Faster, Higher Resolution Spectra

Bruker SMASH Workshop, La Jolla, CA
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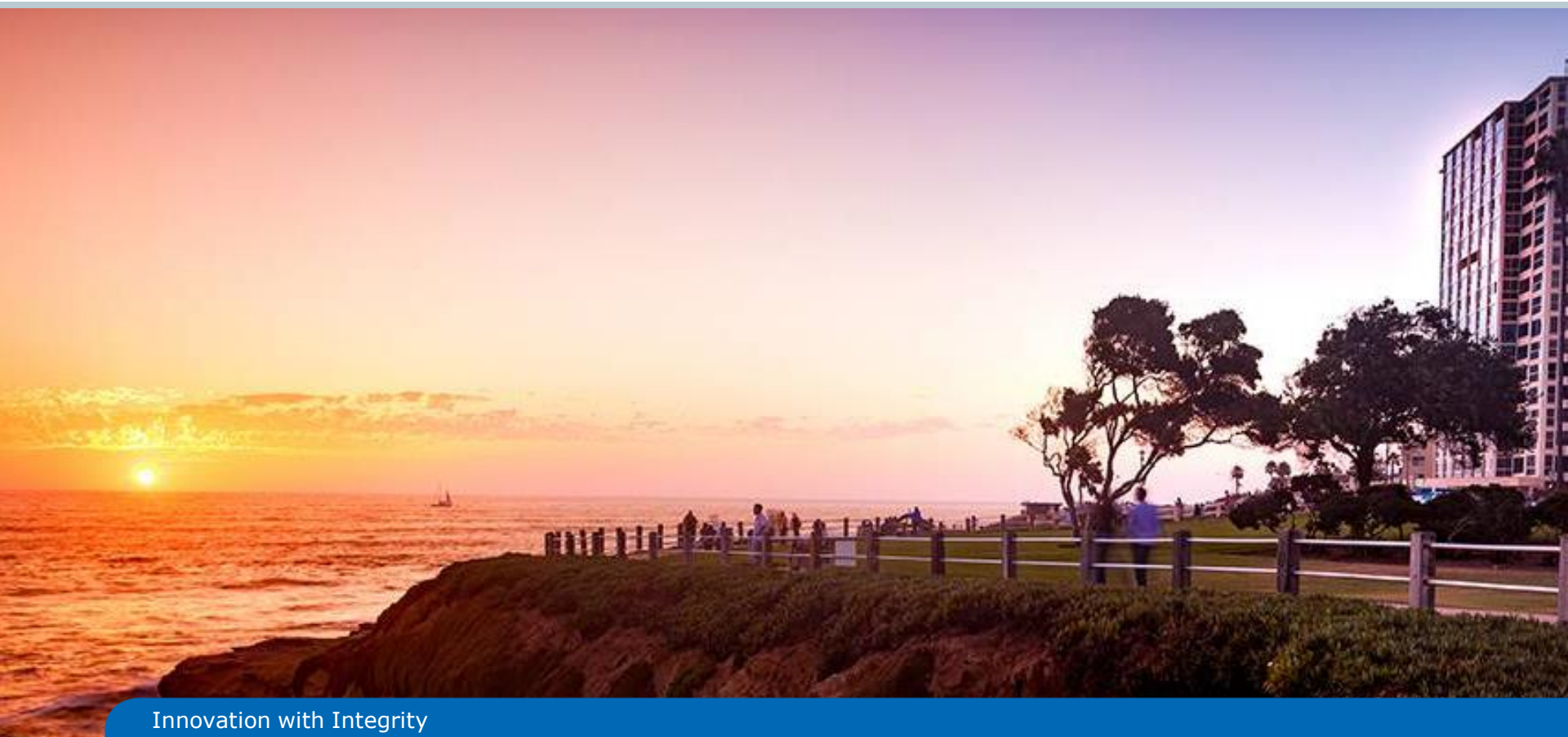
Eric Johnson – Bruker Biospin, Fremont, California



Introduction

- 2D NMR experiments are powerful techniques
- Often a compromise between how much information we want and how much spectrometer time we can afford
- Various techniques can help us get our information faster and/or with higher resolution:
  - NUS – non uniform sampling
  - Selective experiments – focus on a region of our spectrum.

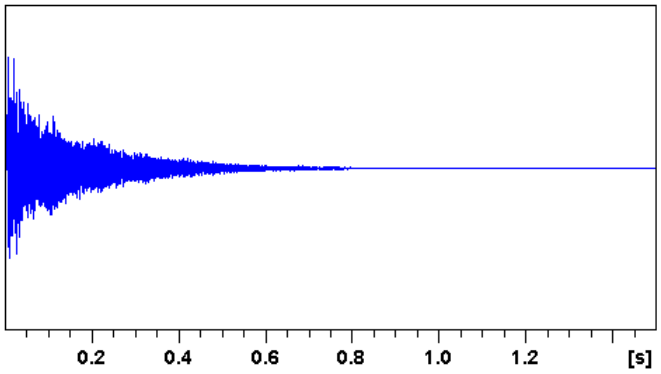
Non Uniform Sampling

… but first a quick review of a few basic concepts …

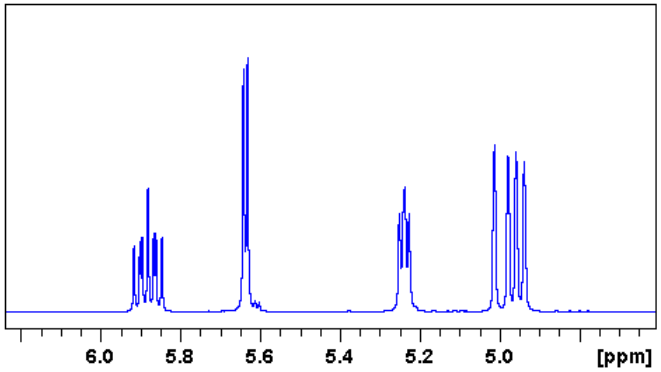
Resolution in a 1D spectrum

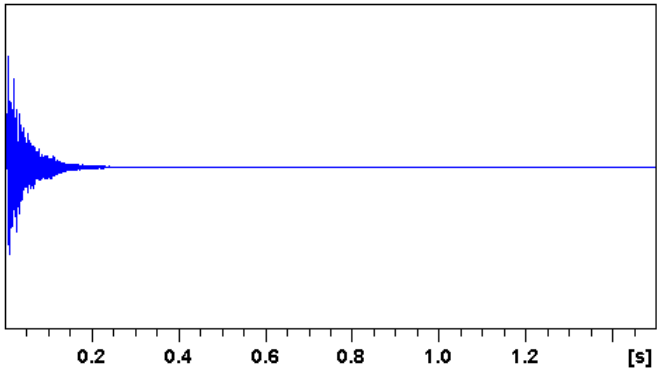
Resolution is primarily defined by

- shimming
- inherent relaxation properties of sample

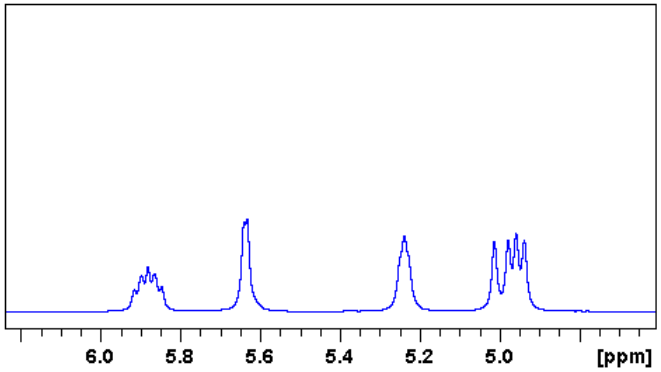


FID decays slowly → sharp peaks





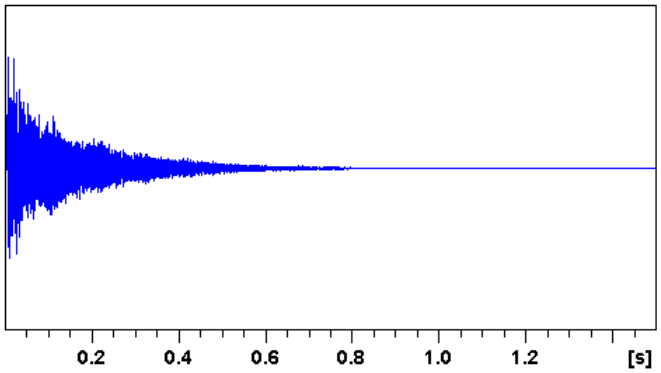
faster relaxation → broader peaks



Acquisition time for a 1D spectrum

It doesn’t take us any extra time to acquire full resolution for a 1D

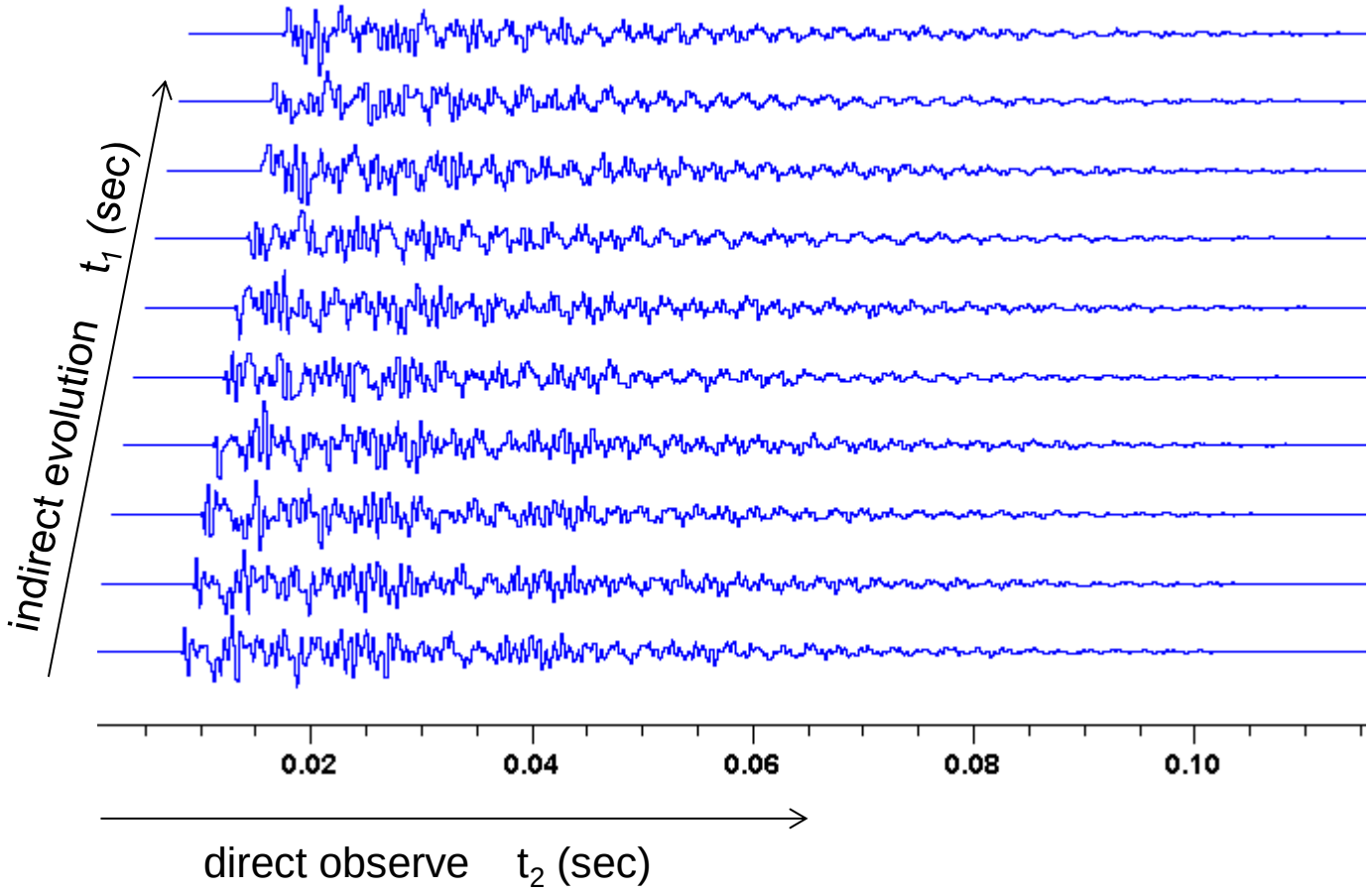
- We’re already waiting a few seconds between scans
- No reason not to acquire FID until it decays to noise



Acquisition time is dominated by the number of scans necessary for the desired signal to noise

Basics of 2D spectra

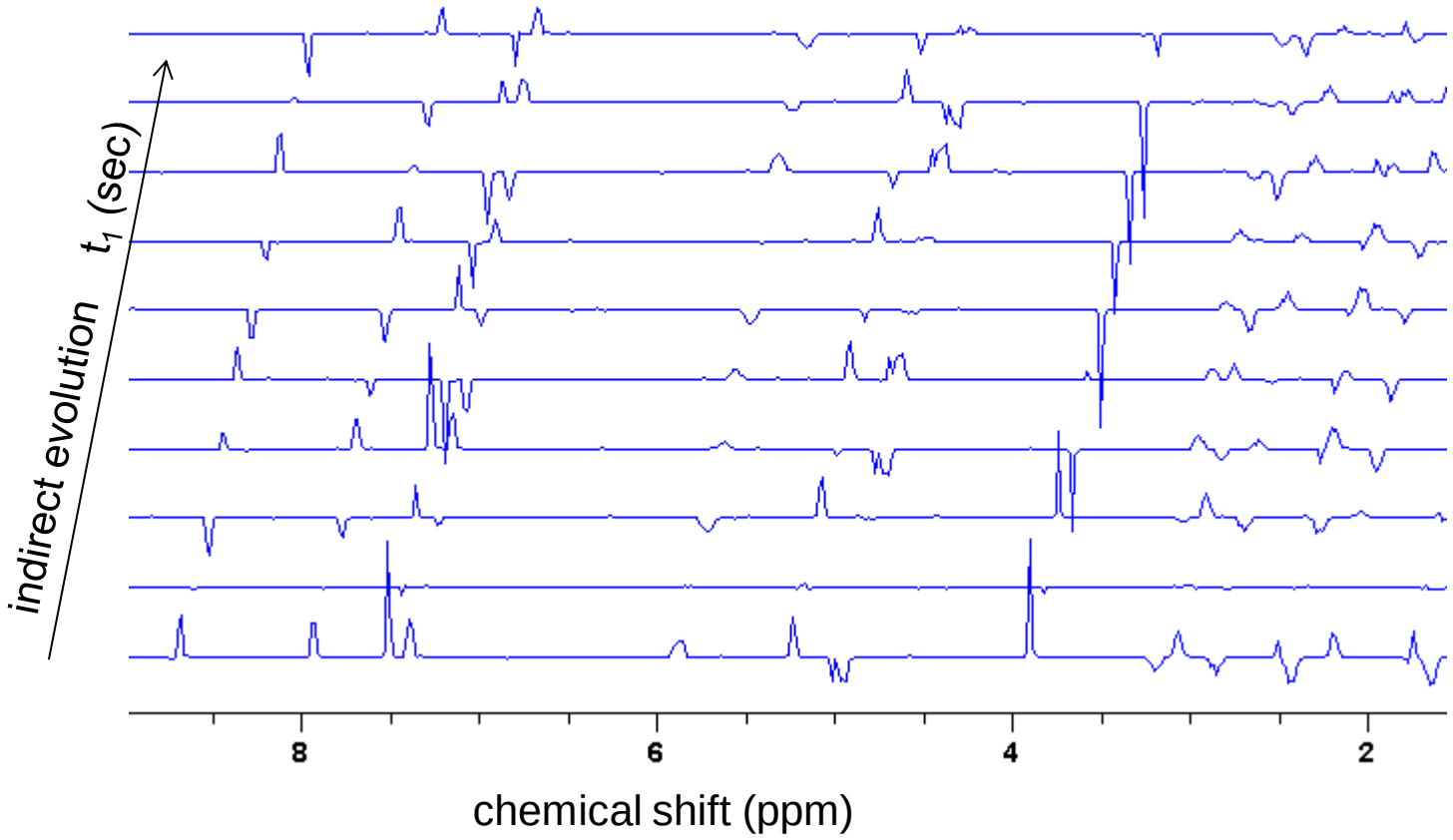
Series of 1D spectra



Basics of 2D spectra

Fourier transform observe dimension

- intensity of peaks oscillates in indirect dimension – indirect “FID”



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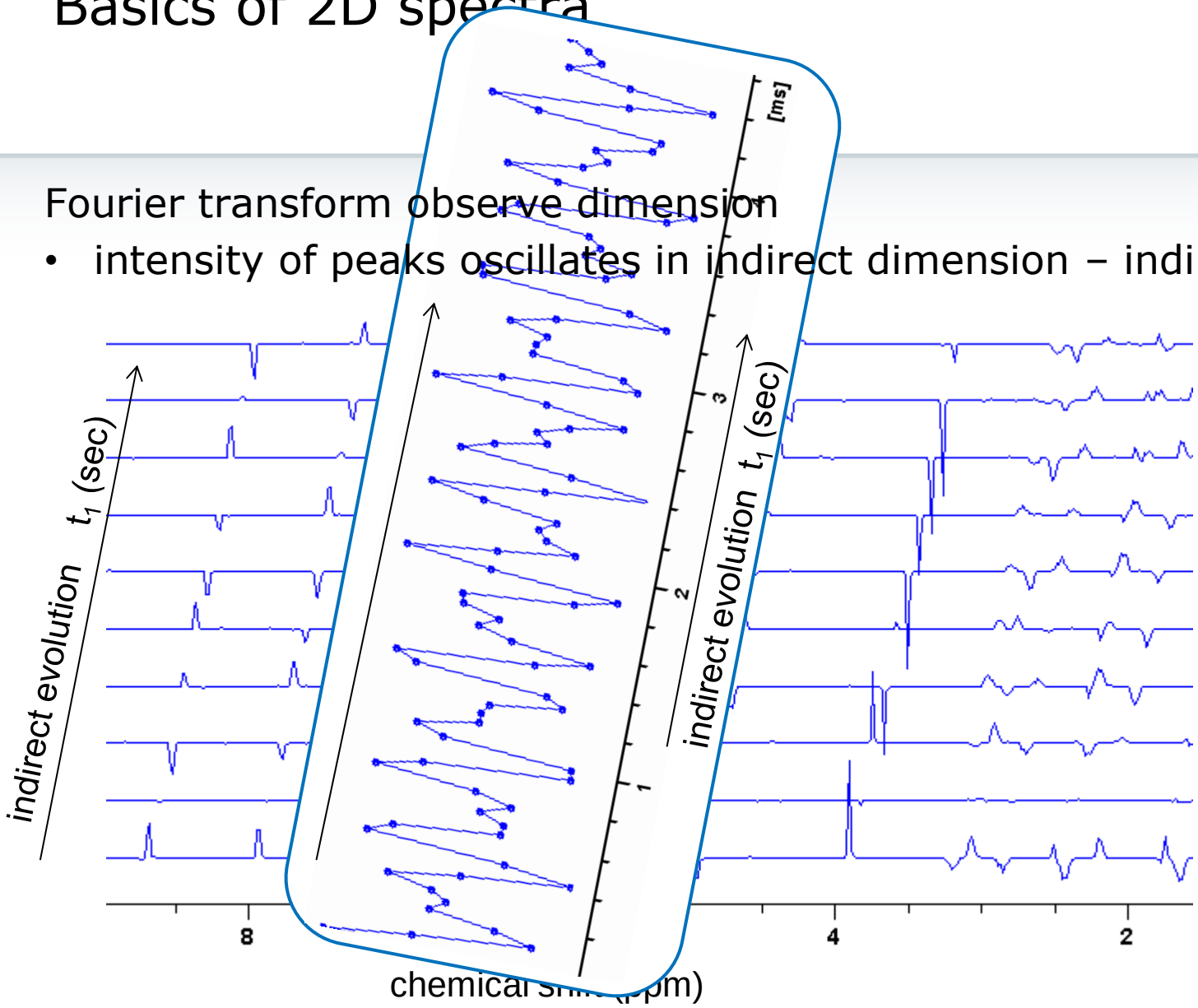
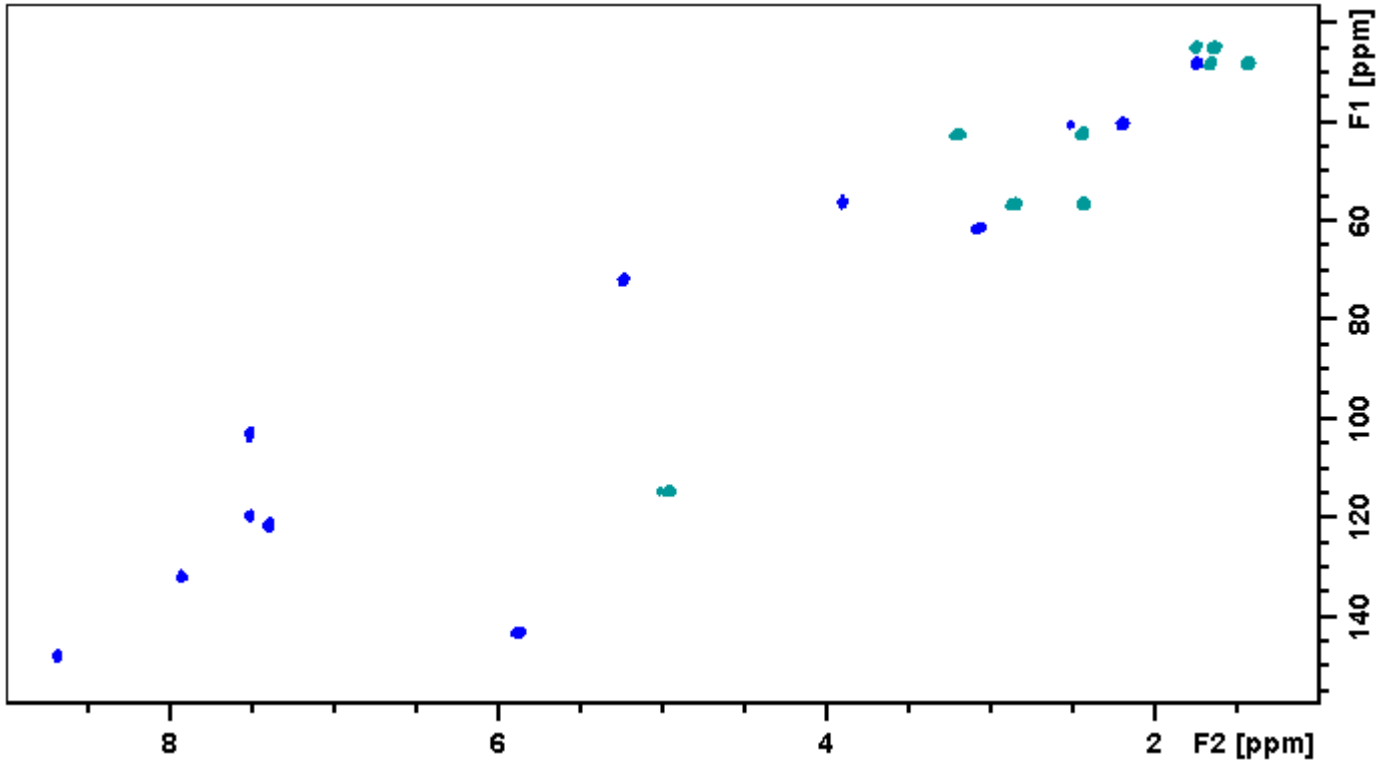


Basics of 2D spectra

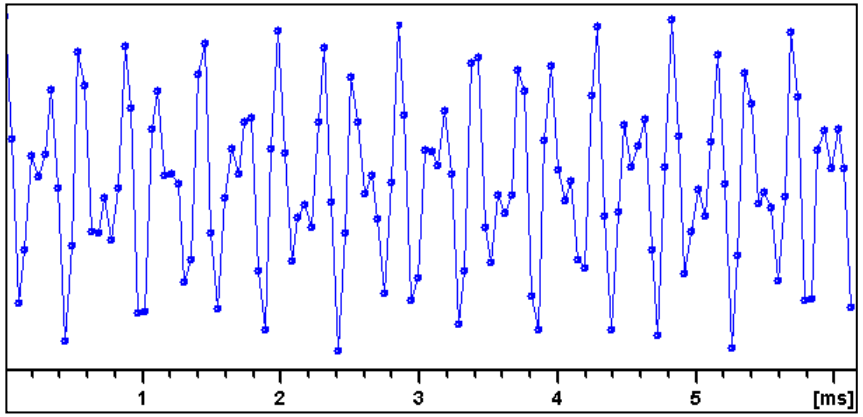
Fourier transform indirect dimension

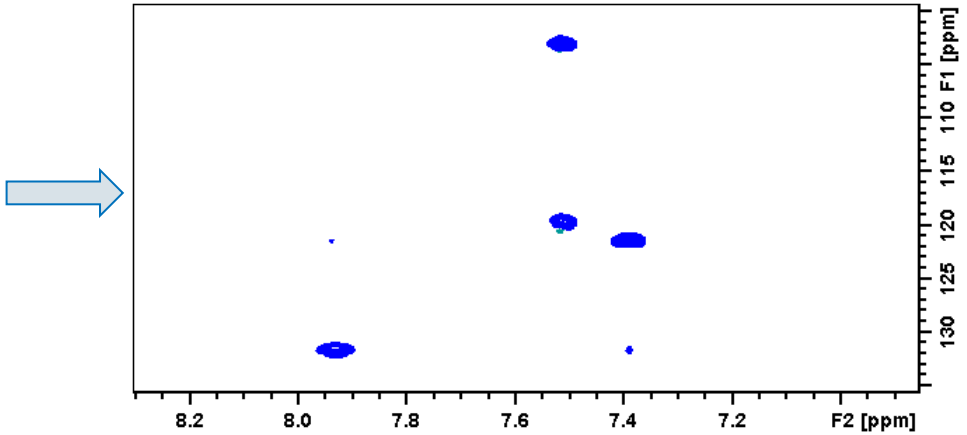
- → 2D spectrum

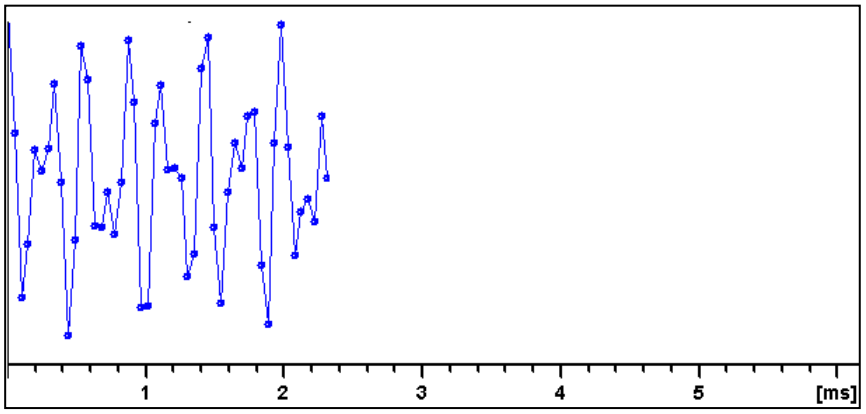


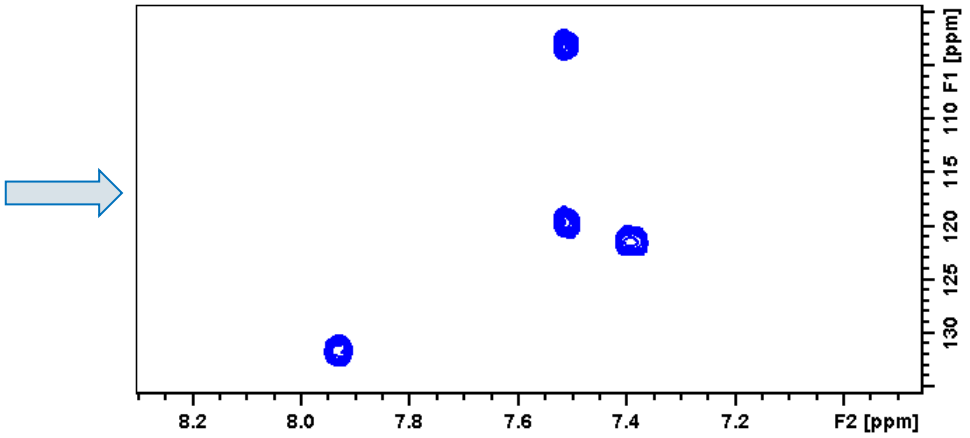
Resolution of 2D spectrum

Resolution in indirect dimension depends on duration of indirect “FID”



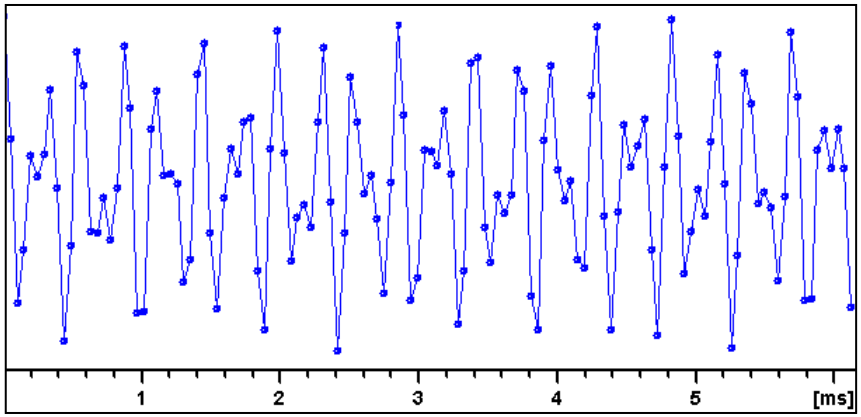






Acquisition time for a 2D spectrum

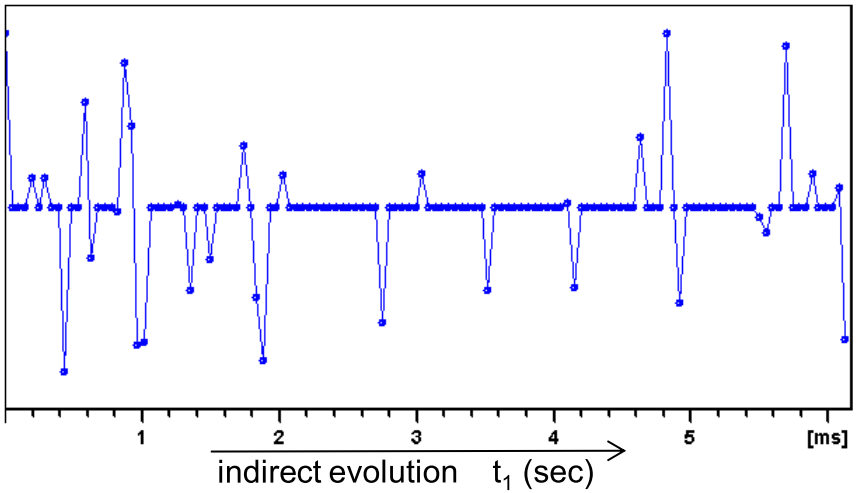
- Each point in the indirect "FID" is a directly observed FID
  → for higher resolution…
    → need to acquire more FID's (larger TD)
      → longer total acquisition time



- Acquisition time is largely determined by the number of FID's required for the desired resolution
- Total number of scans required for desired signal to noise is also important

Do we really need to acquire all these FID's for a 2D (or nD) spectrum?

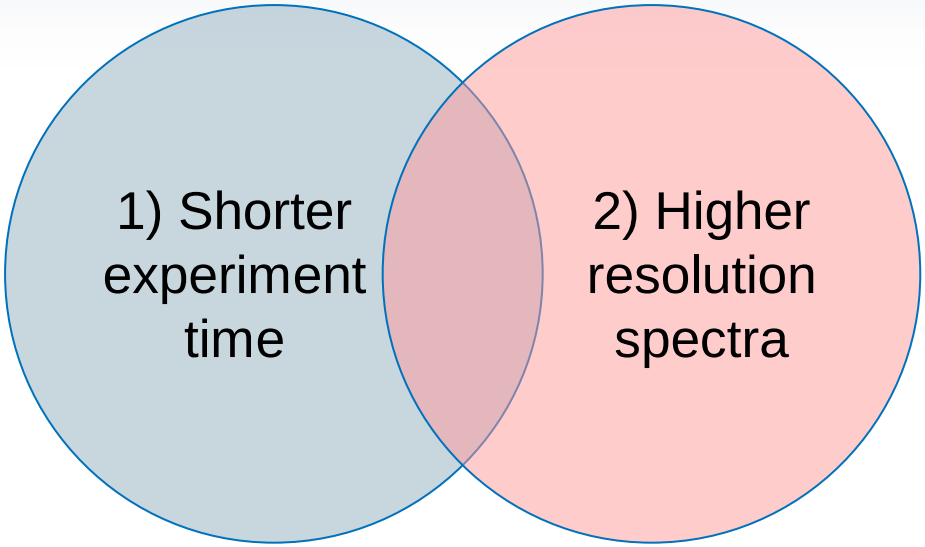
- Not necessarily!!!! NUS – Non Uniform Sampling



- Sparsely sample a fraction of the FID's
- Acquisition and processing* included in Topspin 3.0 and newer…

* I'll mention licenses later…

How can I benefit from using NUS?



1) Acquire a nD spectrum in less time

   or

2) Acquire a spectrum with much higher resolution in the indirect dimension(s)

   or

3) Some combination of the above

What do I need to acquire data with NUS?

Everything you need is already built into Topspin 3.0 and newer!

Some behind the scene details:

- Starting with Topspin 3.0, all standard nD pulse sequence have been modified to be compatible with NUS

- The “mc” macro controls incrementations in the indirect dimension:

  - old mc (Topspin 2.1 and earlier):

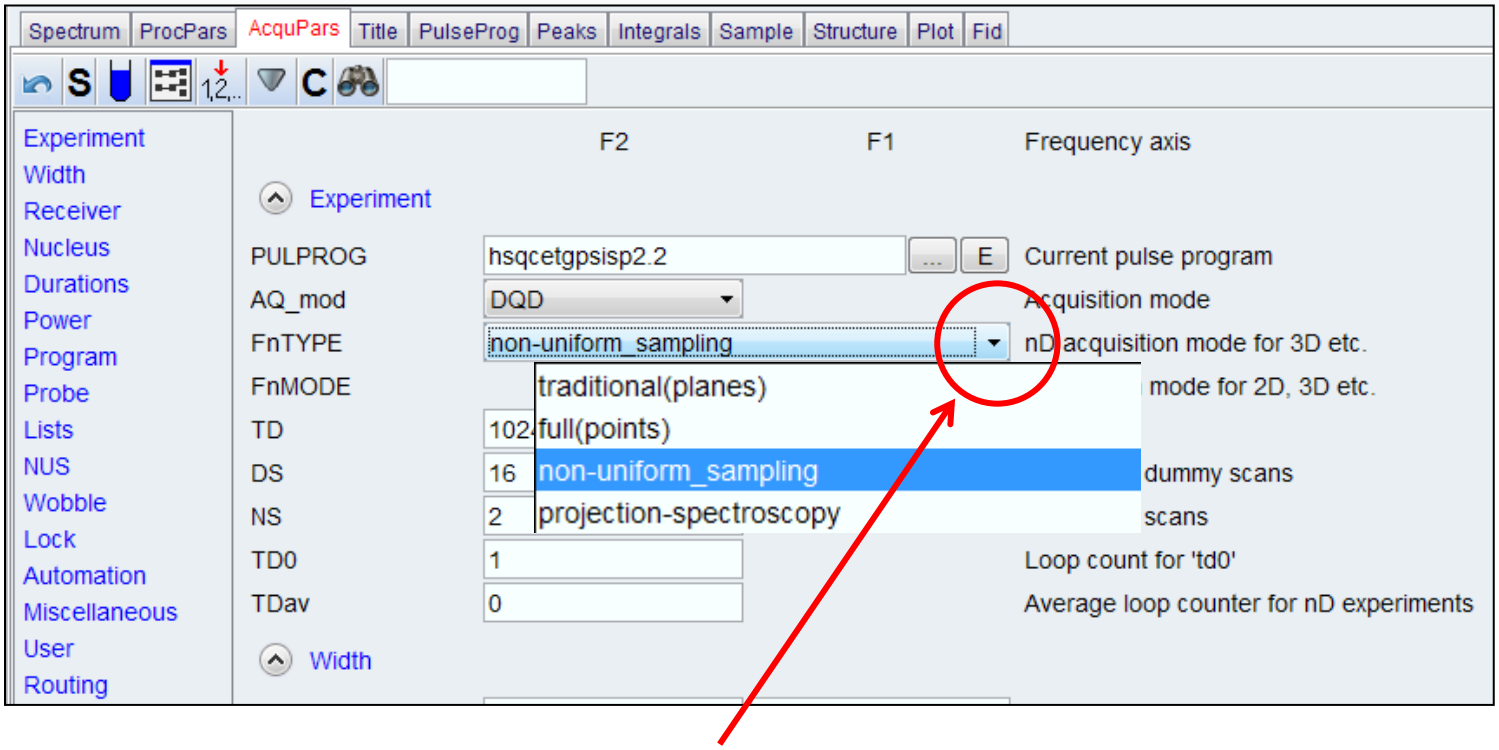
```
d1 do:f2 mc #0 to 2
    F1EA(igrad EA, id0 & ip3*2 & ip6*2 & ip31*2)
```

  - new mc (Topspin 3.0 and newer):

```
d1 do:f2 mc #0 to 2
    F1EA(calgrad(EA), caldel(d0, +in0) & calph(ph3, +180) & calph(ph6,
        +180) & calph(ph31, +180))
```

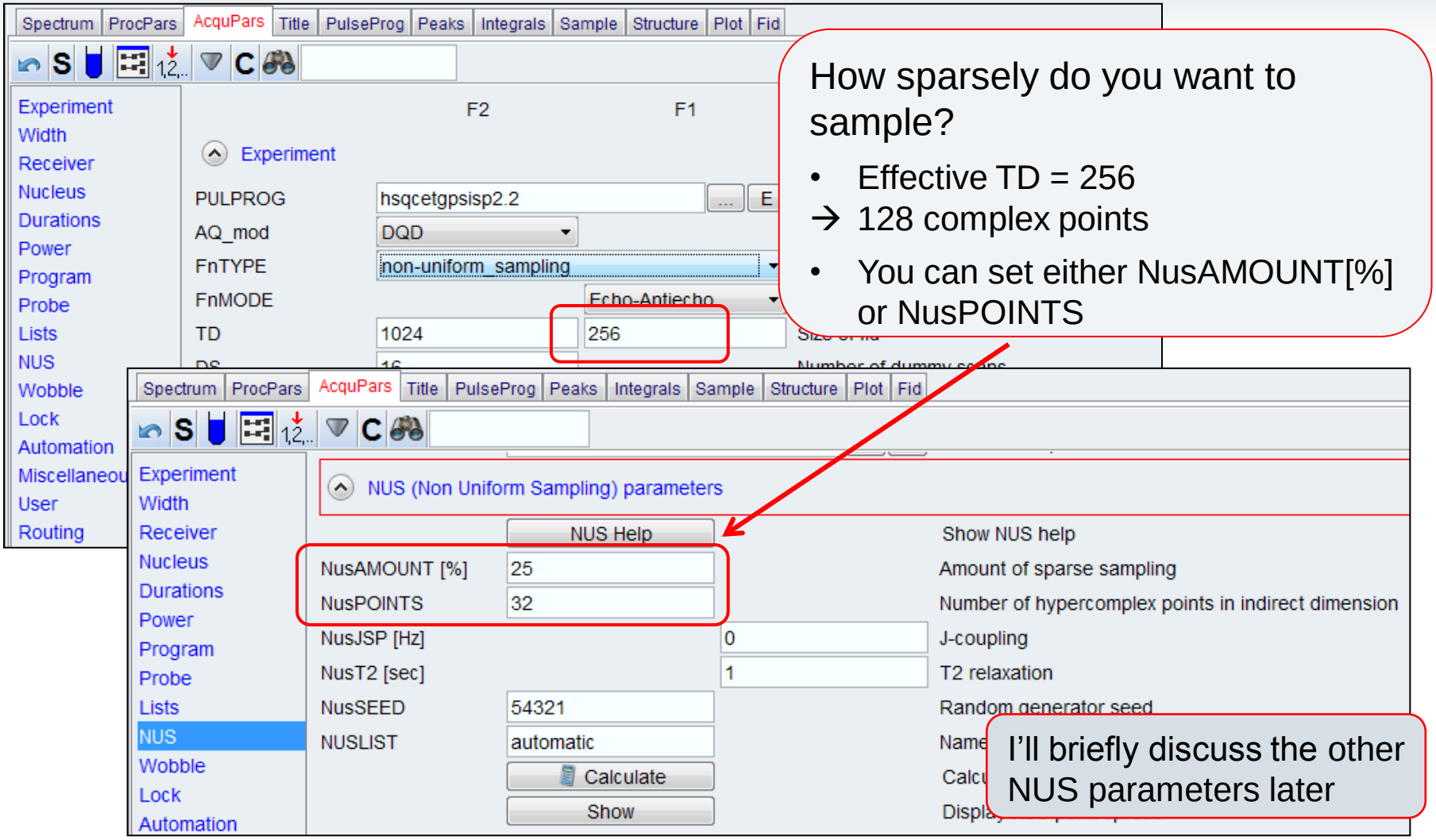
- The old mc macro still works – you just can’t use it for NUS

Turning on NUS

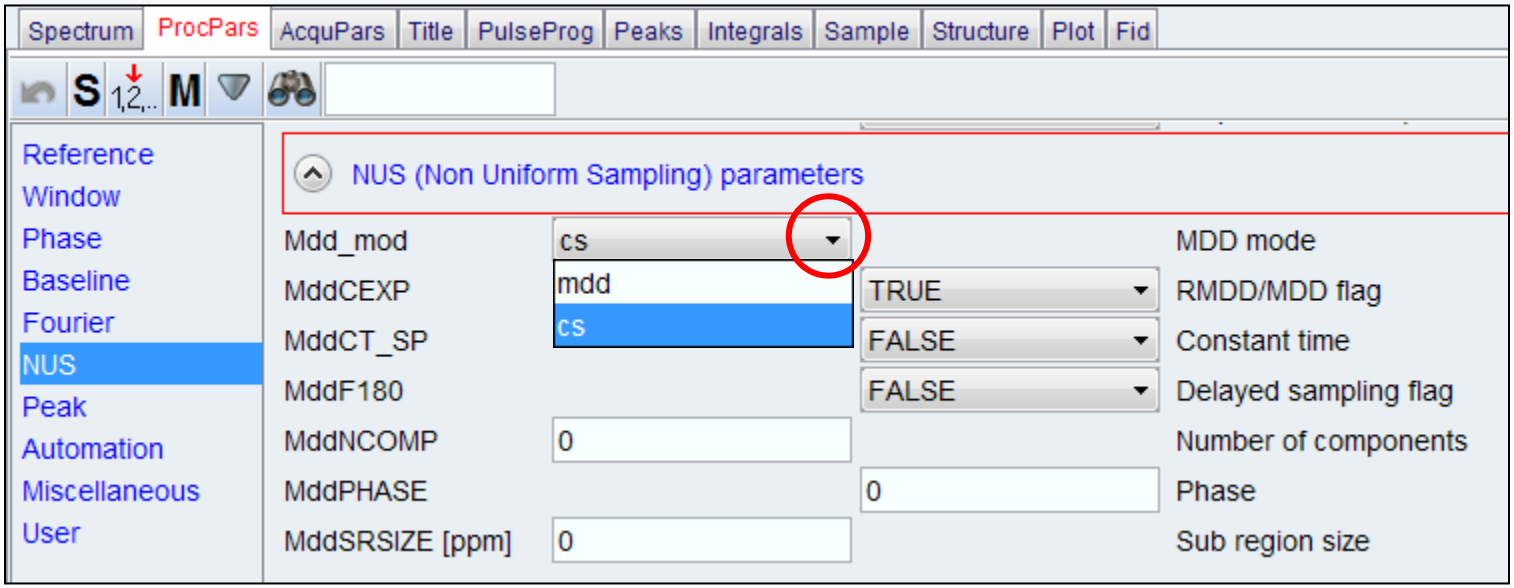


- change FnTYPE from “traditional(planes)” to “non-uniform_sampling”

NUS acquisition parameters



NUS processing parameters

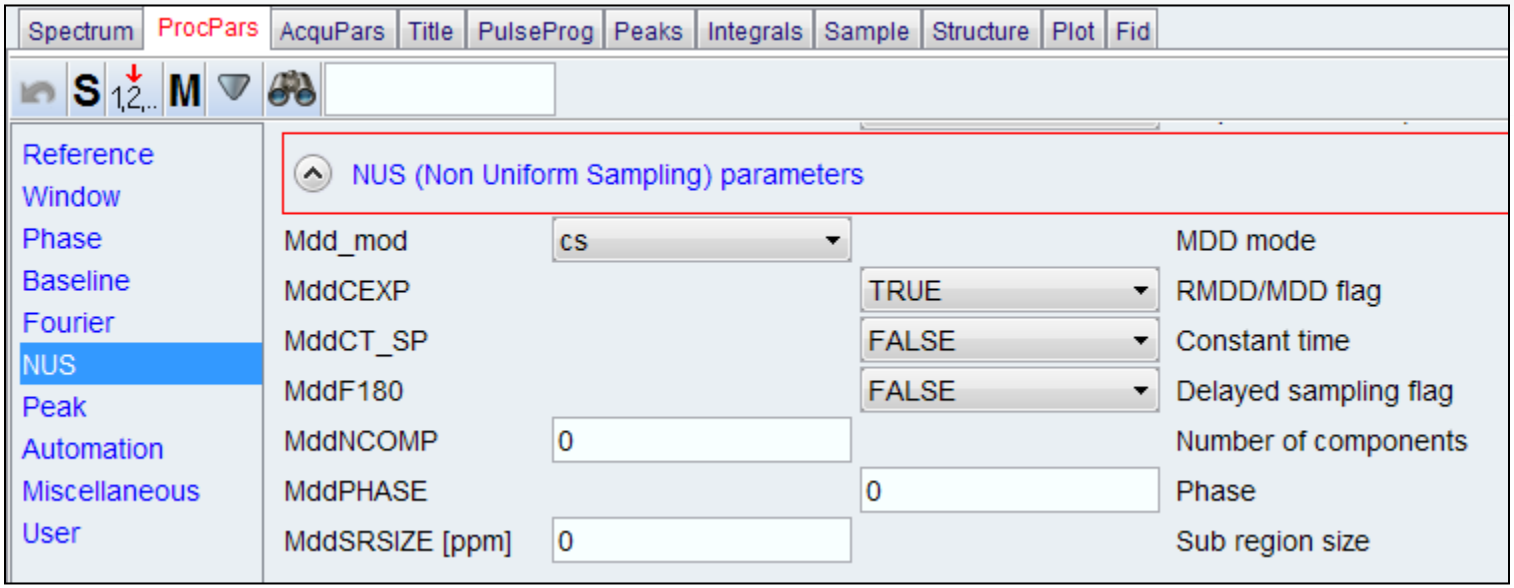


Two processing algorithms are built into Topspin:

1) MDD – Multi Dimensional Deconvolution
2) CS – Compressed Sensing

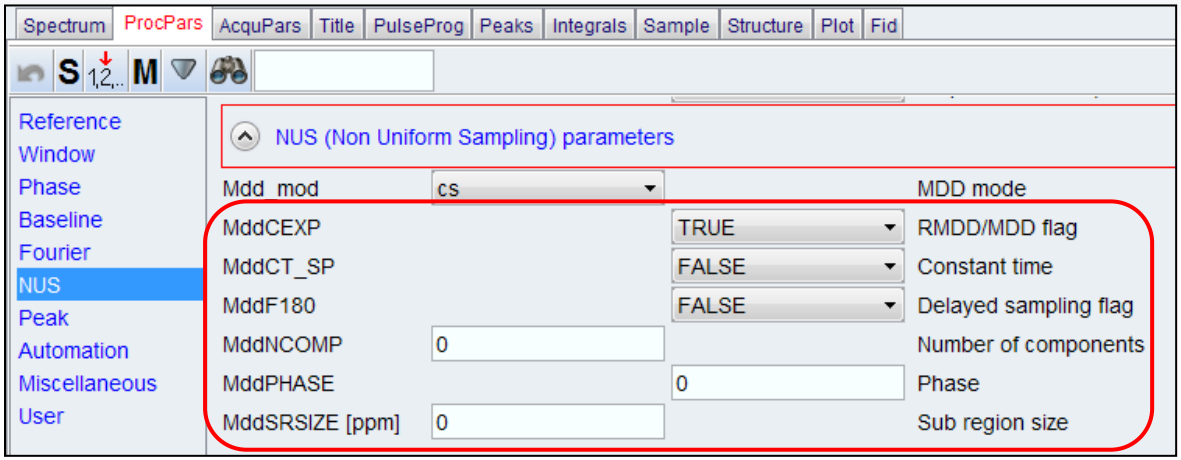
- Several other methods exist (not discussed here…)

NUS processing – Topspin licenses



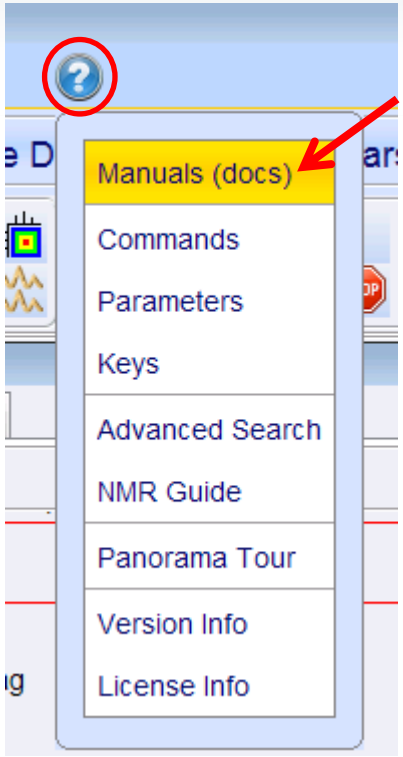
- Originally (TS3.0 until recently), a separate NUS processing license was required
- Starting with Topspin 3.5pl3, basic 2D processing with CS is included with the standard Topspin processing license!!!

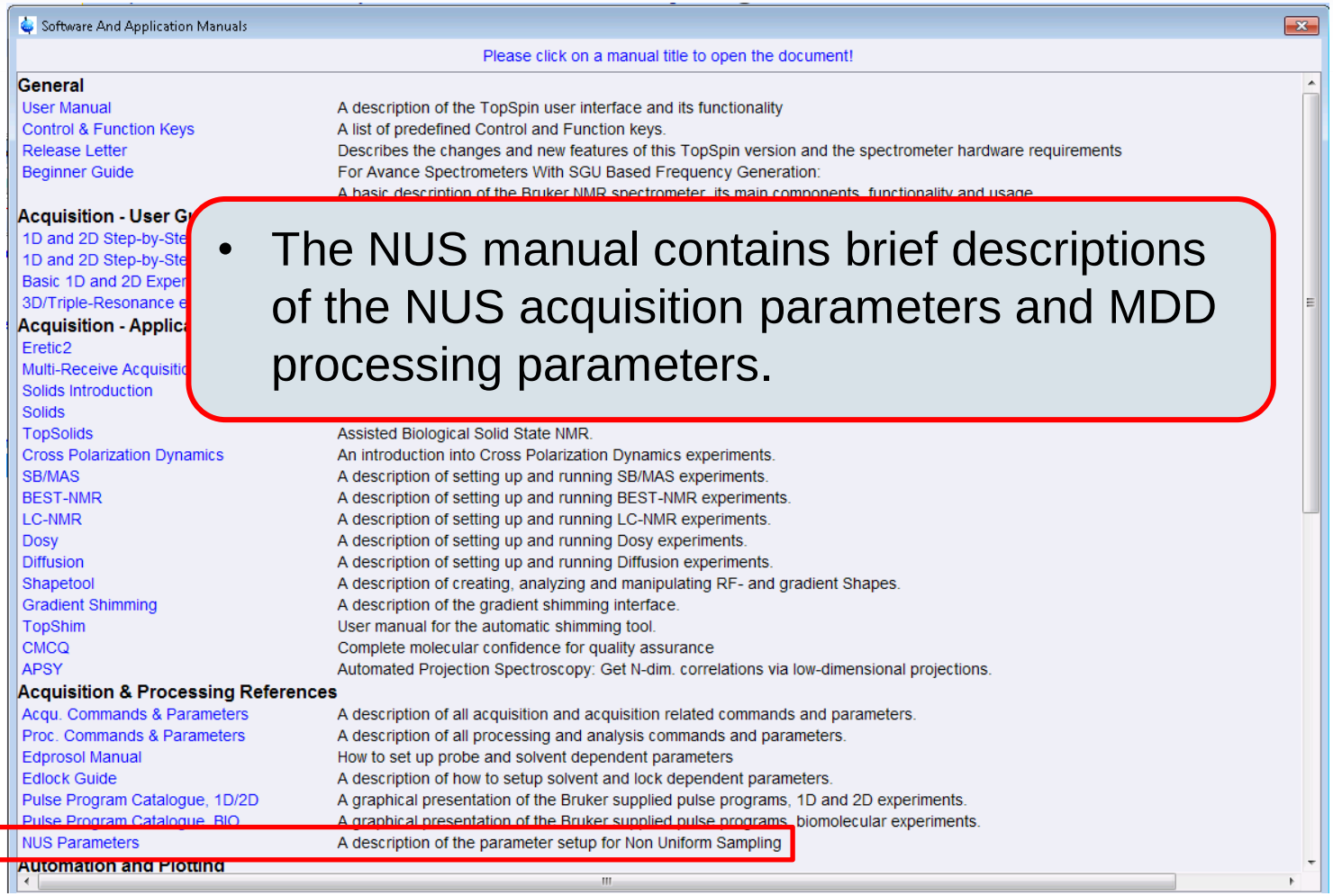
NUS processing parameters



- The NUS/MDD processing parameters should be set appropriately in most standard Topspin parameter sets
- I'll focus mostly on CS processing
  - It tends to preform better than MDD for sparsely samples 2D datasets
  - No additional license is required (TS3.5pl3 and newer)
- The processing algorithm has been improved in TS3.5pl6 – much faster!

NUS processing manual





- The NUS manual contains brief descriptions of the NUS acquisition parameters and MDD processing parameters.

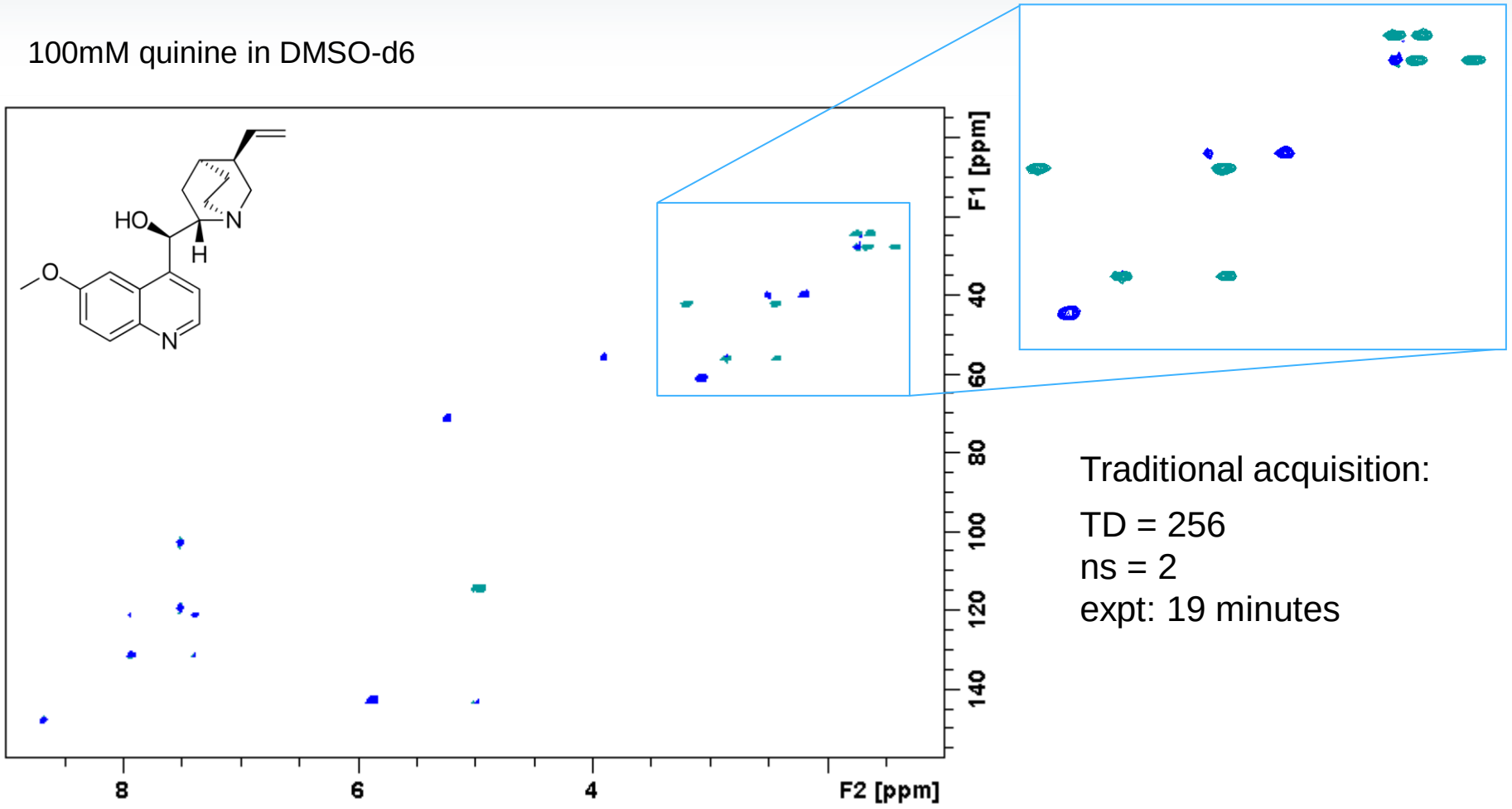
Processing NUS datasets

- Processing using the same Topspin commands as traditionally acquired datasets
  - **`xfb`** and **`xf2`** for 2D datasets
  - **`ftnd`** for 3D and higher dimensionality datasets

- Topspin automatically detects how the data was acquired and processed accordingly
  - Direct dimension is processed with standard FFT
  - “missing” 1D spectra are calculated with MDD or CS
  - indirect dimension(s) are then processed with FFT

First example: edited HSQC - traditional acquisition

100mM quinine in DMSO-d6

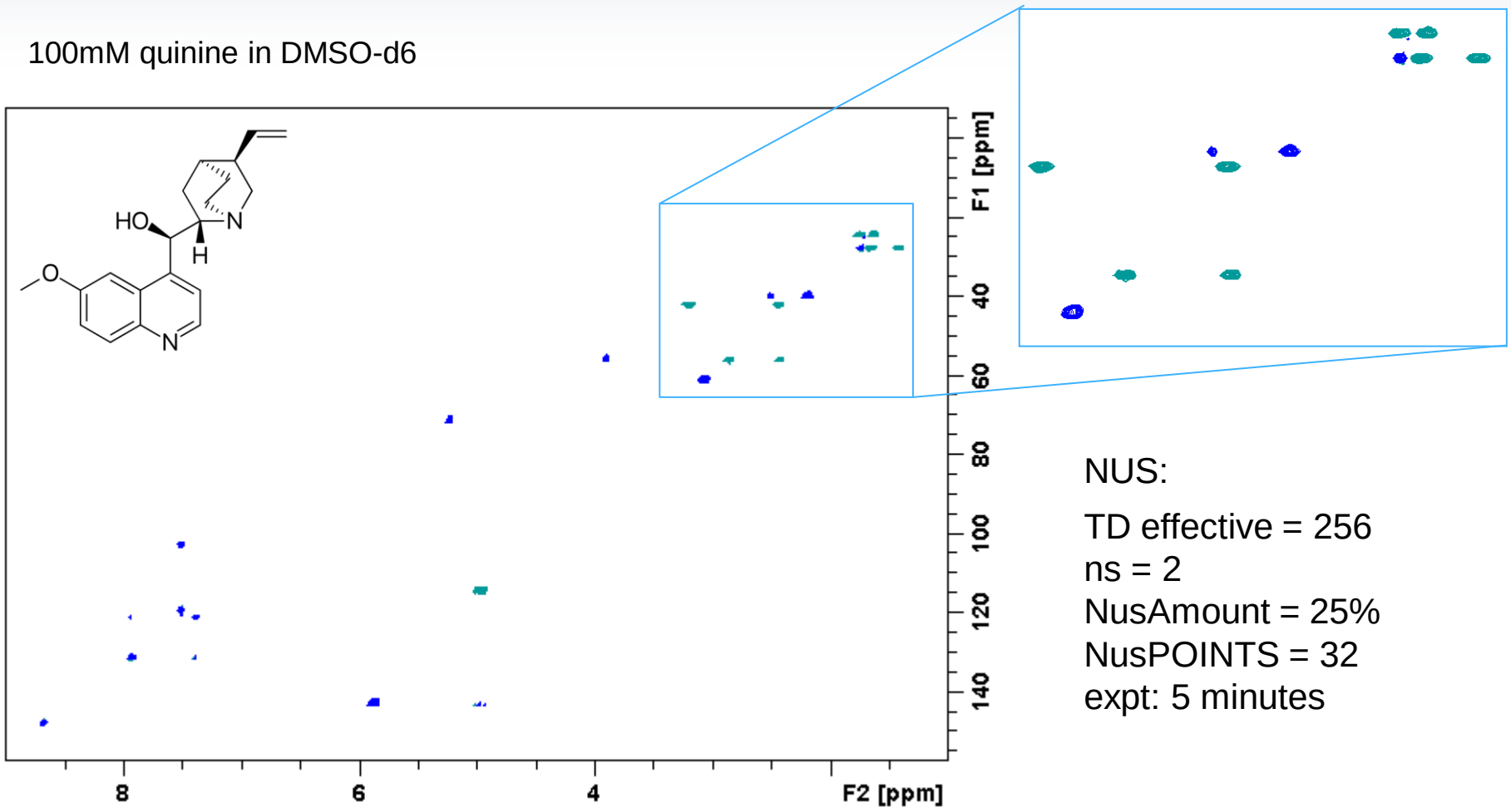


Traditional acquisition:

TD = 256
ns = 2
expt: 19 minutes

First example: edited HSQC
- NUS for faster acquisition

100mM quinine in DMSO-d6

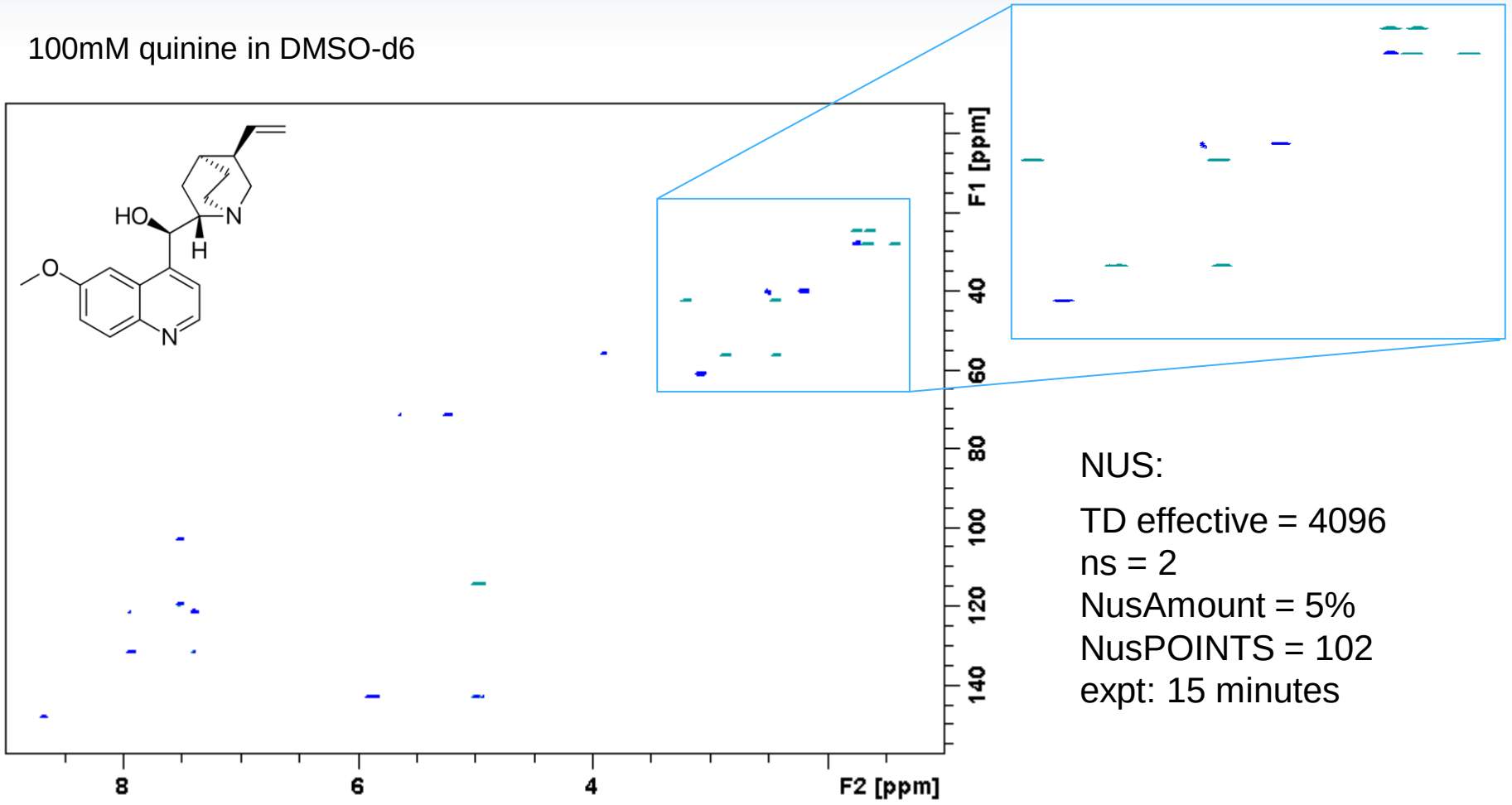


NUS:

TD effective = 256
ns = 2
NusAmount = 25%
NusPOINTS = 32
expt: 5 minutes

First example: edited HSQC
- NUS for higher resolution

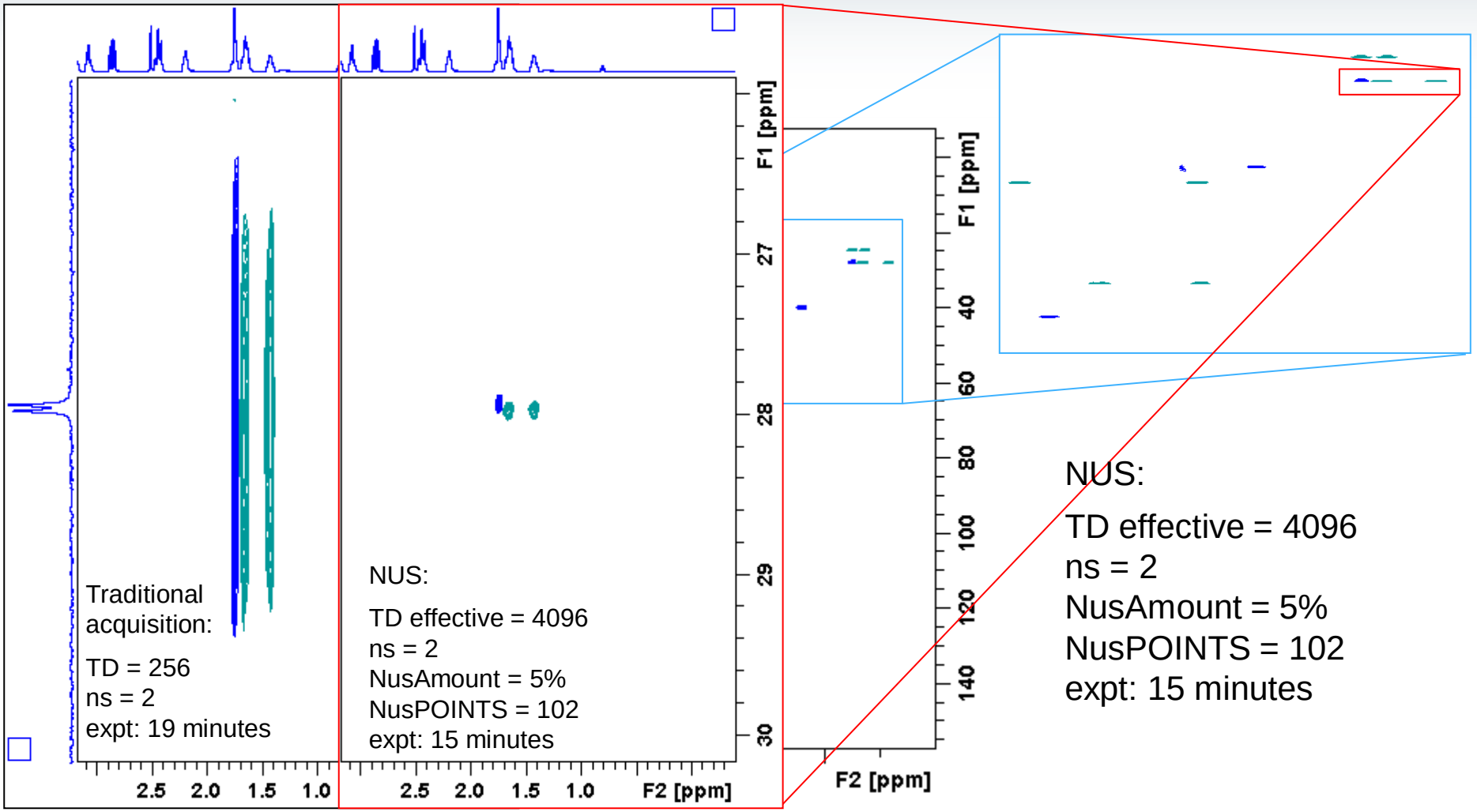
100mM quinine in DMSO-d6



NUS:

TD effective = 4096
ns = 2
NusAmount = 5%
NusPOINTS = 102
expt: 15 minutes

First example: edited HSQC
- NUS for higher resolution



NUS:

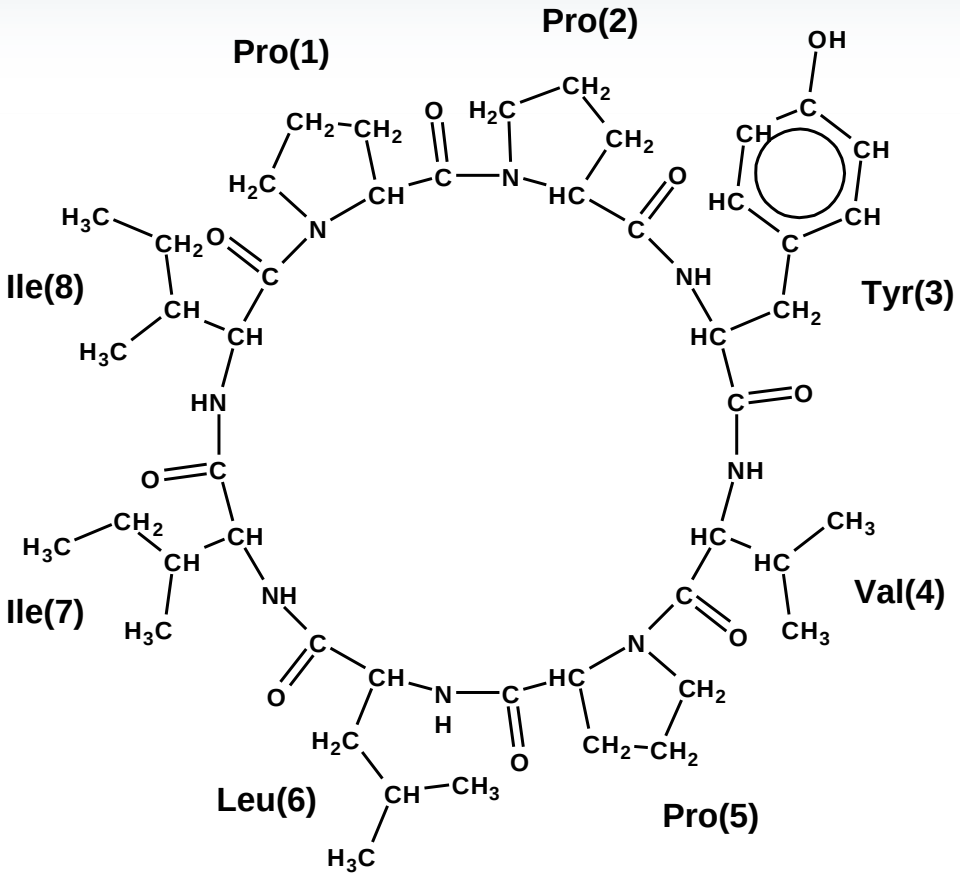
TD effective = 4096
ns = 2
NusAmount = 5%
NusPOINTS = 102
expt: 15 minutes

Applications and limitations of NUS

- Rule of thumb:
  - Minimum number of NusPOINTS should be on the order of the number of frequencies expected in your spectrum
  - For example, we can acquire fewer FID's (much more sparsely sampled data) for an HSQC than an HMBC
  - Don't forget to consider the contributions of weaker (contaminant) peaks
- Artifacts from undersampling the FID's can be more problematic when you have a large dynamic range in peak intensities (for example NOESY spectra)

Additional examples

Hymenistatin



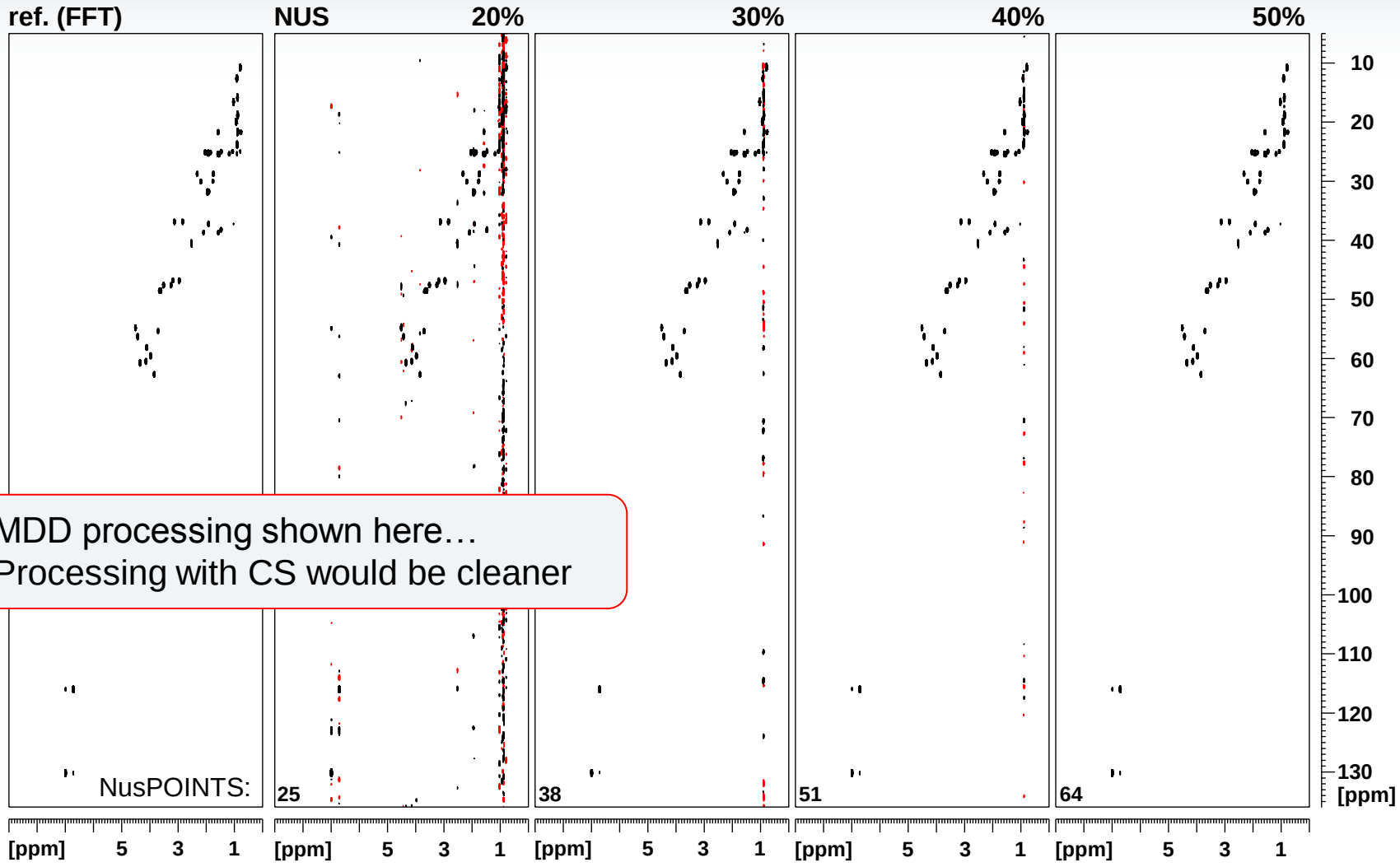
number of cross peaks (estimated maximum):

HSQC (^{13}C)	48
HMBC (^{13}C)	199
COSY	187
TOCSY	339

R.K. Konat, D.F. Mierke, H.Kessler, B. Kutscher, M. Bernd & R. Voegeli, Helv. Chim. Acta 76, 1649 (1993)

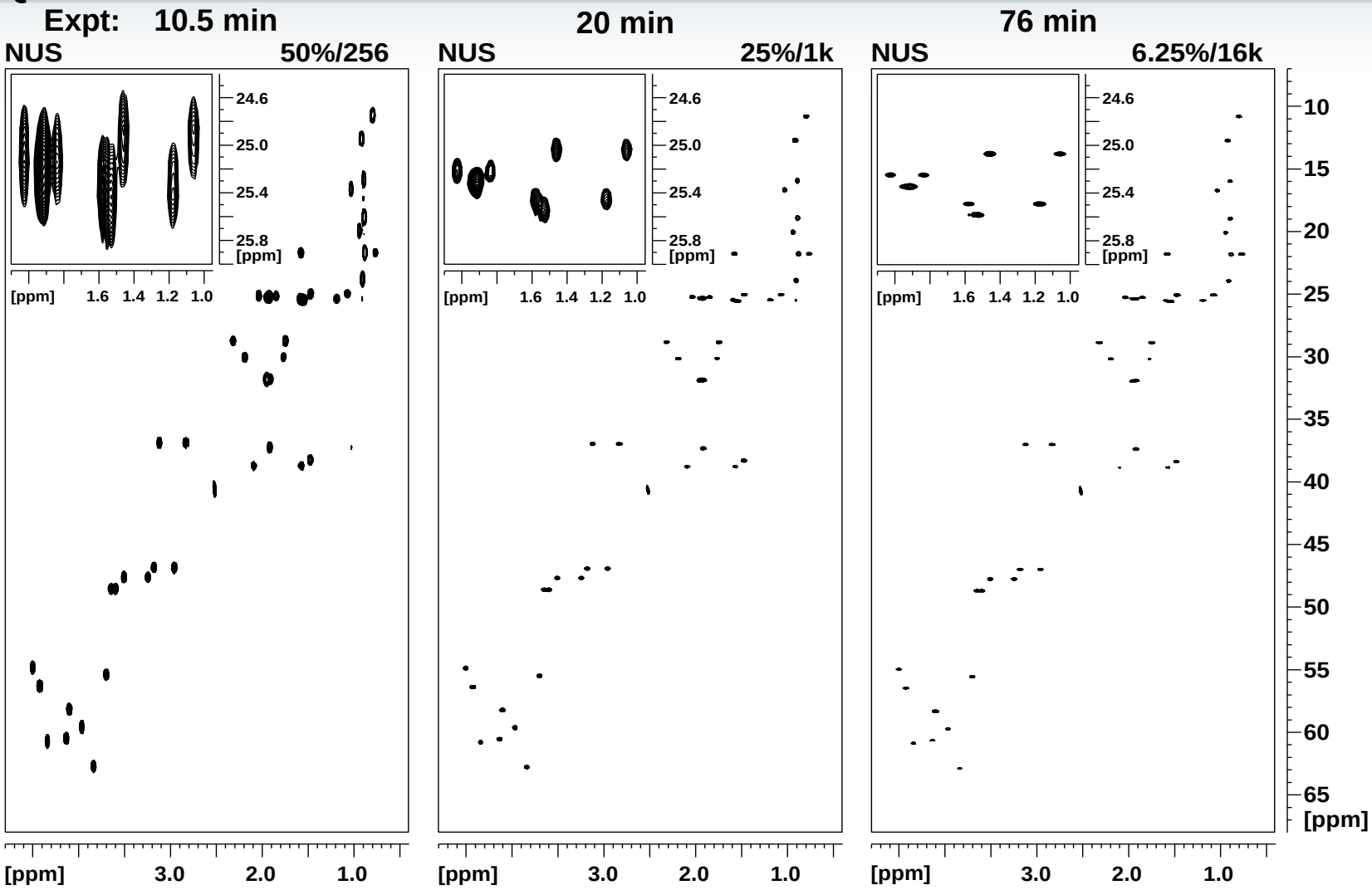
Additional examples – 20mM Hymenistatin

HSQC: td = 256



Additional examples – 20mM Hymenistatin

HSQC



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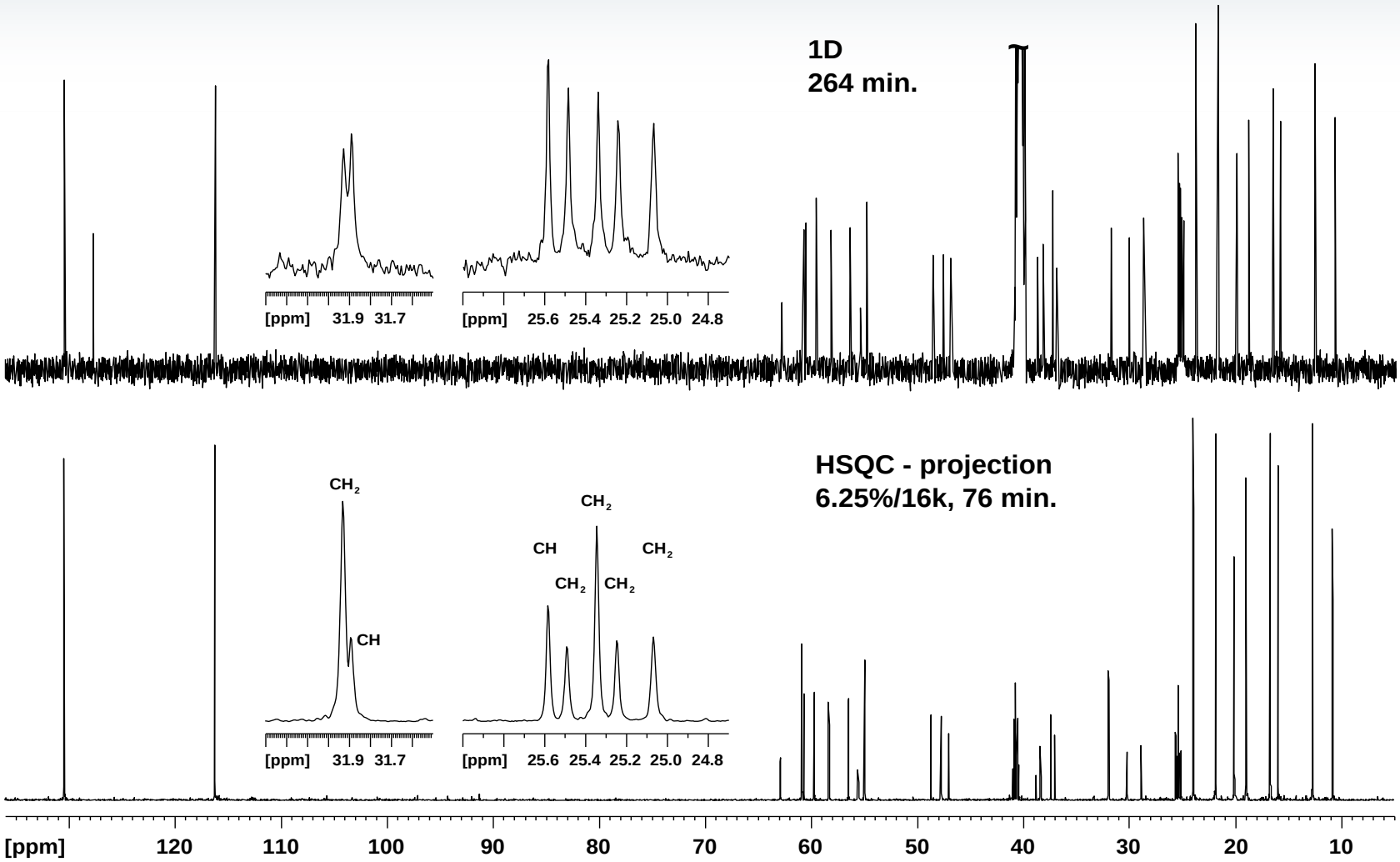
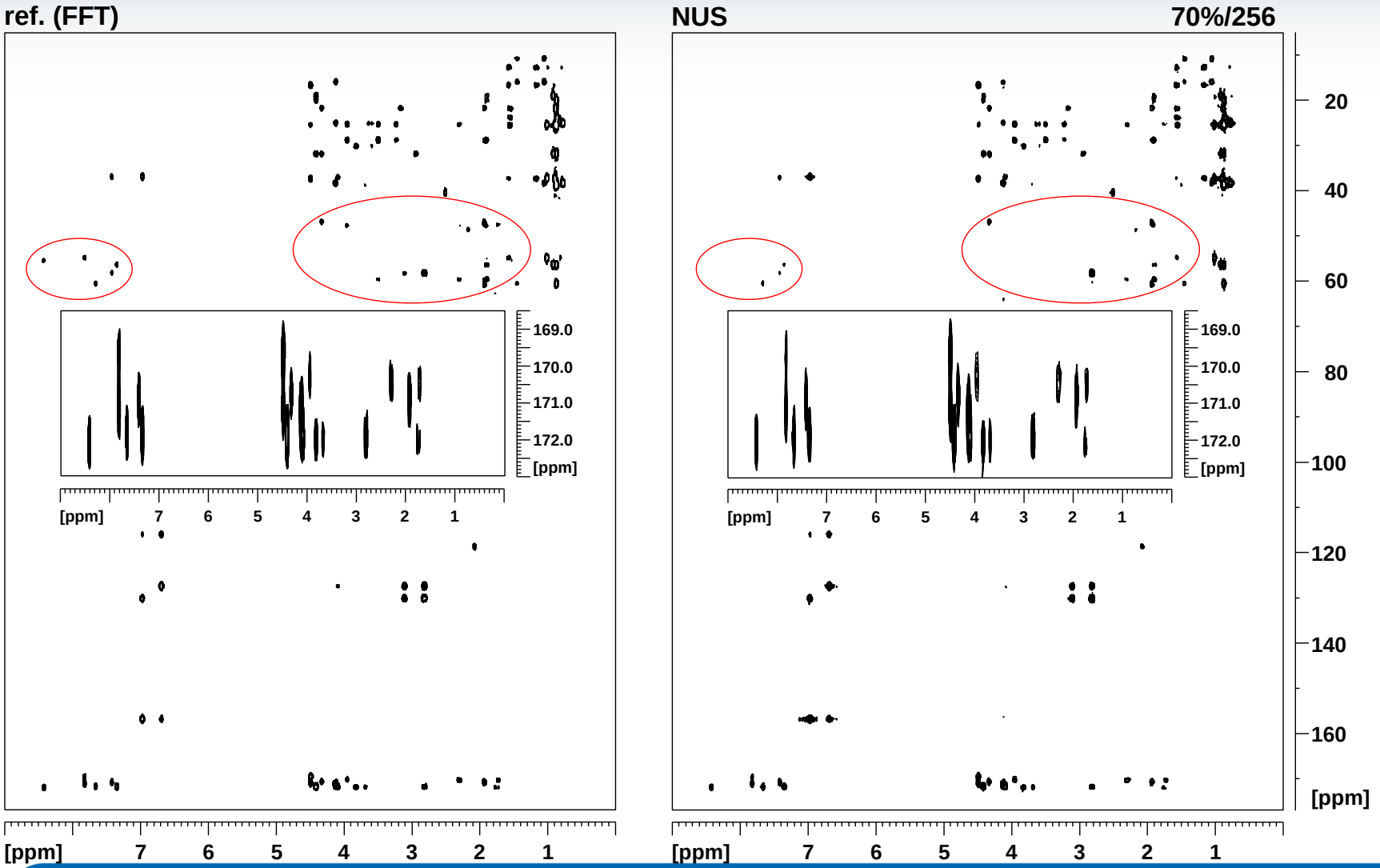


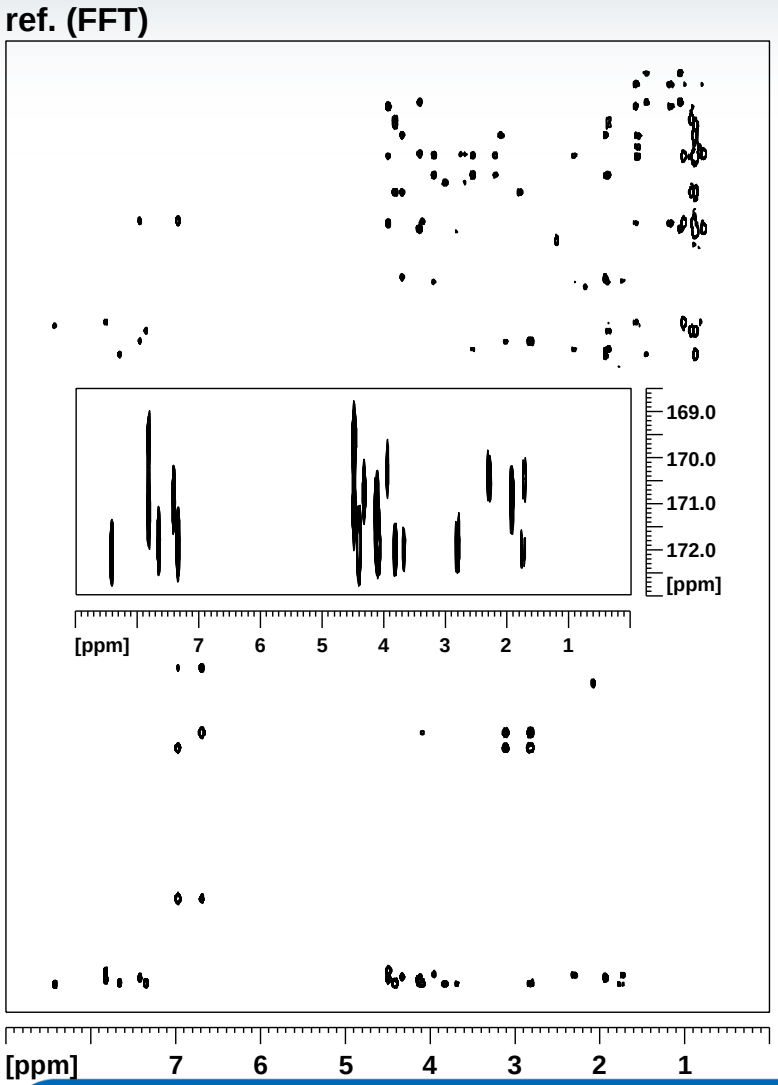
Additional examples – 20mM Hymenistatin

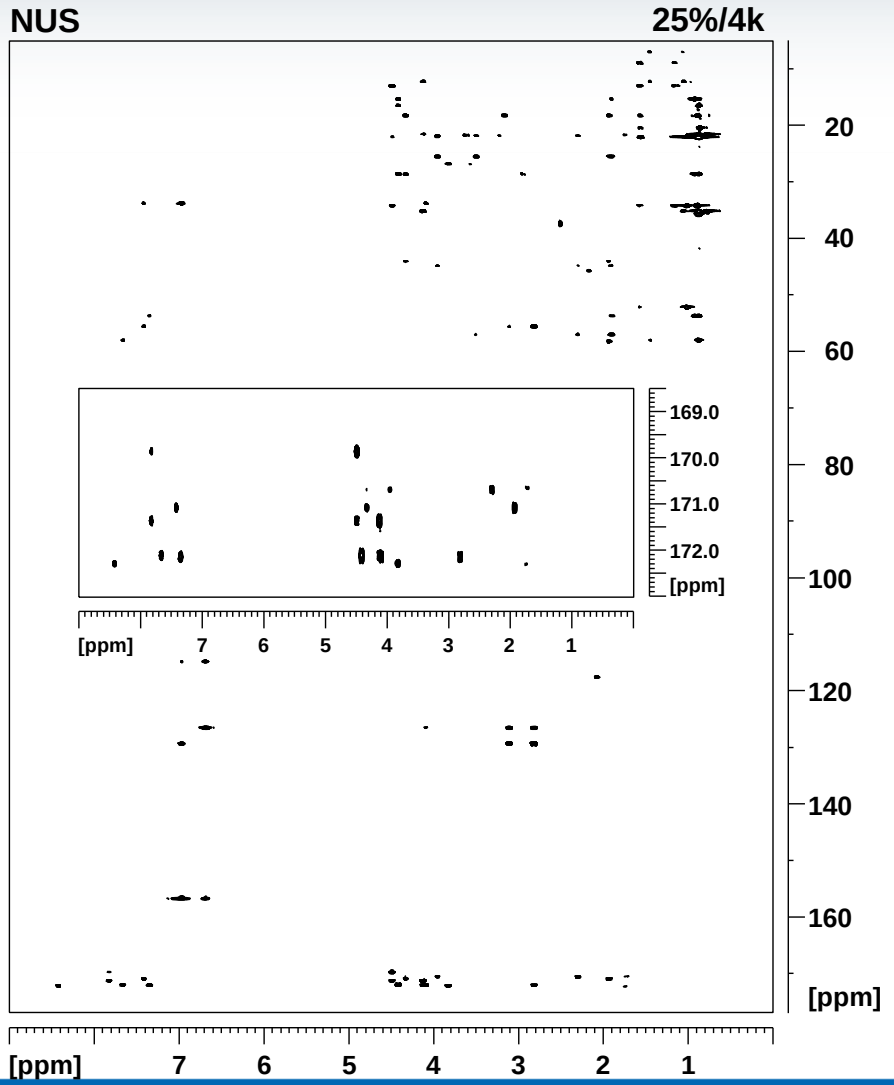
HMBC



Additional examples – 20mM Hymenistatin

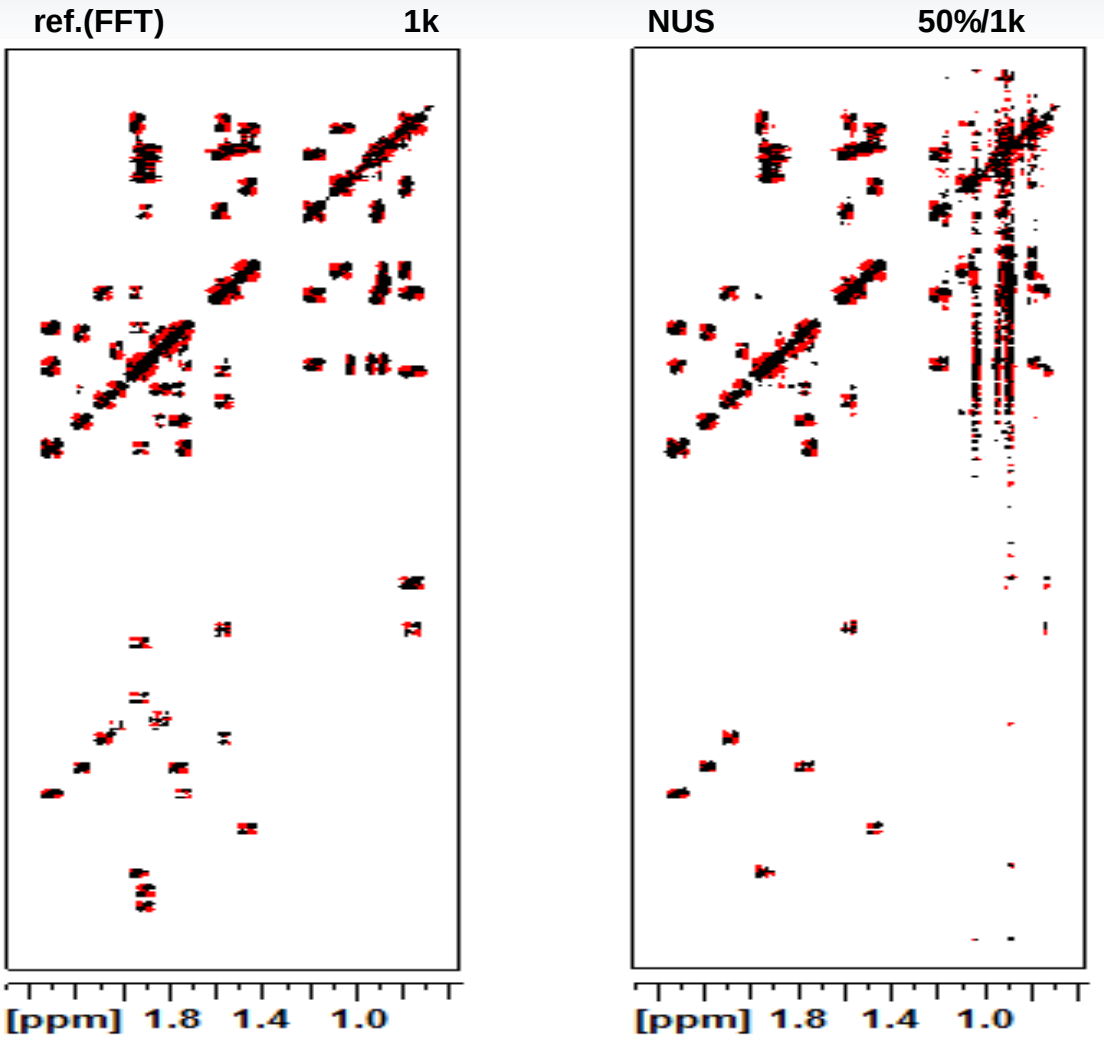
HMBC





Additional examples – 20mM Hymenistatin

double quantum filtered COSY



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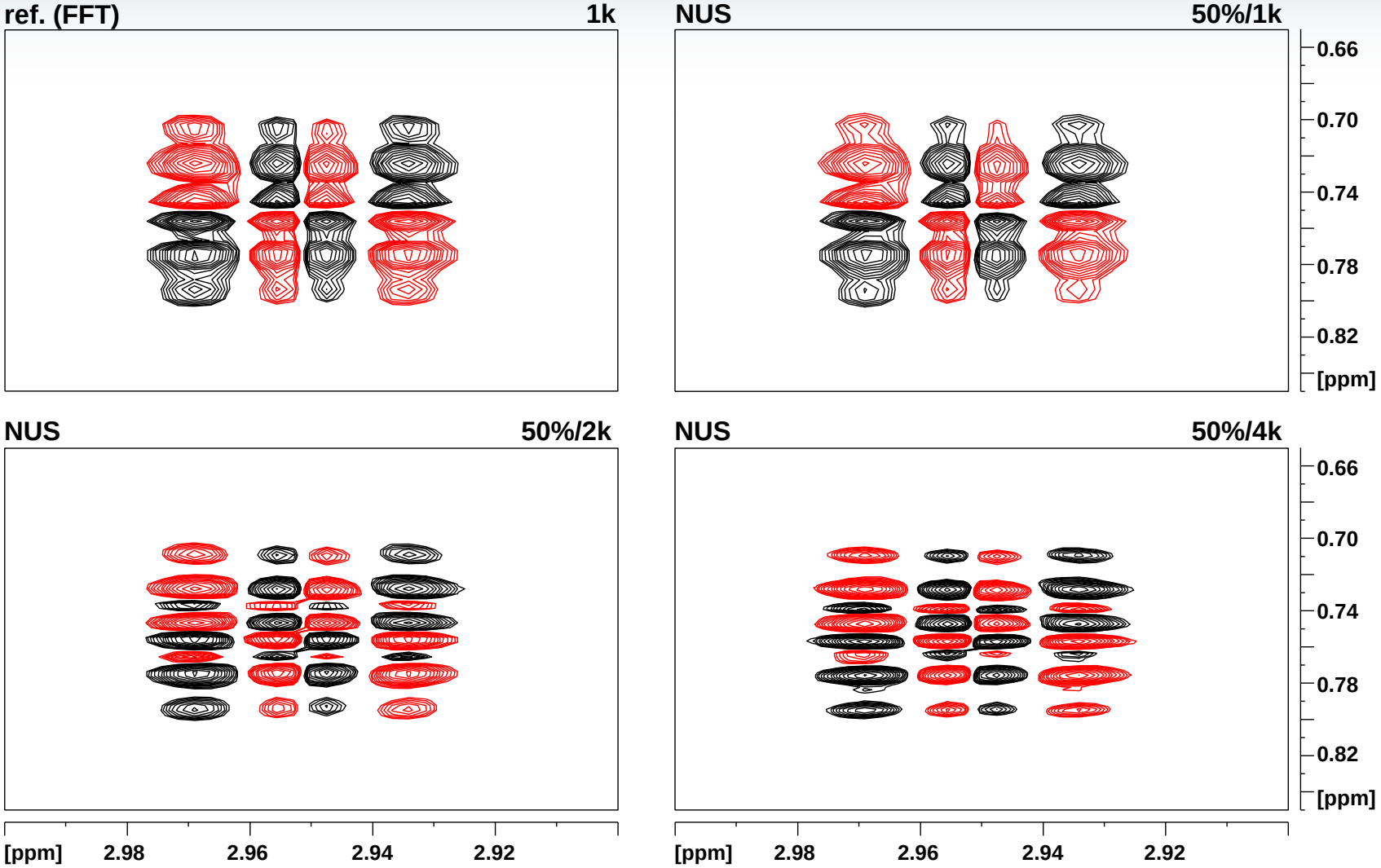

Additional examples – 20mM Hymenistatin

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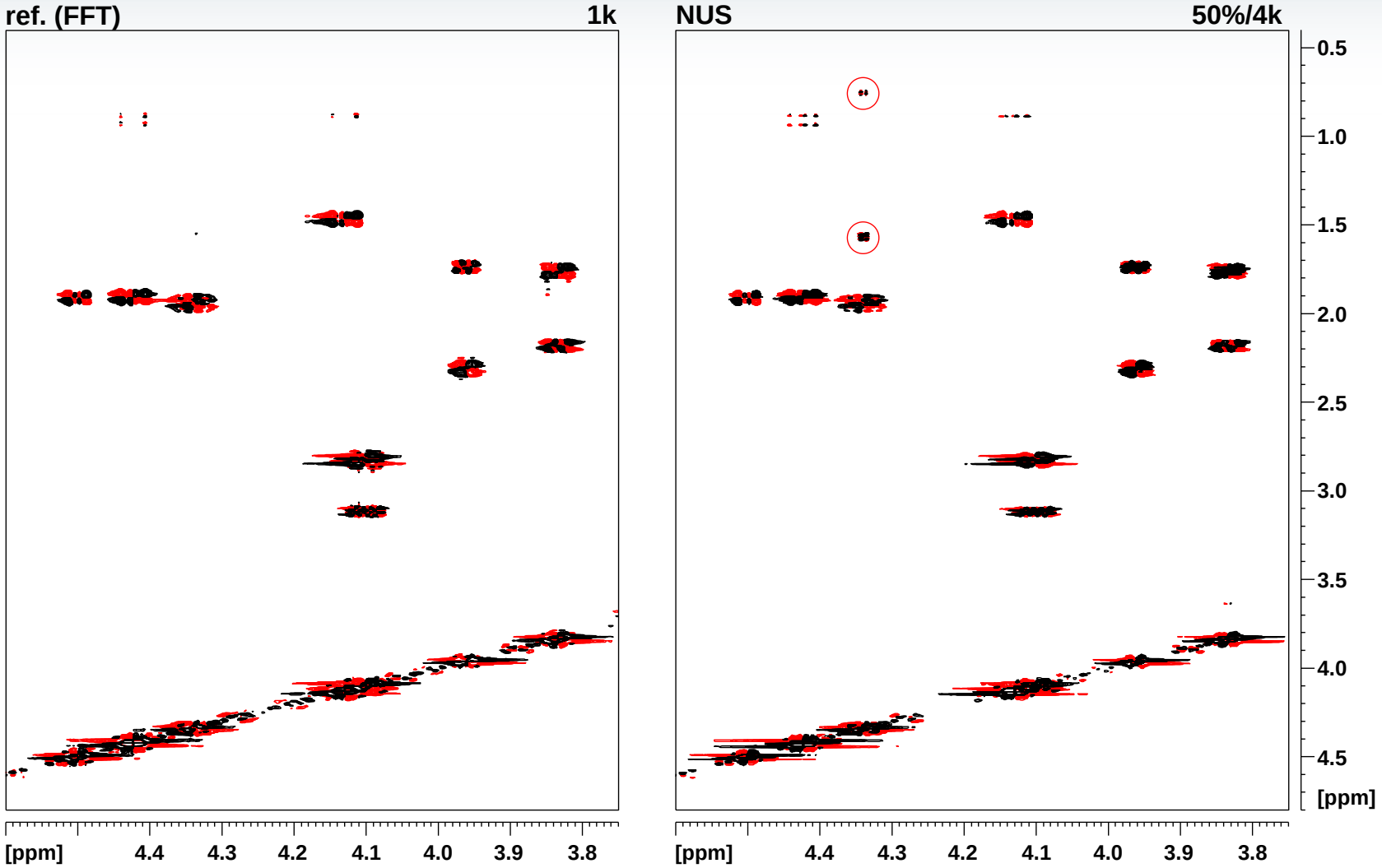
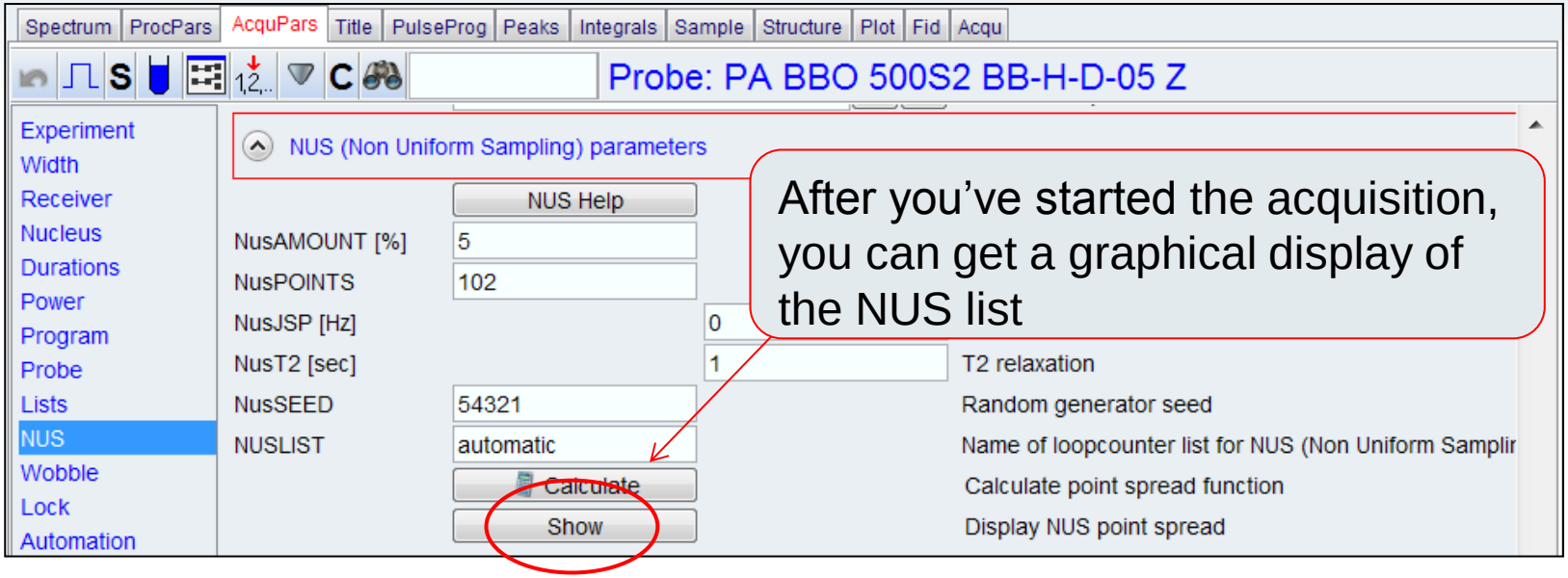
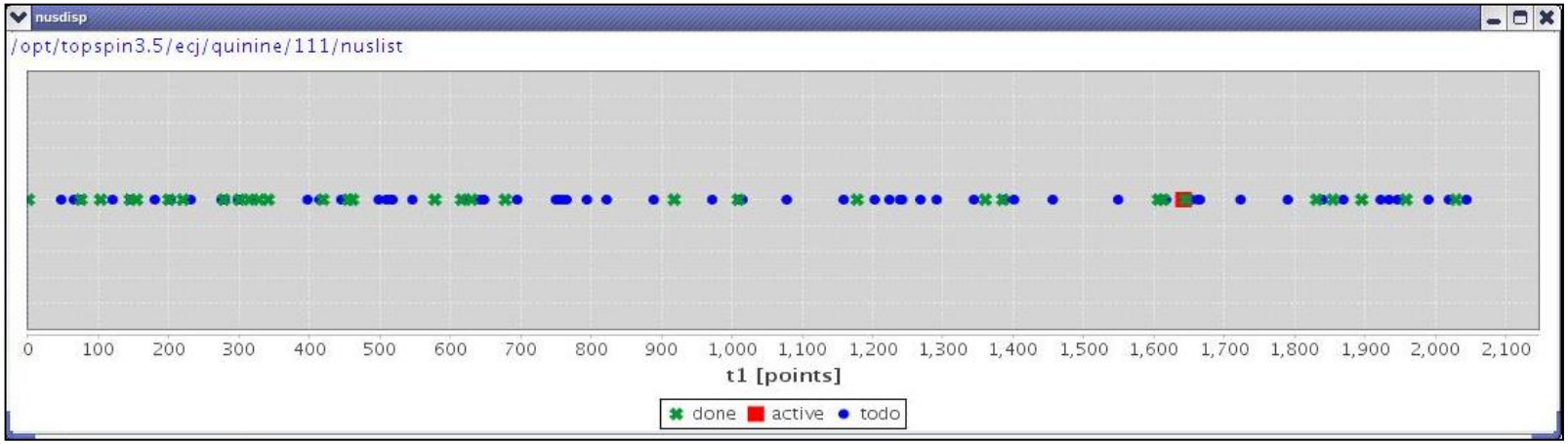


A few more acquisition details





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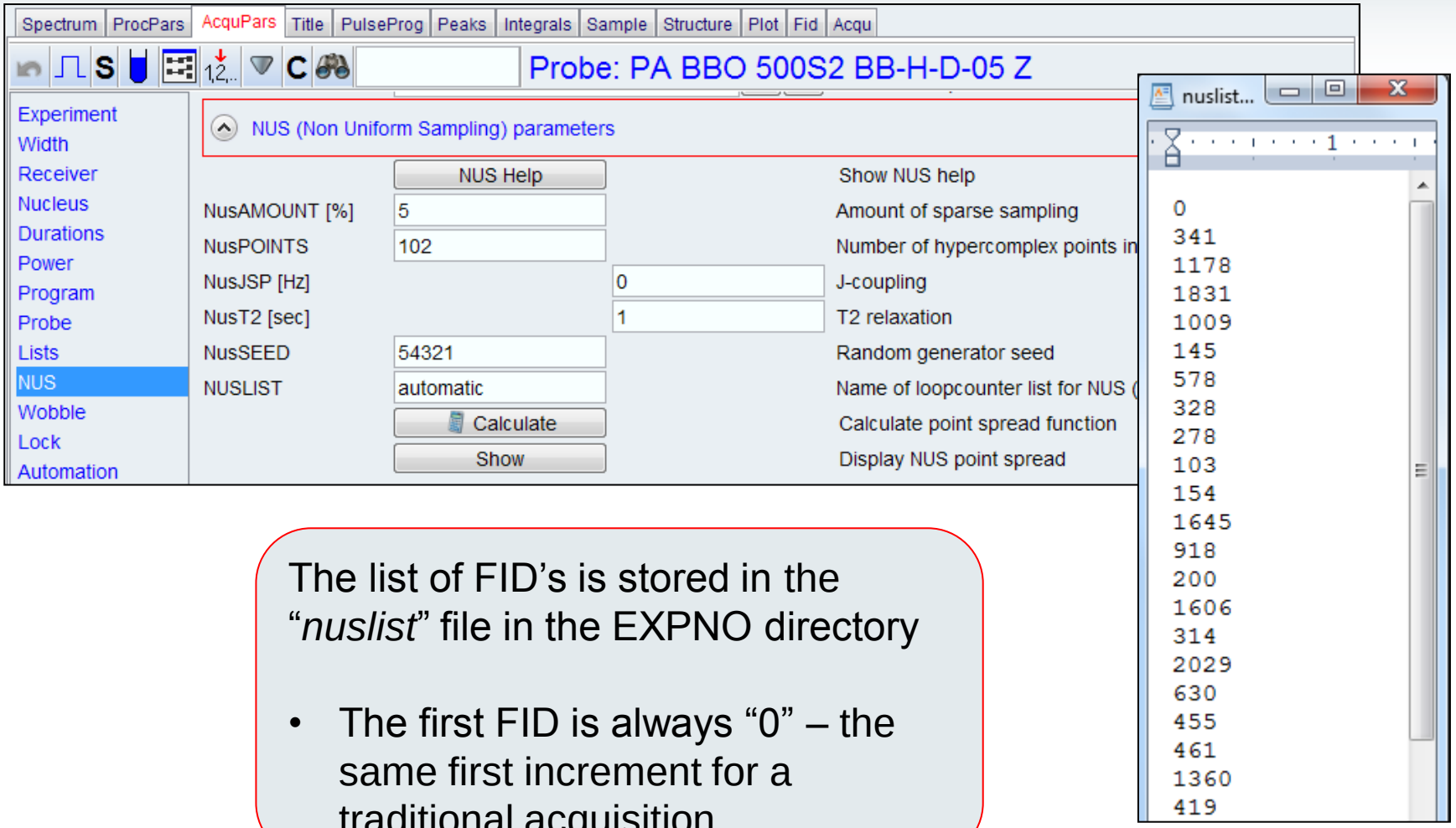
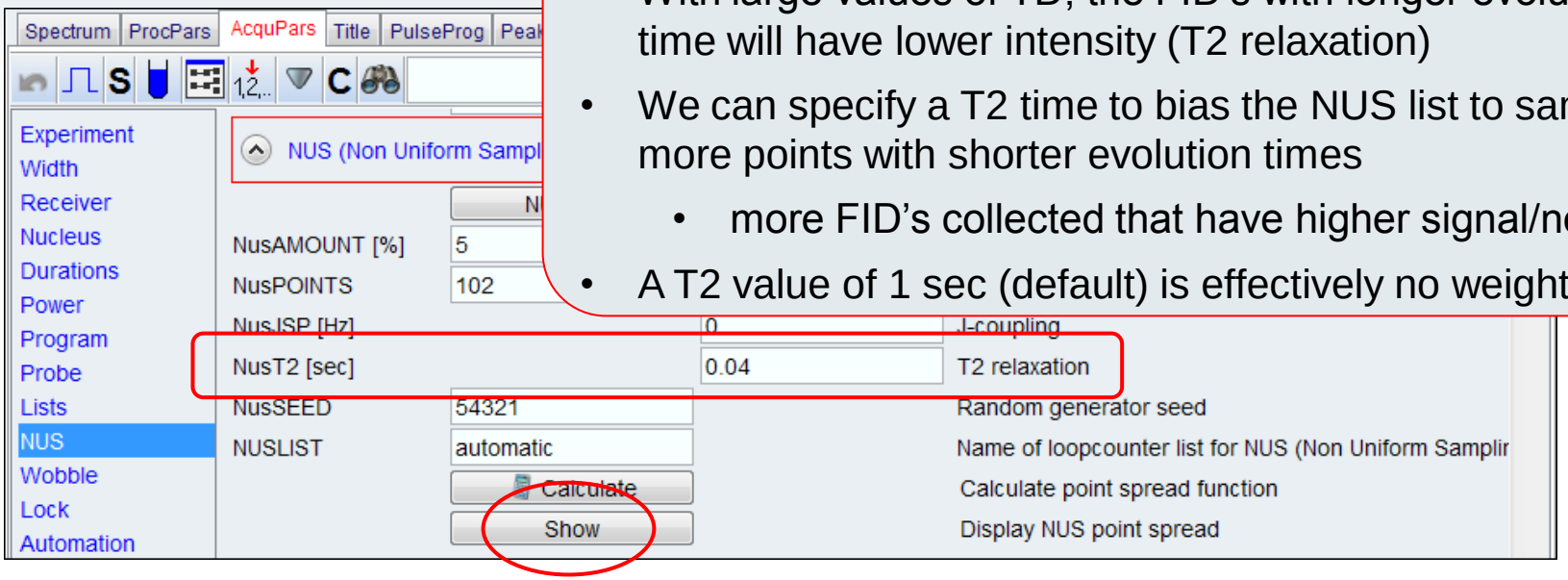


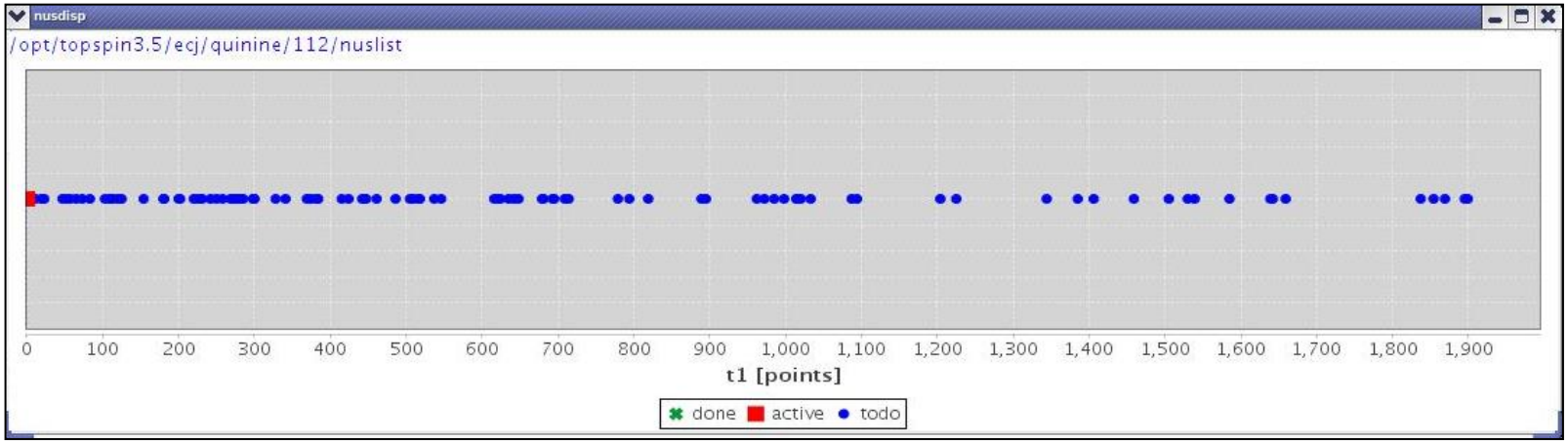
The list of FID's is stored in the "*nuslist*" file in the EXPNO directory

- The first FID is always "0" – the same first increment for a traditional acquisition

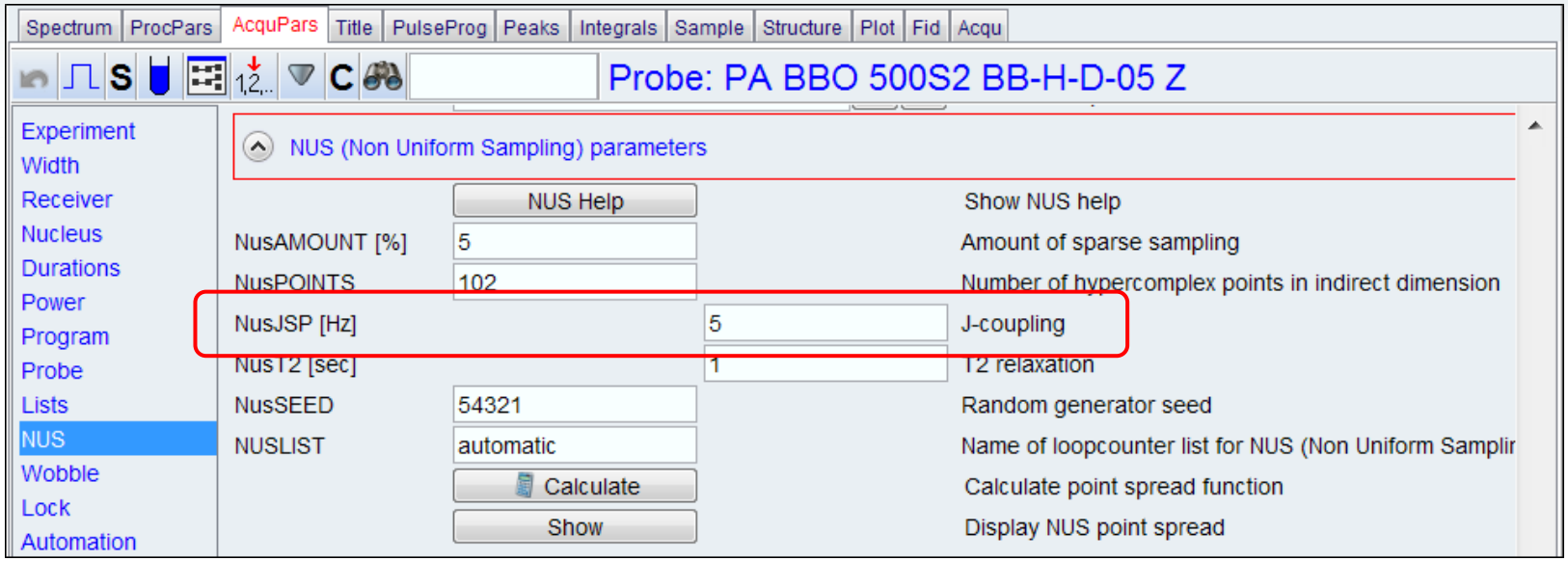
A few more acquisition details

- With large values of TD, the FID's with longer evolution time will have lower intensity (T2 relaxation)
- We can specify a T2 time to bias the NUS list to sample more points with shorter evolution times
  - more FID's collected that have higher signal/noise
- A T2 value of 1 sec (default) is effectively no weighting



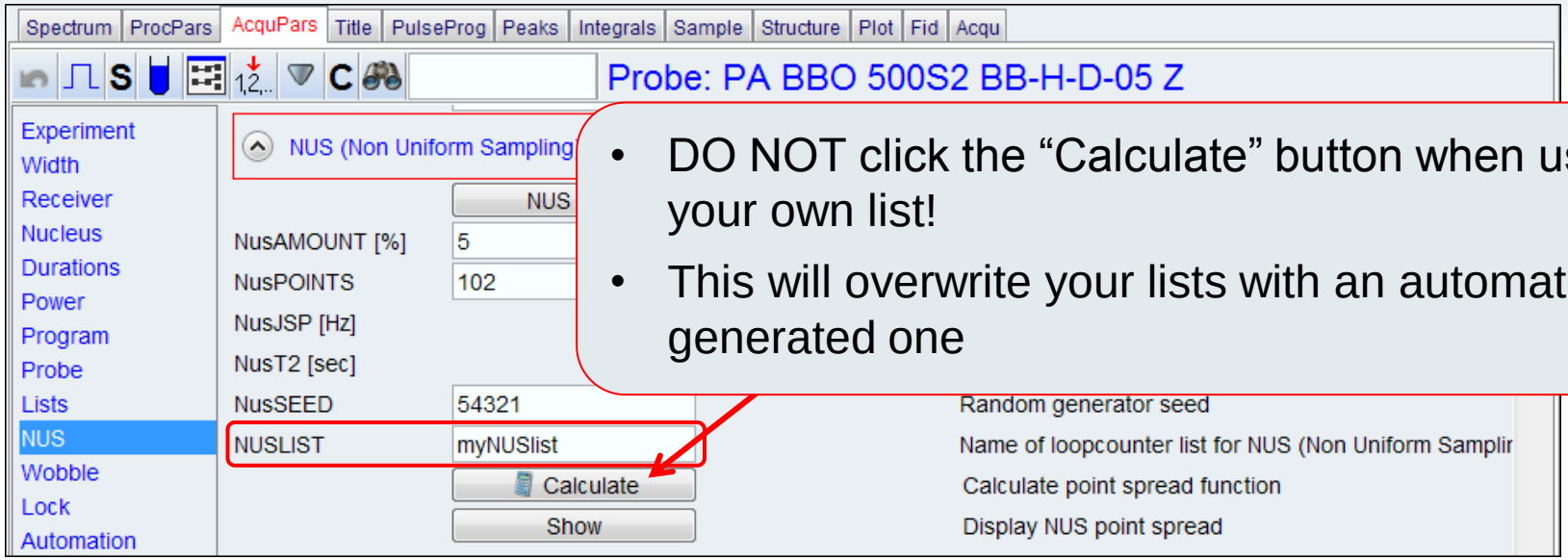


A few more acquisition details



- We can also bias the NUSLIST for datasets in which the intensity of the FID's is modulated by a coupling constant
  - Not common

A few more acquisition details



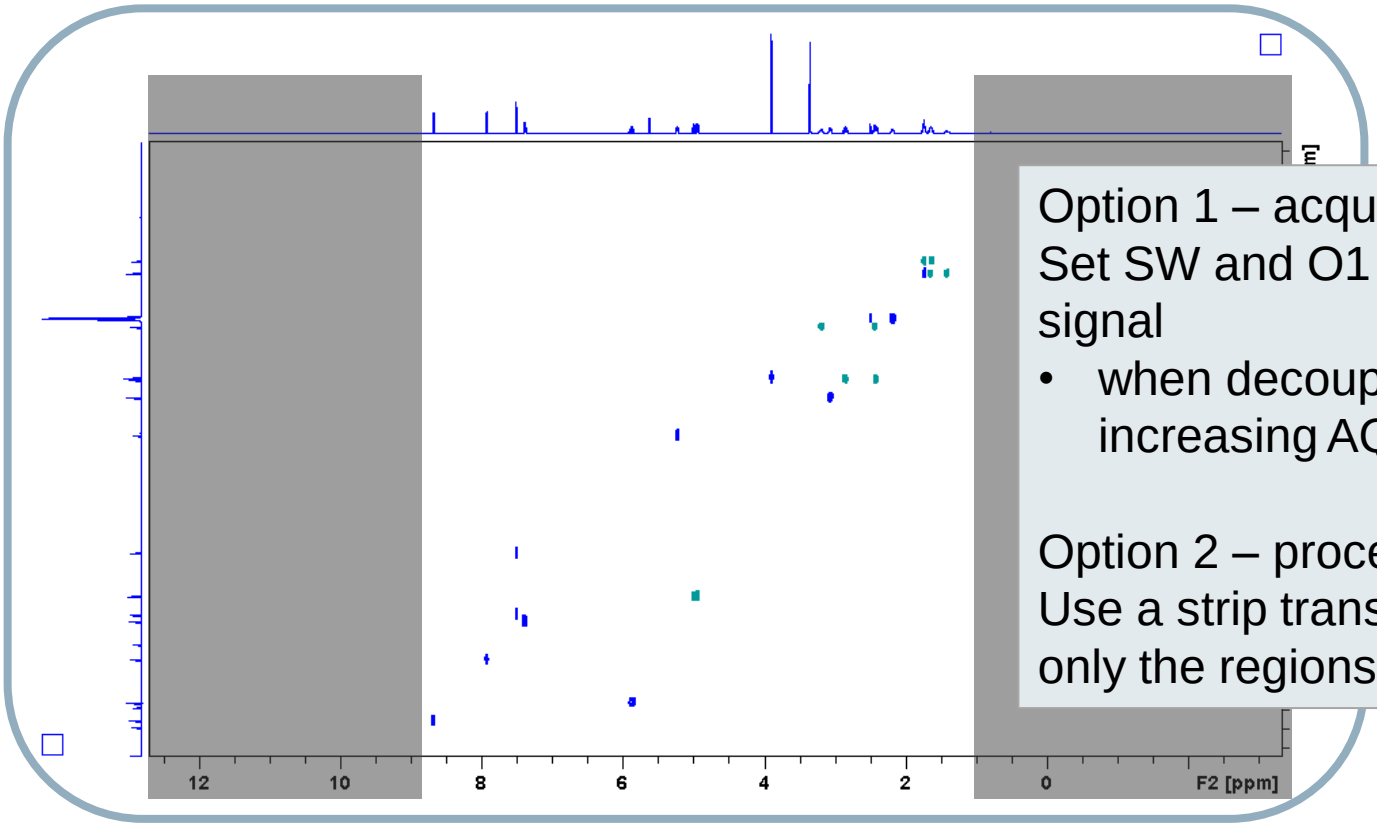
- DO NOT click the “Calculate” button when using your own list!
- This will overwrite your lists with an automatically generated one

- We can define our own NUSLIST
- The list goes in the directory

  `<Topspin>/exp/stan/nmr/lists/vc/`
- When the list name is “automatic”, the list will always be automatically generated when you start the acquisition

A few tips on processing

- To speed up processing, don't process regions with no signal in the observe dimension

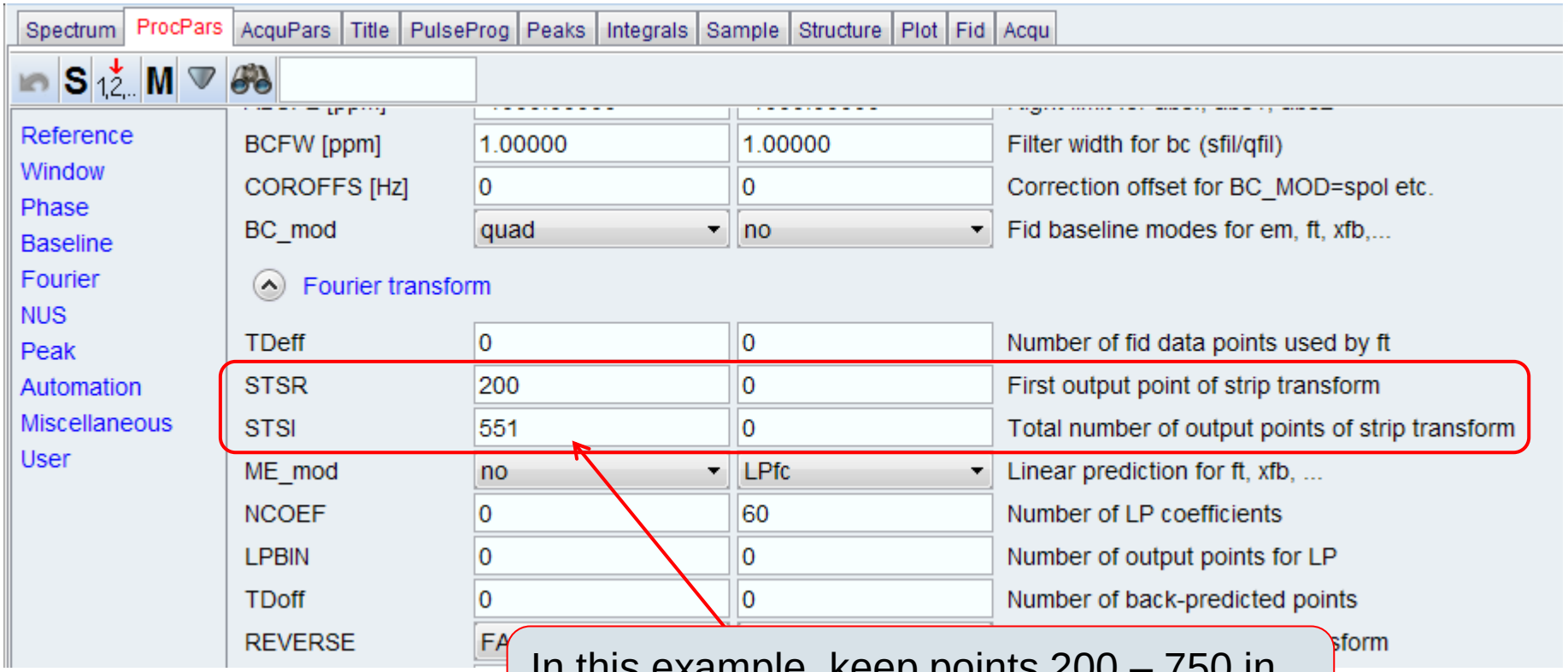


Option 1 – acquisition parameters:
Set SW and O1 for region with signal

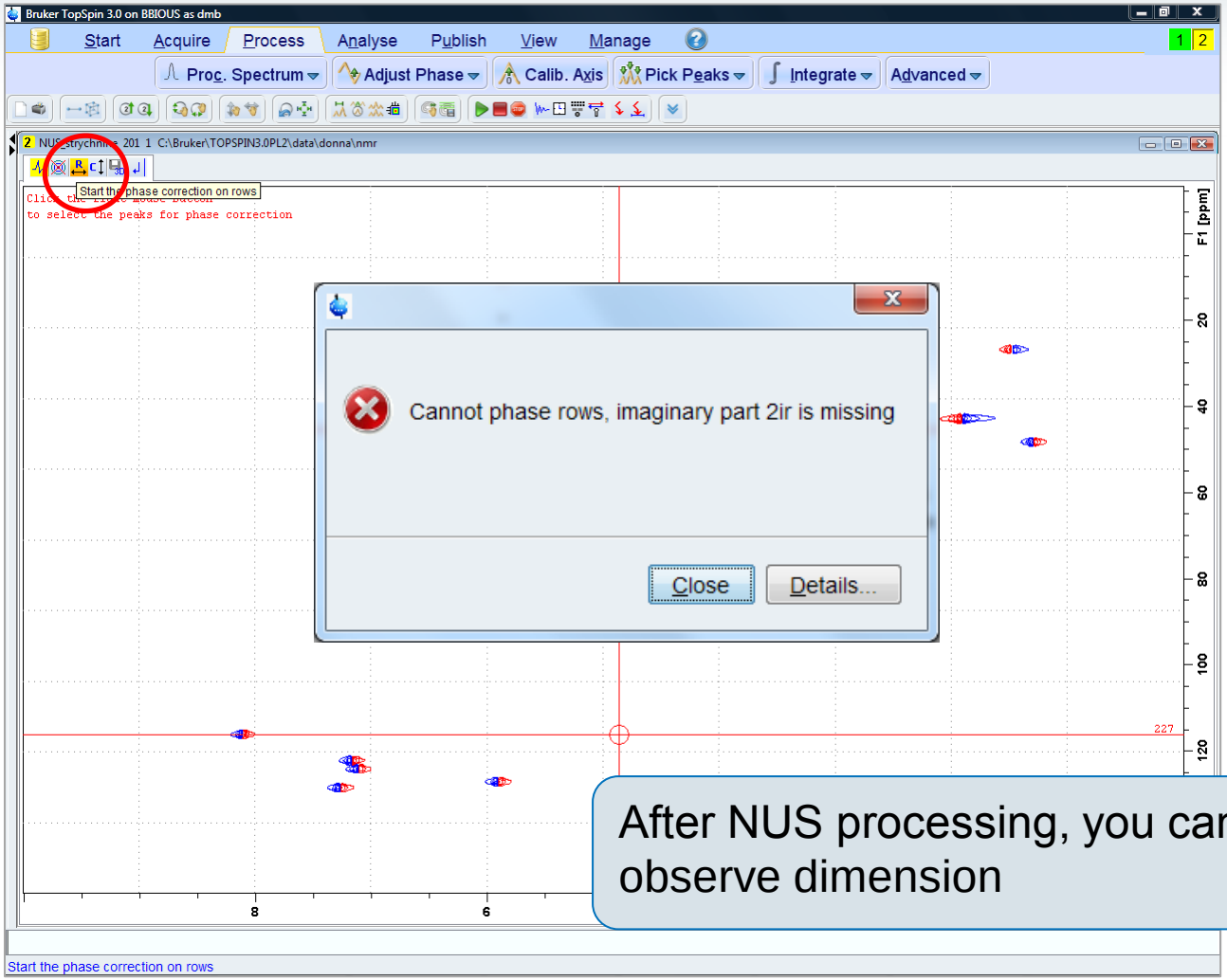
- when decoupling, be careful of increasing AQ too much

Option 2 – processing:
Use a strip transform to process only the regions with signal

A few tips on processing – strip transform



A few tips on processing - phasing



After NUS processing, you can't phase the observe dimension

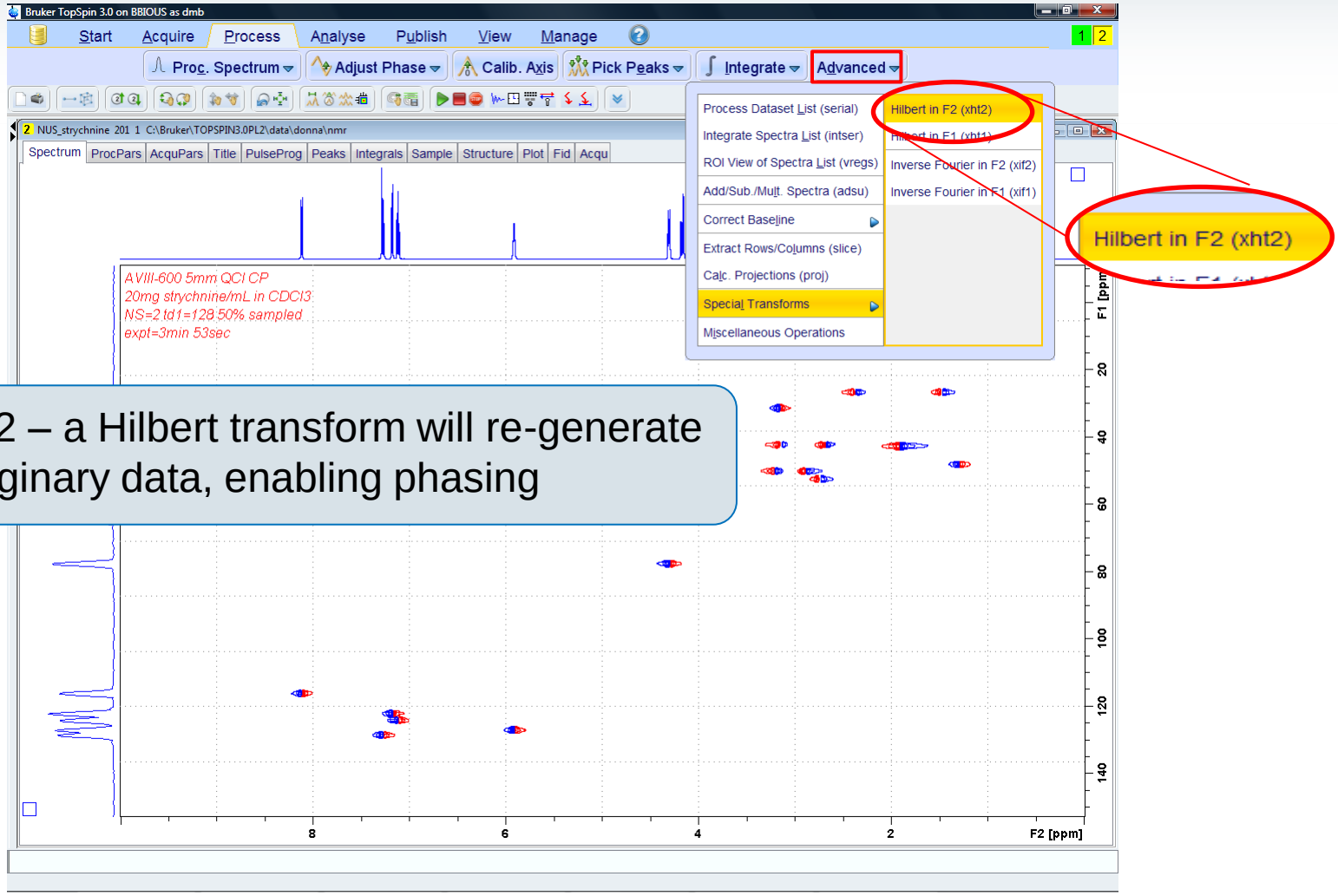
A few tips on processing - phasing

- Option 1: phase the 1st FID and copy PHC0 and PHC1 to the 2D before processing

➢ **`rser 1`**
  - extracts the 1st FID from an nD experiment

For sufficient S/N and using the nuslist from the nussampler, PHC0 and PHC1 for the acquisition dimension can be determined on the 1st FID. If any of the indirect dimensions use Echo-/Antiecho encoding a 90 degree phase shift has to be applied to the phase of the first FID.

A few tips on processing - phasing

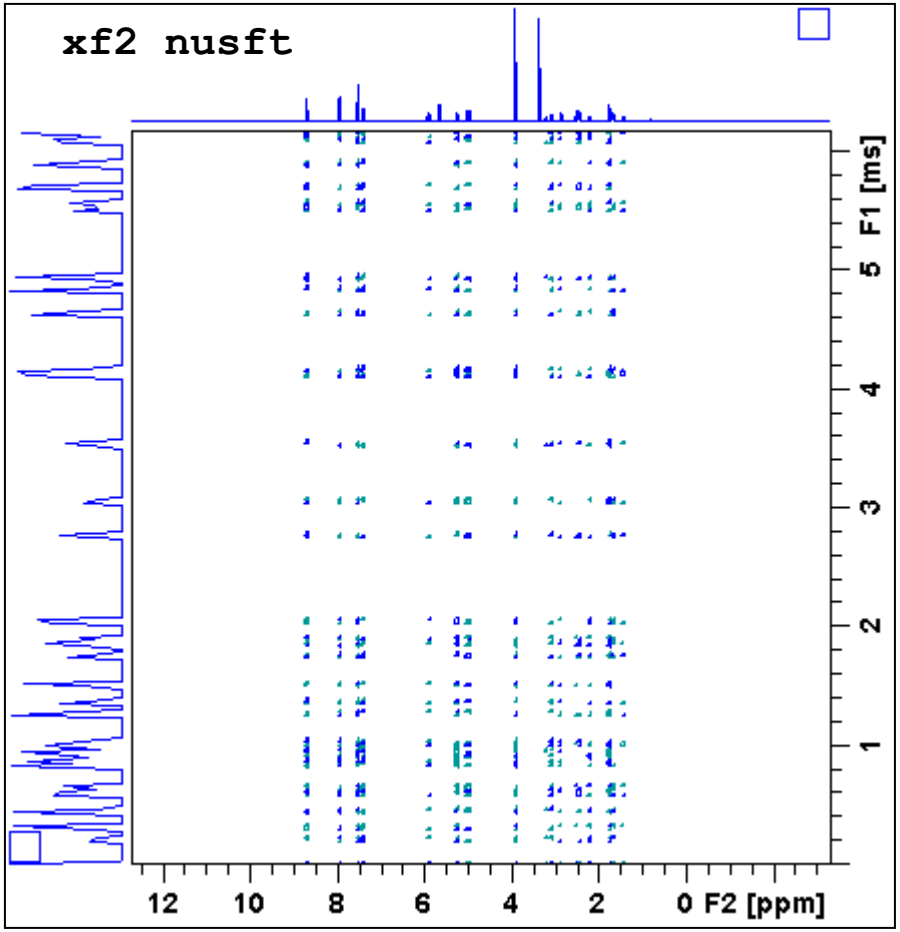


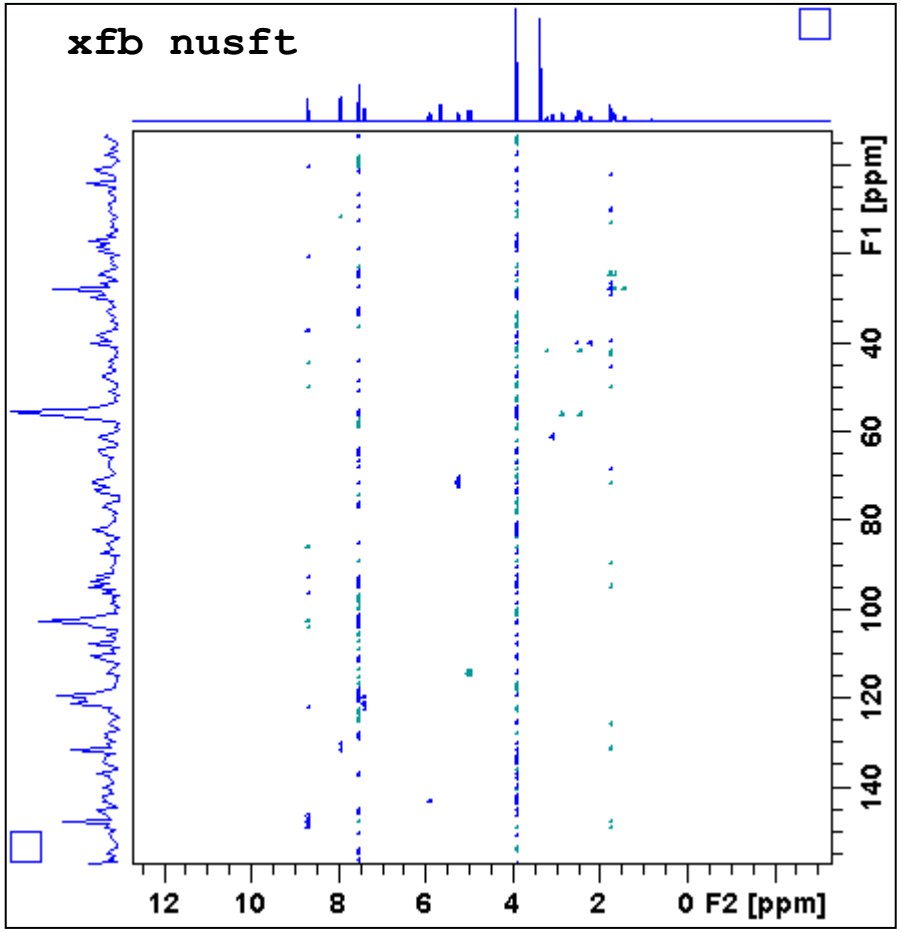
- Option 2 – a Hilbert transform will re-generate the imaginary data, enabling phasing

A sometime useful processing option: "**nusft**"

- "nusft" keyword will fill in the missing FID's with zero's, rather than using NUS reconstruction
  - Examples:
    - **xf2 nusft**
    - **xfb nusft**
    - **ftnd nusft**
- Quick processing
- Usually tons of artifacts, but you might see enough signal for phasing

A sometime useful processing option: "nusft"



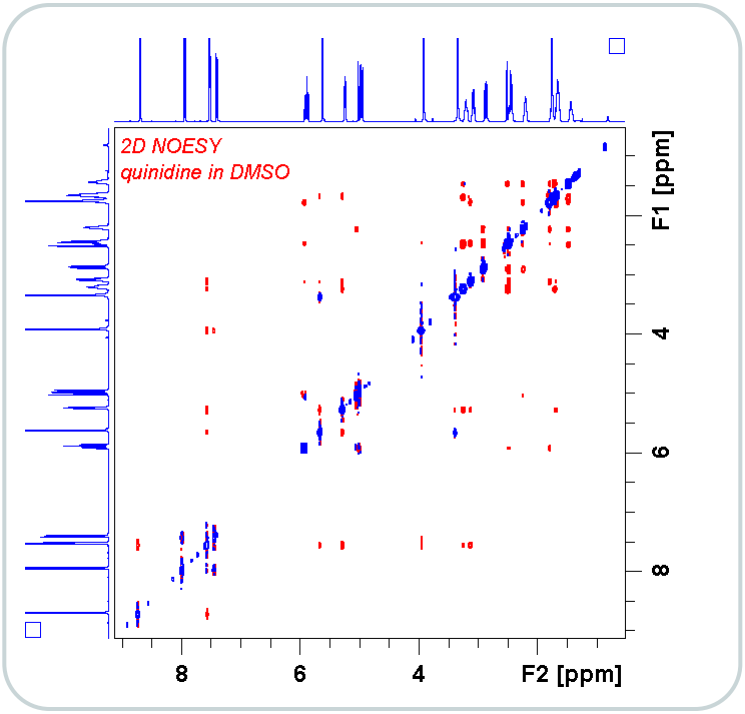


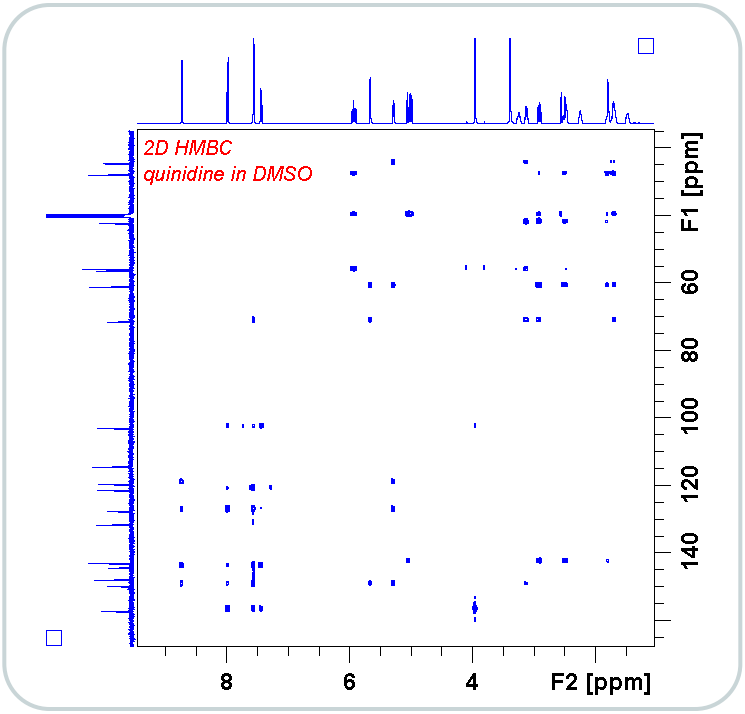
Selective Experiments

focusing on regions of interest in your spectra…

Why use selective experiments?

- 2D homonuclear and 2D heteronuclear experiments are easy to set up and are loaded with information





Individual experiments give correlations throughout the molecule

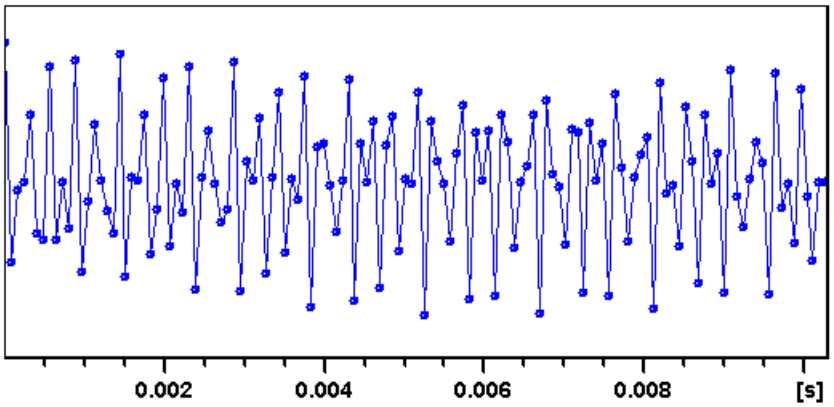
Why use selective experiments?

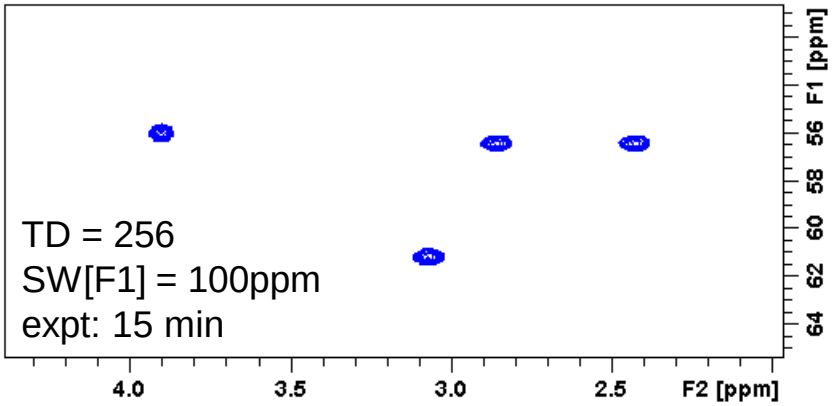
- If we're particularly interested in a few correlations in certain regions of our spectrum, we might be able to get that info faster, with higher resolution using selective experiments

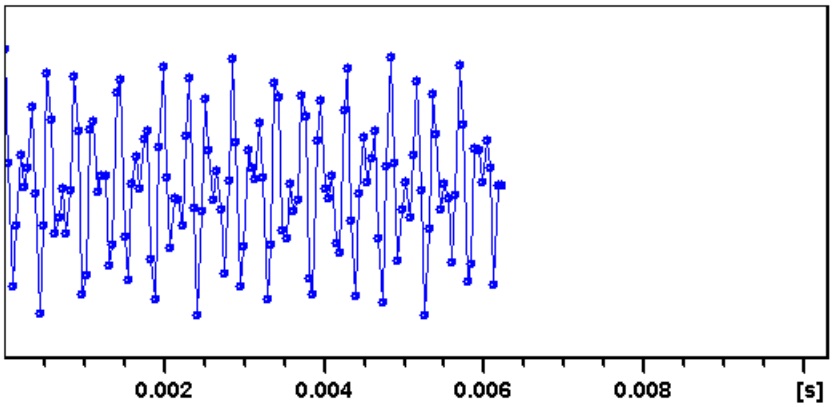
- Built into Topspin:
  - Band-selective versions of 2D HSQC and HMBC
  - 1D selective versions of standard 2D homonuclear experiments
    - COSY
    - TOCSY
    - ROESY
    - NOESY

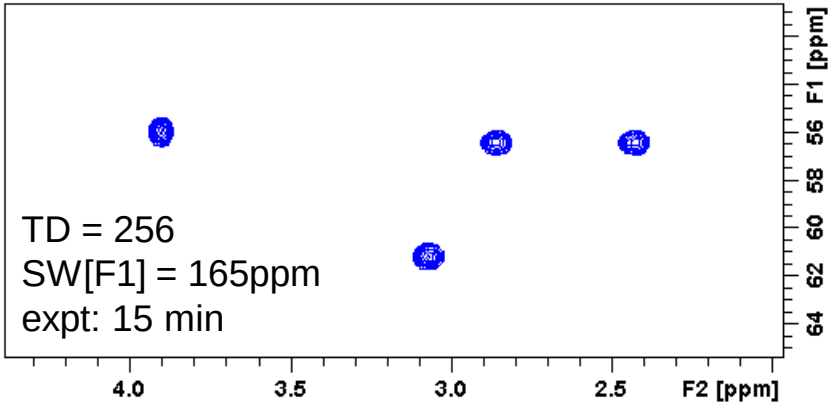
Band selective 2D experiments…
…but first, let's look at resolution again…

- We can increase the resolution of the indirect dimension of a 2D by reducing the sweep width



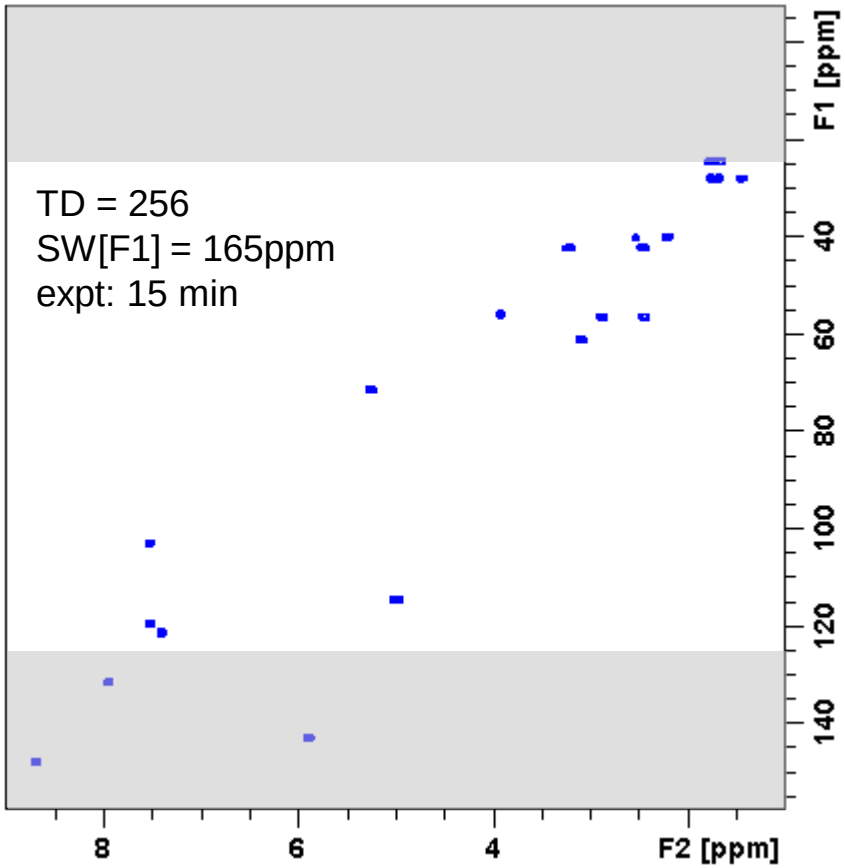


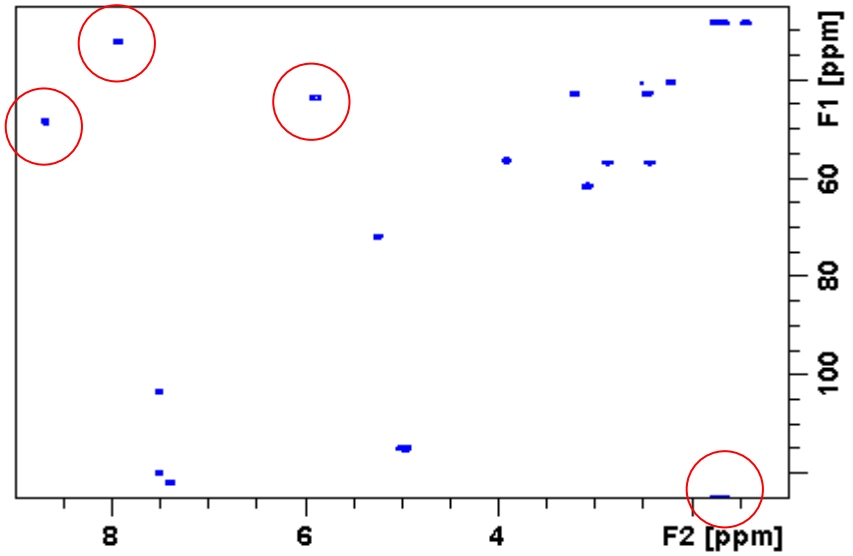




How far can we reduce the sweep width?

- Resolution improves with reduced sweep width, but aliased peaks can cause confusion
- At a certain point, peaks will start to overlap





TD = 256
SW[F1] = 100ppm
expt: 15 min

Band-selective 2D experiments

- What if we can prevent peaks outside our sweep width from folding or aliasing back into our spectrum?

  → Use band-selective excitation or refocusing pulses in the indirect dimension

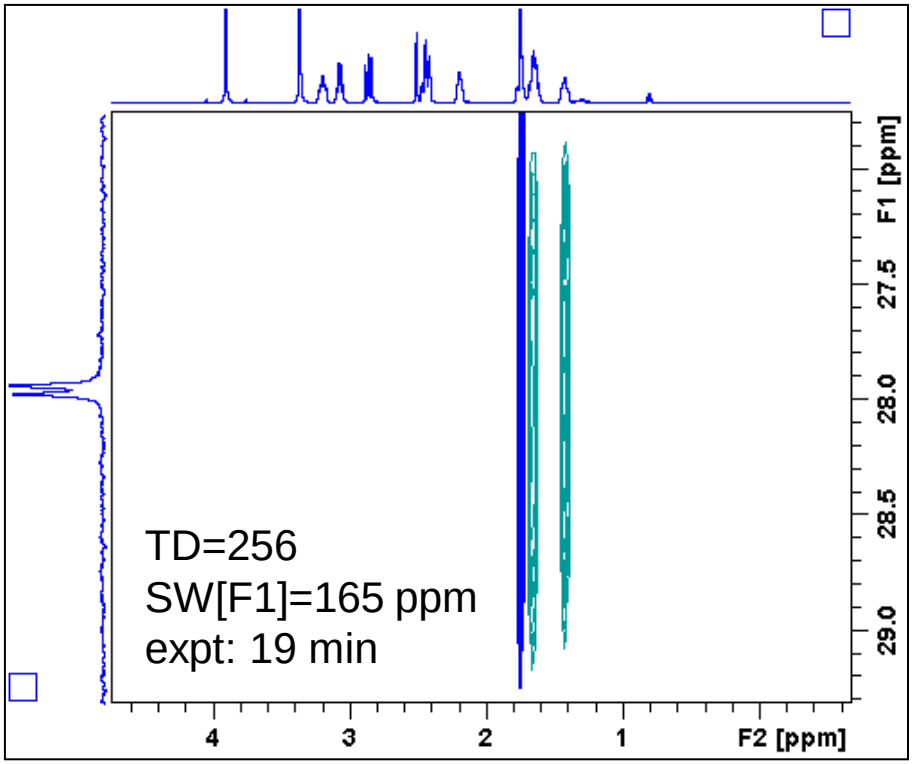
  - We can focus on one region of the spectrum
  - Peaks outside the sweep width are never excited and therefore don't get in our way

- Topspin contains band-selective versions of
  - 2D ^{1}H-^{13}C HSQC and 2D ^{1}H-^{13}C HMBC

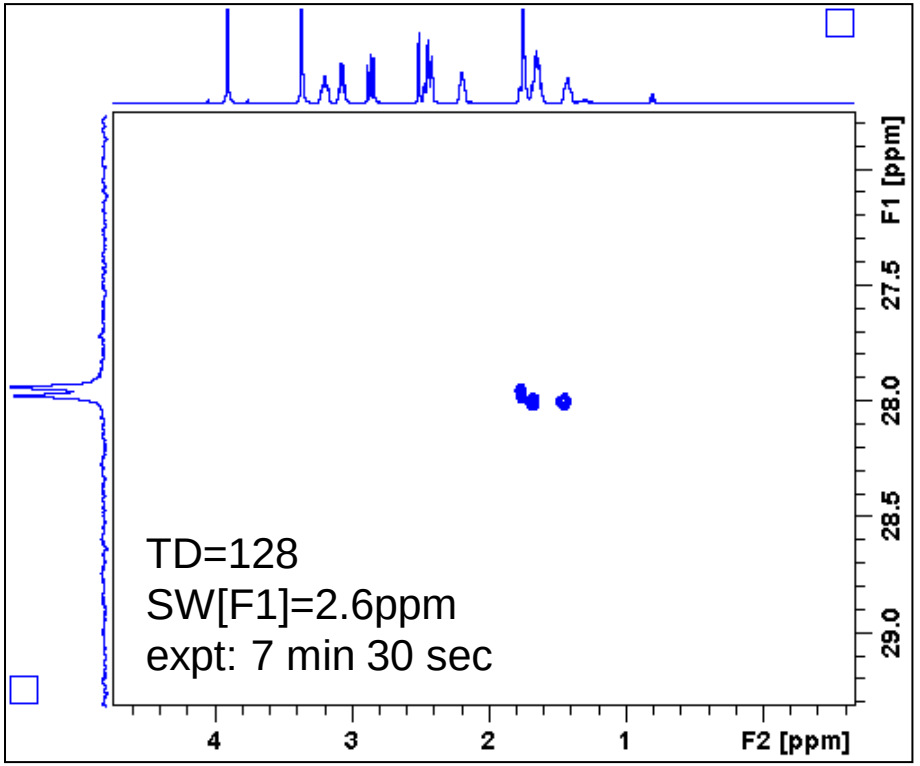
Band-selective 2D HSQC

- Let's focus on the peaks at δ ^{13}C = 28 ppm

standard edited HSQC



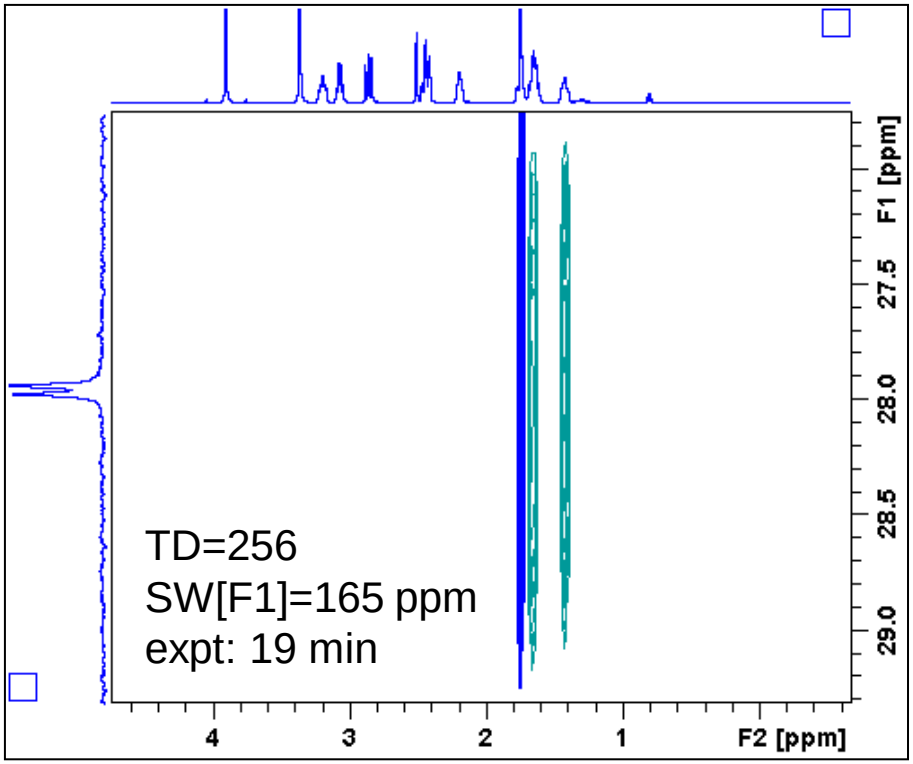
band-selective HSQC



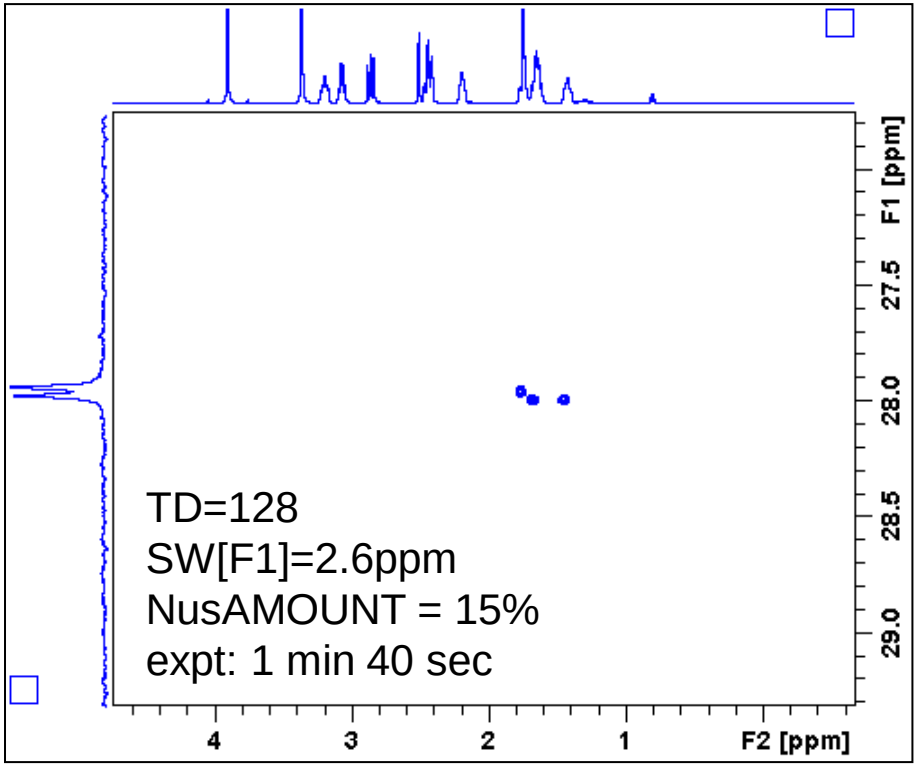
Band-selective 2D HSQC… combined with NUS

- Let's focus on the peaks at δ ^{13}C = 28 ppm

standard edited HSQC



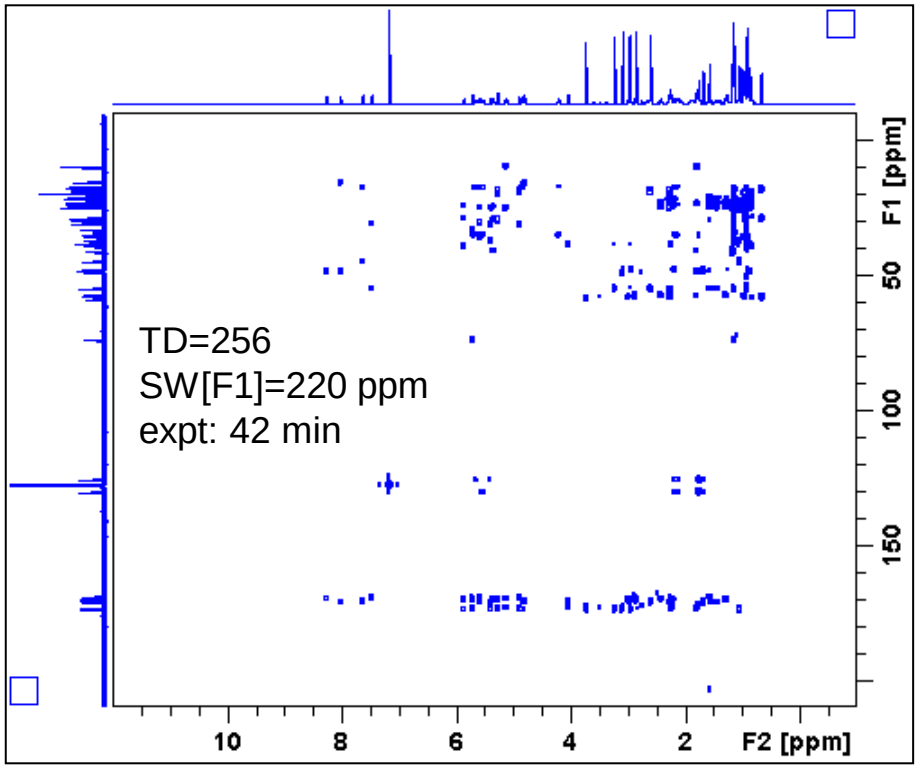
band-selective HSQC, using NUS



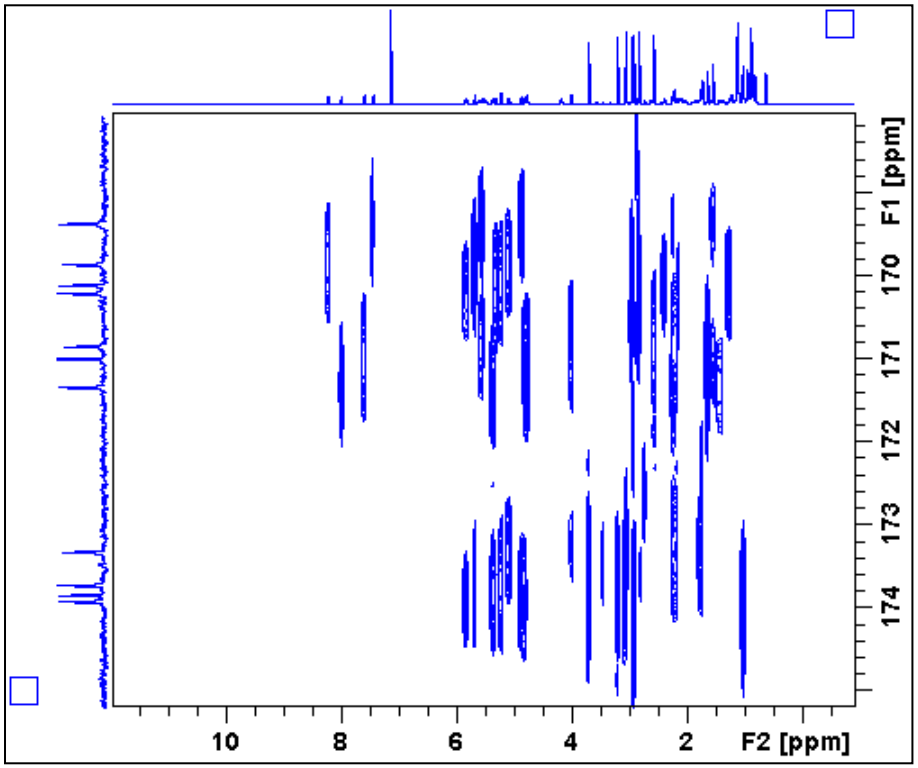
Band-selective 2D HMBC example

- 50mM cyclosporine in benzene-d6

standard HMBC



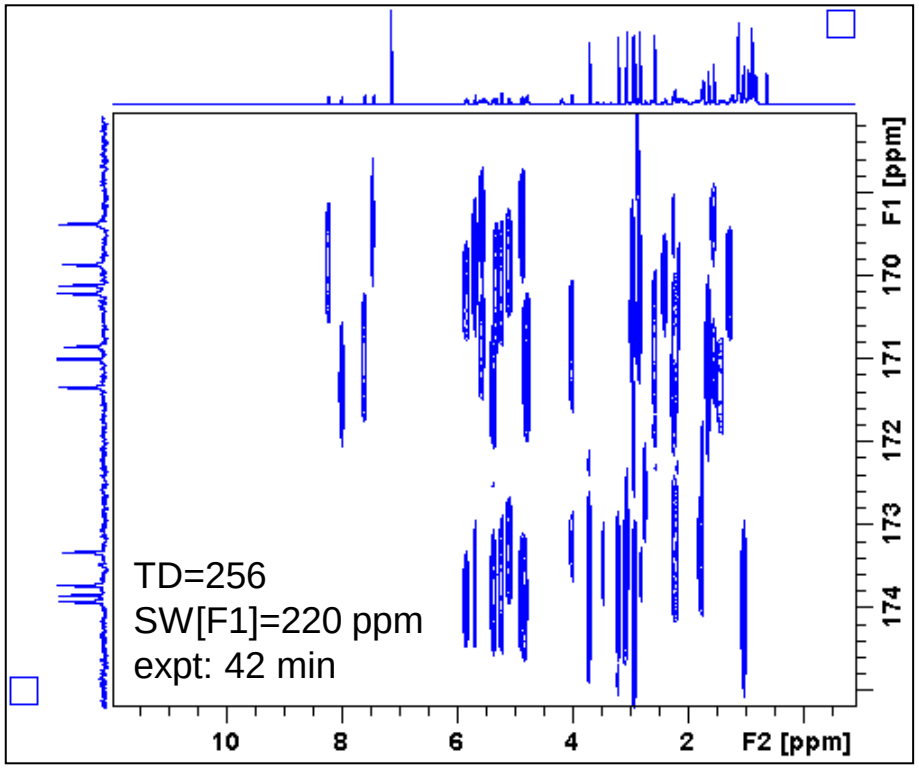
expansion of CO region



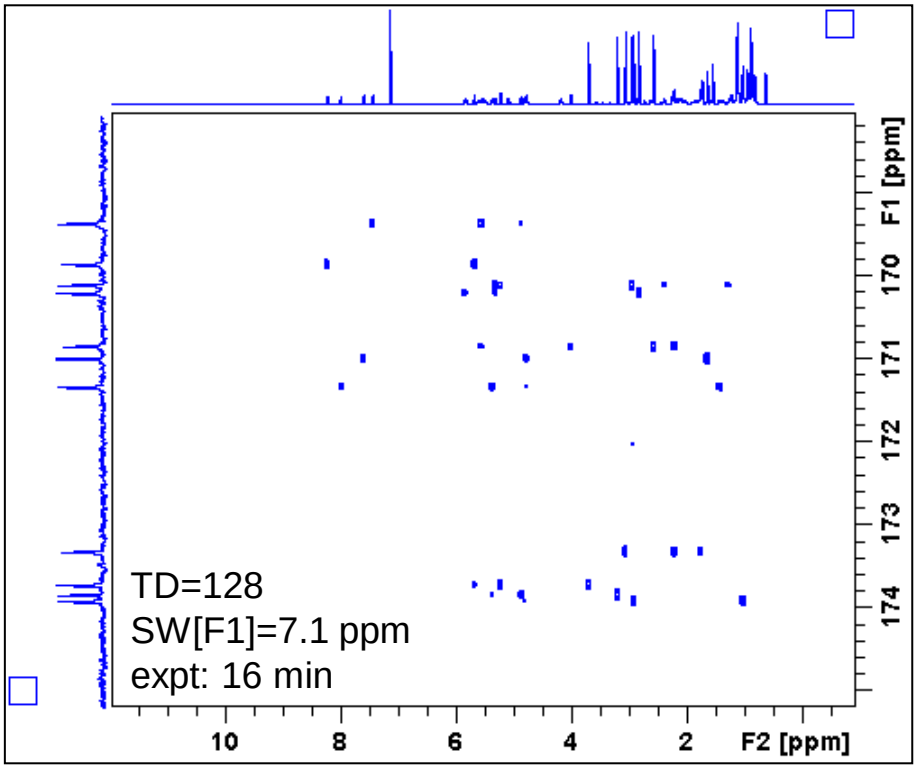
Band-selective 2D HMBC example

- 50mM cyclosporine in benzene-d6

standard HMBC - expansion of CO region

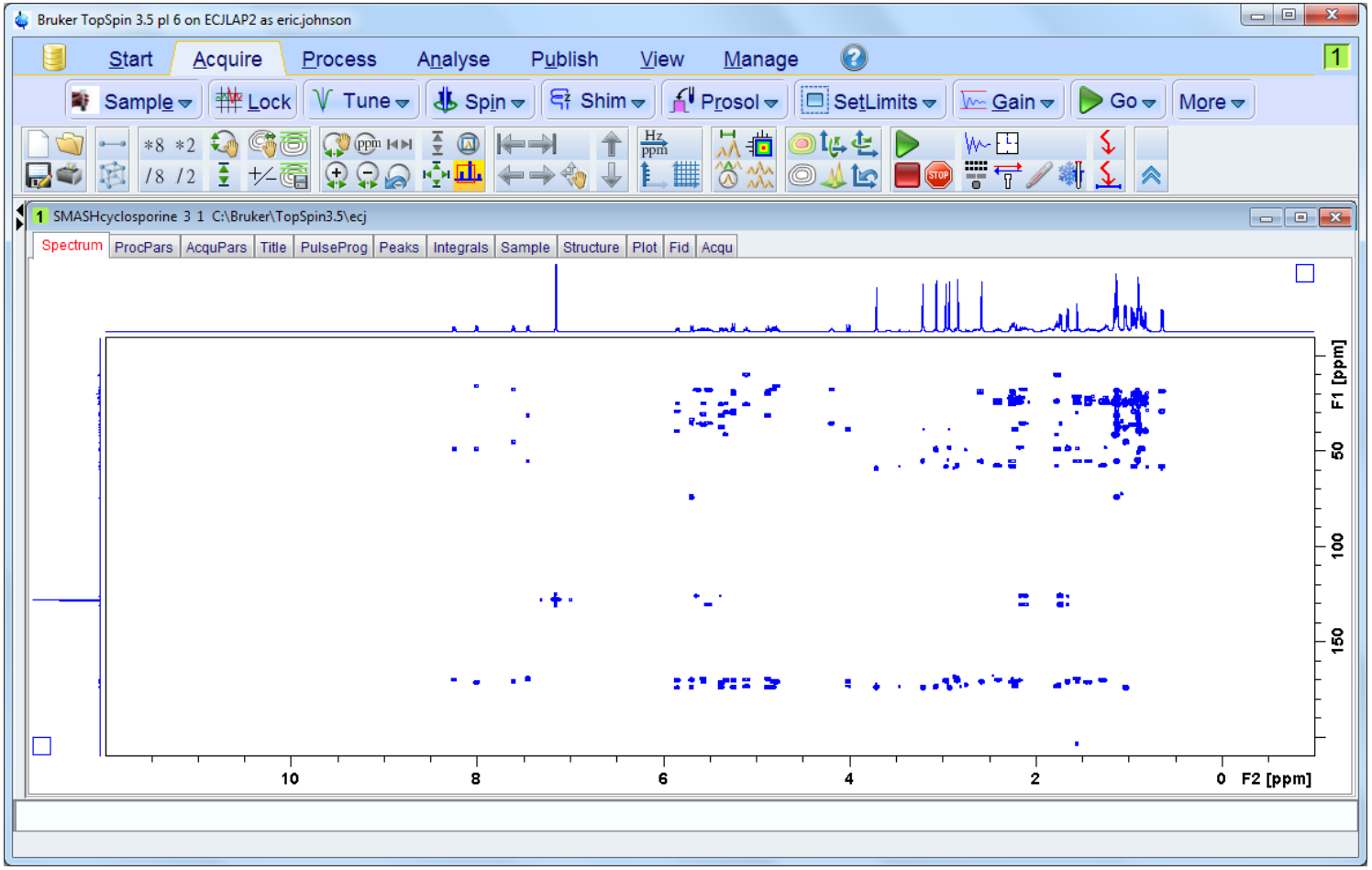


band-selective HMBC



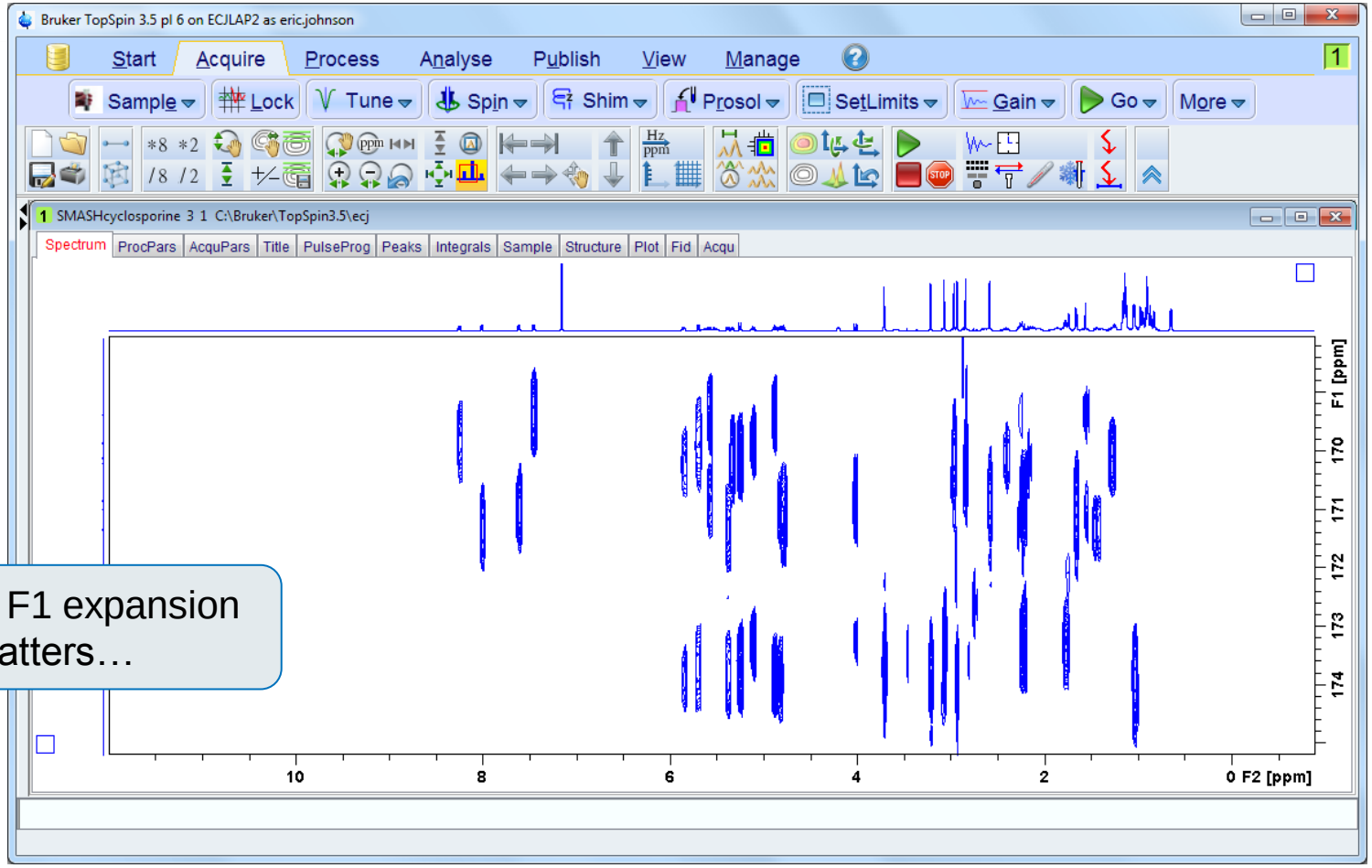
Setting up the band-selective 2D experiments

Method 1 – starting from a non-selective HSQC or HMBC



Setting up the band-selective 2D experiments

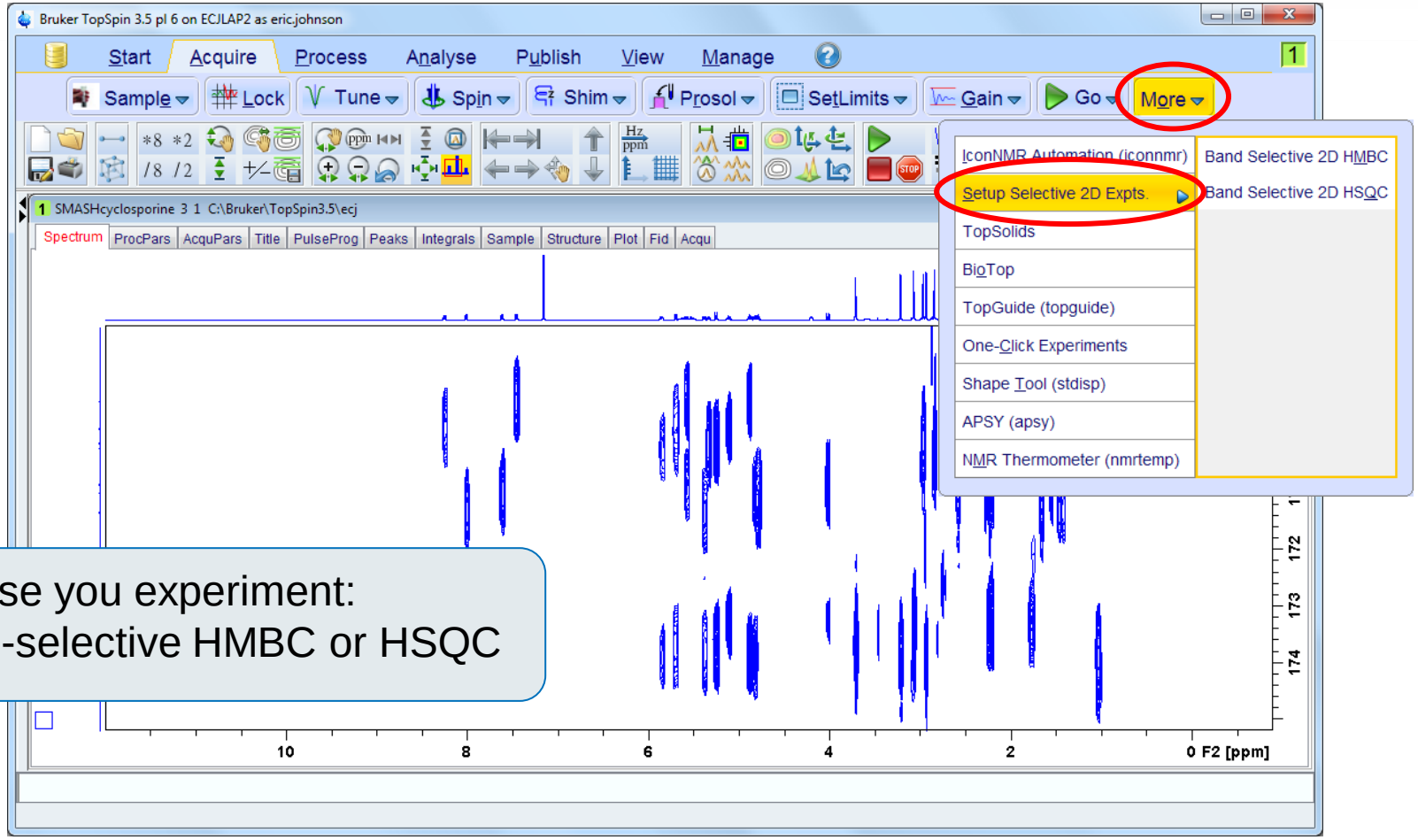
ii) zoom into the spectral region of interest



only the F1 expansion matters…

Setting up the band-selective 2D experiments

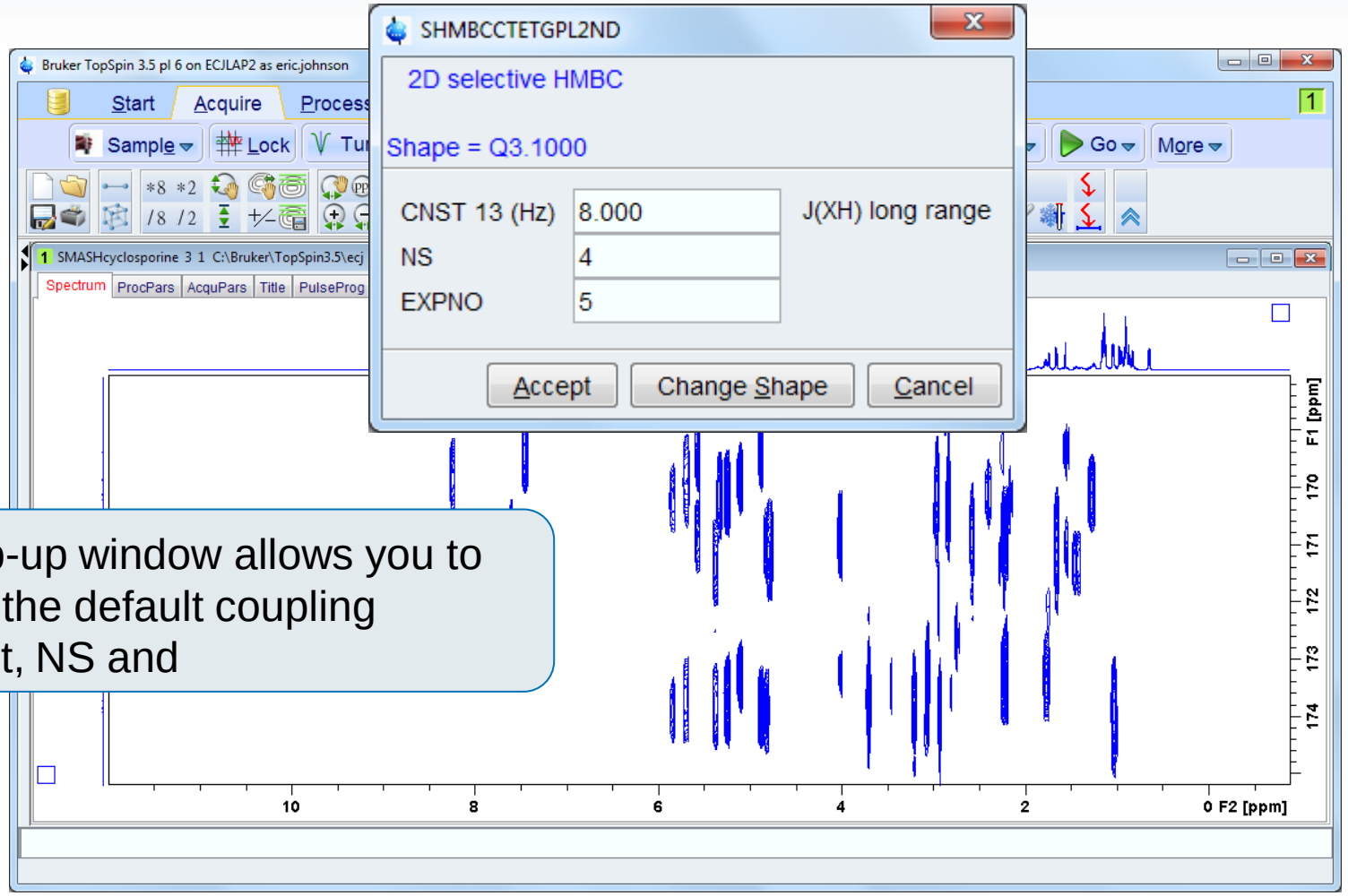
iii) choose “Setup Selective 2D Extps” under “More” in the “Acquire” flow-bar



iv) choose you experiment:

- Band-selective HMBC or HSQC

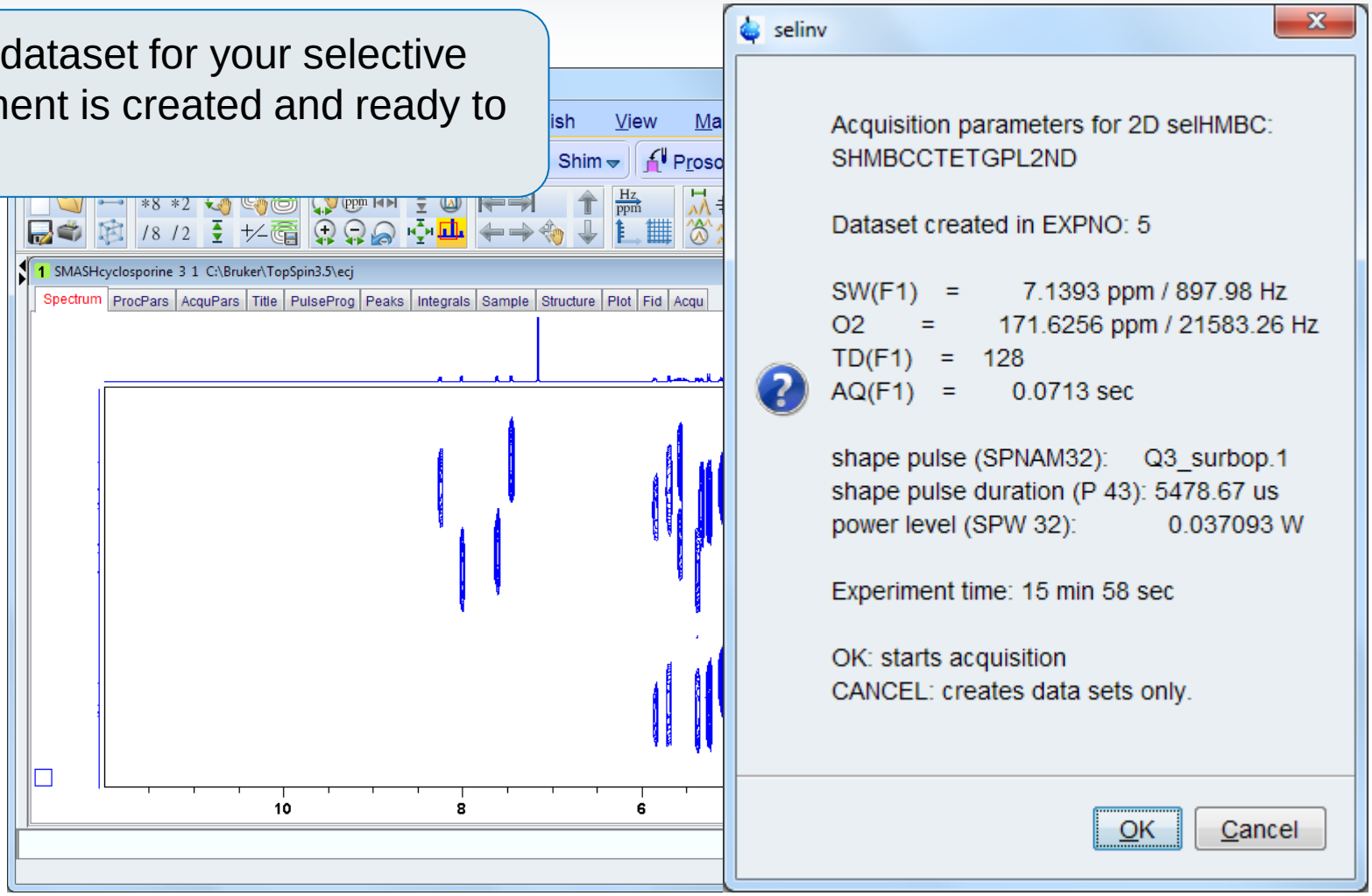
Setting up the band-selective 2D experiments



v) A pop-up window allows you to change the default coupling constant, NS and

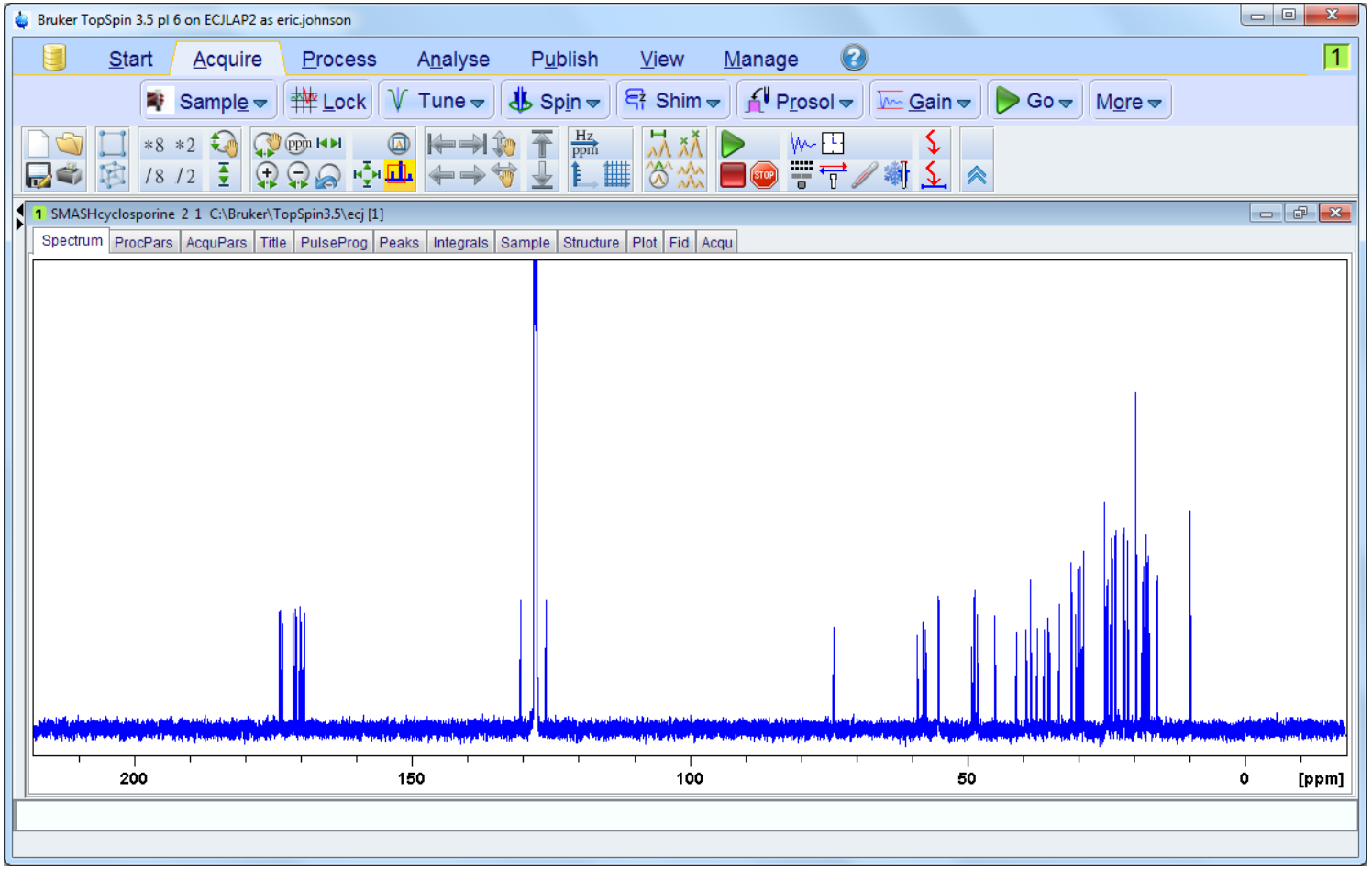
Setting up the band-selective 2D experiments

vi) The dataset for your selective experiment is created and ready to run



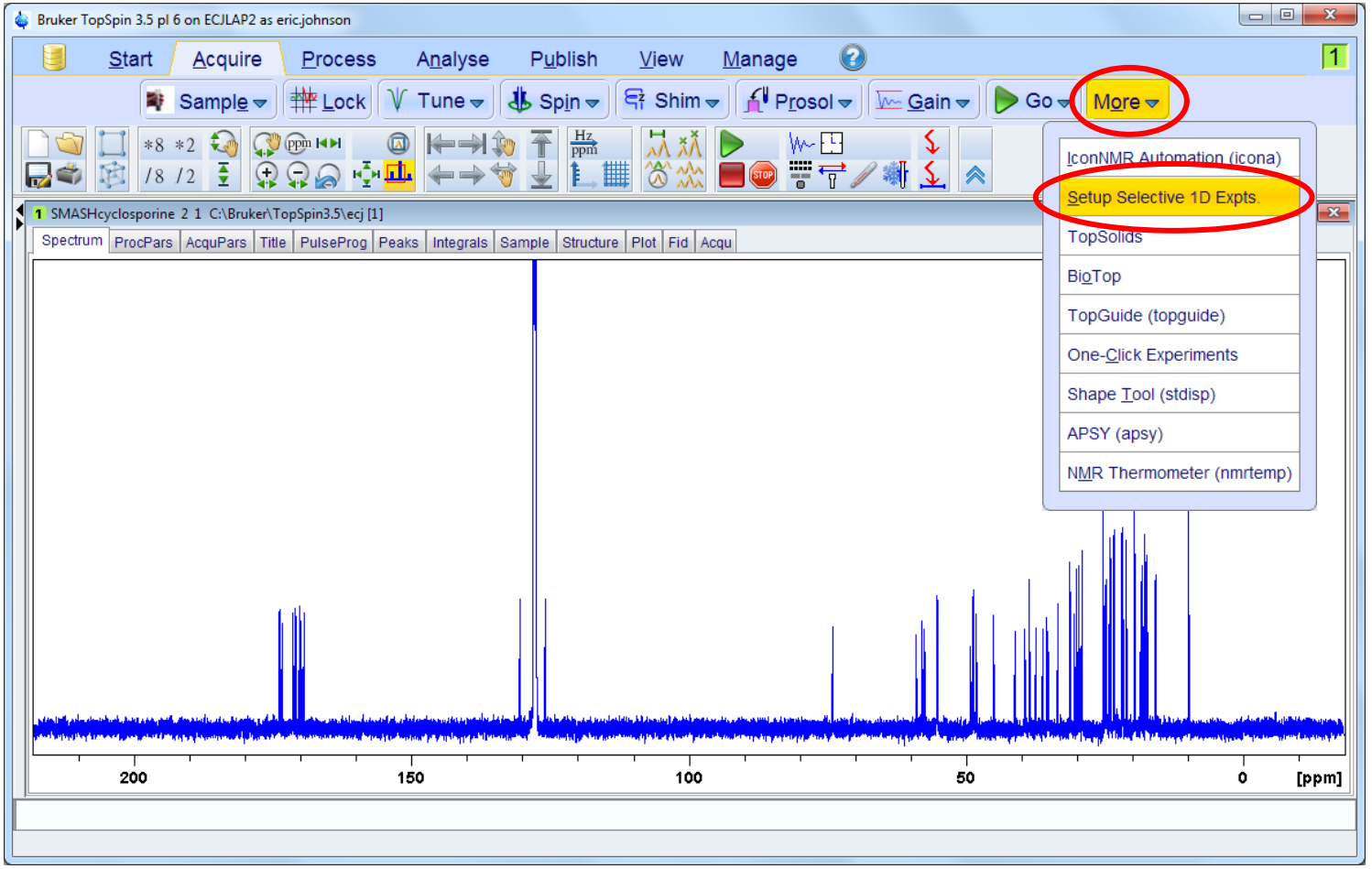
Setting up the band-selective 2D experiments

Method 2 – starting from a 1D 13C spectrum



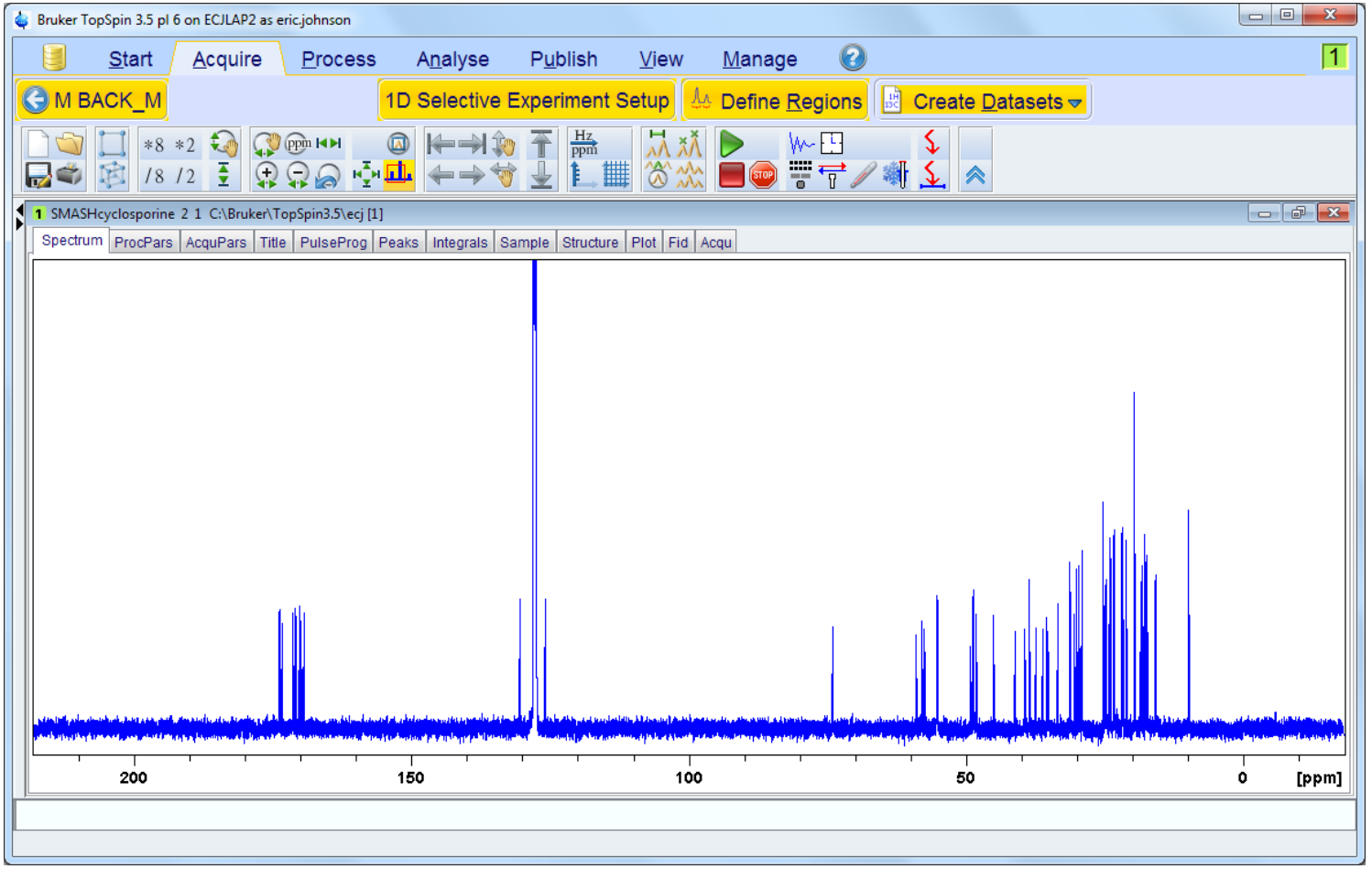
Setting up the band-selective 2D experiments

ii) choose “Setup Selective 1D Extps” under “More” in the “Acquire” flow-bar



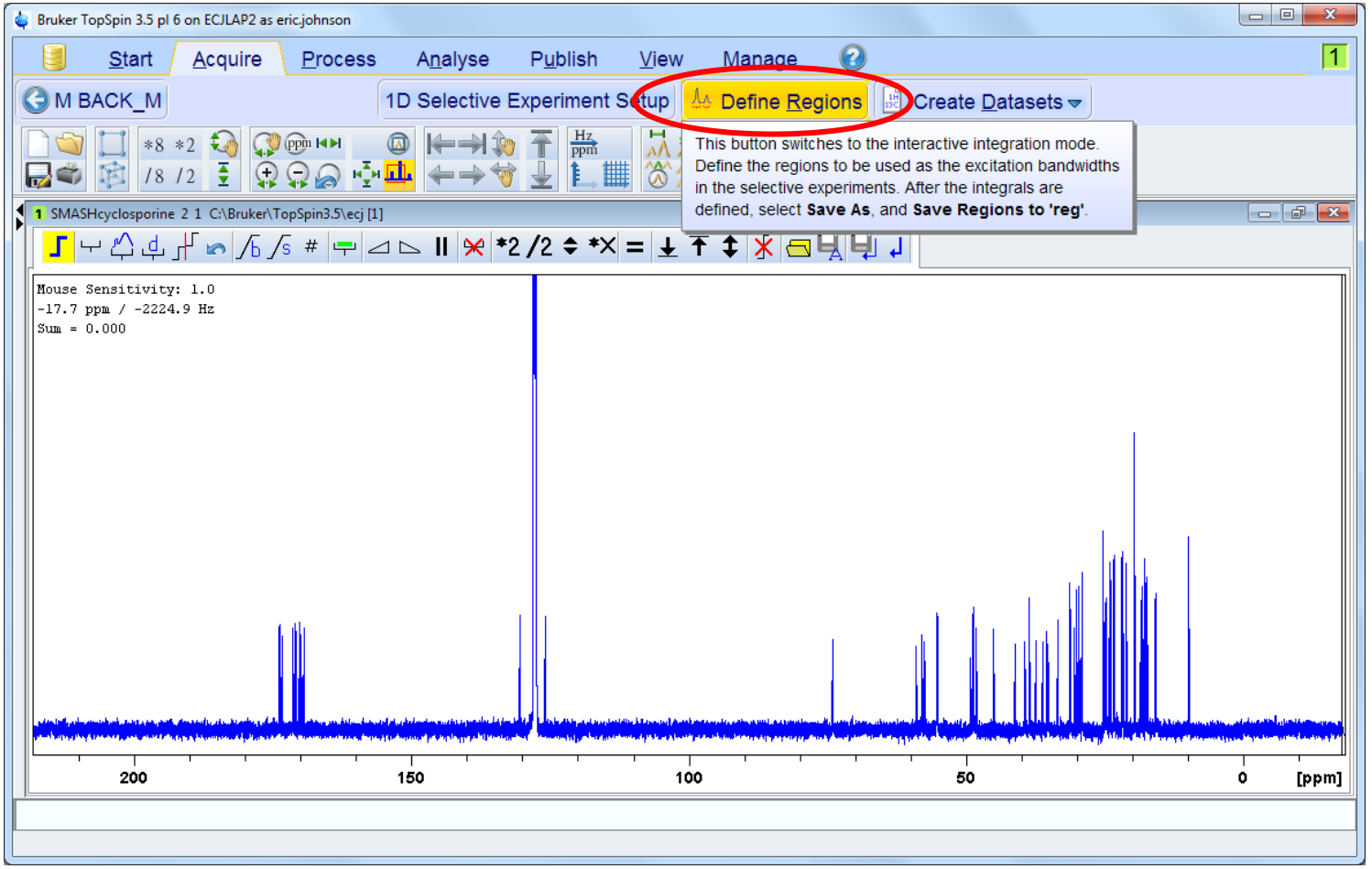
Setting up the band-selective 2D experiments

ii) choose “Setup Selective 1D Extps” under “More” in the “Acquire” flow-bar



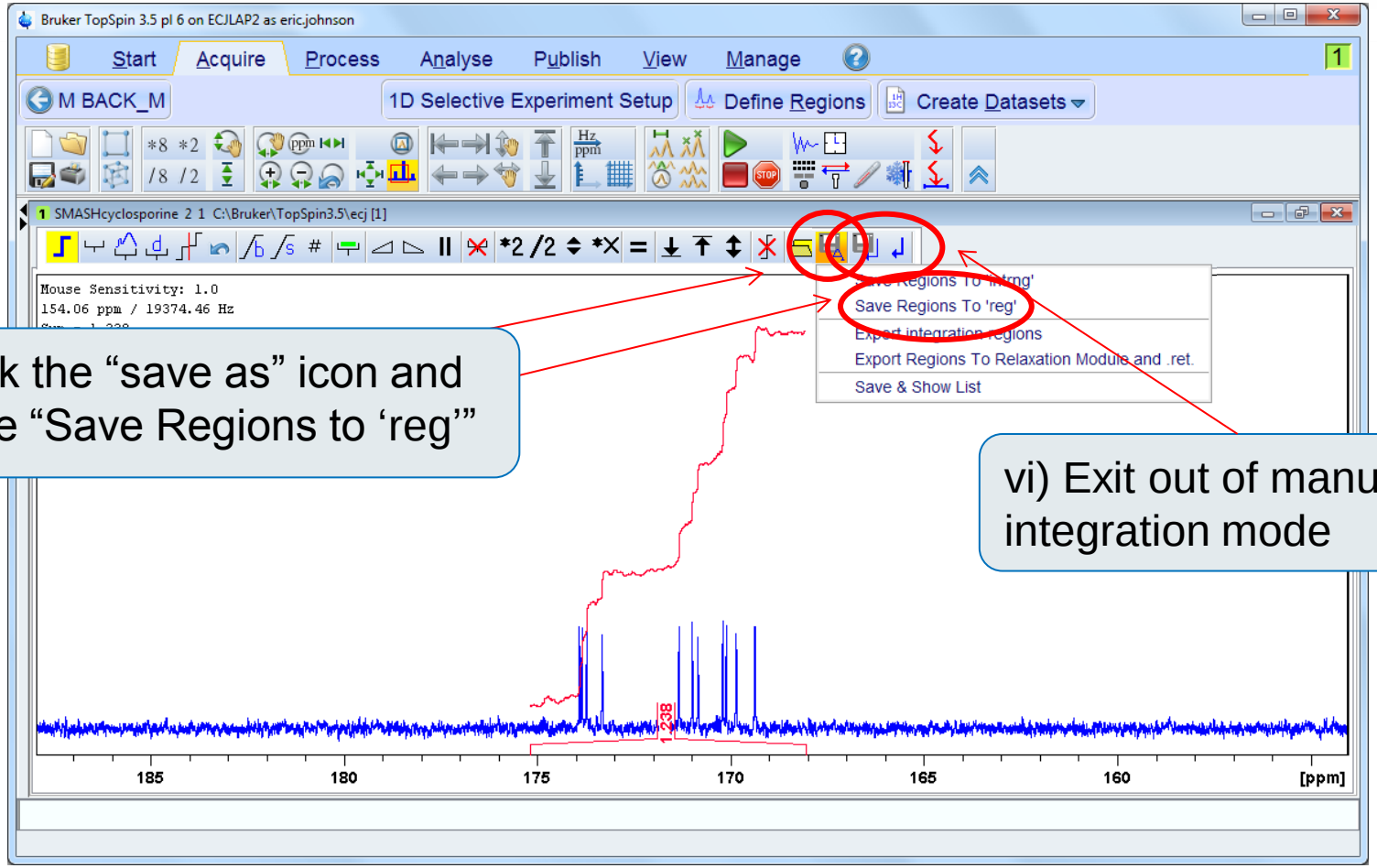
Setting up the band-selective 2D experiments

iii) choose “Define Regions” to start the manual integration mode



Setting up the band-selective 2D experiments

iv) integrate the range to use as your F1 sweep width

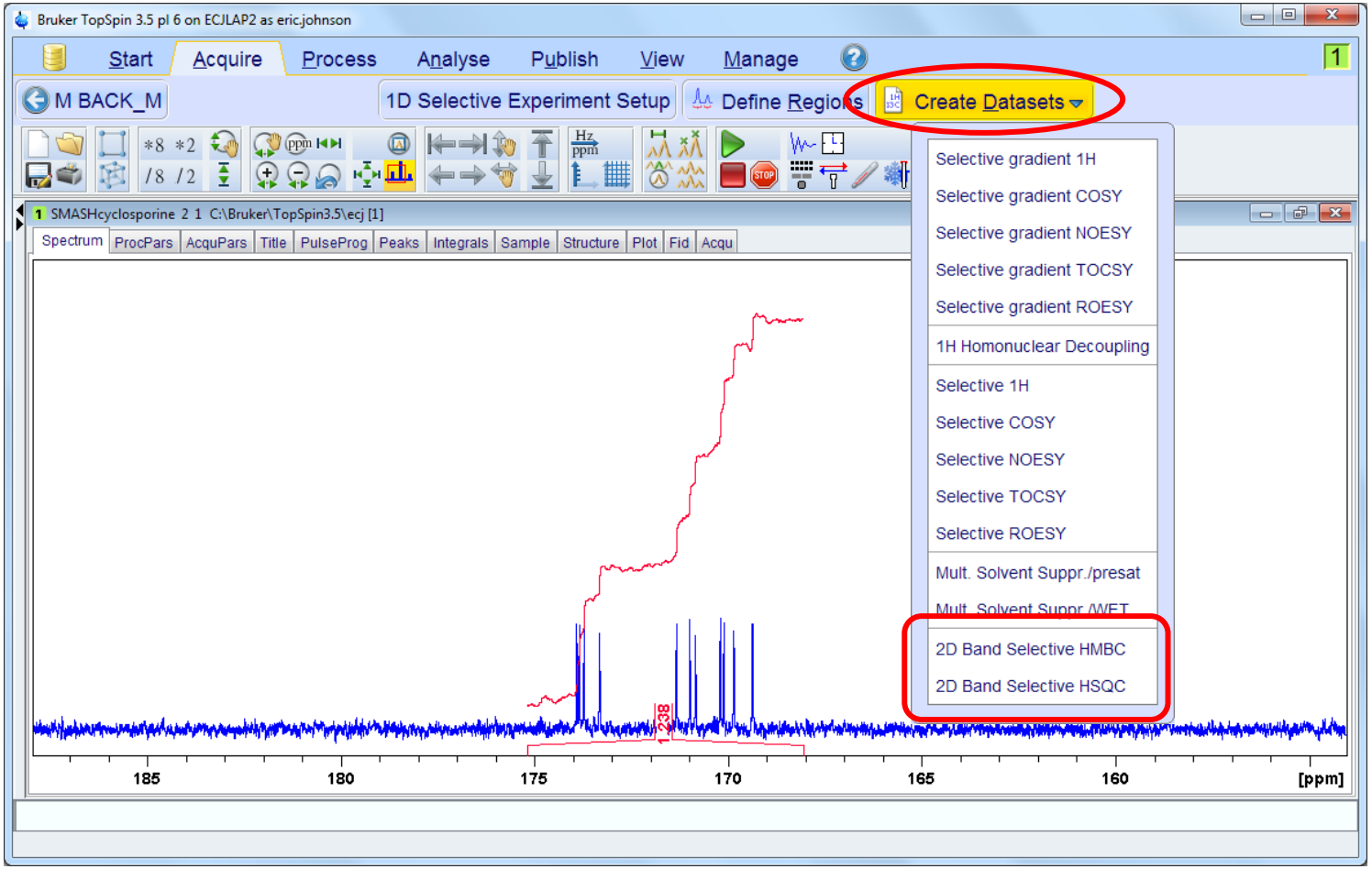


v) Click the “save as” icon and choose “Save Regions to ‘reg’”

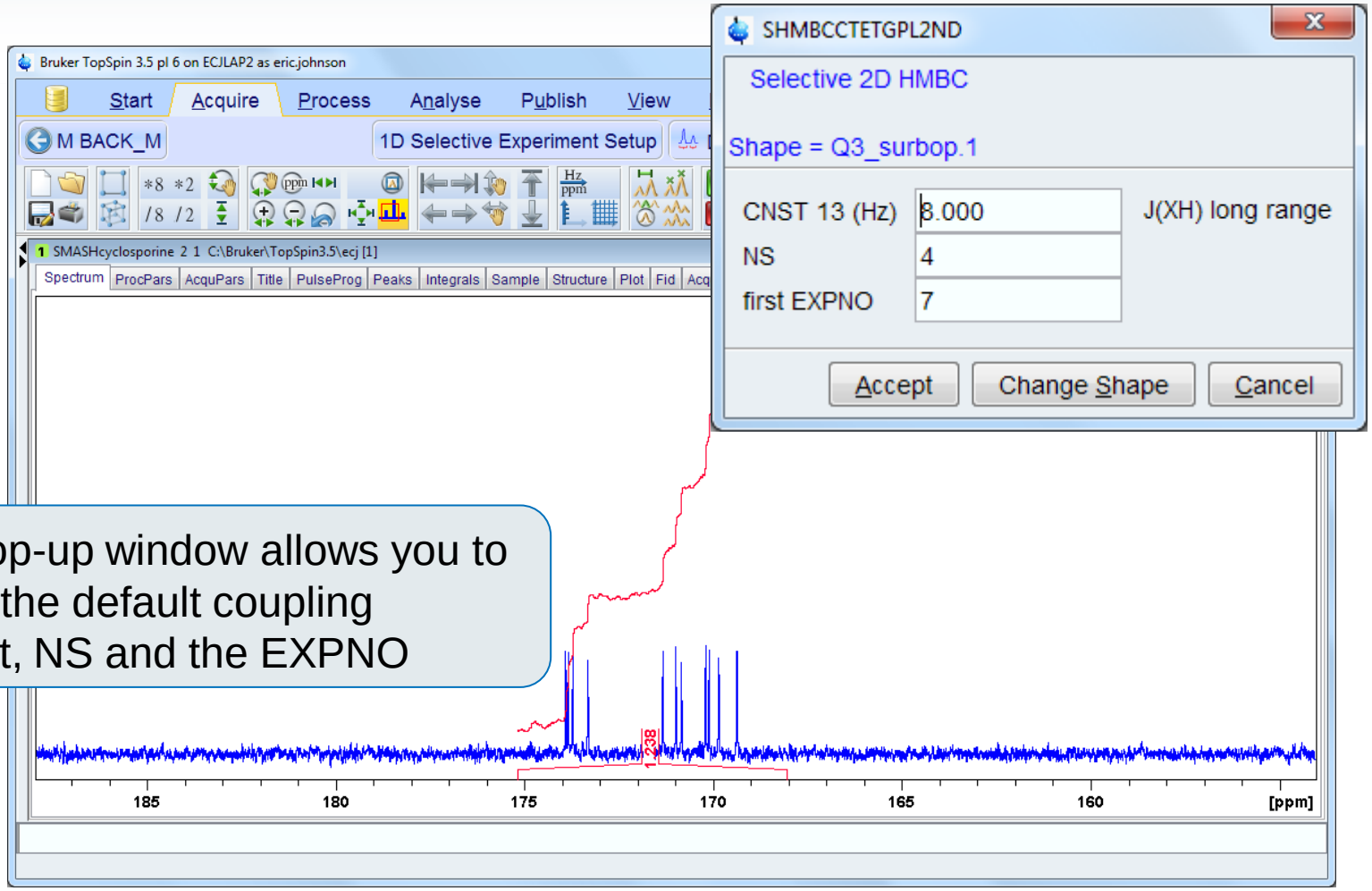
vi) Exit out of manual integration mode

Setting up the band-selective 2D experiments

vii) Select your experiment under the “Created Datasets” tab

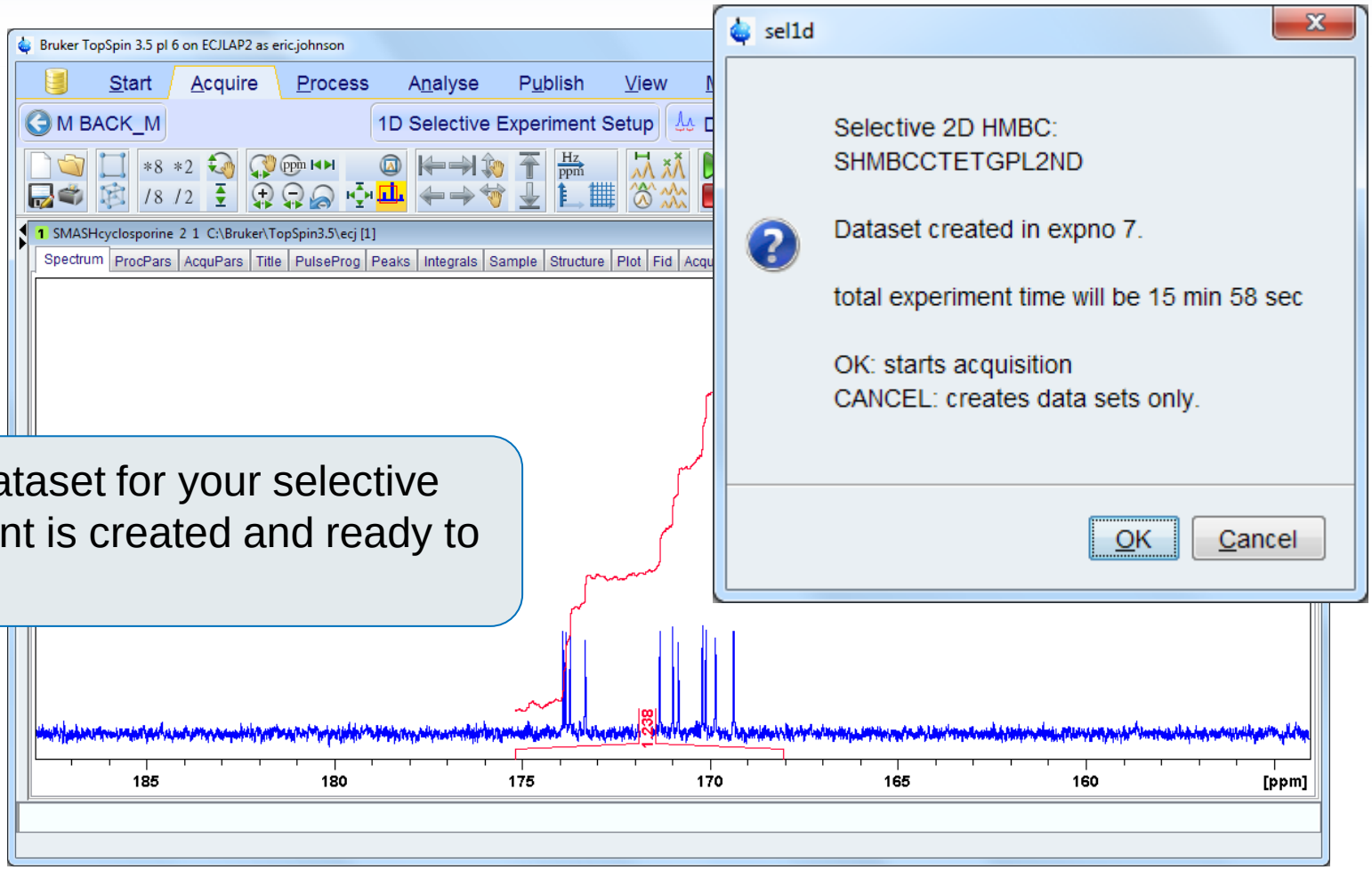


Setting up the band-selective 2D experiments



viii) A pop-up window allows you to change the default coupling constant, NS and the EXPNO

Setting up the band-selective 2D experiments



ix) The dataset for your selective experiment is created and ready to run

A few tips for the band-selective 2D experiments

- Keep your SW[F1] greater than a couple ppm
  - as the SW decreases, the duration of the band-selective pulse increases
  - at a certain point, you'll start loosing signal due to relaxation and diffusion

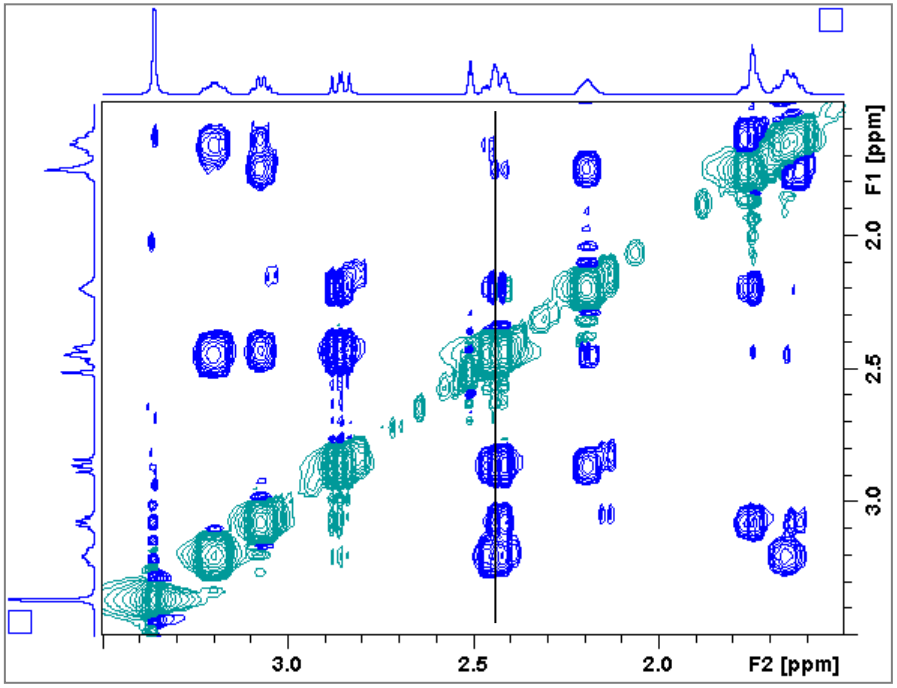
- Don't run "getprosol" on your selective dataset after it's been created with this interface
  - "getprosol" will read default selective pulse parameters from the prosol table, overwriting those calculated based on your SW

- Don't over-interpret the presence or absence of peaks near the edges of your band-selective experiment in the F1 dimension
  - The band-selective pulse isn't a "perfect" shape
    - real correlations near the edges may be attenuated
    - strong correlation just outside the SW may be aliased in

Why use 1D selective experiments?

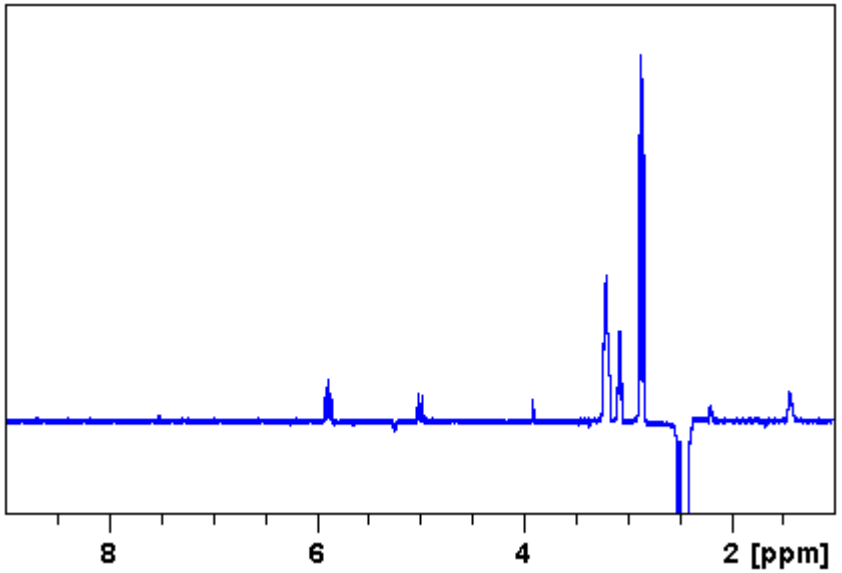
- When we're looking for a specific correlation
  - Much shorter acquisition time than full 2D

Expansion of 2D NOESY



Expt: 1hr 36 min

1D selective NOESY



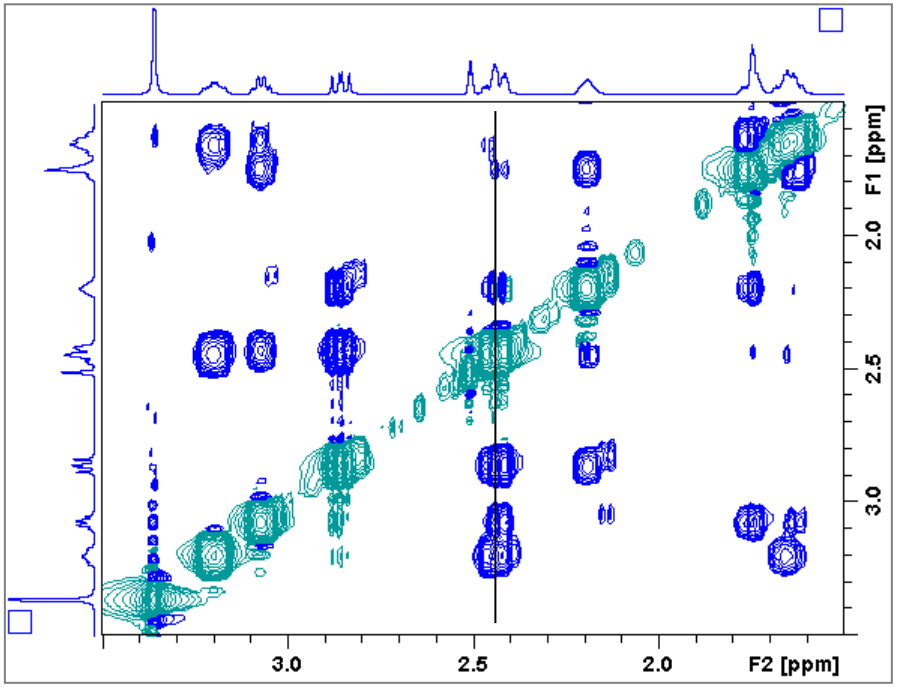
Expt: 1 min 50 sec

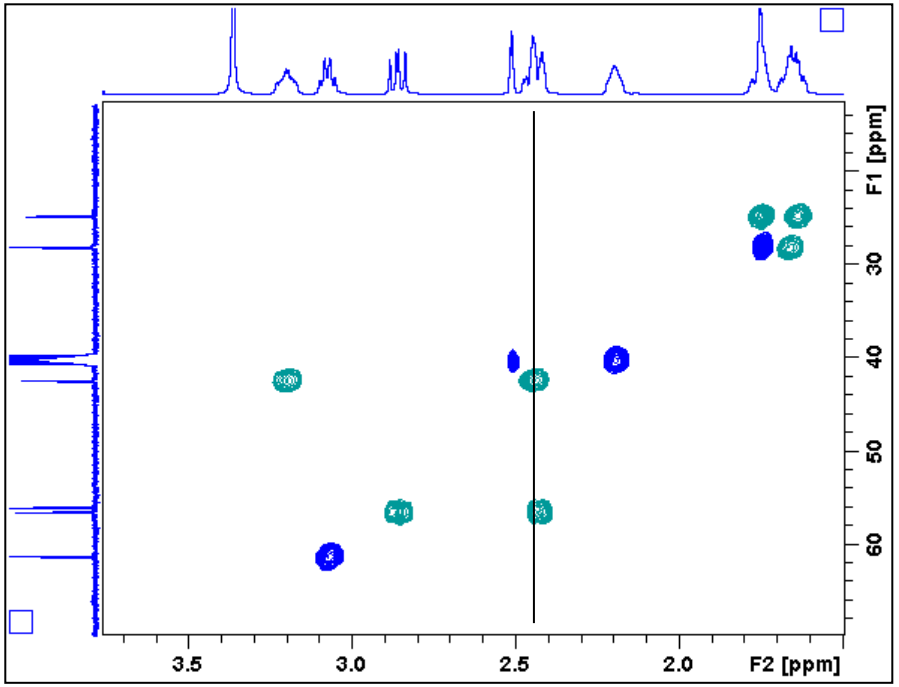
100mM quinine in DMSO-d6

1D selective NOESY example

From the HSQC, we can clearly see that the peak at 2.5ppm has 2 overlapped multiplets

Expansion of 2D NOESY



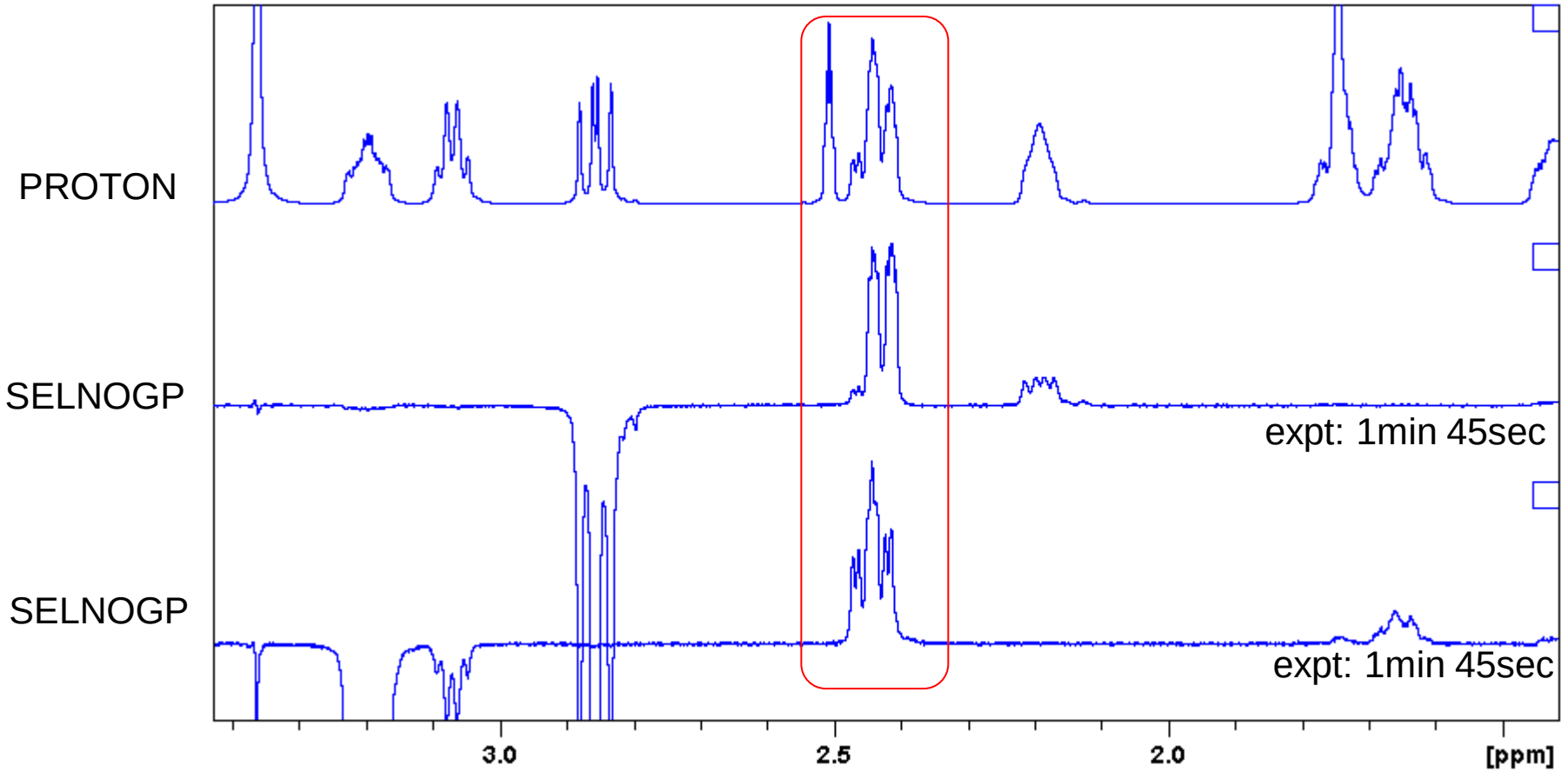


Expt: 1hr 36 min

100mM quinine in DMSO-d6

1D selective NOESY example

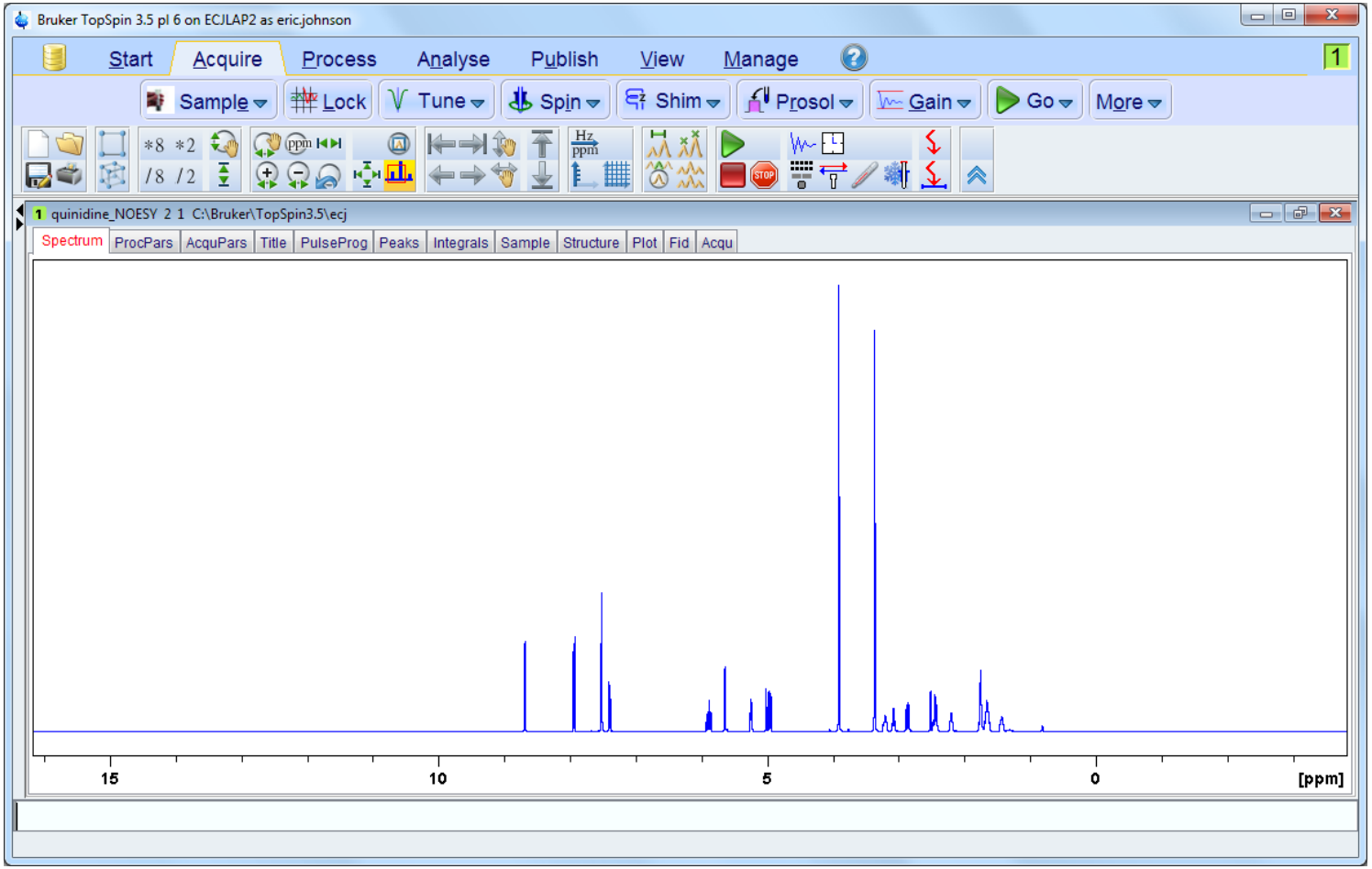
- Much higher resolution correlations in much less time!



100mM quinine in DMSO-d6

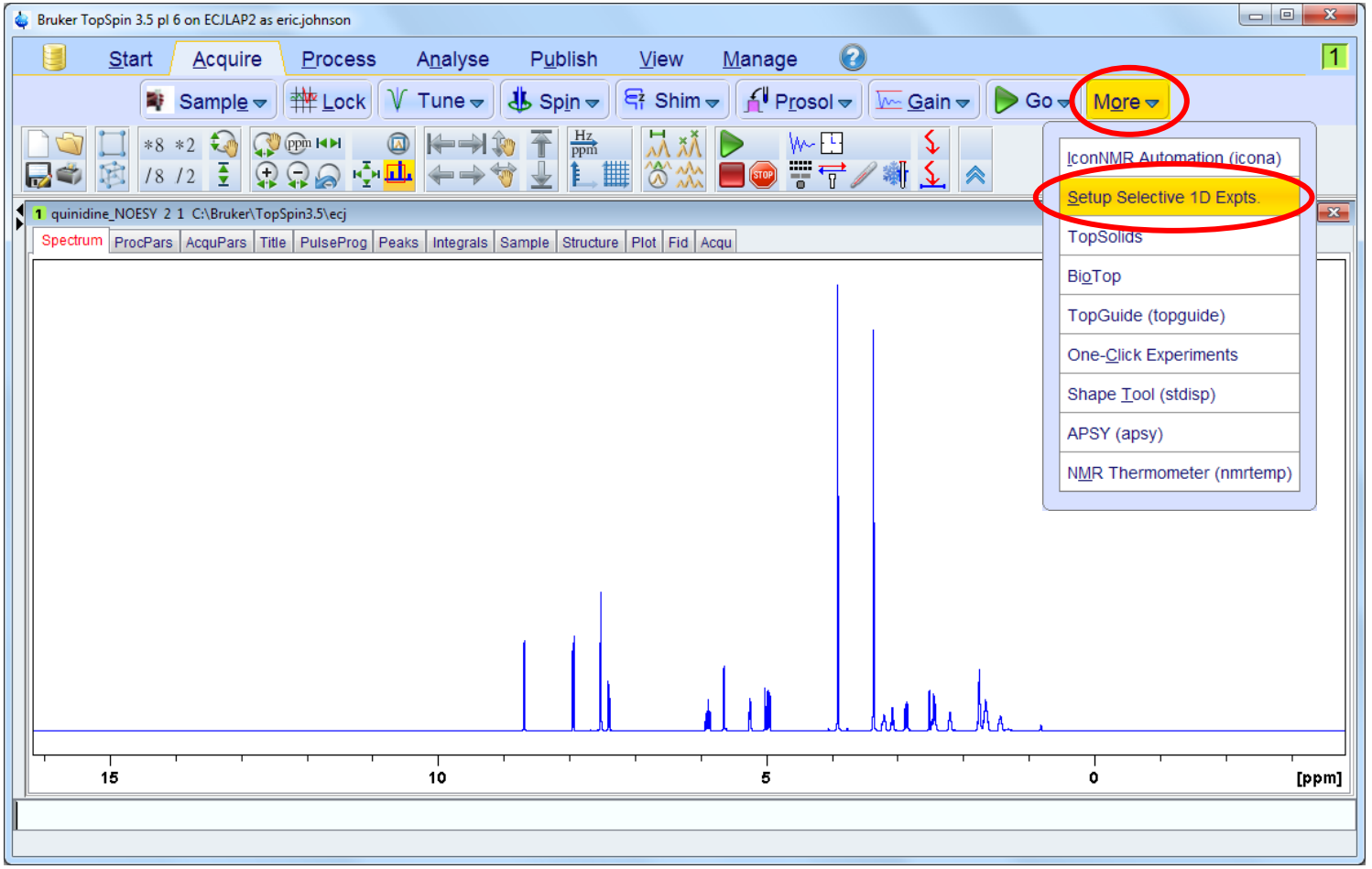
Setting up the 1D selective experiments

i) start from a 1D PROTON spectrum



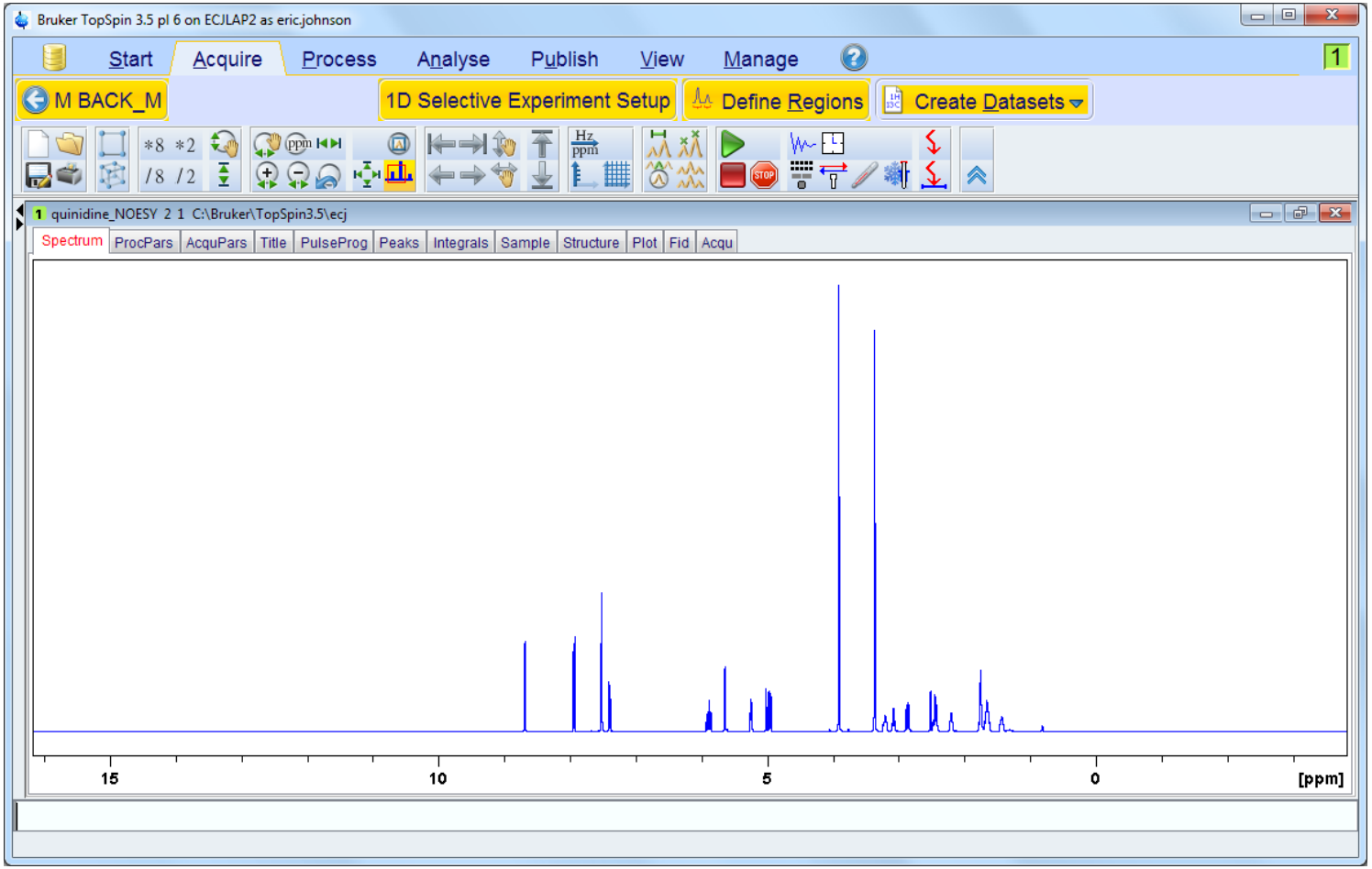
Setting up the 1D selective experiments

ii) choose “Setup Selective 1D Extps” under “More” in the “Acquire” flow-bar



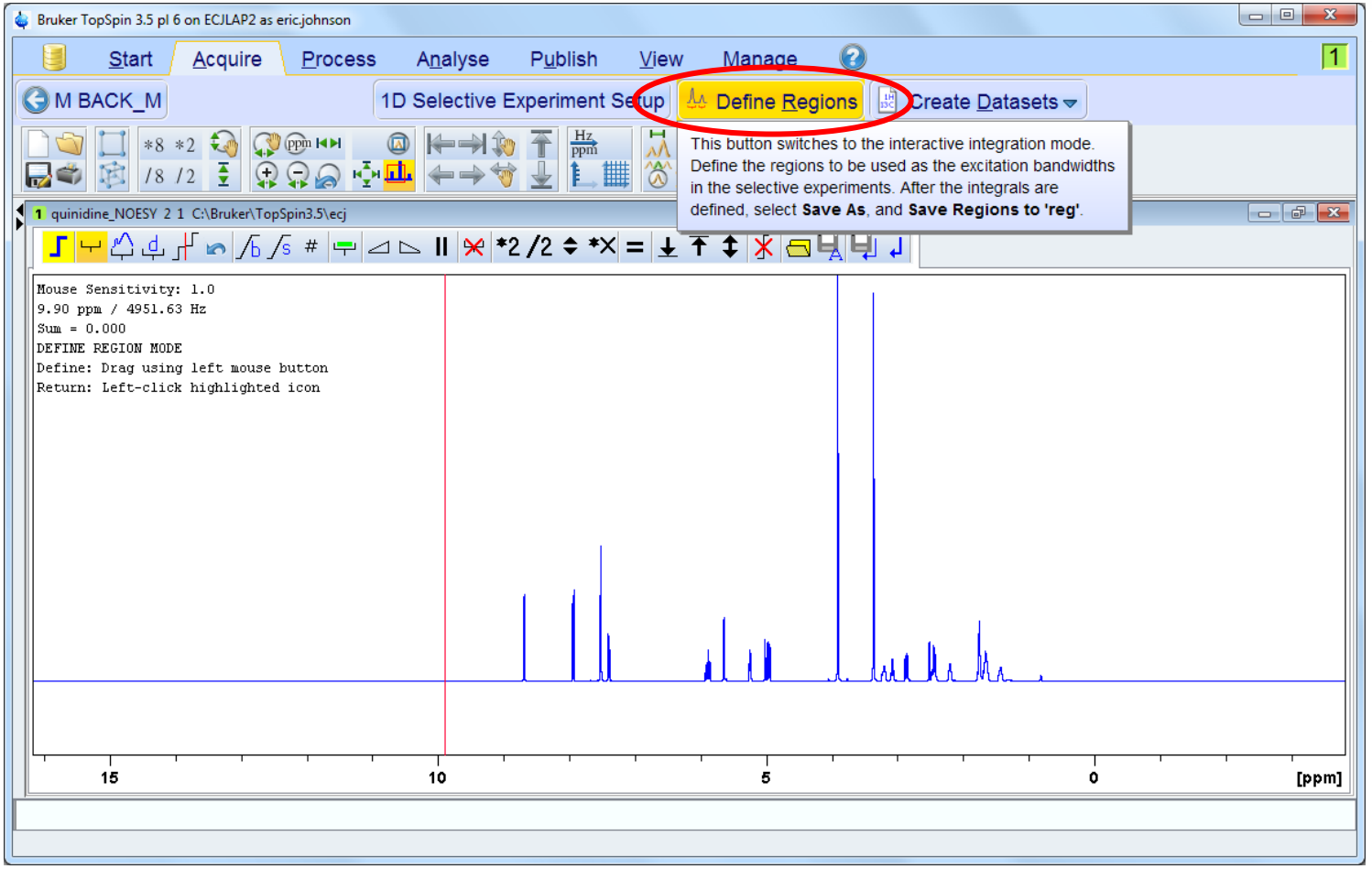
Setting up the 1D selective experiments

ii) choose “Setup Selective 1D Extps” under “More” in the “Acquire” flow-bar



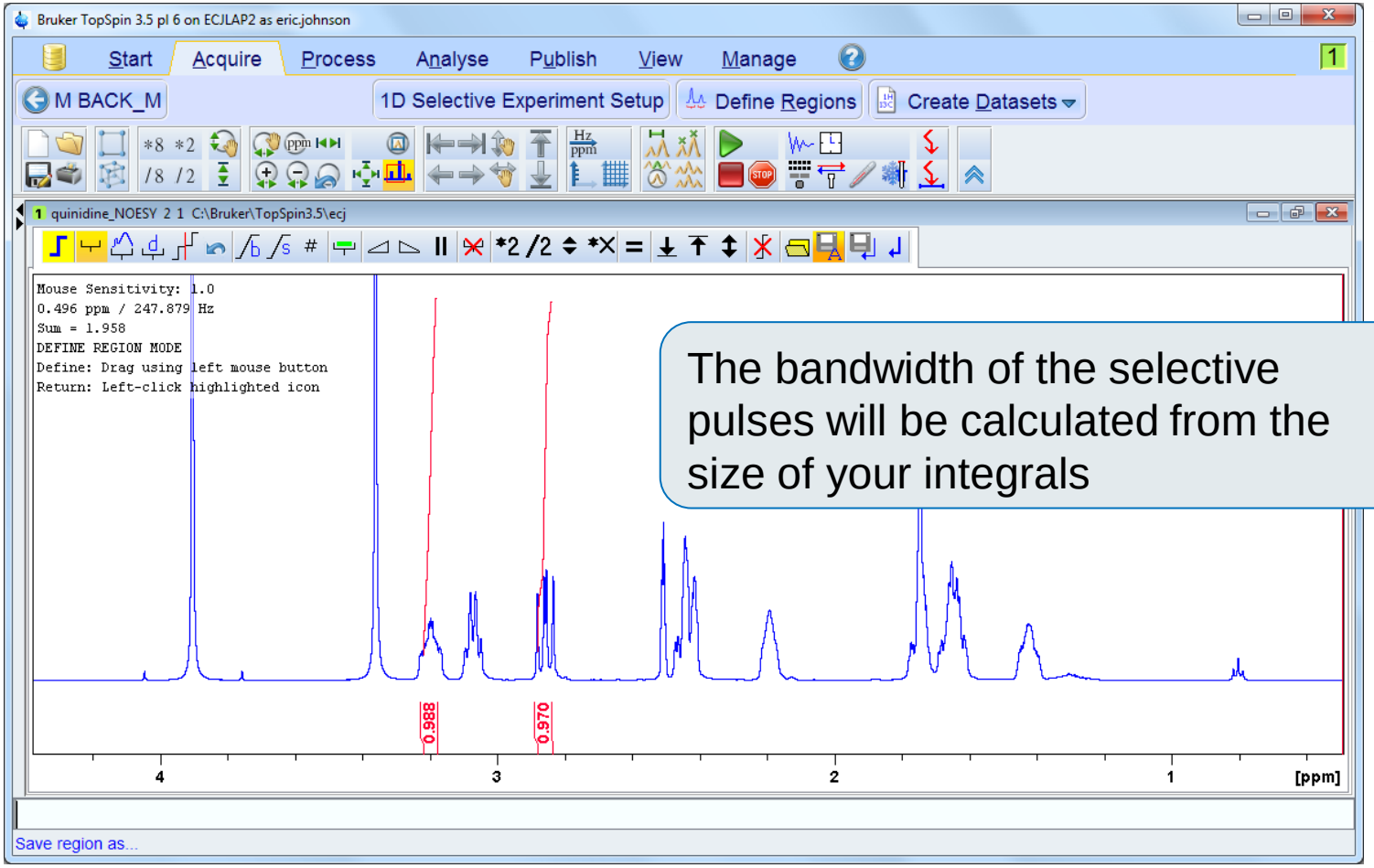
Setting up the 1D selective experiments

iii) choose “Define Regions” to start the manual integration mode

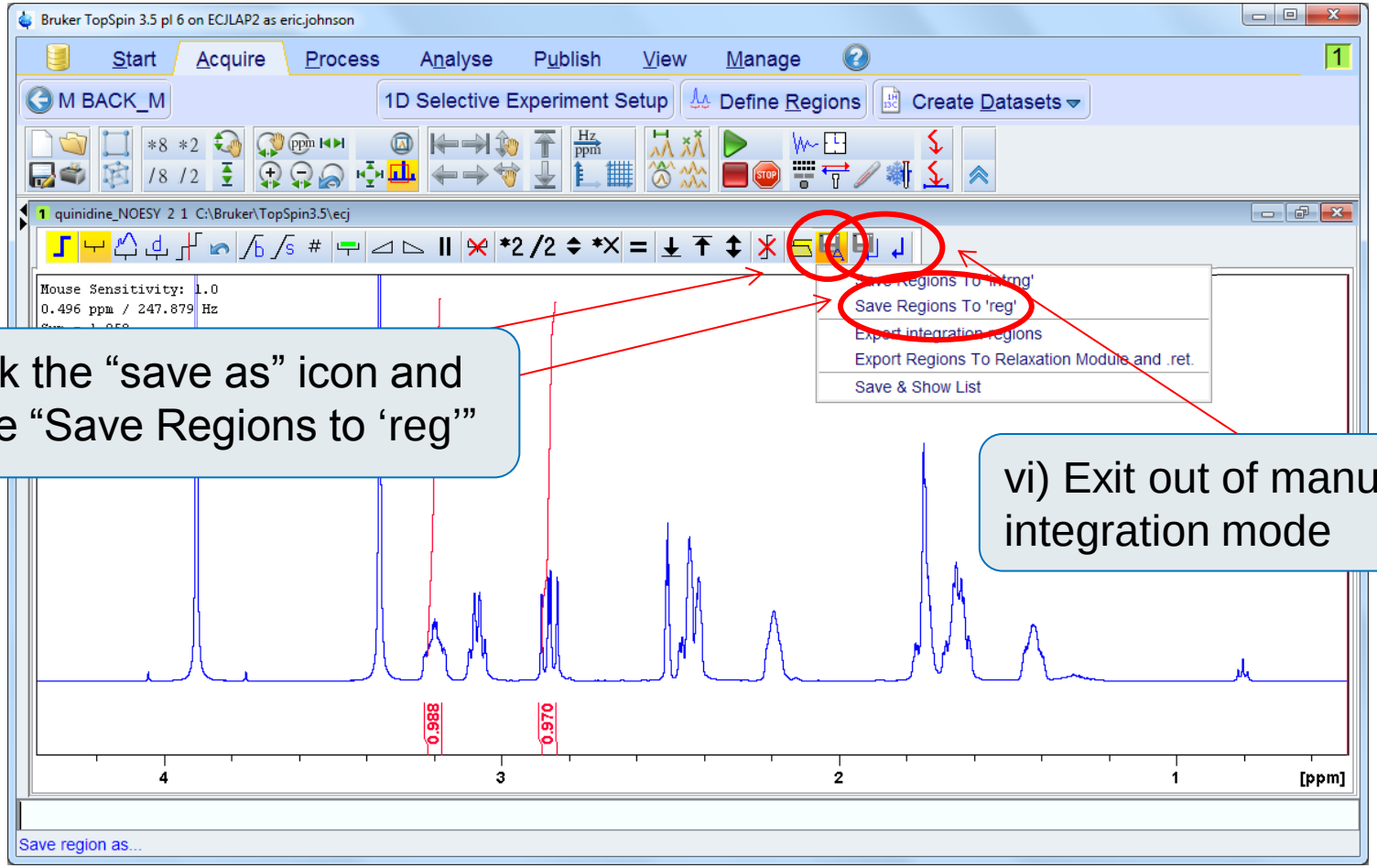


Setting up the 1D selective experiments

iv) integrate the peaks for selective excitation



Setting up the 1D selective experiments

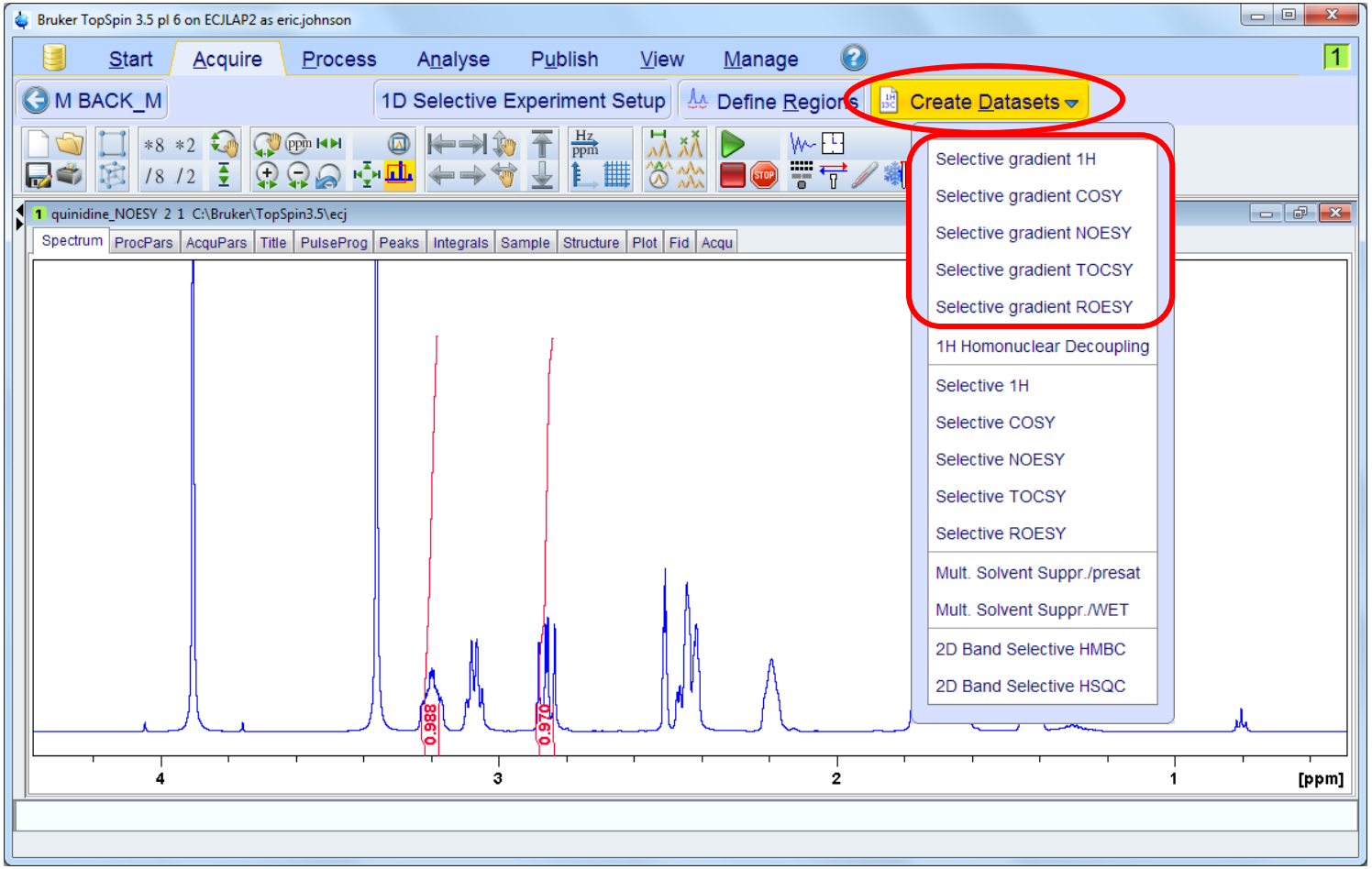


v) Click the “save as” icon and choose “Save Regions to ‘reg’”

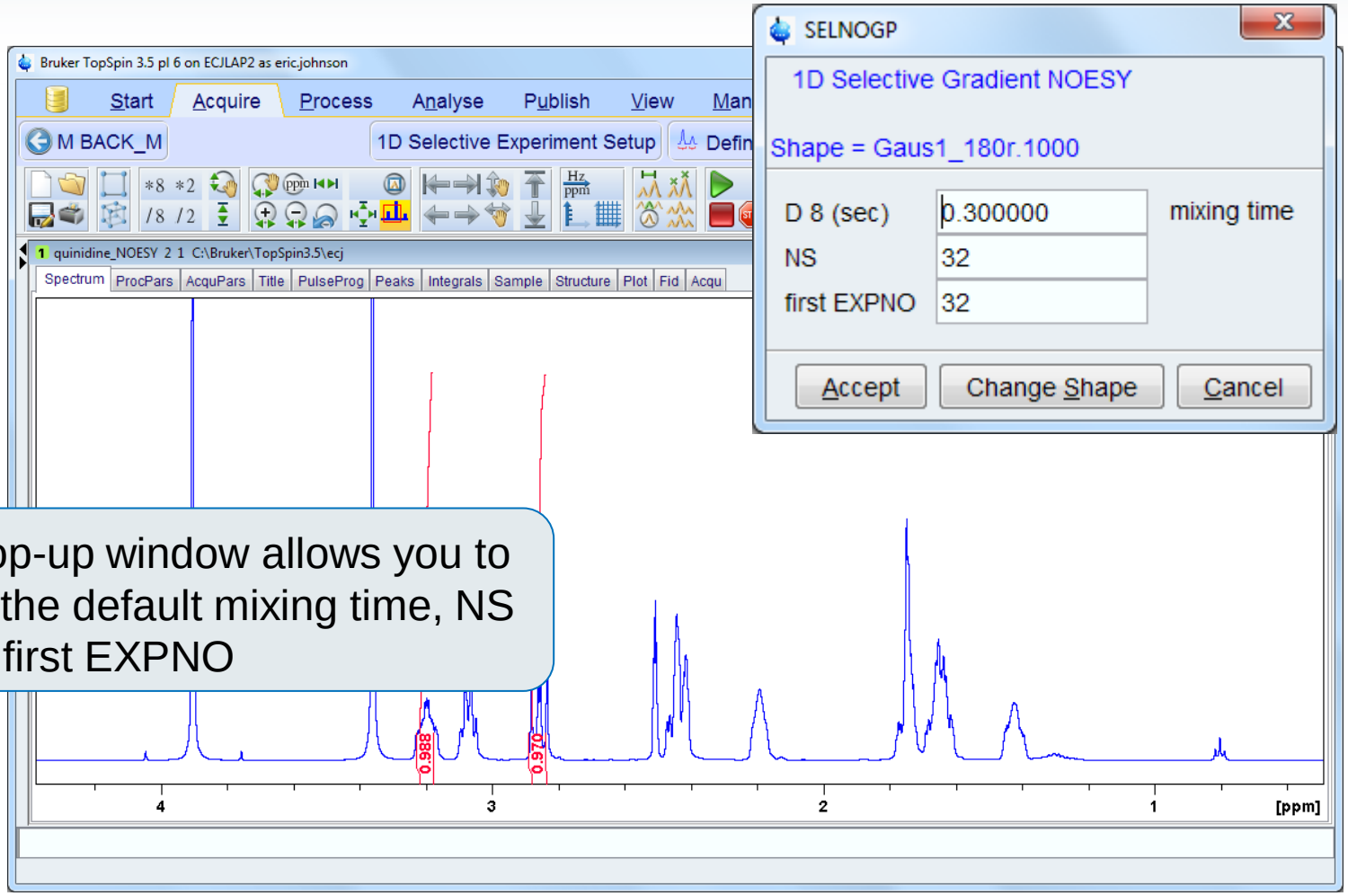
vi) Exit out of manual integration mode

Setting up the 1D selective experiments

vii) Select your experiment under the “Created Datasets” tab

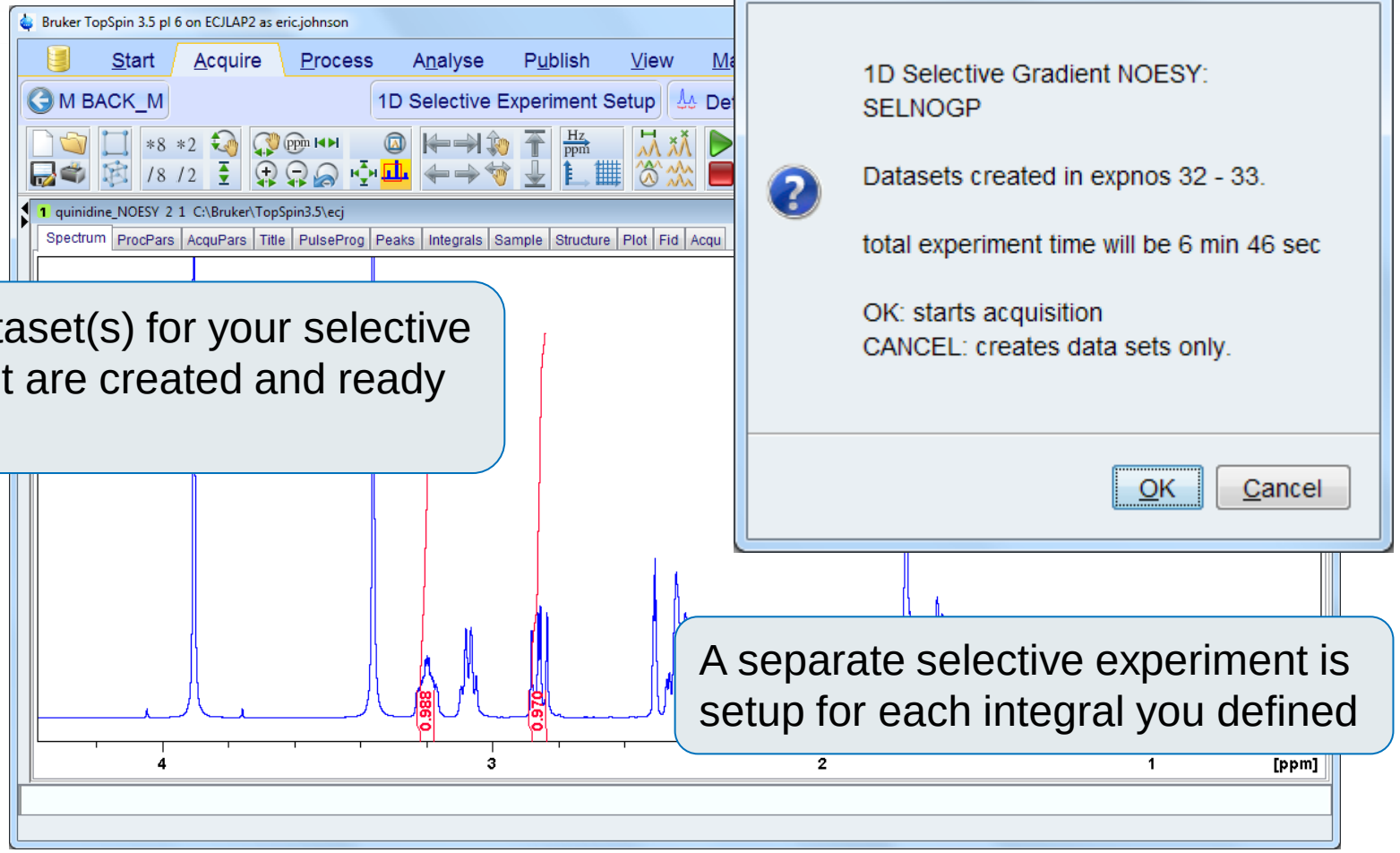


Setting up the 1D selective experiments



viii) A pop-up window allows you to change the default mixing time, NS and the first EXPNO

Setting up the 1D selective experiments



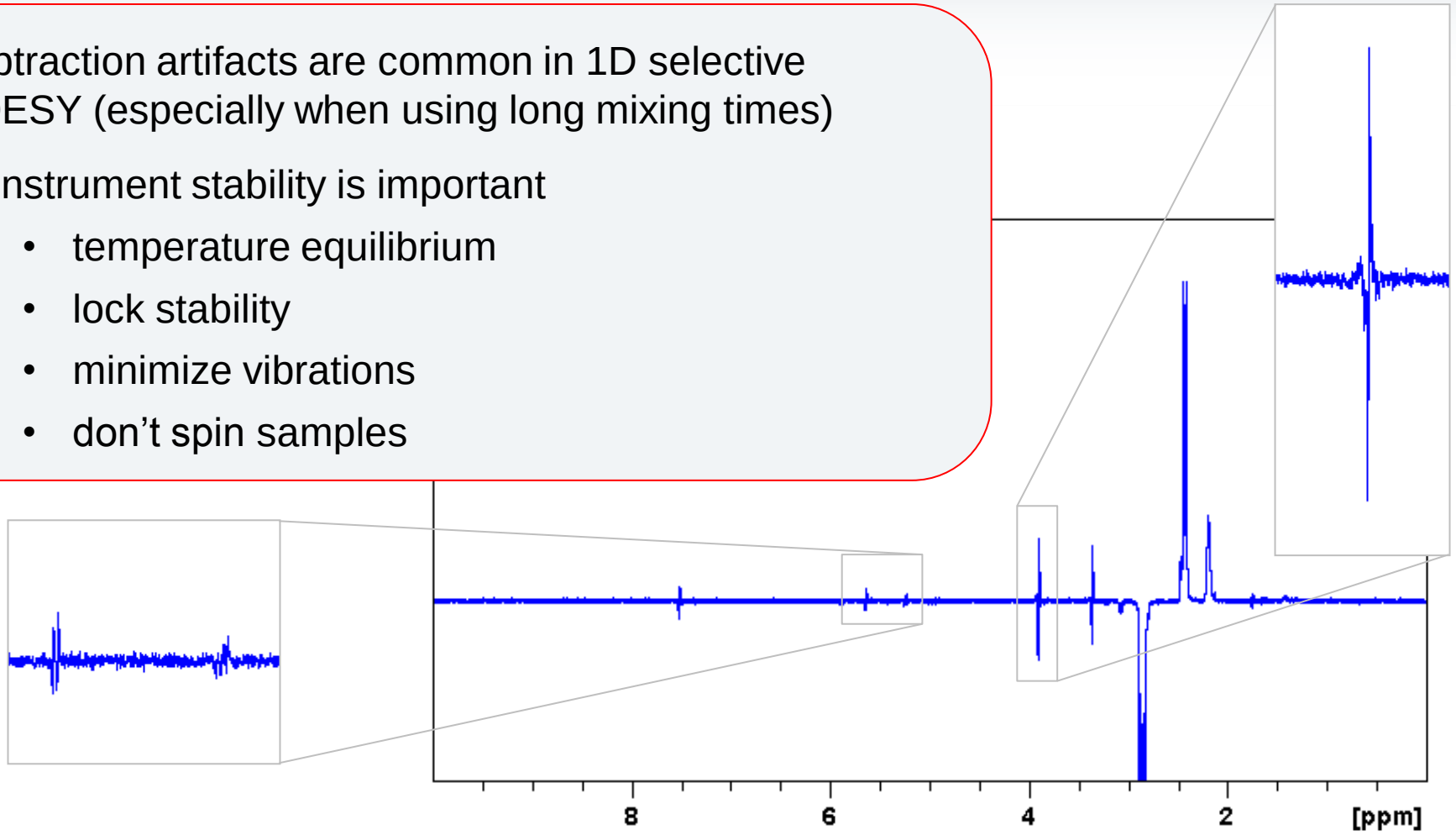
ix) The dataset(s) for your selective experiment are created and ready to run

A separate selective experiment is setup for each integral you defined

Tips on selective 1D's

Subtraction artifacts are common in 1D selective NOESY (especially when using long mixing times)

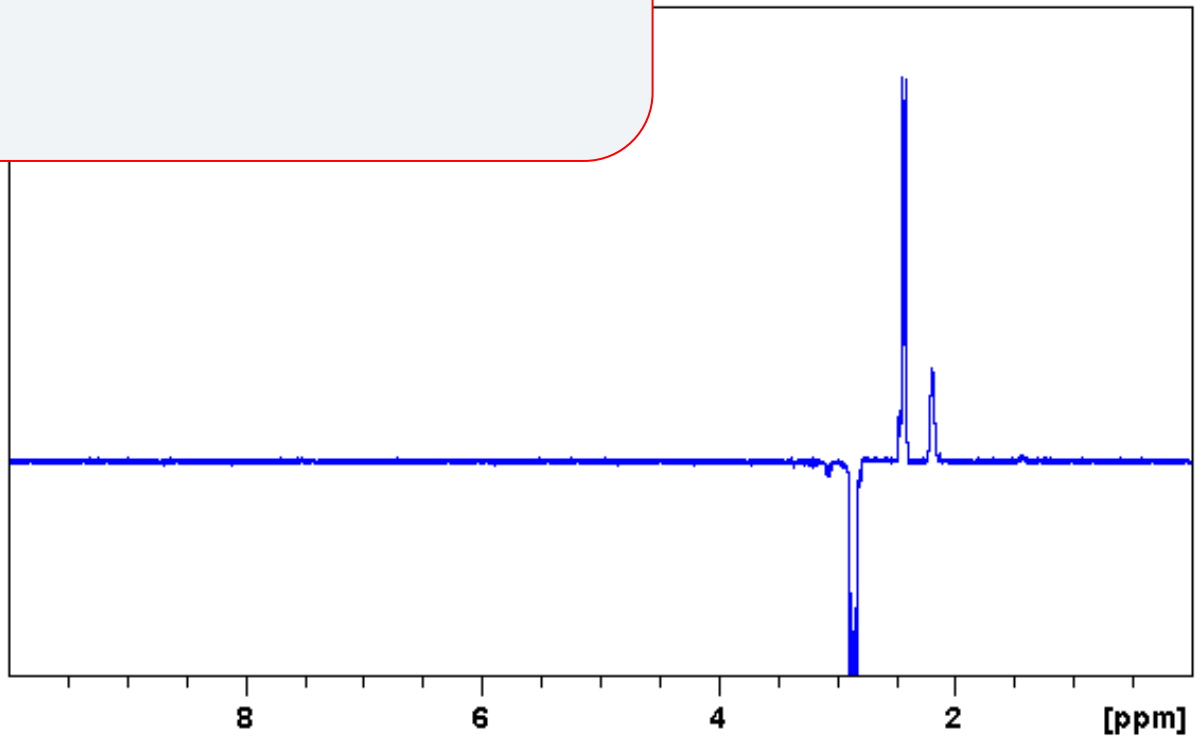
- instrument stability is important
  - temperature equilibrium
  - lock stability
  - minimize vibrations
  - don't spin samples



Tips on selective 1D’s

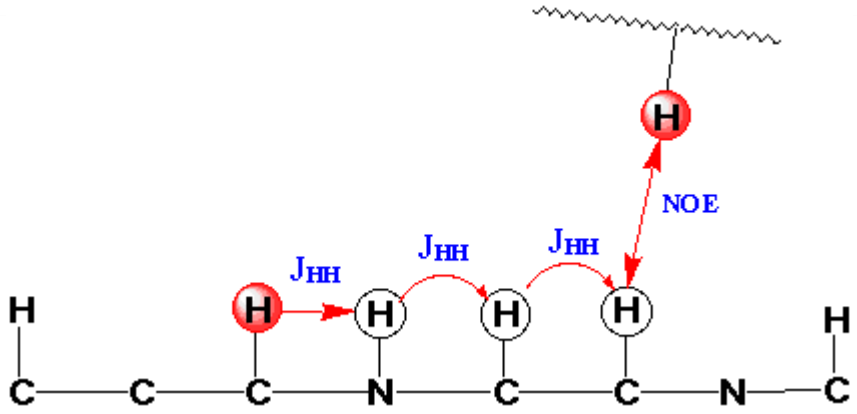
Subtraction artifacts are common in 1D selective NOESY (especially when using long mixing times)

- a modified pulse program can help
- available upon request



What if the peak I want to use for the selective NOESY is overlapped?

stepNOESY – “selective TOCSY edited preparation NOESY”



Doubly selective:

1. selective TOCSY transfer from well-resolved multiplet to overlapped peak
   - The previously overlapped peak should be well-resolved in the selective TOCSY
2. selective NOESY from this newly well-resolved peak

Hu et al, Magn Reson. 2004 Dec;171(2):201-6.

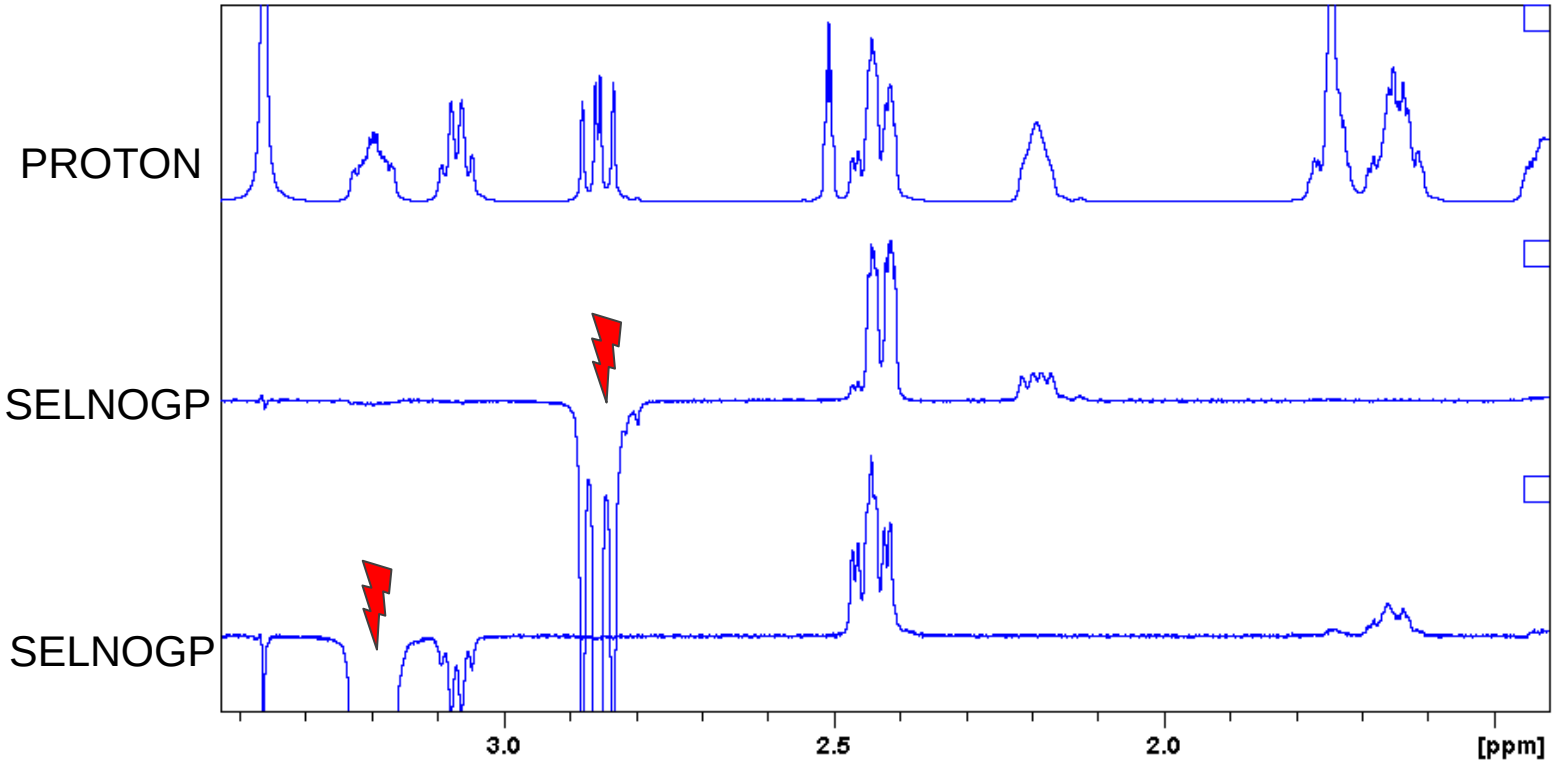
stepNOESY example – stereochemical assignment in a highly overlapped spectrum

Example from the original presentation has been removed until those results are published…

I'll add an example of some stepNOESY data on quinine on the next few slides.

stepNOESY – 1D selective NOESY from overlapped peaks

- I previously showed the quinine multiplet at 2.45ppm is an overlap of 2 peaks
- We can use selective experiments starting on other spins to show correlations to the individual components of this multiplet



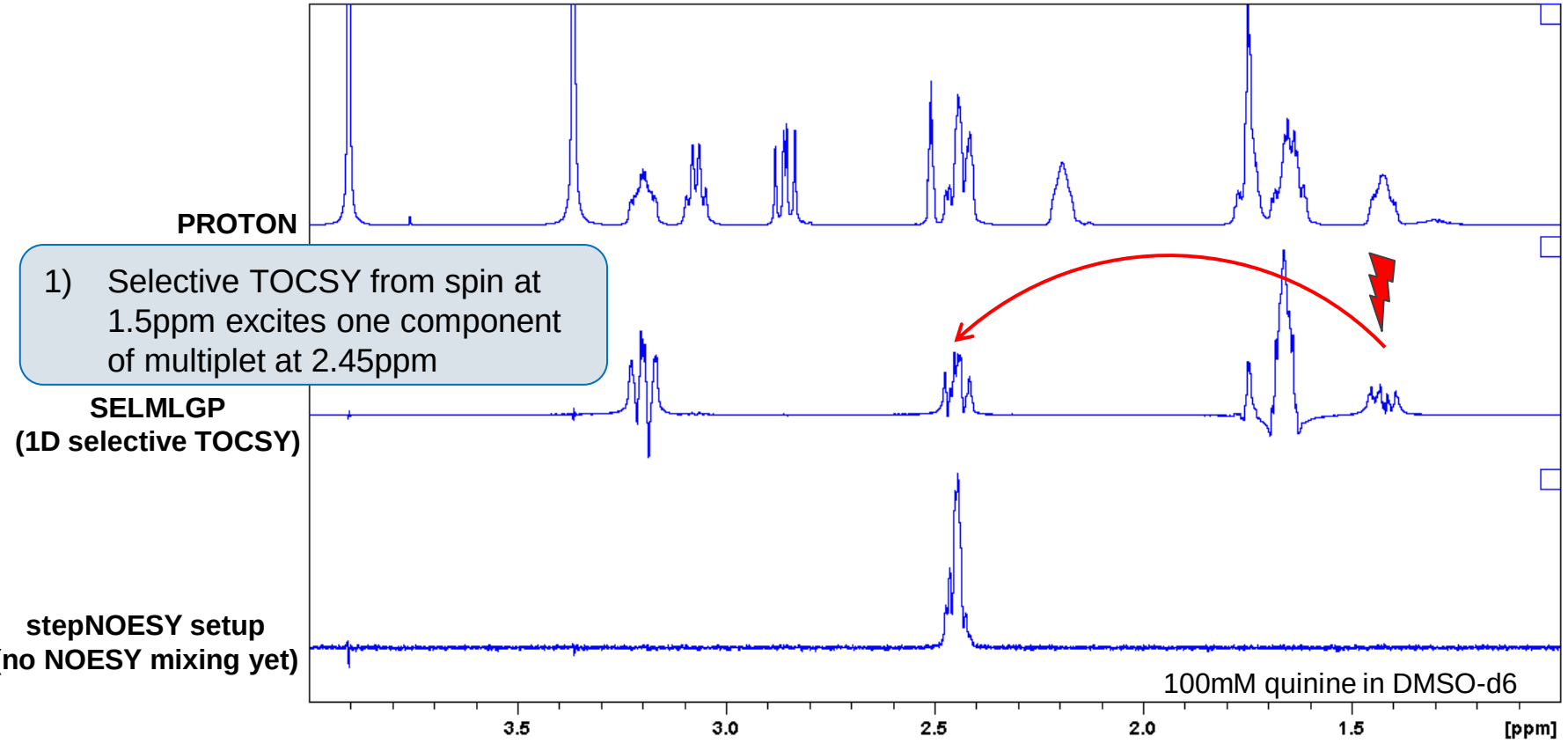
100mM quinine in DMSO-d6

stepNOESY – 1D selective NOESY from overlapped peaks

To get selective NOESY from individual components of peak at 2.45ppm

We can't selectively excite these separately – they're too overlapped

We can do a TOCSY transfer to them from another spin



stepNOESY – 1D selective NOESY from overlapped peaks

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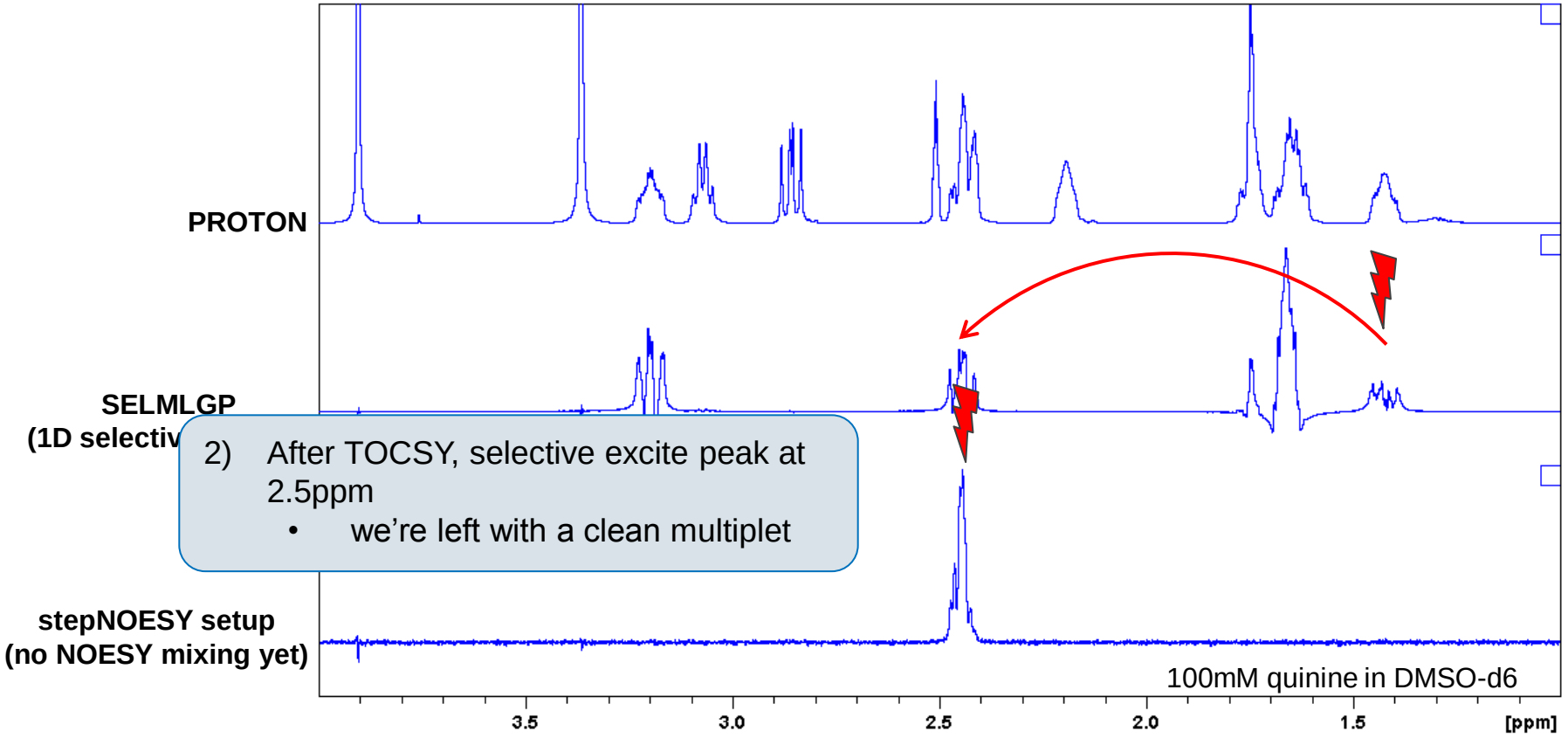
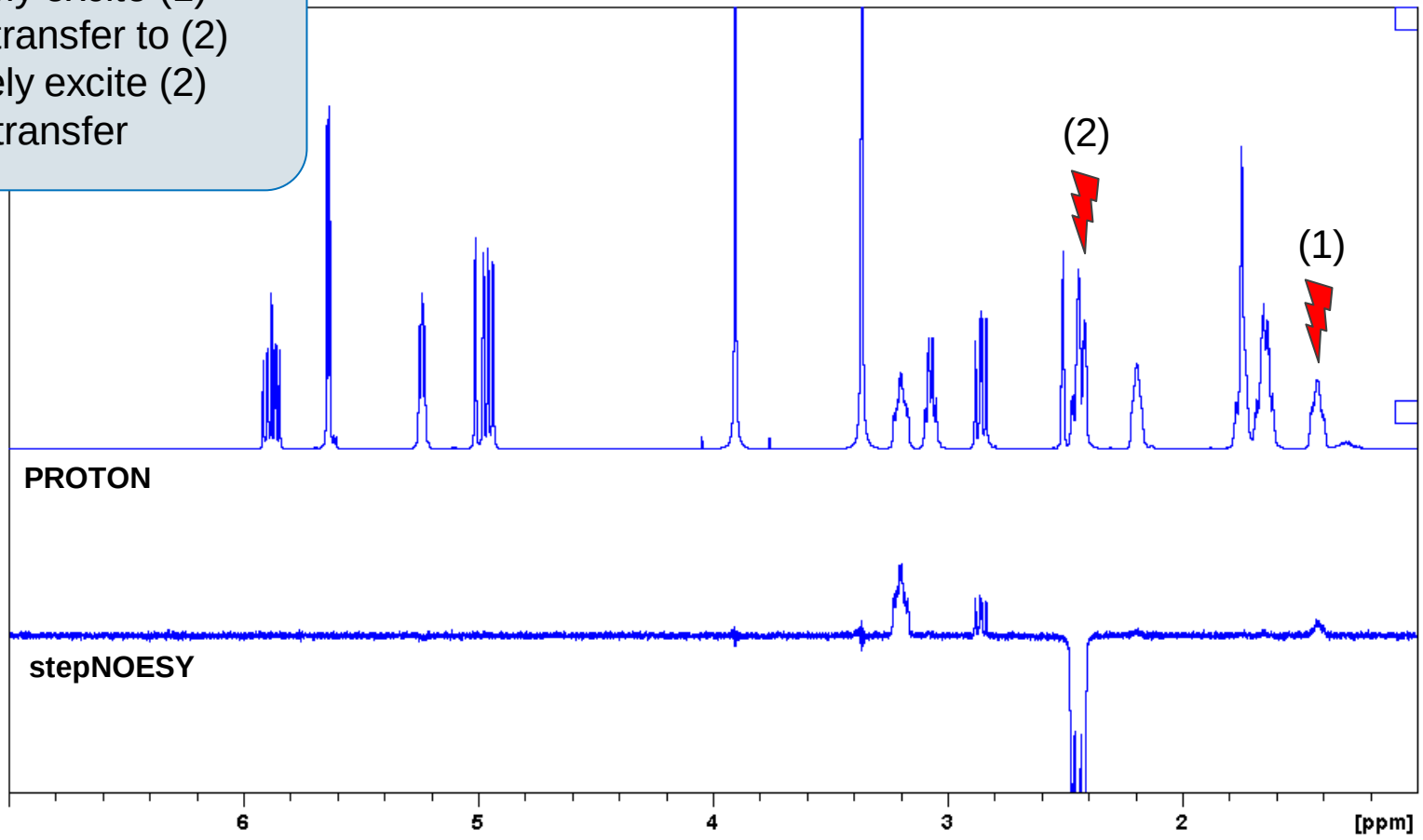


stepNOESY – 1D selective NOESY from overlapped peaks

1. Selectively excite (1)
2. TOCSY transfer to (2)
3. Selectively excite (2)
4. NOESY transfer



100mM quinine in DMSO-d6

stepNOESY – 1D selective NOESY from overlapped peaks

1. Selectively excite (1)
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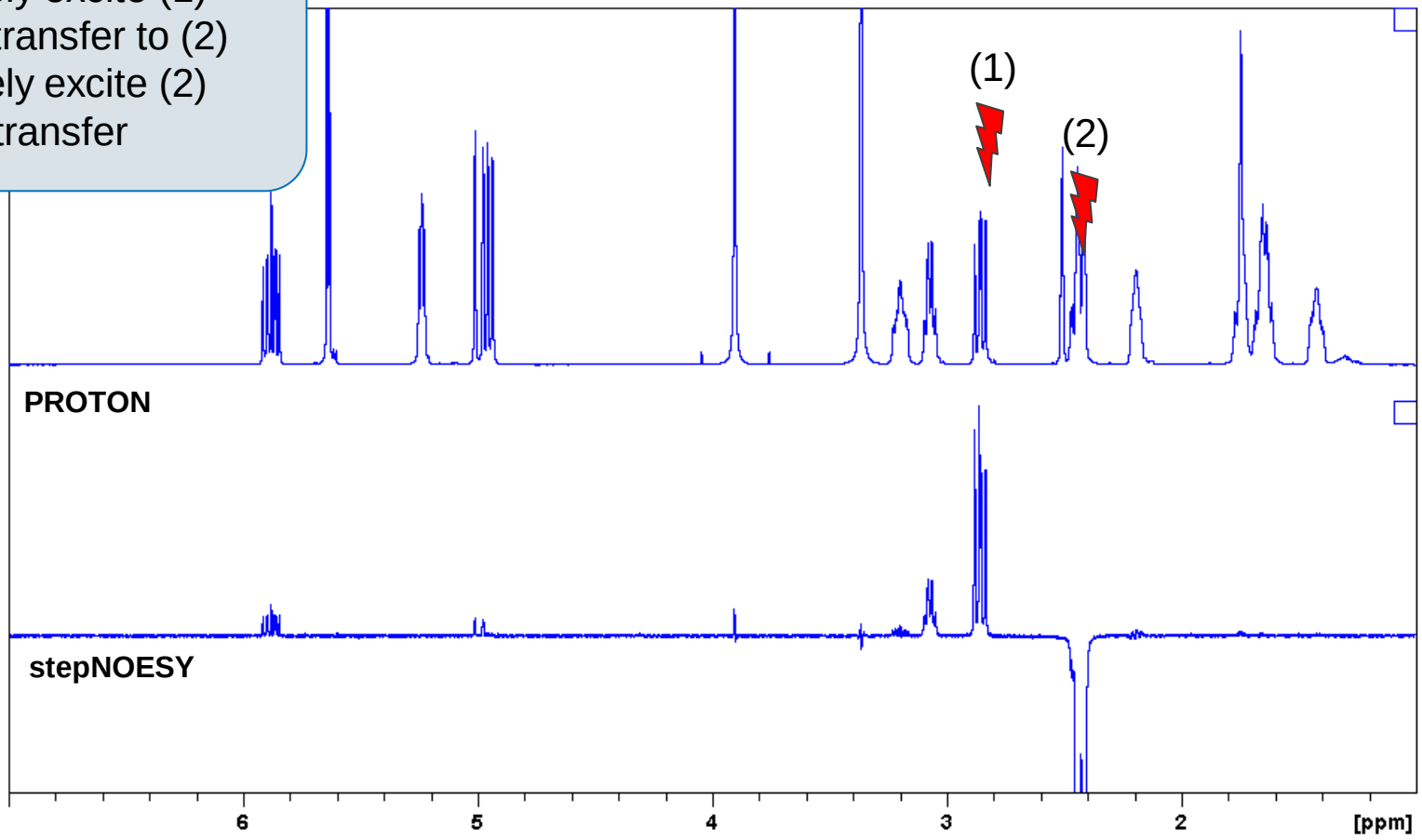
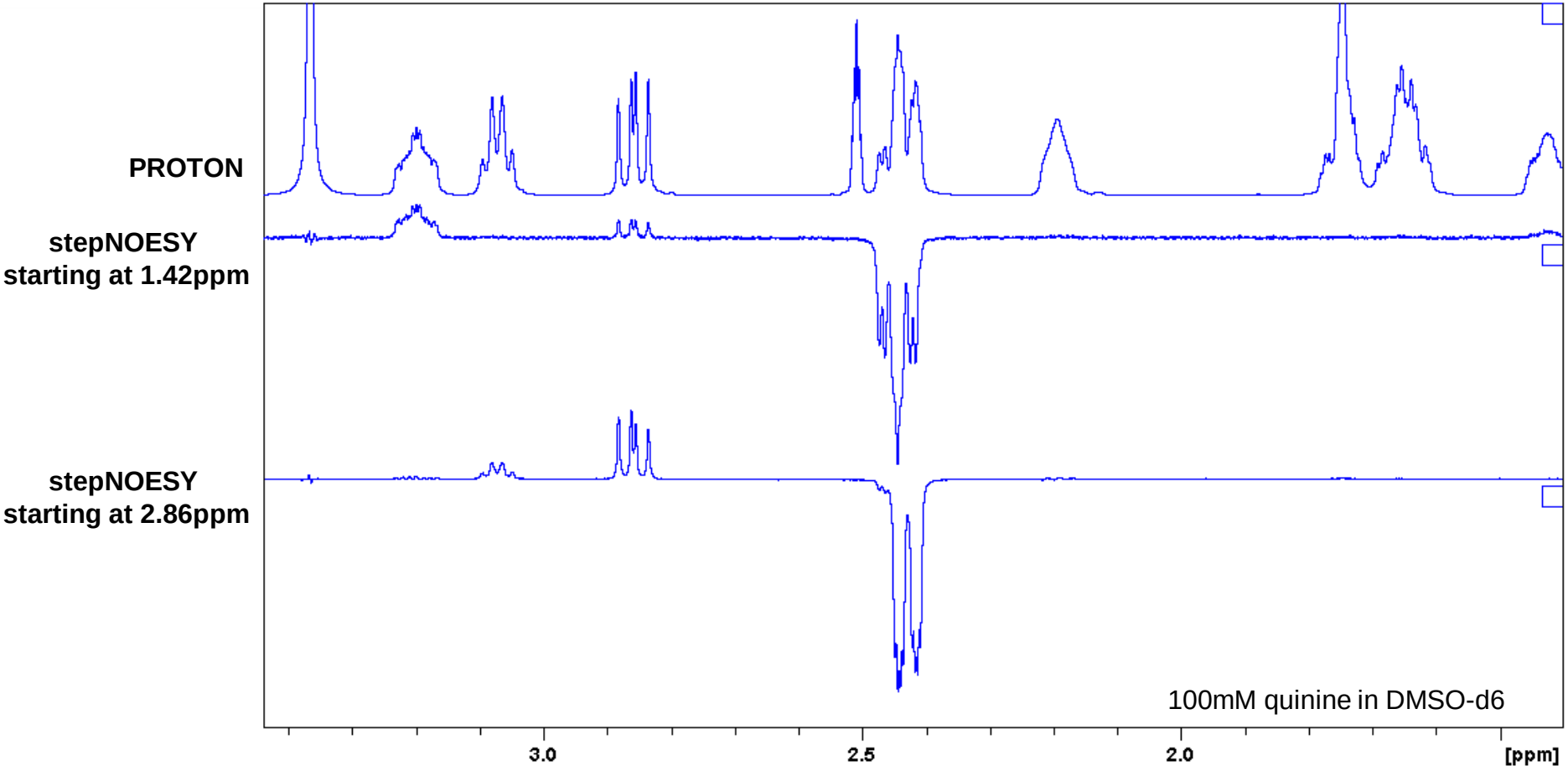
4. NOESY transfer



100mM quinine in DMSO-d6

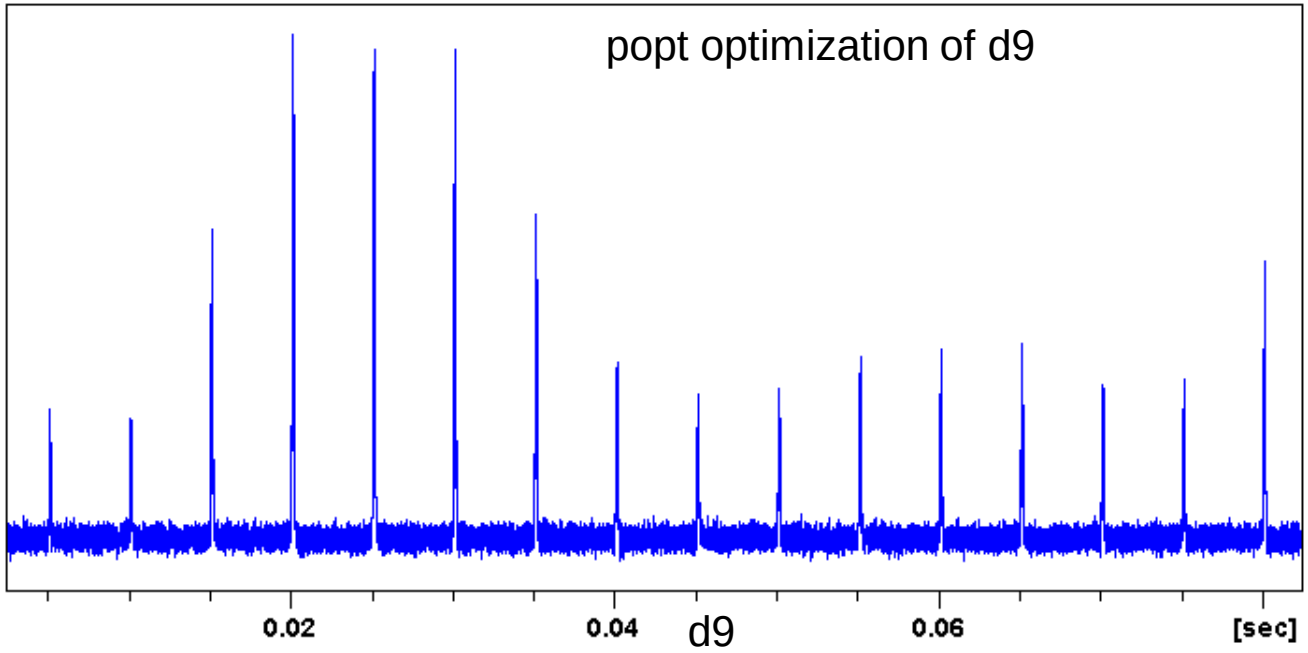
stepNOESY – 1D selective NOESY from overlapped peaks

- Showing how cleanly the stepNOESY is exciting each component of the multiplet at 2.45ppm



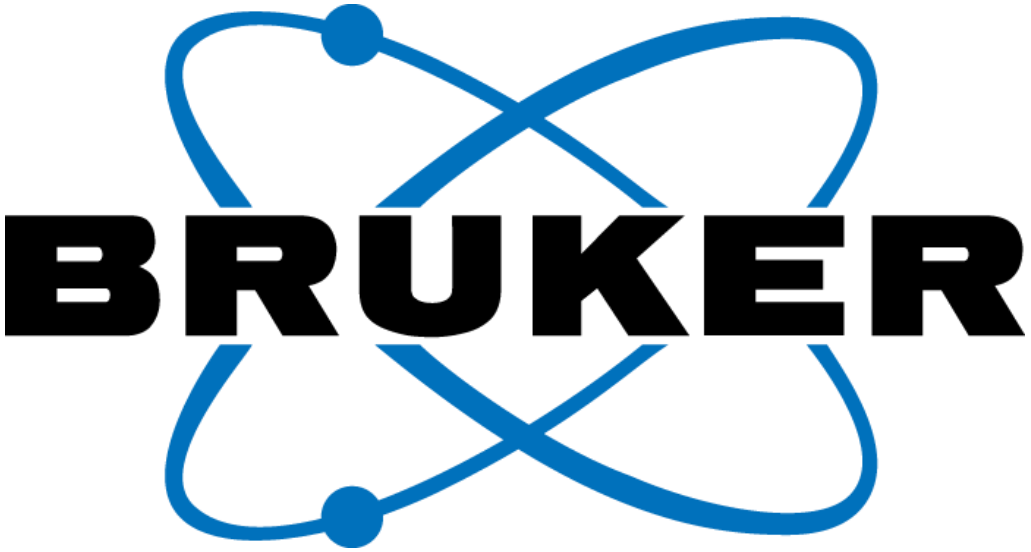
stepNOESY – optimizing parameters

- The stepNOESY is much less sensitive than the standard selective NOESY
  - Signal losses during both TOCSY and NOESY parts
- When running the TOCSY, we usually keep the mixing time relatively long to transfer magnetization to the whole spin system
- He can optimize the mixing time “d9” to maximize the transfer of magnetization to the single spin of interest



stepNOESY

- The stepNOESY is not yet in the Topspin pulse program library or in the acquisition flowbar setup tool
  - hopefully it will be soon…
- pulse program available upon request



Innovation with Integrity