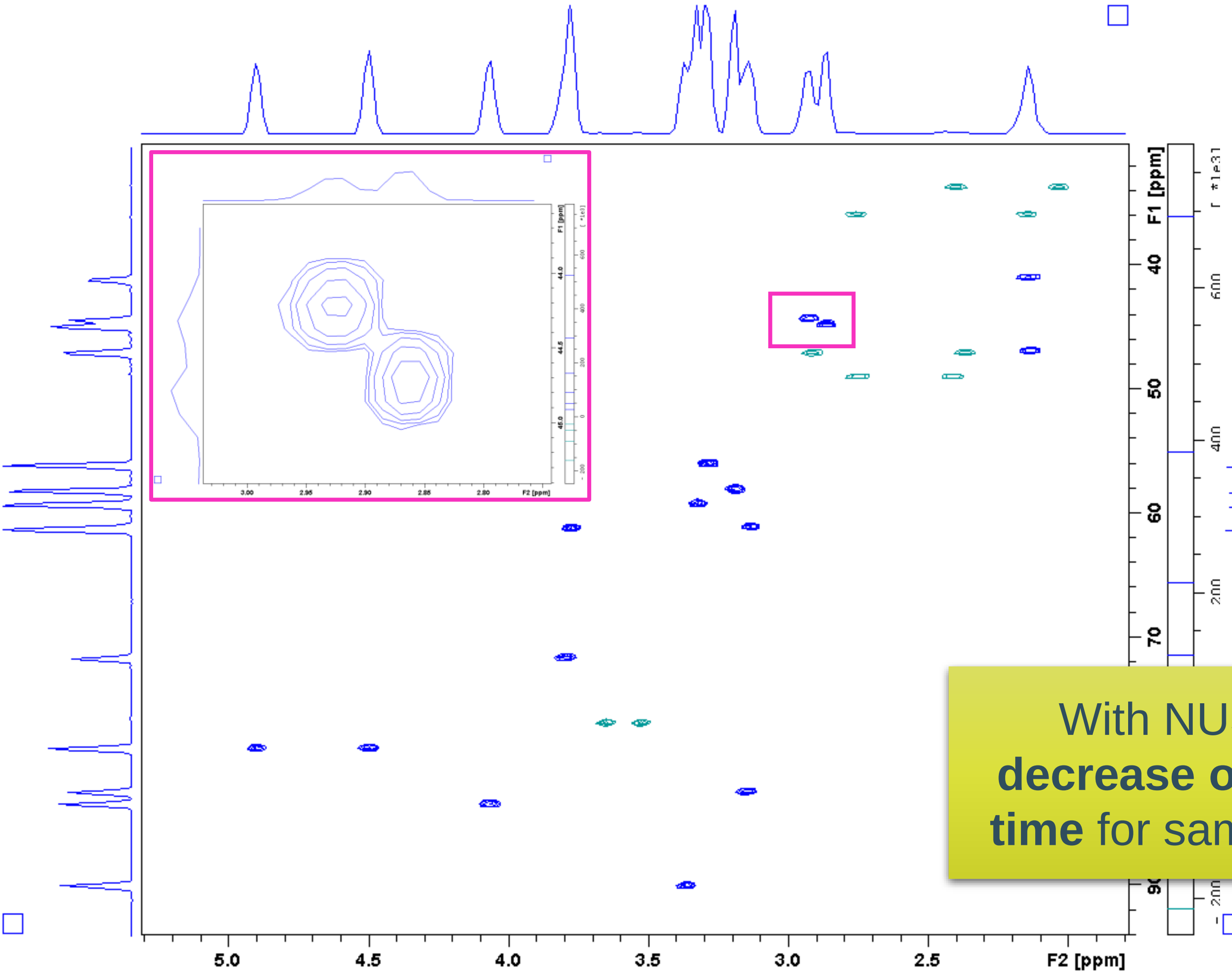
Example 3

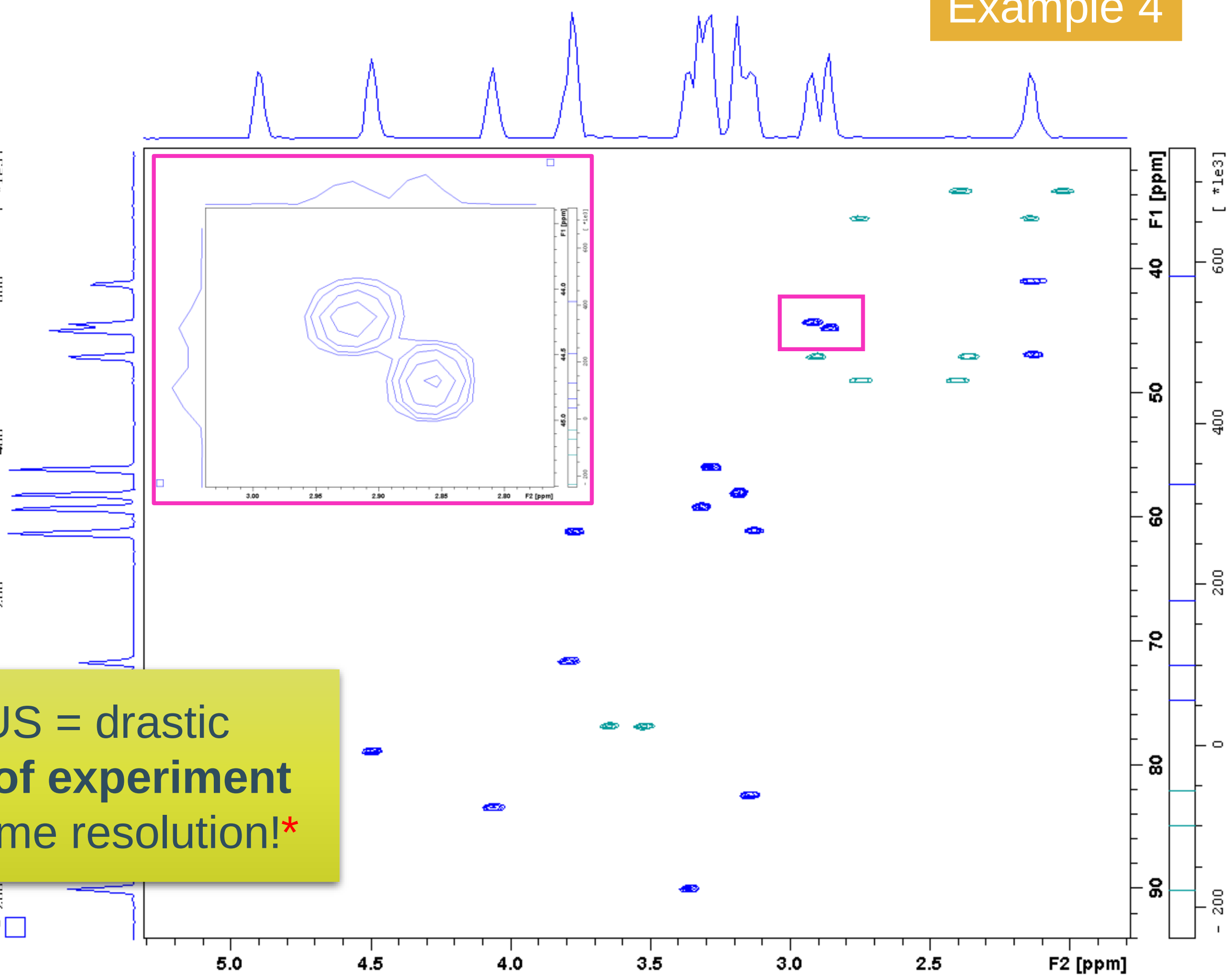
TD(F1) 1024, regular ~ 2 hours
TD(F1) 1024, 25% NUS ~ 31 min
TD(F1) 1024, 12.5% NUS ~ 15 min
TD(F1) 1024, 6.25% NUS ~ 8 min



HMBC (hmbcgplpndqf) of 4.7 mg galanthamine in $CDCl_3$ (33 mM)

Example 4





With NUS = drastic **decrease of experiment time** for same resolution!*

Regular HSQC
TD(F1) 512 ~ 56 min

HSQC with NUS
TD(F1) 512, 25% NUS ~ 15 min

HSQC (hsqcedetgpsisp2.2) of 5.6 mg aconitine in $CDCl_3$ (17 mM).
* Sometimes some form of resolution enhancement may be observed as well!

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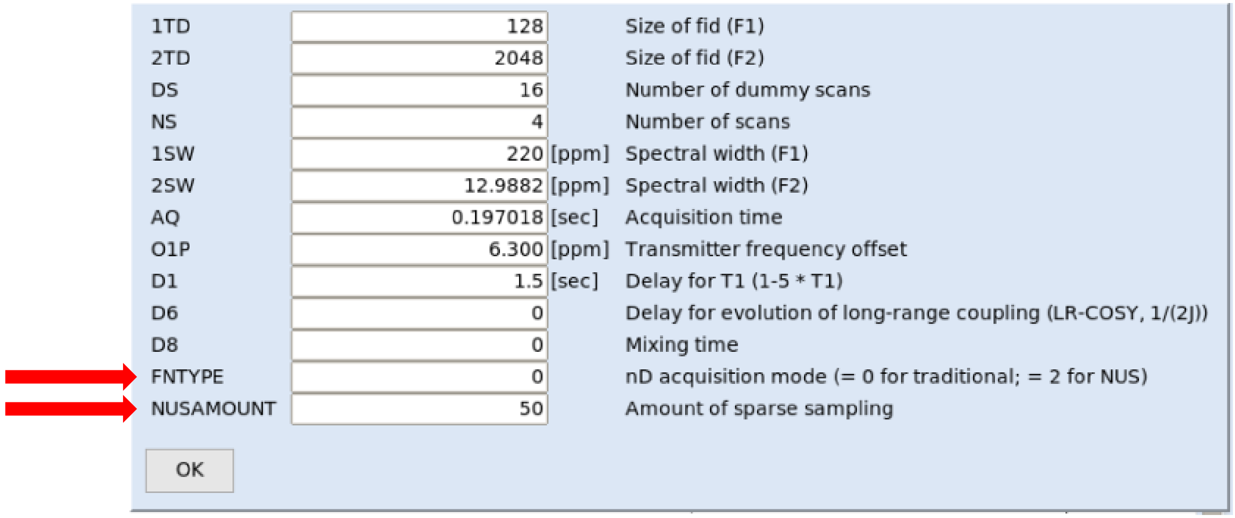
Time-optimization in 2D: NUS

- Acquisition in Icon-NMR
  - Select appropriate 2D experiment
  - Set FNTYPE = 2 (standard 0 = traditional acquisition, 2 = NUS acquisition)
  - Set NUSAMOUNT = <desired percentage of NUS sampling>
    - Percentage (0-100)
    - Amount of NUS sampling
    - Fraction of TD(F1) that will really be recorded



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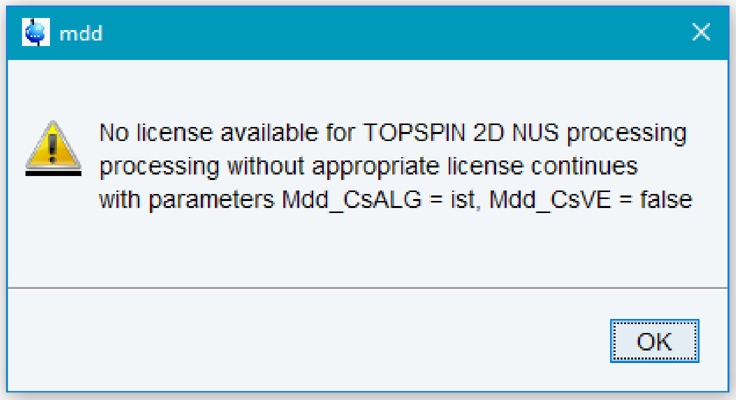
Time-optimization in 2D: NUS

- Processing
  - Regular processing can be used
  - No license needed for basic 2D NUS processing (≥ TopSpin 3.5pl7)
  - TopSpin will display warning, saying you have no license for NUS processing, so the standard settings will be used (Compressed Sensing (CS) algorithm *via* Iterative Soft Thresholding (IST)). Just click OK.



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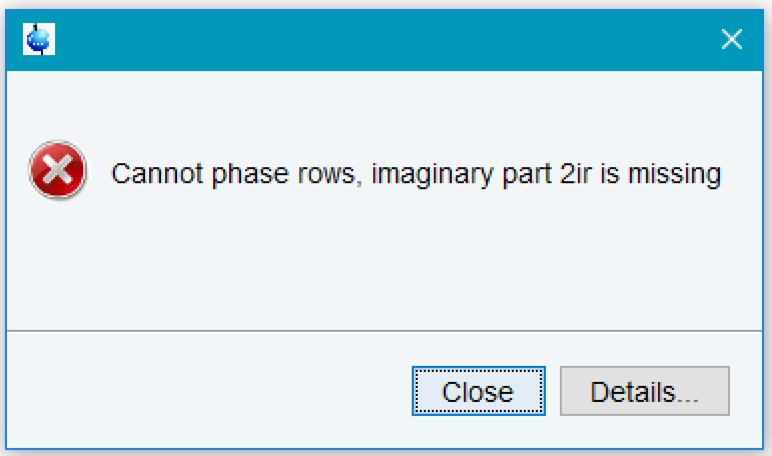
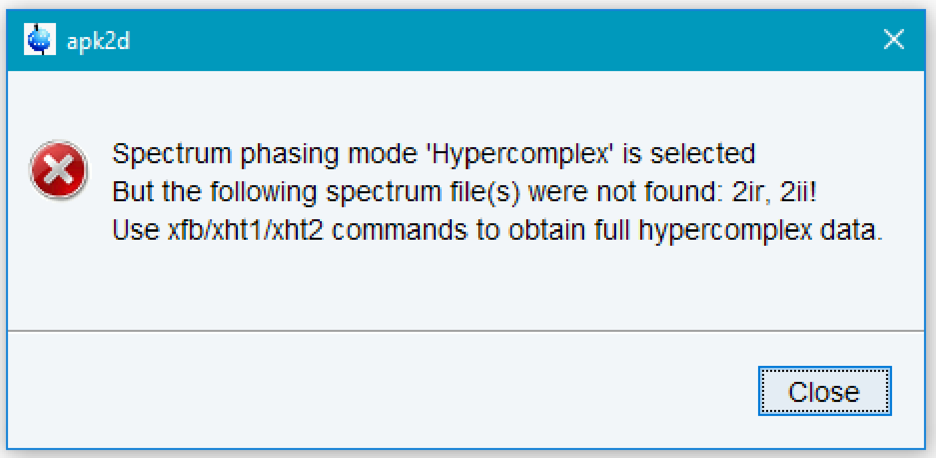
Time-optimization in 2D: NUS

- Processing
  - If spectrum is phase-sensitive, no phasing can be performed immediately after NUS processing, as imaginary parts of 2D processed data (2ir, 2ii) are not saved after NUS reconstruction.
  - To solve this problem: use Hilbert transform in F2 (**xht2**) to recalculate imaginary data
  - Now phasing is possible
  - Hilbert transform is necessary before phasing after Fourier transform





Time-optimization in 2D: NUS

- When can I use NUS?
  - Sufficient SINO
  - Small to medium dynamic range of peaks
    - Large dynamic range can result in artifacts when undersampling
    - Don’t forget to take impurities into consideration!

  $\Rightarrow$ HSQC of pure compound: definitely!

  $\Rightarrow$ NOESY of mixture: more challenging
- How many FIDs should I record?
  - NUSPOINTS = $\dfrac{\frac{NUSAMOUNT}{100} * TD(F1)}{2}$
  - NUSPOINTS should be ≥ number of peaks in the spectrum (again, don’t forget to take peaks from impurities into consideration!)

Thank you