

Faster, Higher Resolution Spectra



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- 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.

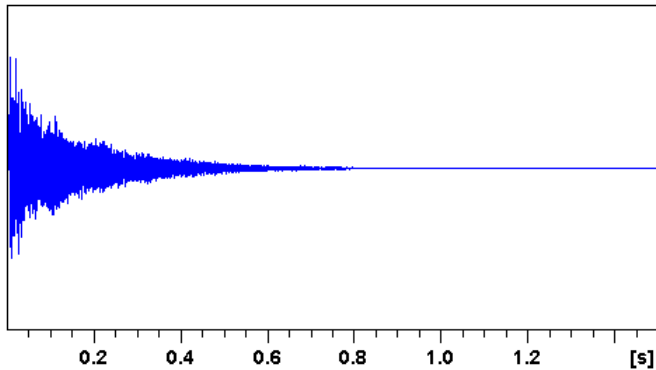
Non Uniform Sampling

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

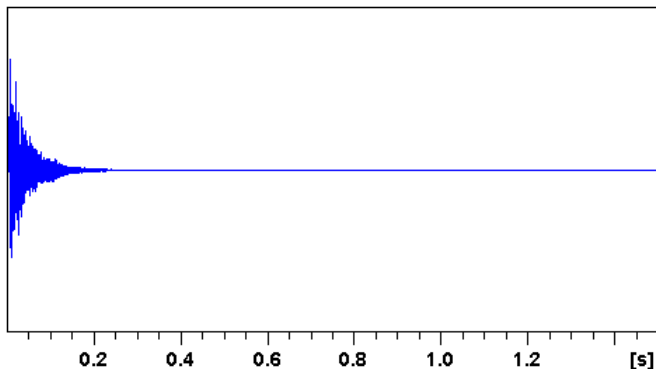
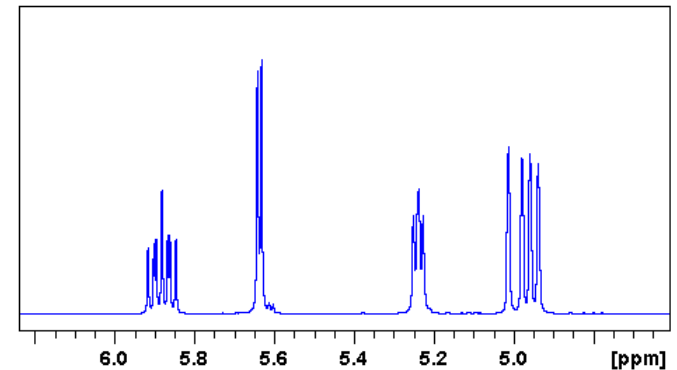
Resolution in a 1D spectrum

Resolution is primarily defined by

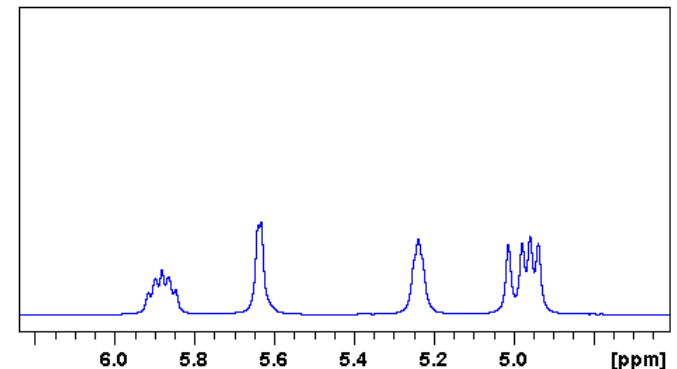
- shimming
- inherent relaxation properties of sample



FID decays slowly → sharp peaks



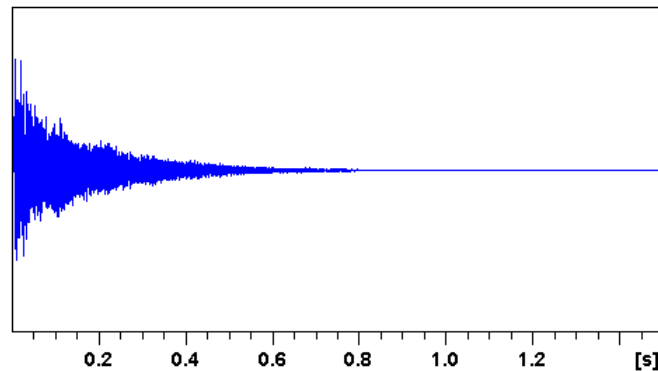
faster relaxation → broader peaks



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

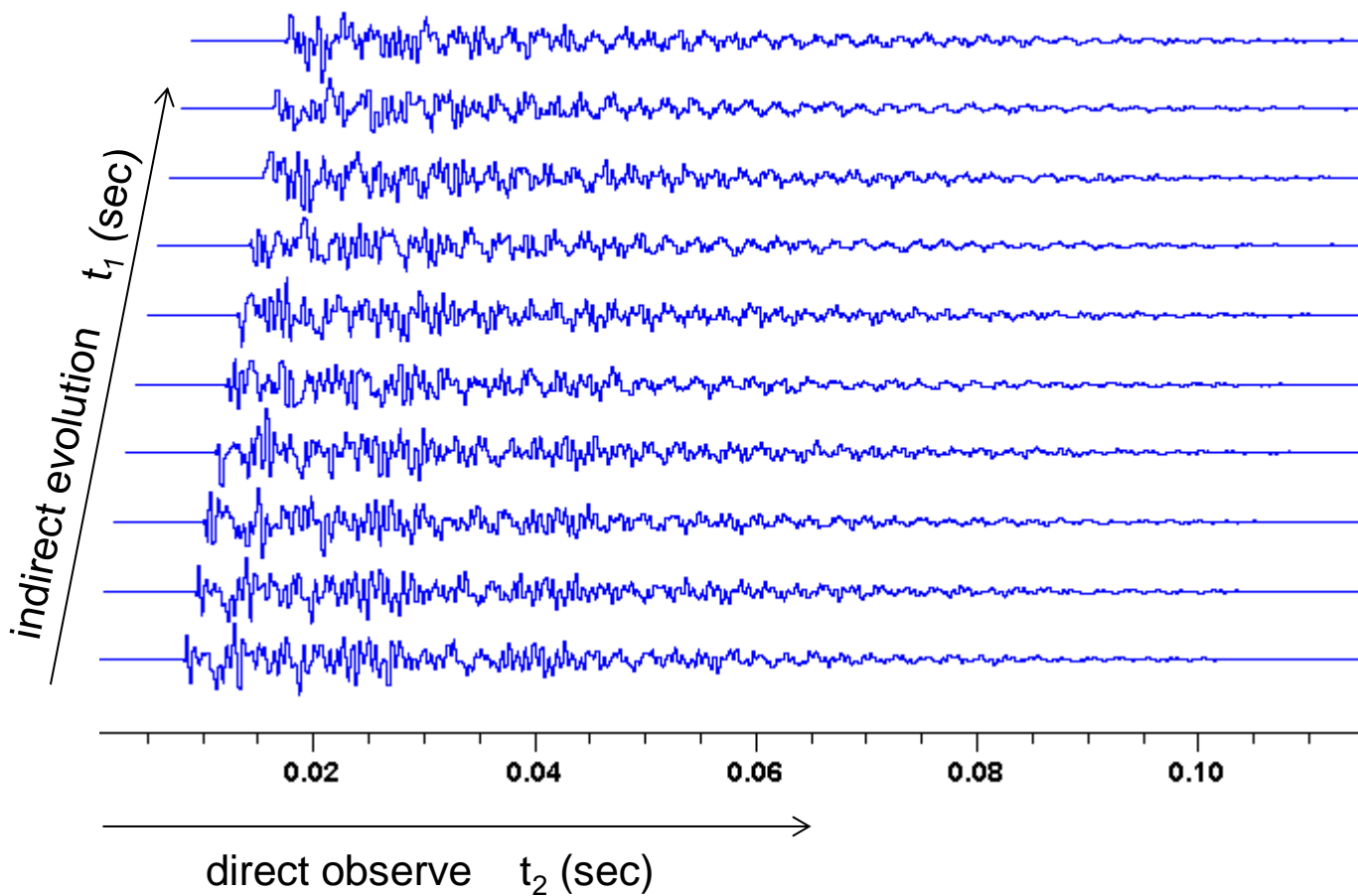


Acquisition time is dominated by the number of scans necessary for the desired signal to noise

Basics of 2D spectra



Series of 1D spectra

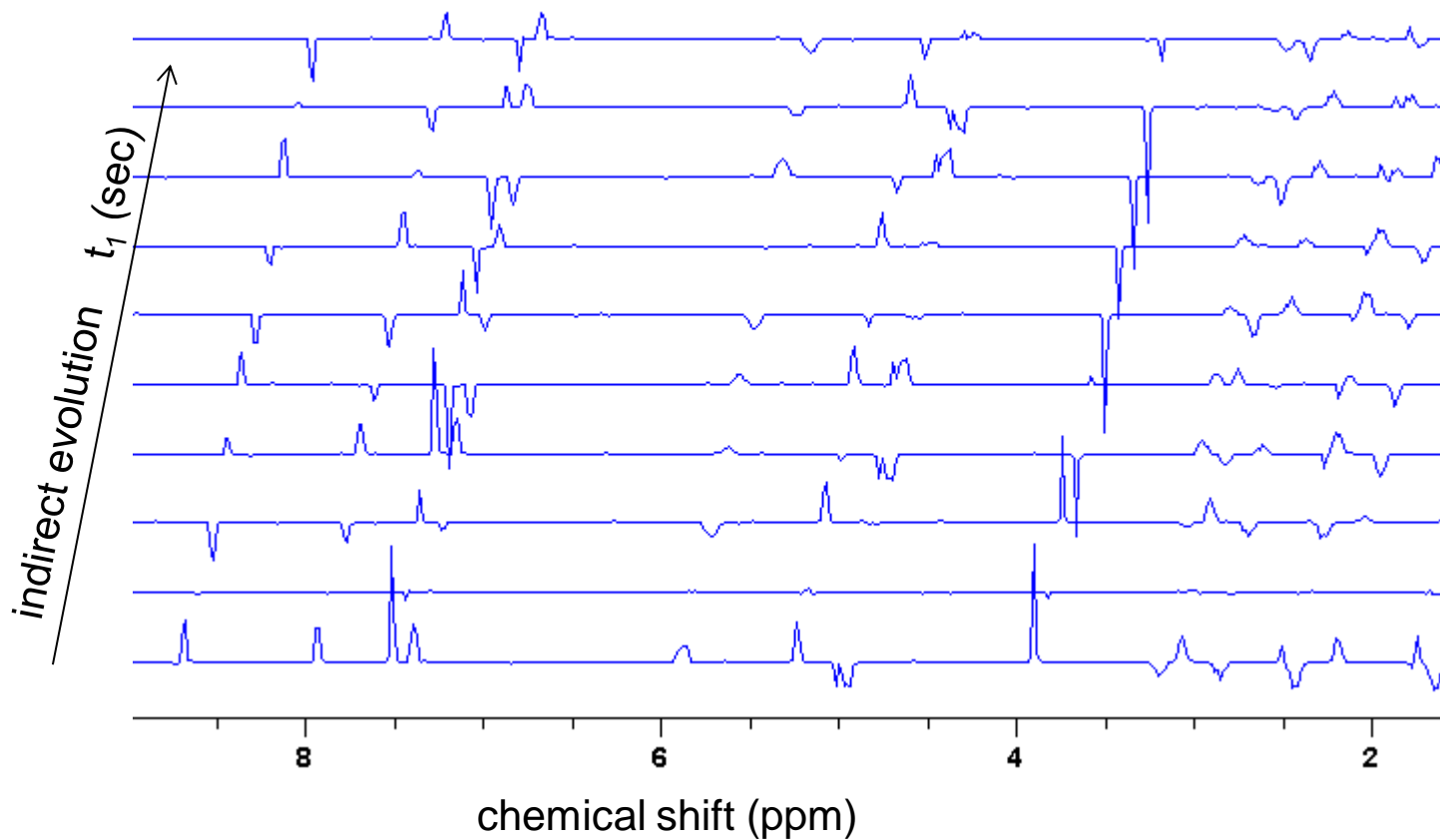


Basics of 2D spectra



Fourier transform observe dimension

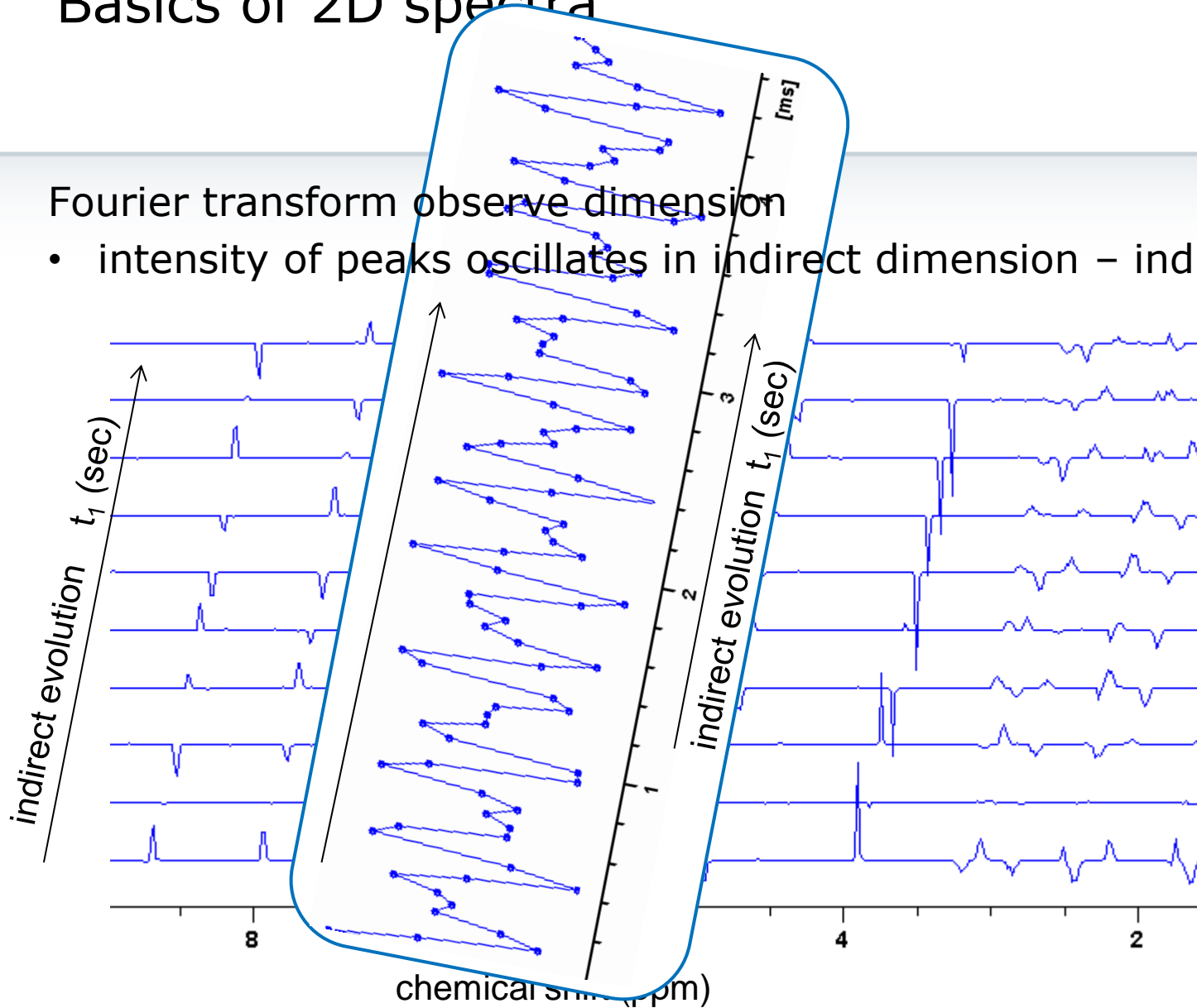
- intensity of peaks oscillates in indirect dimension – indirect "FID"



Basics of 2D spectra

Fourier transform observe dimension

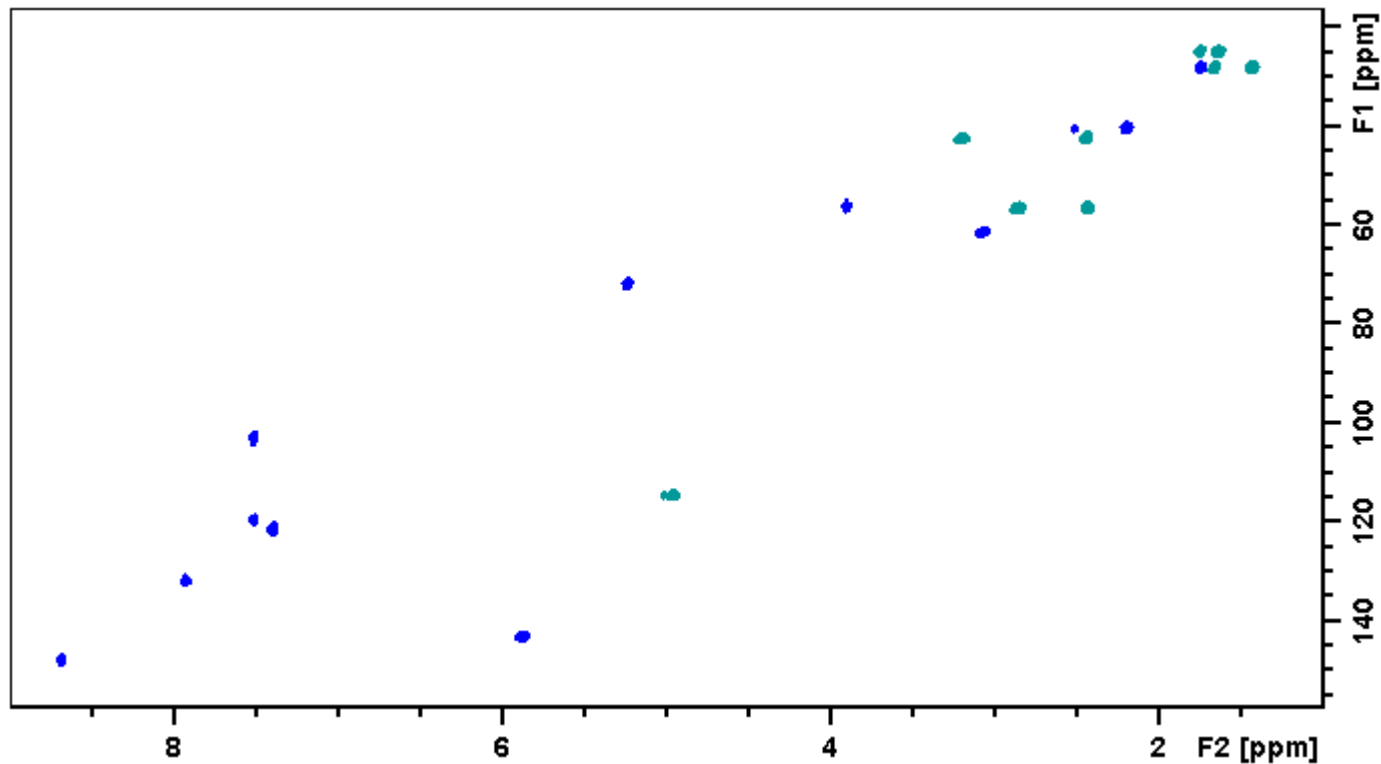
- intensity of peaks oscillates in indirect dimension – indirect “FID”



Basics of 2D spectra

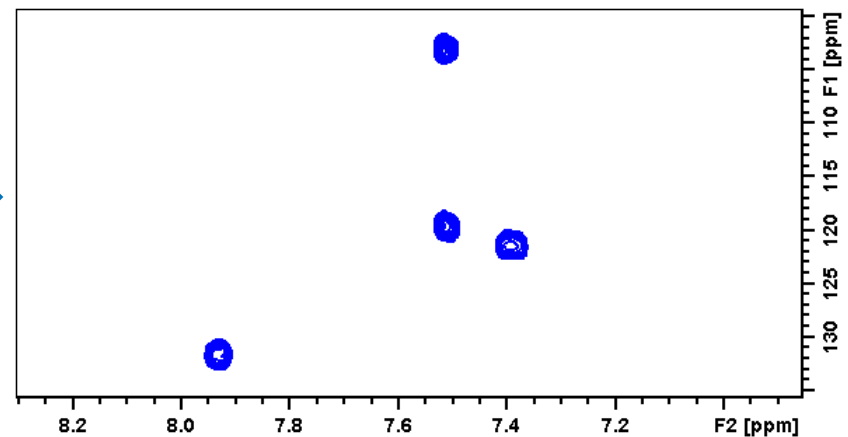
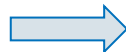
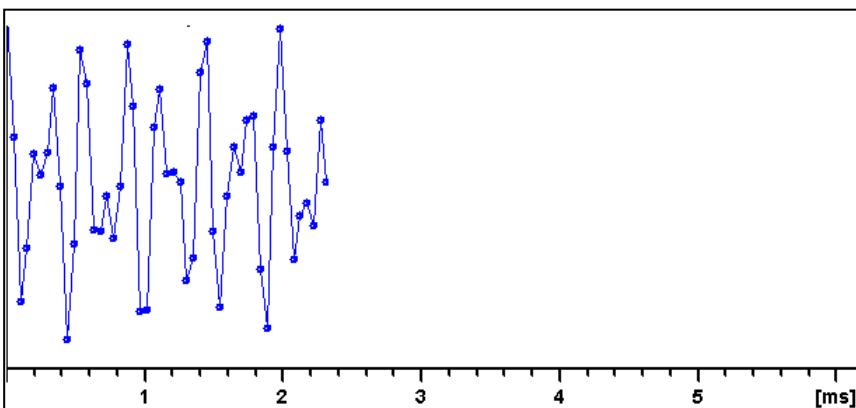
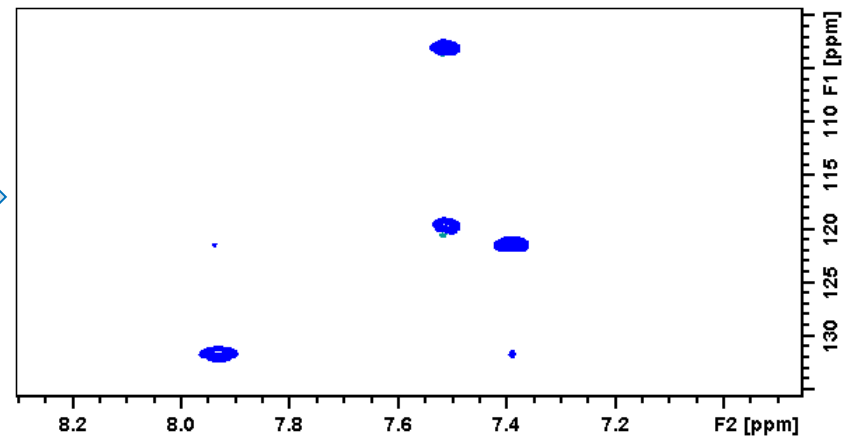
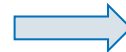
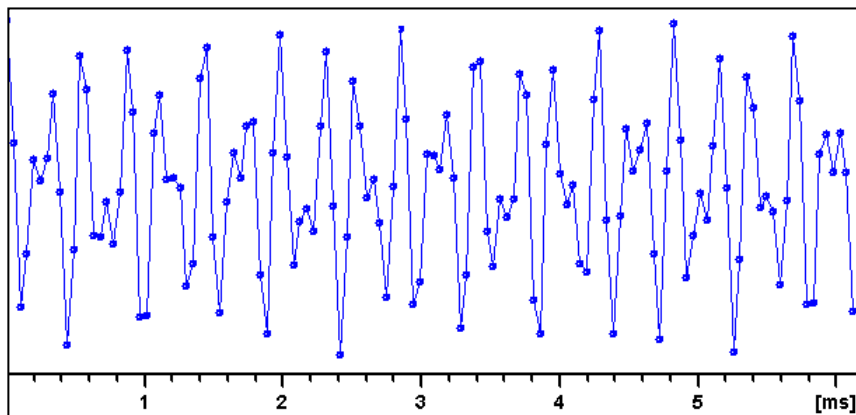
Fourier transform indirect dimension

- → 2D spectrum



Resolution of 2D spectrum

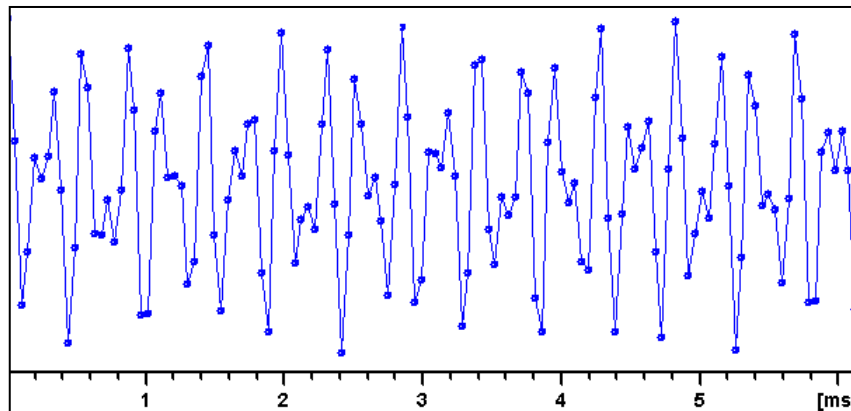
Resolution in indirect dimension depends on duration of indirect "FID"



Acquisition time for a 2D spectrum



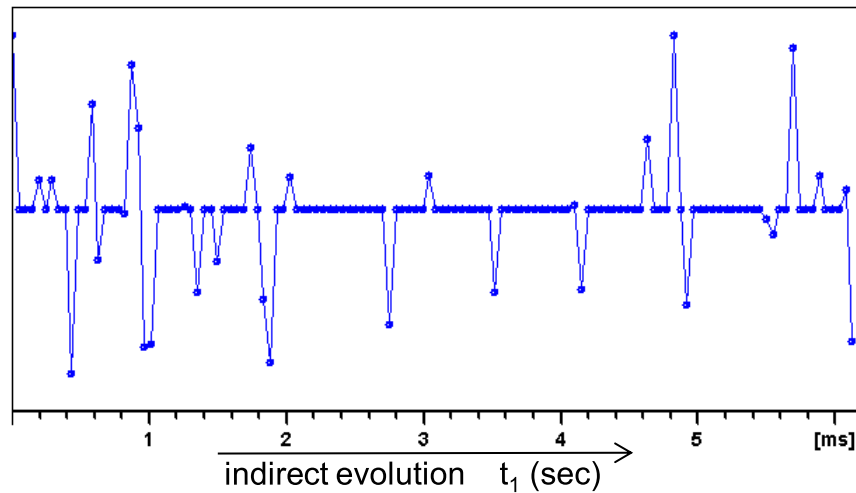
- Each point in the indirect "FID" is a directly observed FID
→ for higher resolution...
 - need to acquire more FID's (larger TD)
 - longer total acquisition time



- 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?

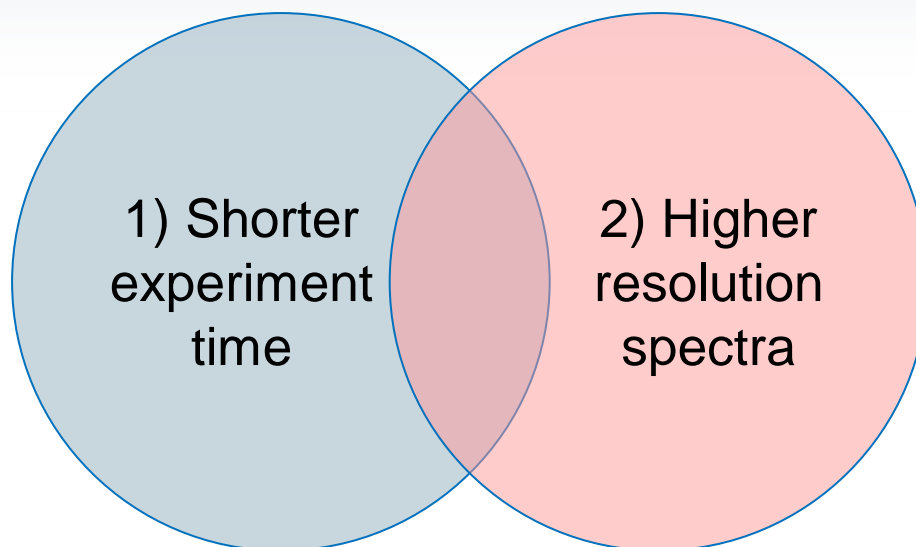
- Not necessarily!!!! NUS – Non Uniform Sampling



- 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



The screenshot shows the Bruker software interface with the 'AcquPars' tab selected. The 'FnTYPE' dropdown menu is open, showing the following options:

Option	Description
traditional(planes)	mode for 2D, 3D etc.
full(points)	
non-uniform_sampling	dummy scans
projection-spectroscopy	scans

A red circle highlights the 'non-uniform_sampling' option, and a red arrow points to it from below. The 'FnTYPE' field is currently set to 'non-uniform_sampling'. Other parameters visible include PULPROG: hsqcetgpsisp2.2, AQ_mod: DQD, TD: 102, DS: 16, NS: 2, TD0: 1, and TDav: 0.

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

NUS acquisition parameters



How sparsely do you want to sample?

- Effective TD = 256
→ 128 complex points
- You can set either NusAMOUNT[%] or NusPOINTS

The screenshot displays two windows from the Bruker software interface. The top window, titled 'AcquPars', shows the 'non-uniform sampling' method selected. The 'TD' parameter is set to 1024, and a red box highlights the value 256. The bottom window, titled 'NUS (Non Uniform Sampling) parameters', shows 'NusAMOUNT [%]' set to 25 and 'NusPOINTS' set to 32, both highlighted with red boxes. A red arrow points from the '256' value in the top window to the 'NusPOINTS' value in the bottom window. The right side of the bottom window contains a help section with the following text:

Show NUS help
Amount of sparse sampling
Number of hypercomplex points in indirect dimension
J-coupling
T2 relaxation
Random generator seed
Name
Calcul
Display

I'll briefly discuss the other NUS parameters later

NUS processing parameters



The screenshot shows the 'ProcParams' window in Bruker Topspin, specifically the 'NUS (Non Uniform Sampling) parameters' section. The 'Mdd_mod' parameter is set to 'cs', which is highlighted with a red circle. Other parameters include 'MddCEXP' (mdd), 'MddCT_SP' (cs), 'MddF180' (FALSE), 'MddNCOMP' (0), 'MddPHASE' (0), and 'MddSRSIZE [ppm]' (0). The right side of the window shows the corresponding descriptions for these parameters: 'MDD mode', 'RMDD/MDD flag', 'Constant time', 'Delayed sampling flag', 'Number of components', 'Phase', and 'Sub region size'.

Two processing algorithms are built into Topspin:

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

- Several other methods exist (not discussed here...)

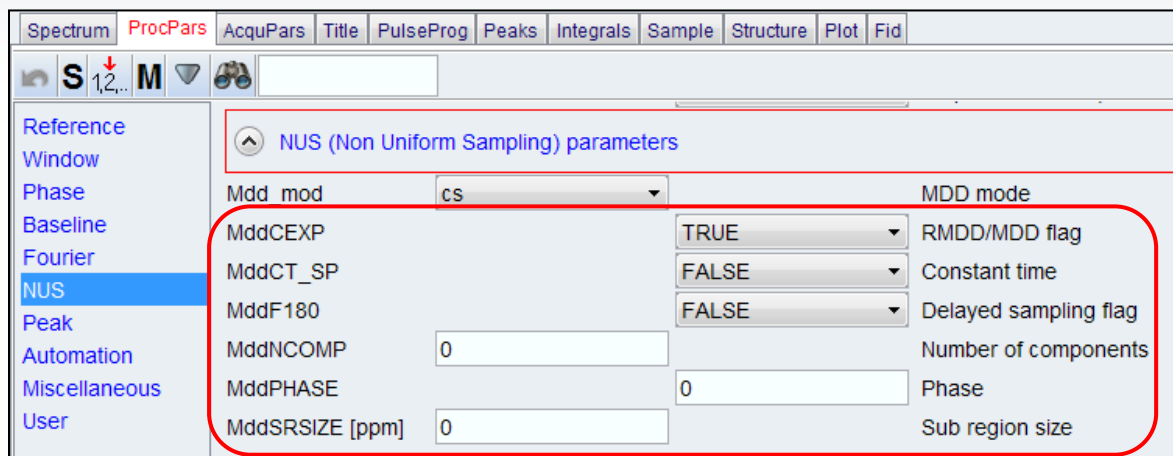
NUS processing – Topspin licenses

A screenshot of the Bruker Topspin software interface. The top menu bar includes "Spectrum", "ProcPars", "AcquPars", "Title", "PulseProg", "Peaks", "Integrals", "Sample", "Structure", "Plot", and "Fid". Below the menu bar is a toolbar with icons for "S", "1,2,..", "M", and a magnifying glass. On the left is a vertical navigation pane with categories: Reference, Window, Phase, Baseline, Fourier, NUS (highlighted in blue), Peak, Automation, Miscellaneous, and User. The main window displays the "NUS (Non Uniform Sampling) parameters" section, which is outlined in red. It contains several parameters with their values and descriptions:

Parameter	Value	Description
Mdd_mod	cs	MDD mode
MddCEXP	TRUE	RMDD/MDD flag
MddCT_SP	FALSE	Constant time
MddF180	FALSE	Delayed sampling flag
MddNCOMP	0	Number of components
MddPHASE	0	Phase
MddSRSIZE [ppm]	0	Sub region size

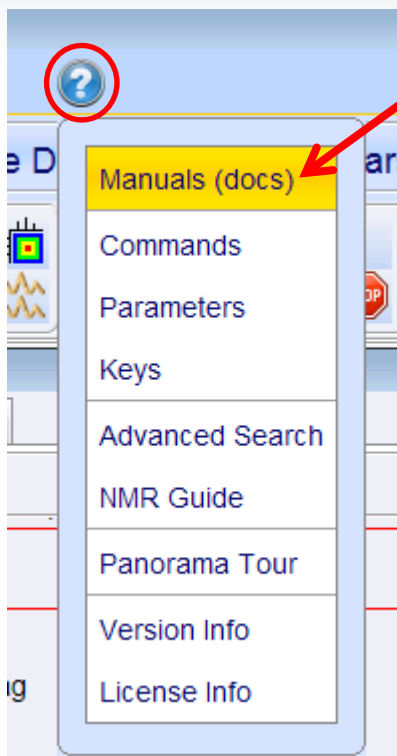
- Originally (TS3.0 until recently), a separate NUS processing license was required
- Starting with Topspin 3.5pl3, basic 2D processing with CS is included with the standard Topspin processing license!!!

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 perform better than MDD for sparsely sampled 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



Software And Application Manuals

Please click on a manual title to open the document!

General	
User Manual	A description of the TopSpin user interface and its functionality
Control & Function Keys	A list of predefined Control and Function keys.
Release Letter	Describes the changes and new features of this TopSpin version and the spectrometer hardware requirements
Beginner Guide	For Avance Spectrometers With SGU Based Frequency Generation: A basic description of the Bruker NMR spectrometer, its main components, functionality and usage
Acquisition - User Guide	
1D and 2D Step-by-Step	
1D and 2D Step-by-Step	
Basic 1D and 2D Experiments	
3D/Triple-Resonance Experiments	
Acquisition - Applications	
Eretic2	
Multi-Receive Acquisition	
Solids Introduction	
Solids	
TopSolids	Assisted Biological Solid State NMR.
Cross Polarization Dynamics	An introduction into Cross Polarization Dynamics experiments.
SB/MAS	A description of setting up and running SB/MAS experiments.
BEST-NMR	A description of setting up and running BEST-NMR experiments.
LC-NMR	A description of setting up and running LC-NMR experiments.
Dosy	A description of setting up and running Dosy experiments.
Diffusion	A description of setting up and running Diffusion experiments.
Shapetool	A description of creating, analyzing and manipulating RF- and gradient Shapes.
Gradient Shimming	A description of the gradient shimming interface.
TopShim	User manual for the automatic shimming tool.
CMCQ	Complete molecular confidence for quality assurance
APSY	Automated Projection Spectroscopy: Get N-dim. correlations via low-dimensional projections.
Acquisition & Processing References	
Acqu. Commands & Parameters	A description of all acquisition and acquisition related commands and parameters.
Proc. Commands & Parameters	A description of all processing and analysis commands and parameters.
Edprosol Manual	How to set up probe and solvent dependent parameters
Edlock Guide	A description of how to setup solvent and lock dependent parameters.
Pulse Program Catalogue, 1D/2D	A graphical presentation of the Bruker supplied pulse programs, 1D and 2D experiments.
Pulse Program Catalogue, BIO	A graphical presentation of the Bruker supplied pulse programs, biomolecular experiments.
NUS Parameters	A description of the parameter setup for Non Uniform Sampling
Automation and Plotting	

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

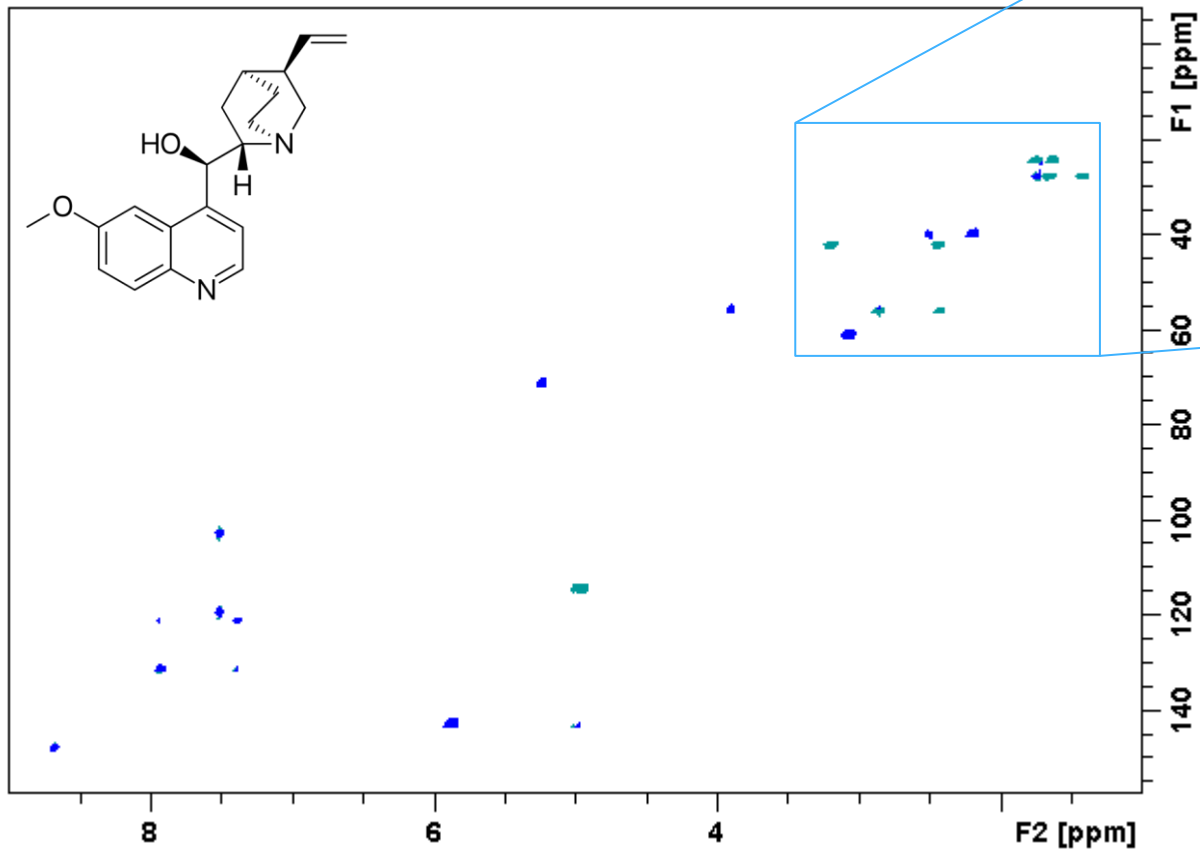
Processing NUS datasets



- 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

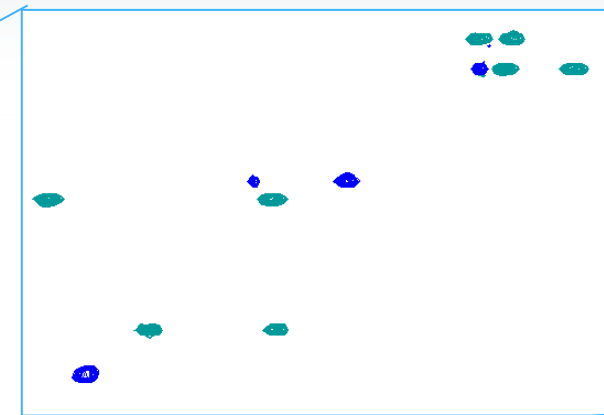
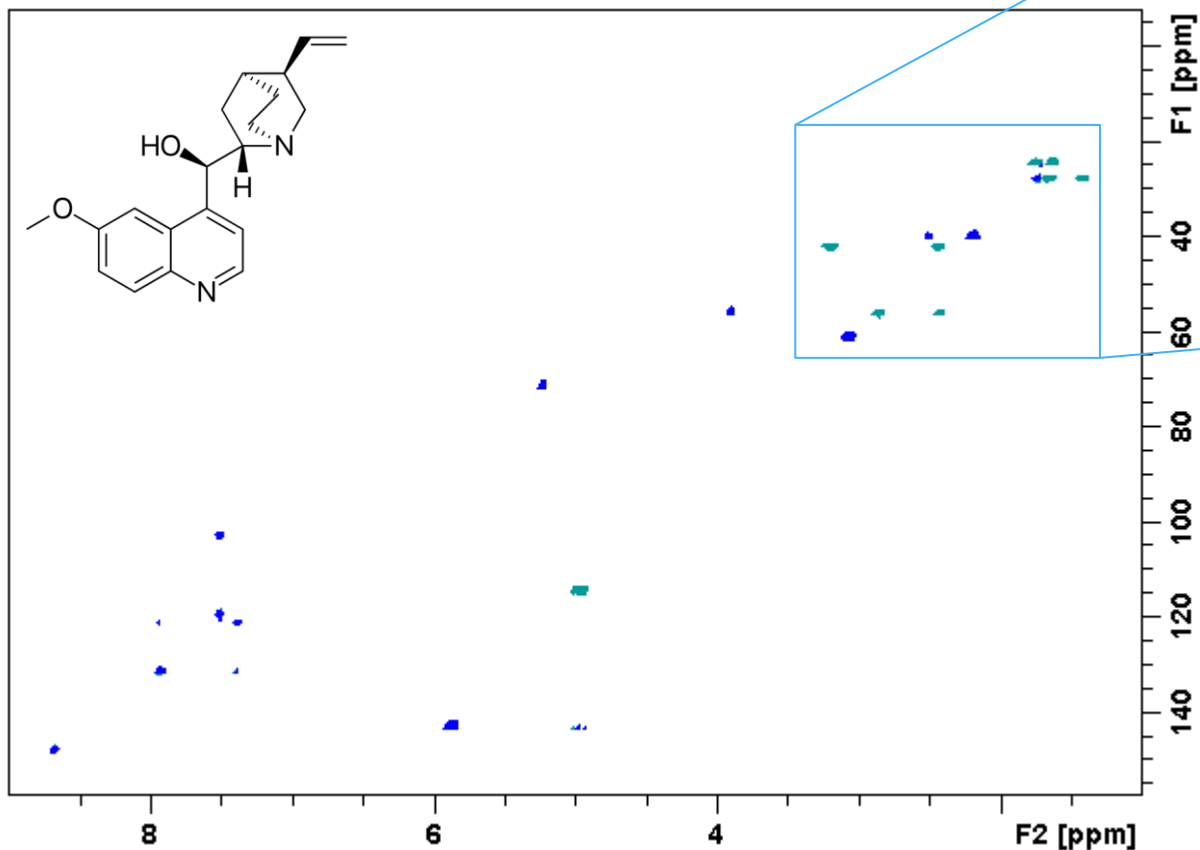
100mM quinine in DMSO-d6



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

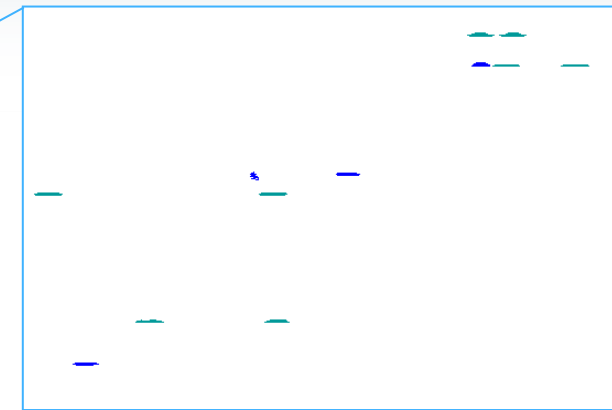
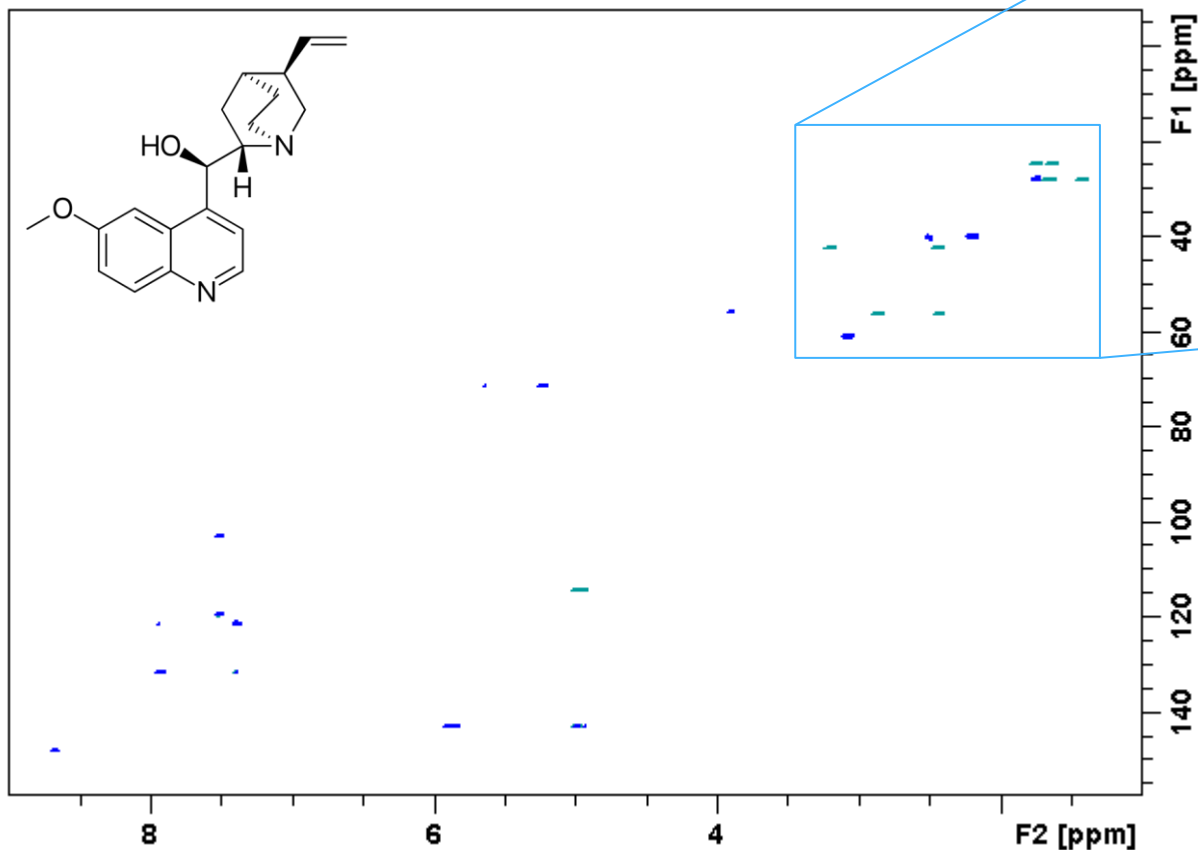
NusAmount = 25%

NusPOINTS = 32

expt: 5 minutes

First example: edited HSQC - NUS for higher resolution

100mM quinine in DMSO-d6



NUS:

TD effective = 4096

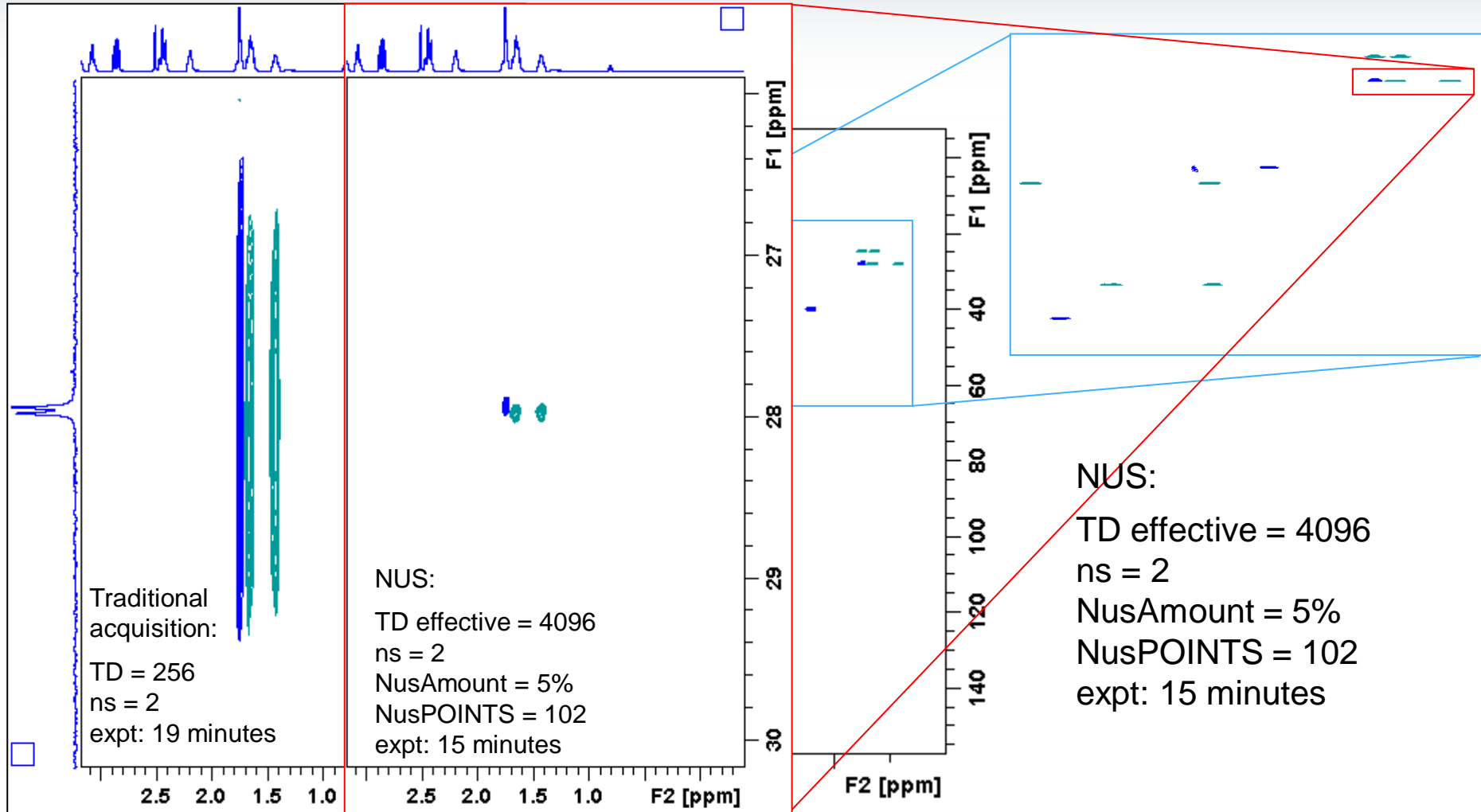
ns = 2

NusAmount = 5%

NusPOINTS = 102

expt: 15 minutes

First example: edited HSQC - NUS for higher resolution



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)

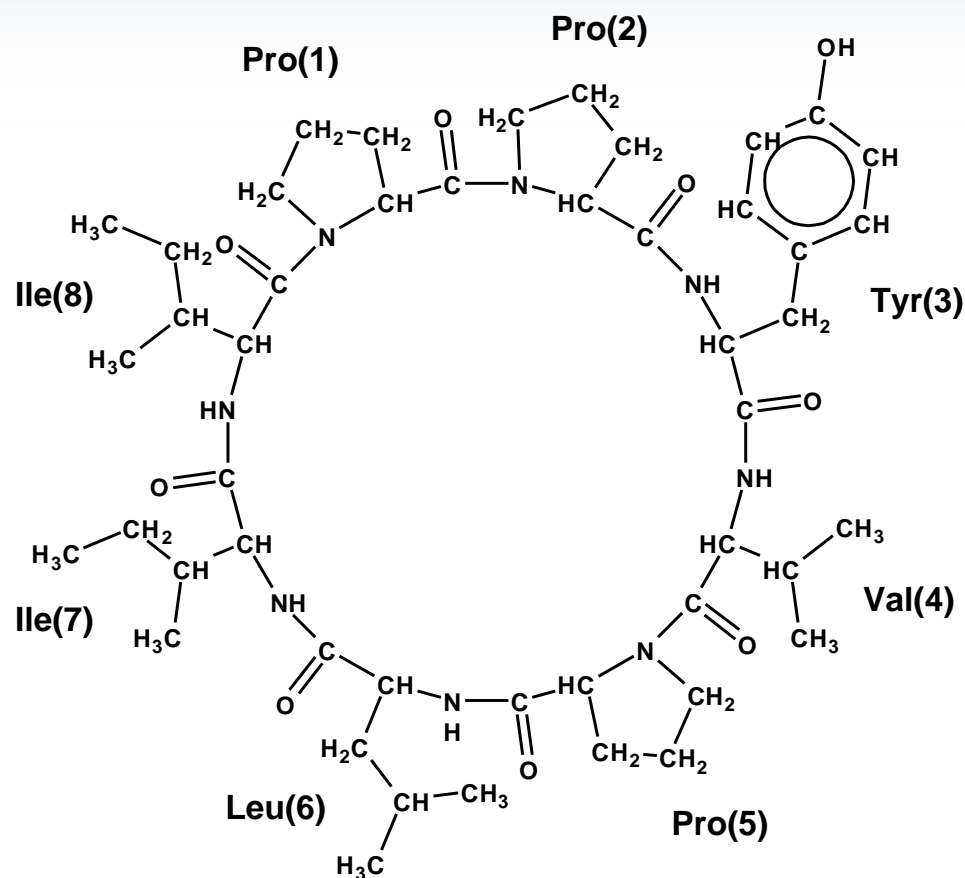
Additional examples



Hymenistatin

number of cross peaks
(estimated maximum):

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

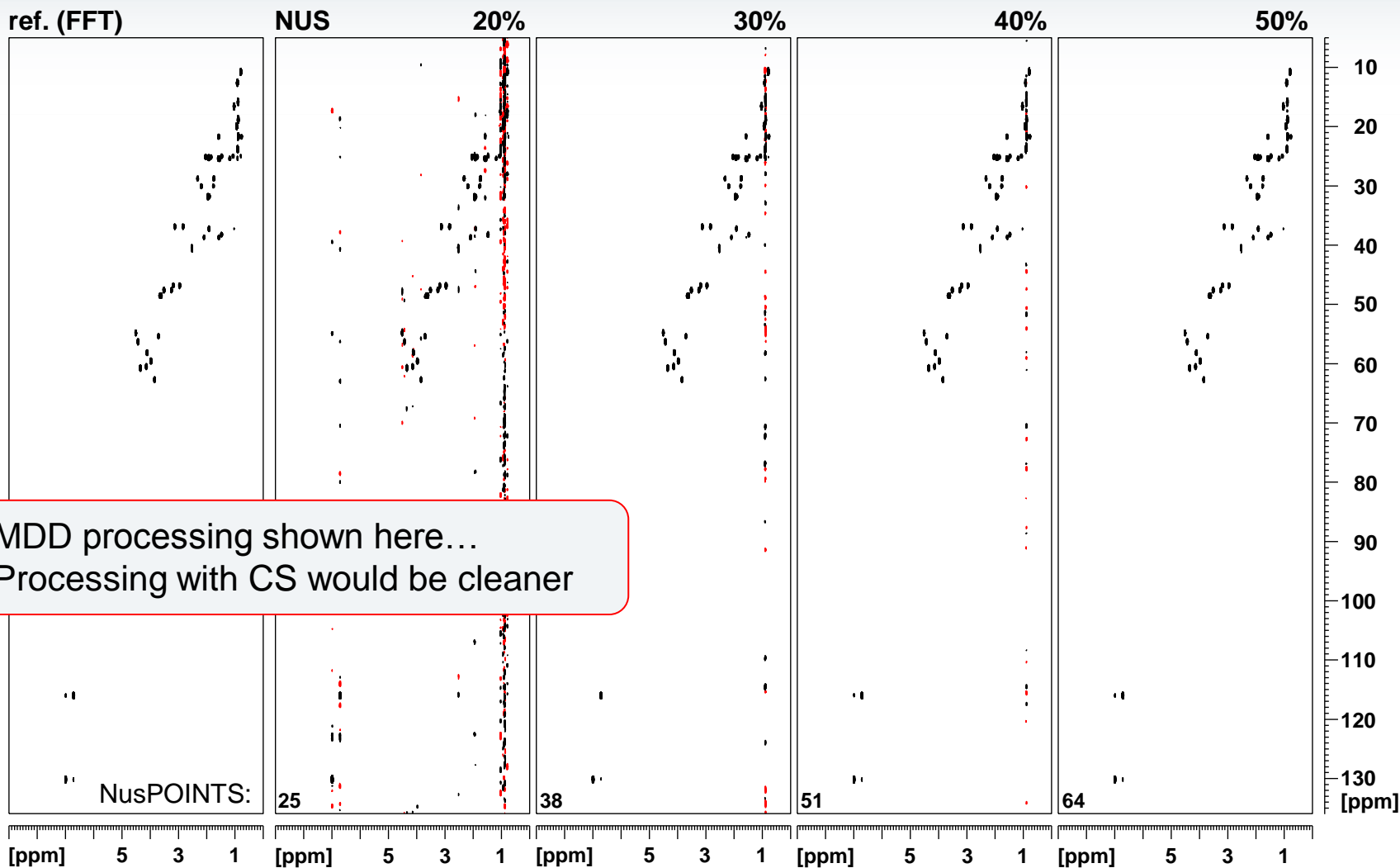


R.K. Konat, D.F. Mierke, H.Kessler, B. Kutscher, M. Bernd
& R. Voegeli, *Helv. Chim. Acta* **76**, 1649 (1993)

Additional examples – 20mM Hymenistatin



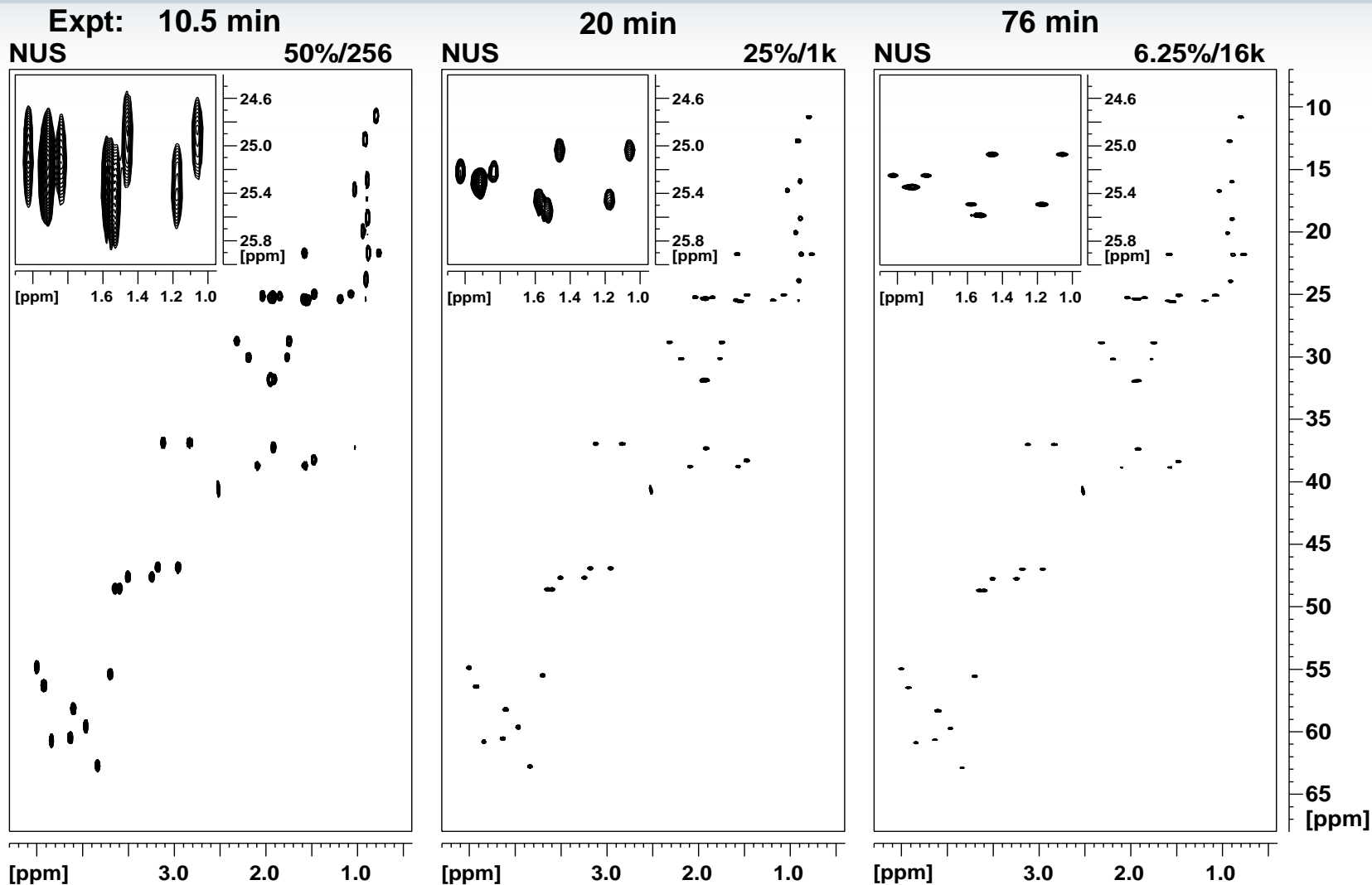
HSQC: td = 256



Additional examples – 20mM Hymenistatin



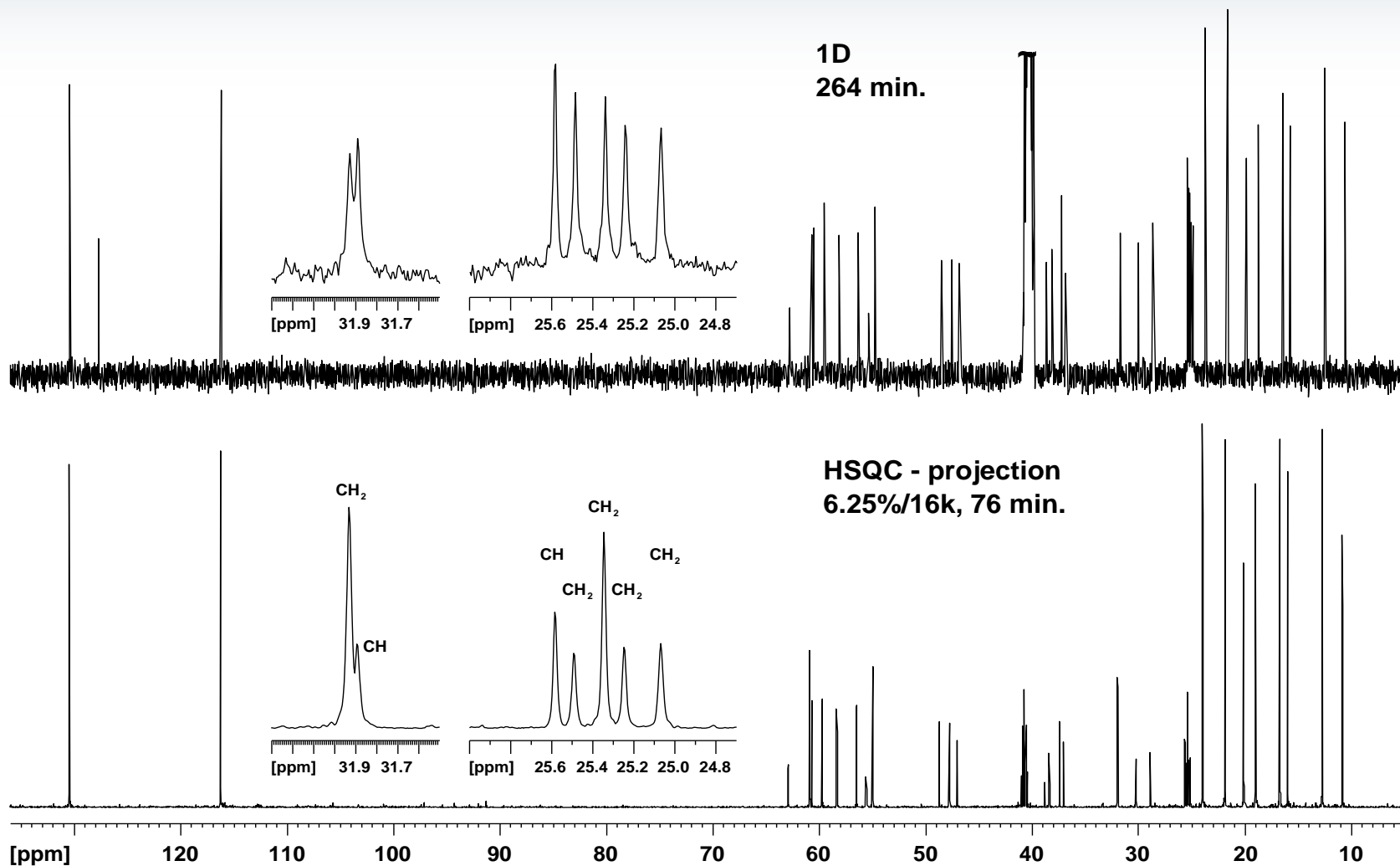
HSQC



Additional examples – 20mM Hymenistatin



HSQC

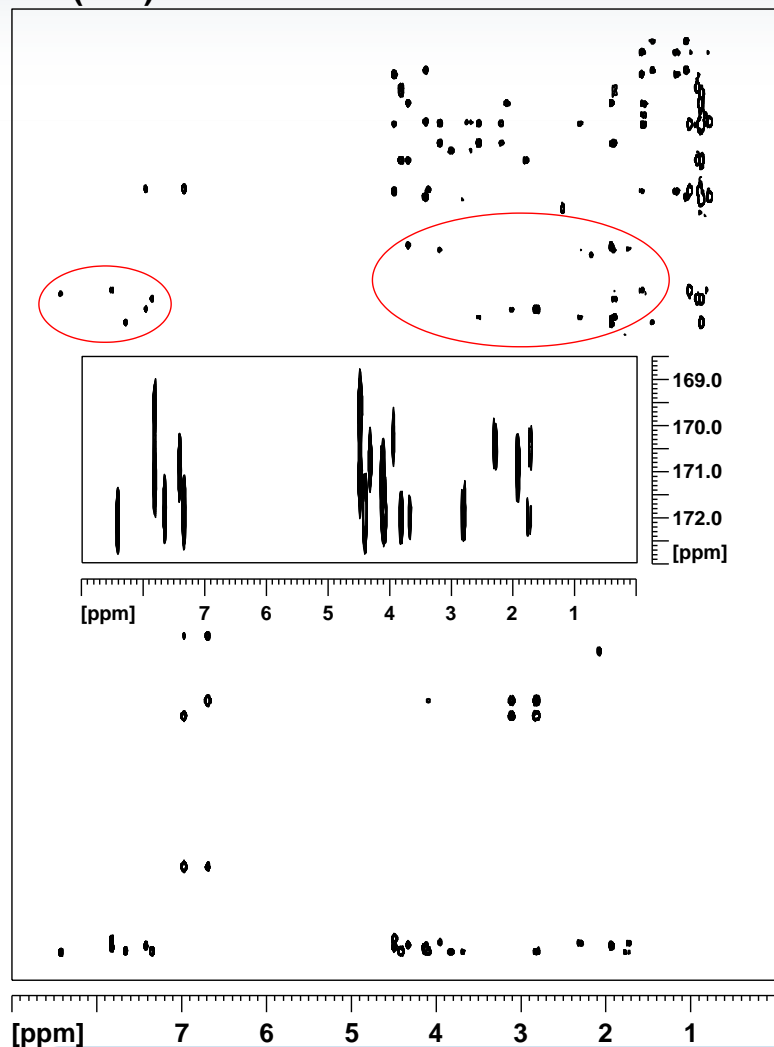


Additional examples – 20mM Hymenistatin



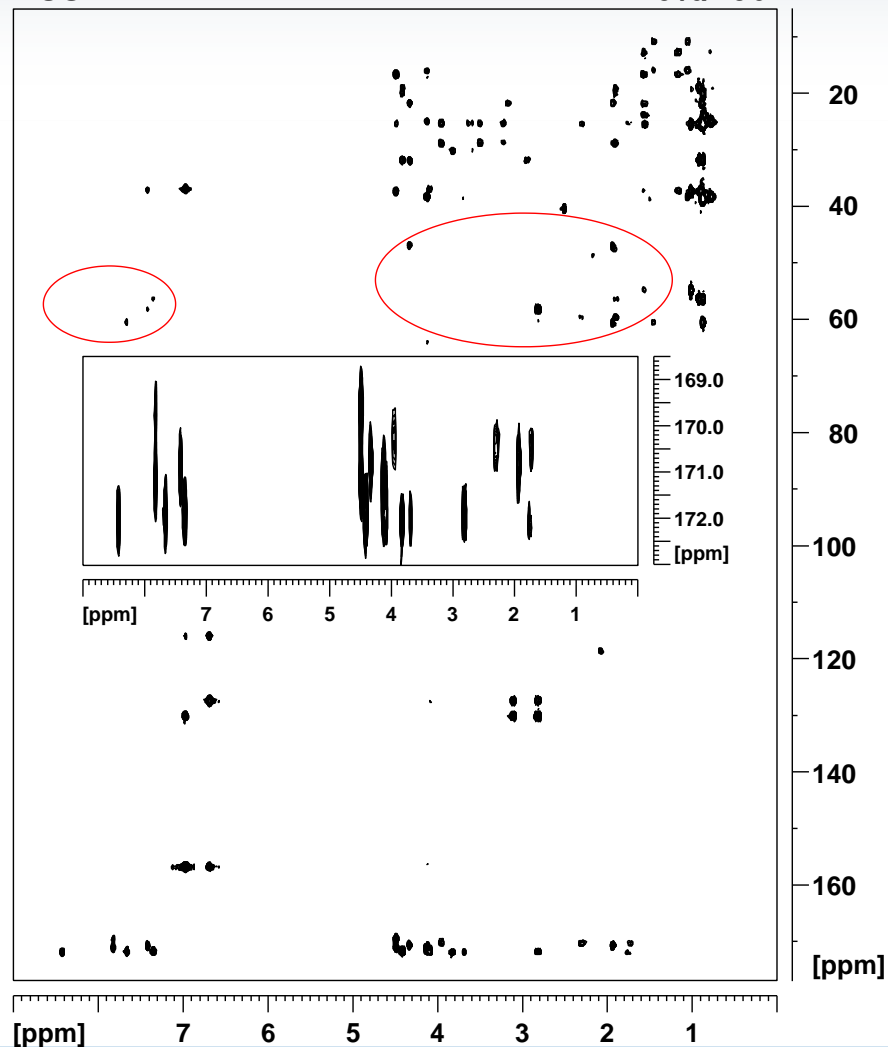
HMBC

ref. (FFT)



NUS

70%/256

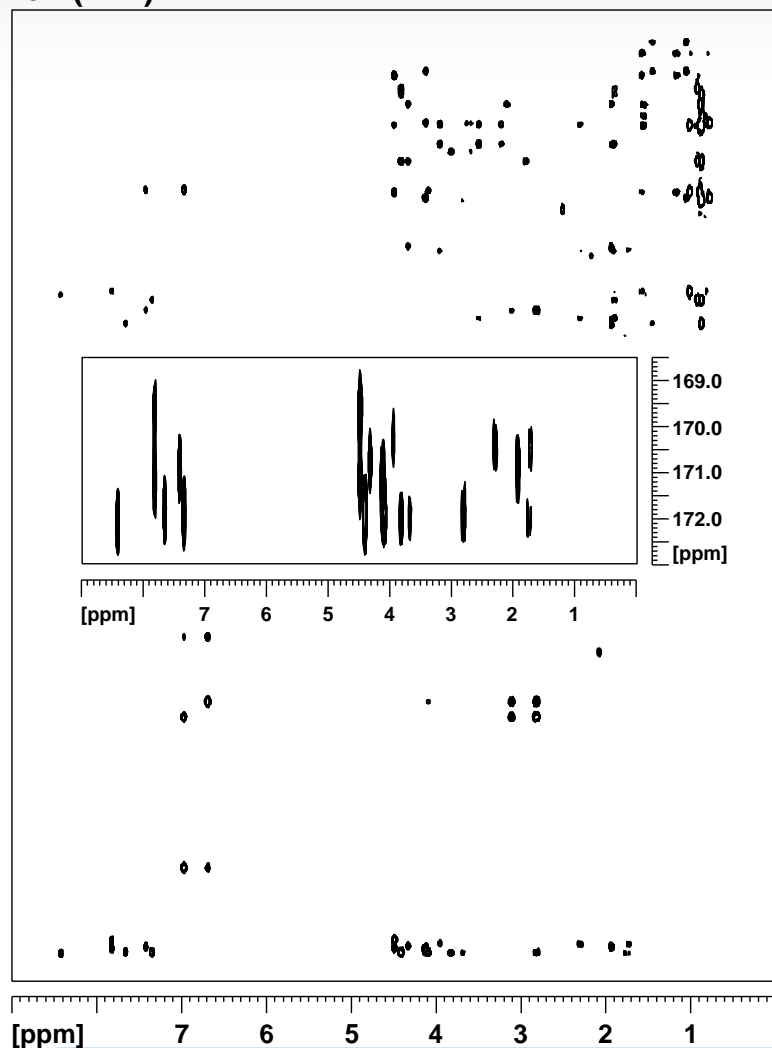


Additional examples – 20mM Hymenistatin



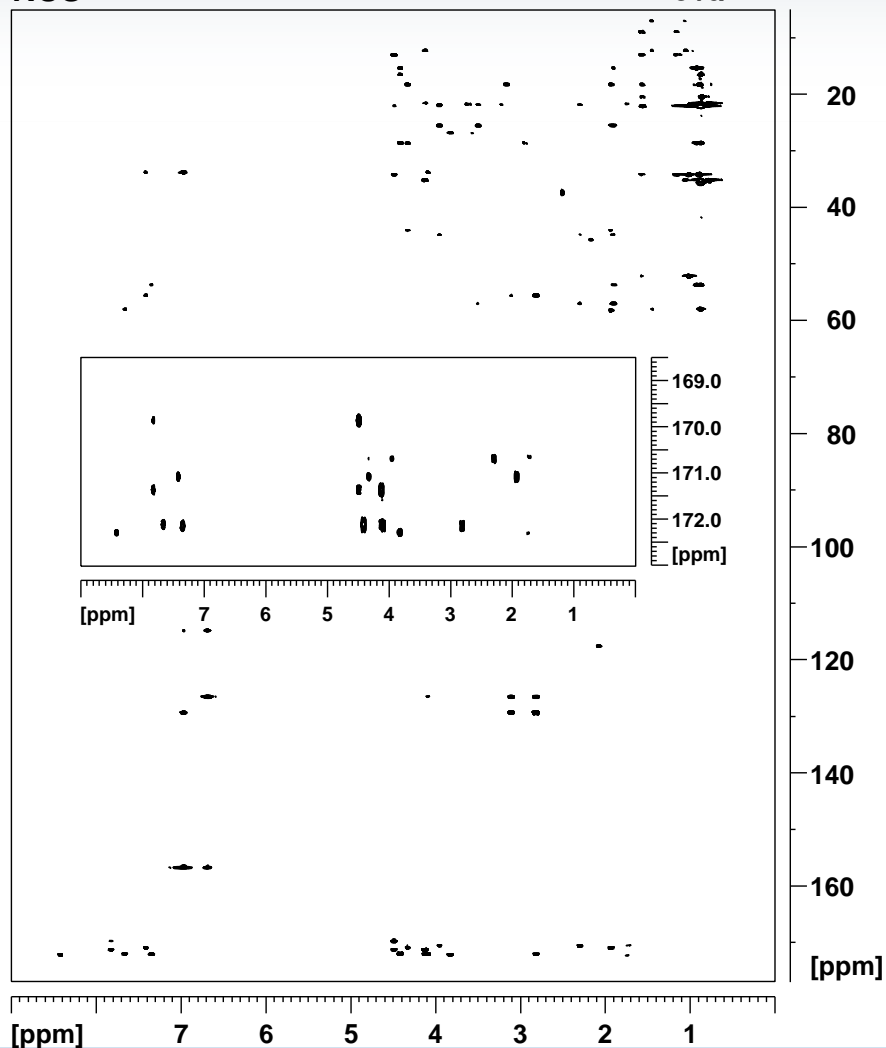
HMBC

ref. (FFT)



NUS

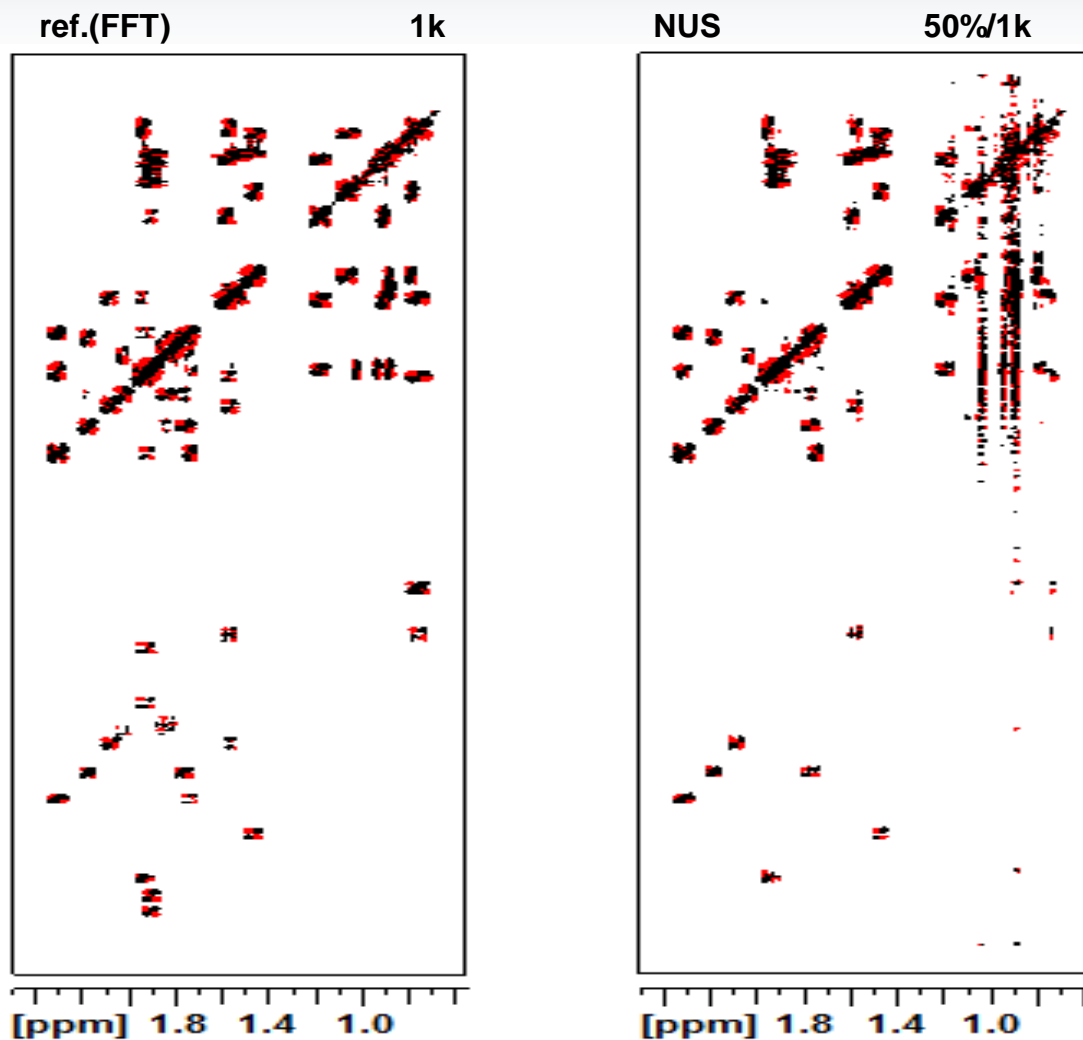
25%/4k



Additional examples – 20mM Hymenistatin



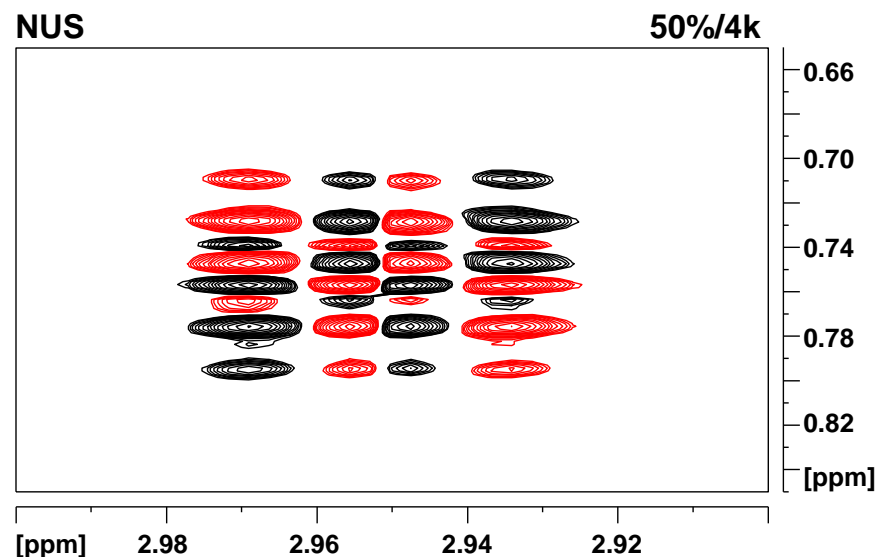
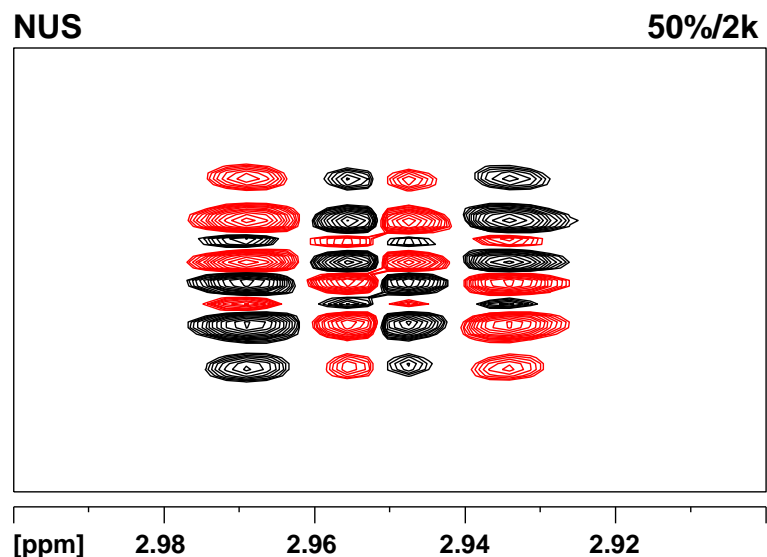
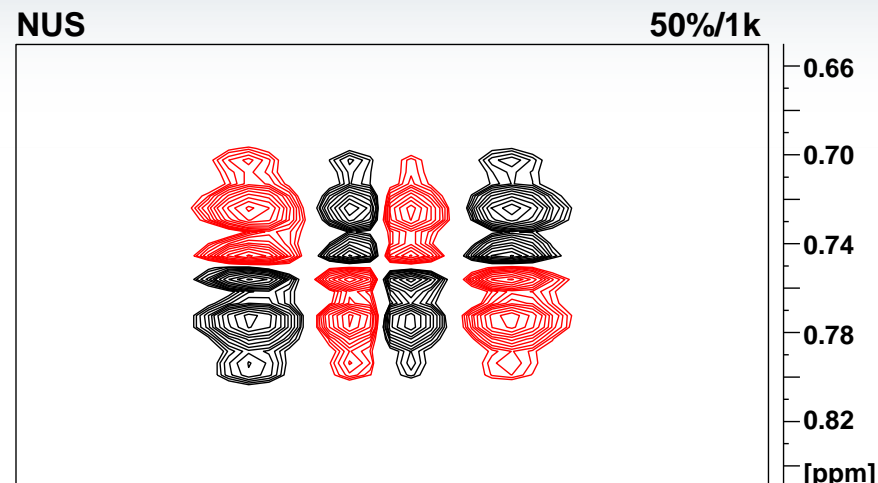
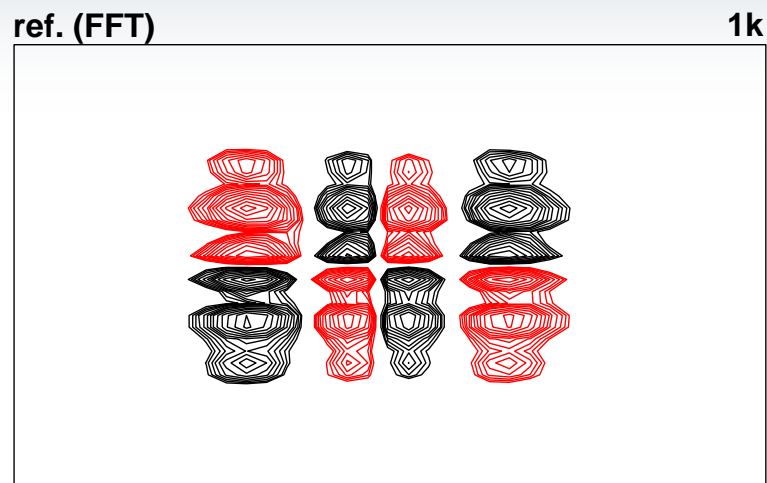
double quantum filtered COSY



Additional examples – 20mM Hymenistatin



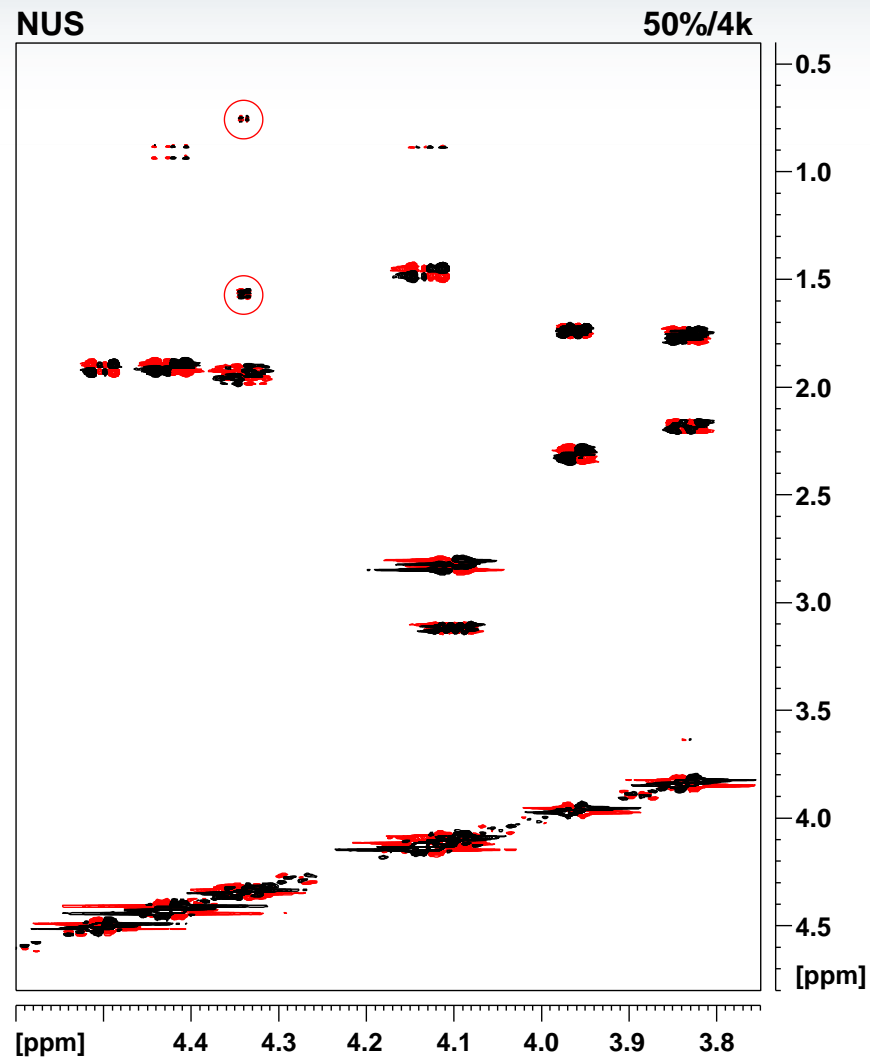
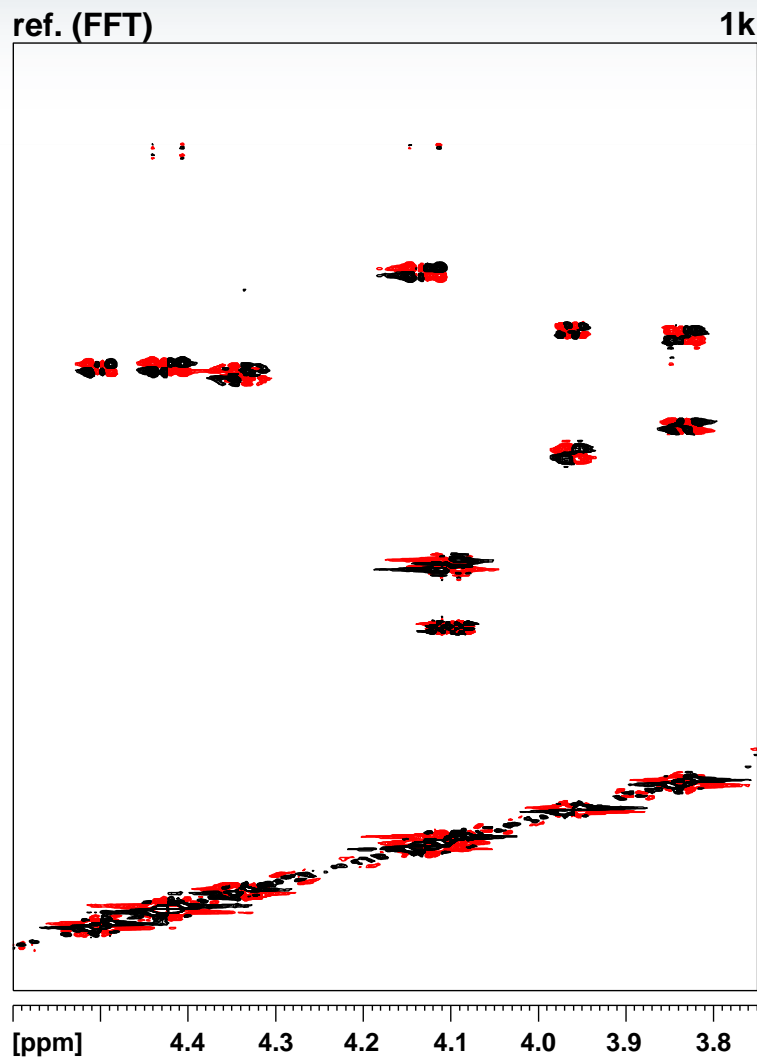
double quantum filtered COSY



Additional examples – 20mM Hymenistatin



double quantum filtered COSY



A few more acquisition details



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

Probe: PA BBO 500S2 BB-H-D-05 Z

Experiment
Width
Receiver
Nucleus
Durations
Power
Program
Probe
Lists
NUS
Wobble
Lock
Automation

^ NUS (Non Uniform Sampling) parameters

NUS Help

NusAMOUNT [%] 5

NusPOINTS 102

NusJSP [Hz] 0

NusT2 [sec] 1 T2 relaxation

NusSEED 54321 Random generator seed

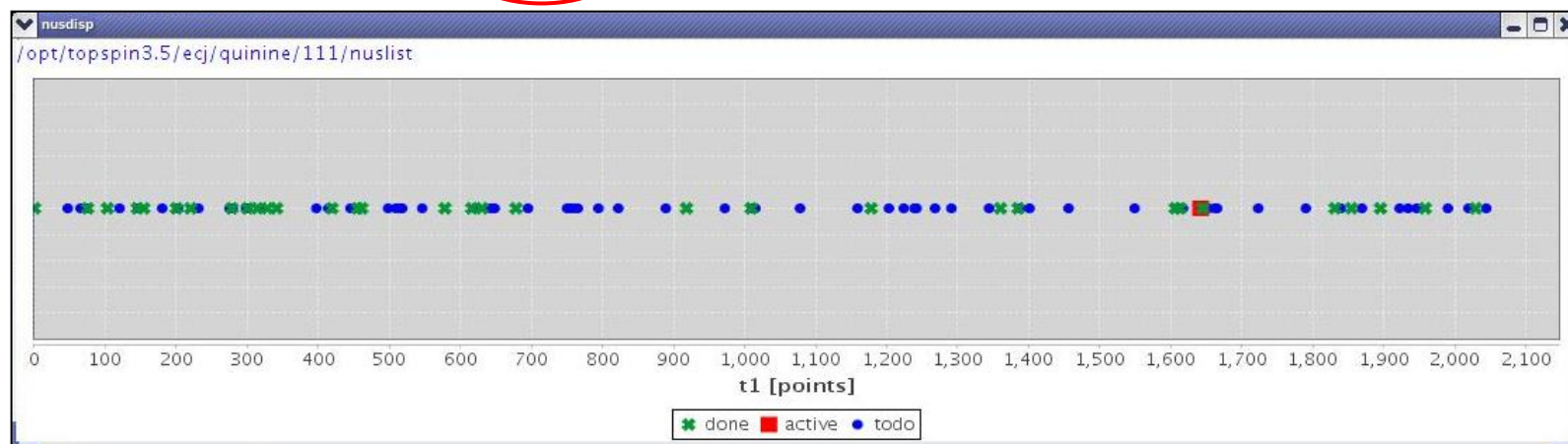
NUSLIST automatic Name of loopcounter list for NUS (Non Uniform Samplir

Calculate

Show

Display NUS point spread

After you've started the acquisition, you can get a graphical display of the NUS list



A few more acquisition details



The screenshot shows the Bruker software interface. The main window displays the 'AcquPars' tab with 'NUS (Non Uniform Sampling) parameters'. The parameters are as follows:

Parameter	Value	Description
NusAMOUNT [%]	5	Amount of sparse sampling
NusPOINTS	102	Number of hypercomplex points in
NusJSP [Hz]	0	J-coupling
NusT2 [sec]	1	T2 relaxation
NusSEED	54321	Random generator seed
NUSLIST	automatic	Name of loopcounter list for NUS

Buttons include 'NUS Help', 'Calculate', and 'Show'. A 'nuslist...' window is open on the right, displaying a list of FID numbers:

```
0
341
1178
1831
1009
145
578
328
278
103
154
1645
918
200
1606
314
2029
630
455
461
1360
419
```

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

Experiment
Width
Receiver
Nucleus
Durations
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NUS
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Automation

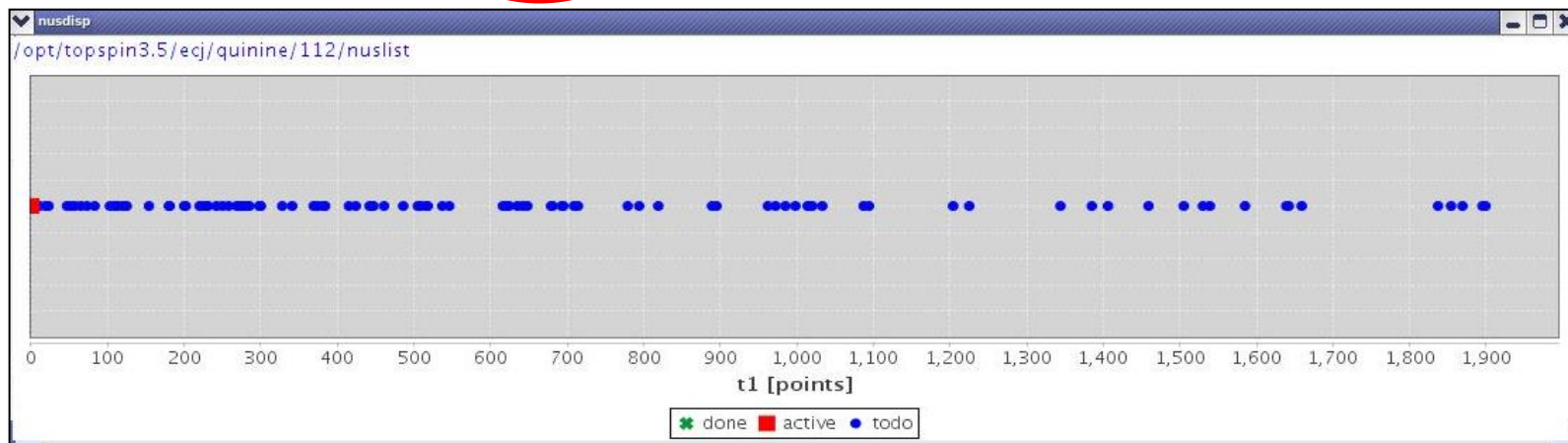
AcquPars

NUS (Non Uniform Sampling)

NusAMOUNT [%] 5
NusPOINTS 102
NusISP [Hz] 0
NusT2 [sec] 0.04
NusSEED 54321
NUSLIST automatic

J-coupling
T2 relaxation
Random generator seed
Name of loopcounter list for NUS (Non Uniform Sampling)
Calculate point spread function
Display NUS point spread

Calculate
Show



A few more acquisition details



Probe: PA BBO 500S2 BB-H-D-05 Z

^ NUS (Non Uniform Sampling) parameters

NusAMOUNT [%]	5	NUS Help	Show NUS help
NusPOINTS	102		Amount of sparse sampling
NusJSP [Hz]	5		Number of hypercomplex points in indirect dimension
NusT2 [sec]	1		J-coupling
NusSEED	54321		T2 relaxation
NUSLIST	automatic		Random generator seed
		Calculate	Name of loopcounter list for NUS (Non Uniform Samplir
		Show	Calculate point spread function
			Display NUS point spread

- 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



Probe: PA BBO 500S2 BB-H-D-05 Z

Experiment
Width
Receiver
Nucleus
Durations
Power
Program
Probe
Lists
NUS
Wobble
Lock
Automation

NUS (Non Uniform Sampling)

NUS

NusAMOUNT [%] 5

NusPOINTS 102

NusJSP [Hz]

NusT2 [sec]

NusSEED 54321 Random generator seed

NUSLIST myNUSlist Name of loopcounter list for NUS (Non Uniform Samplir

Calculate Calculate point spread function

Show Display NUS point spread

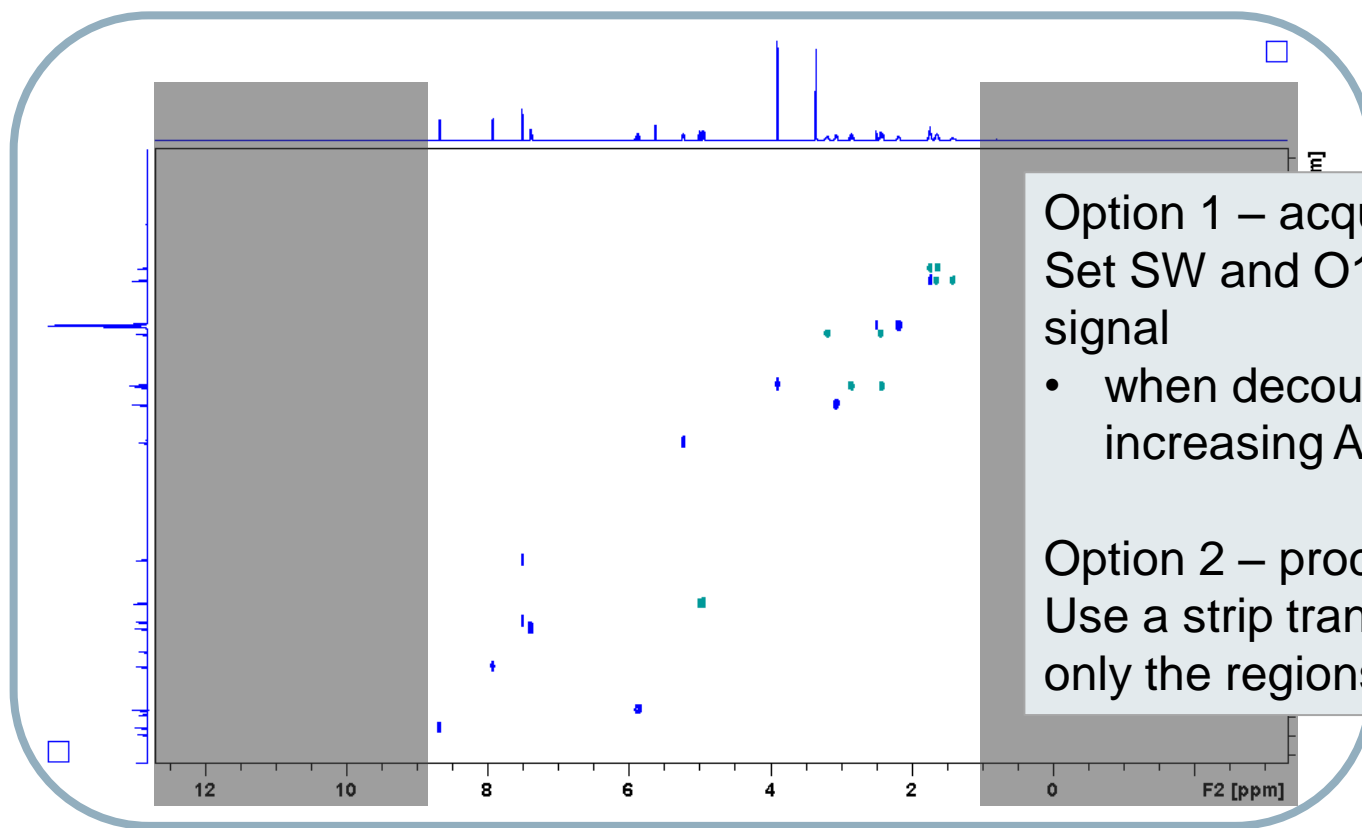
- DO NOT click the “Calculate” button when using your own list!
- This will overwrite your lists with an automatically generated one

- 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



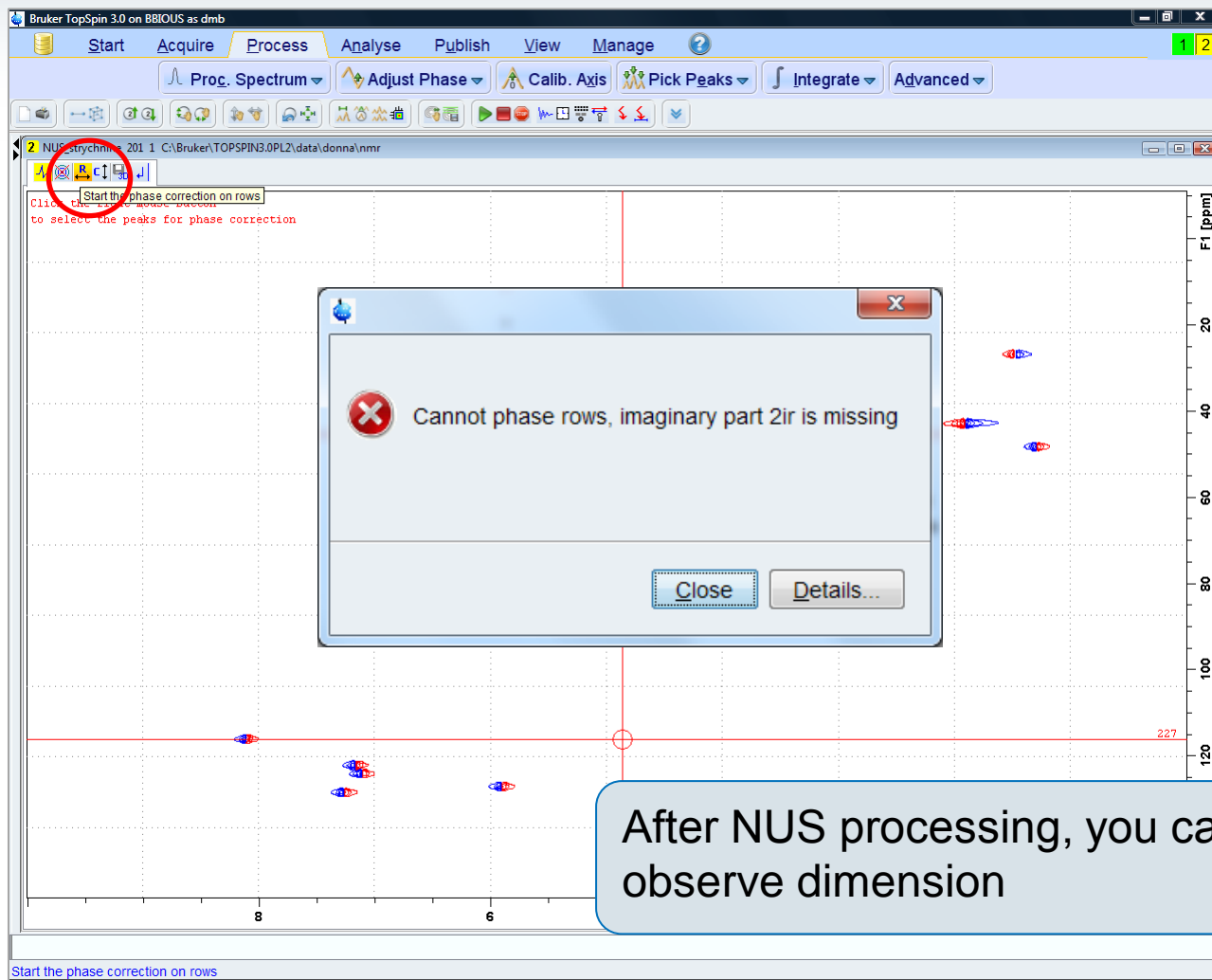
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu

S 1,2... M

BCFW [ppm]	1.00000	1.00000	Filter width for bc (sfil/qfil)
COROFFS [Hz]	0	0	Correction offset for BC_MOD=spol etc.
BC_mod	quad	no	Fid baseline modes for em, ft, xfb,...
Fourier transform			
TDeff	0	0	Number of fid data points used by ft
STSR	200	0	First output point of strip transform
STSI	551	0	Total number of output points of strip transform
ME_mod	no	LPfc	Linear prediction for ft, xfb, ...
NCOEF	0	60	Number of LP coefficients
LPBIN	0	0	Number of output points for LP
TDoff	0	0	Number of back-predicted points
REVERSE	FA		transform

In this example, keep points 200 – 750 in observe dimension.

A few tips on processing - phasing



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

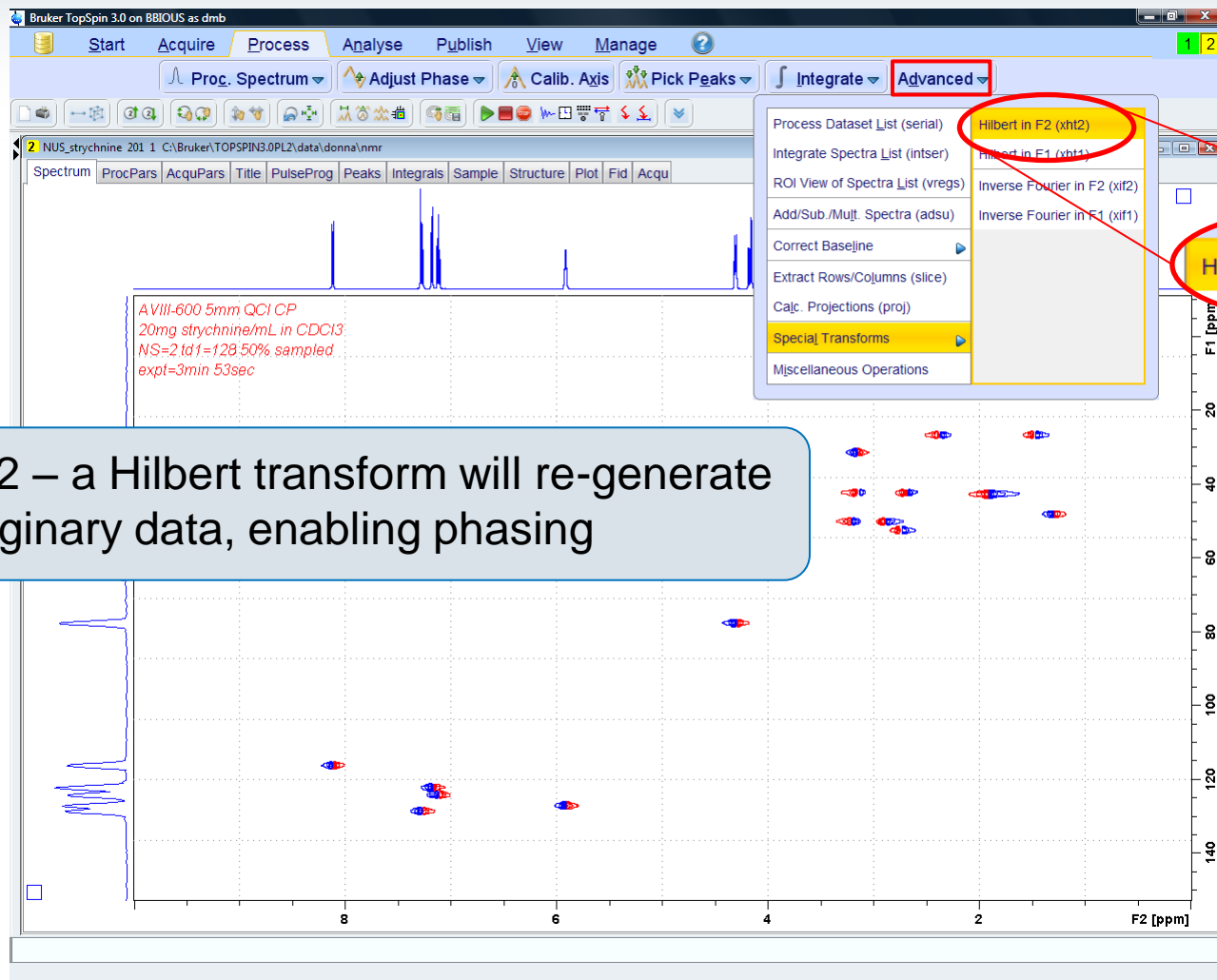
A few tips on processing - phasing



- 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

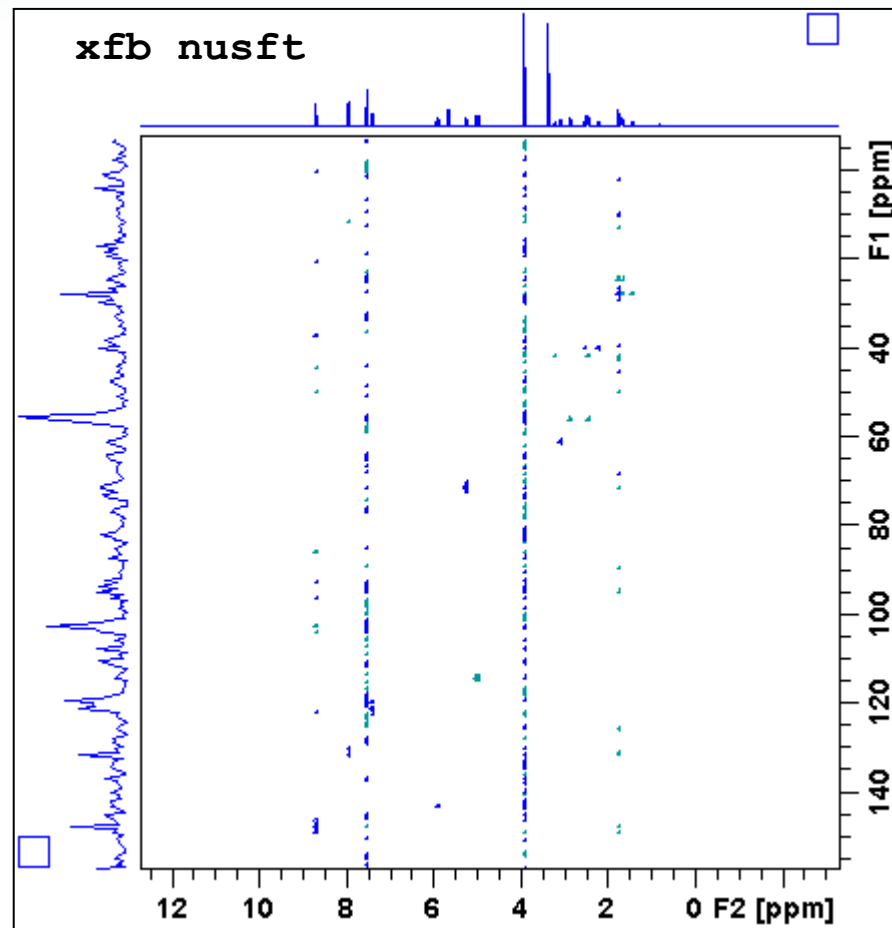
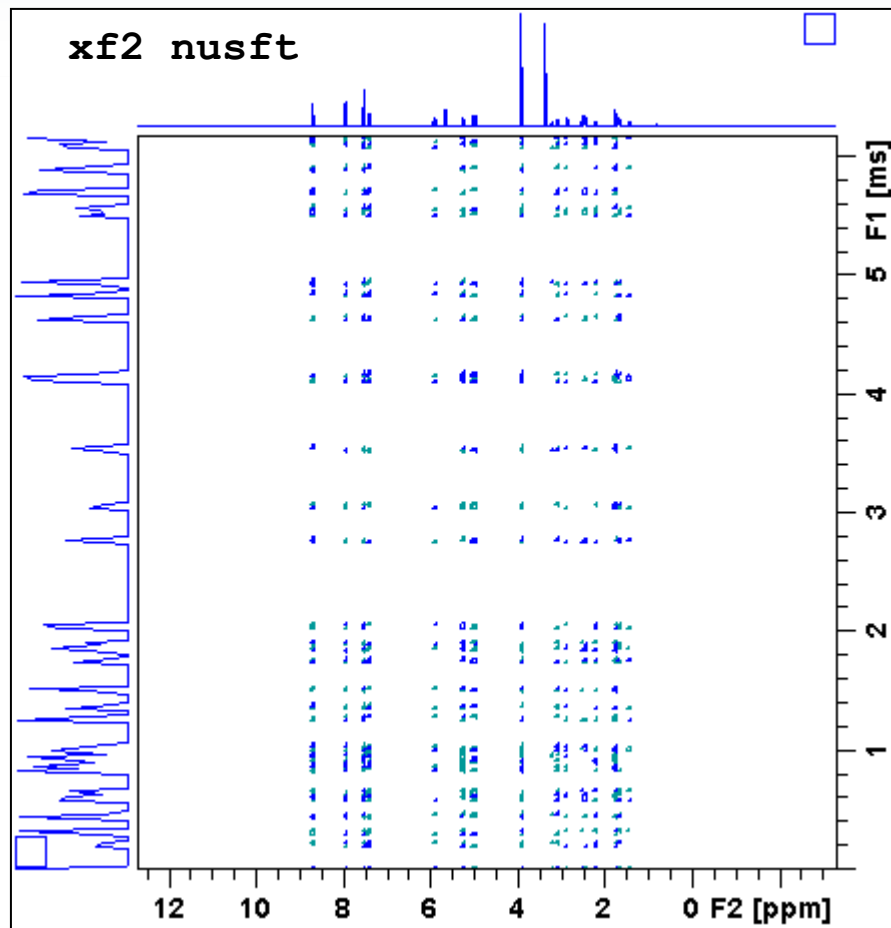


- Option 2 – a Hilbert transform will re-generate the imaginary data, enabling phasing

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"



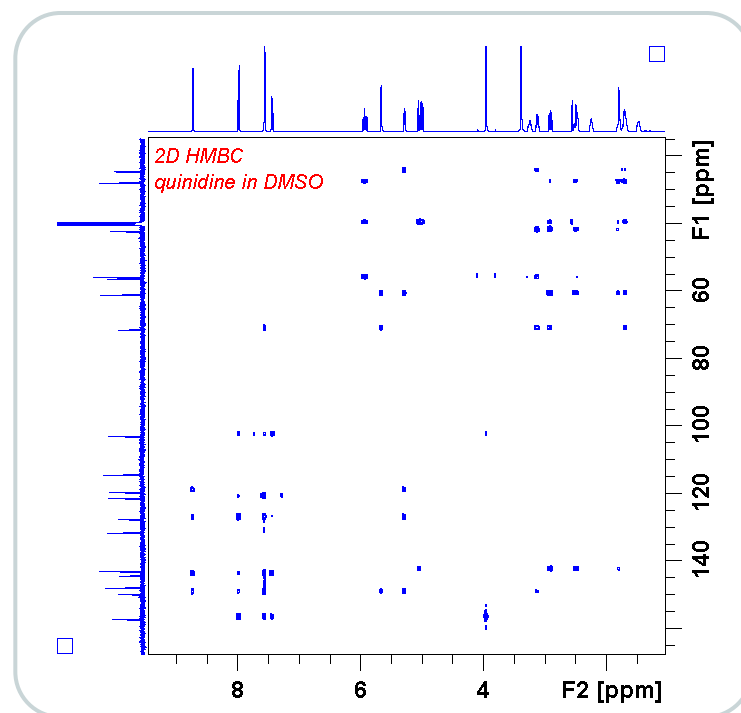
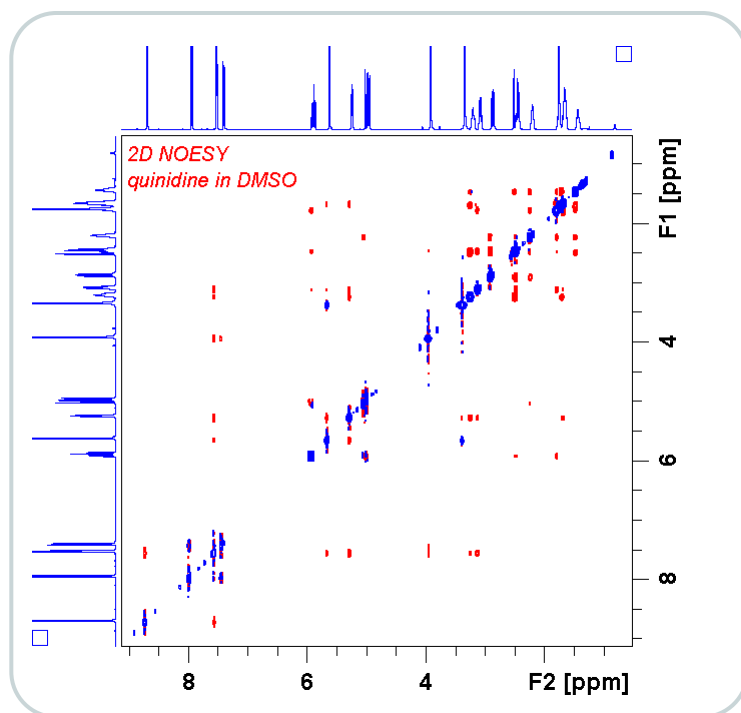
Selective Experiments

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?

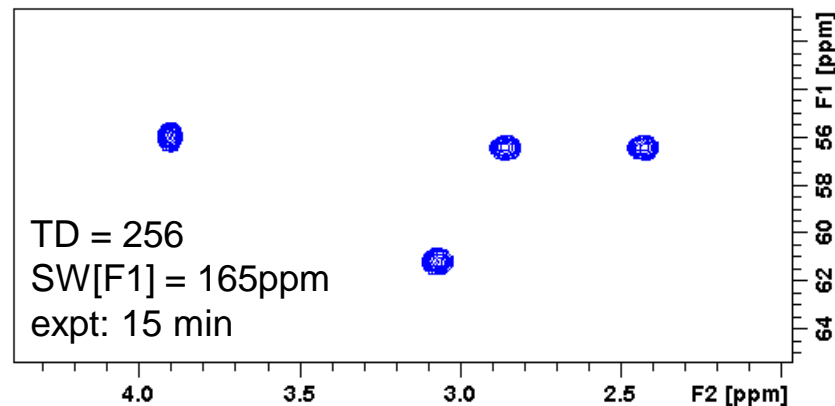
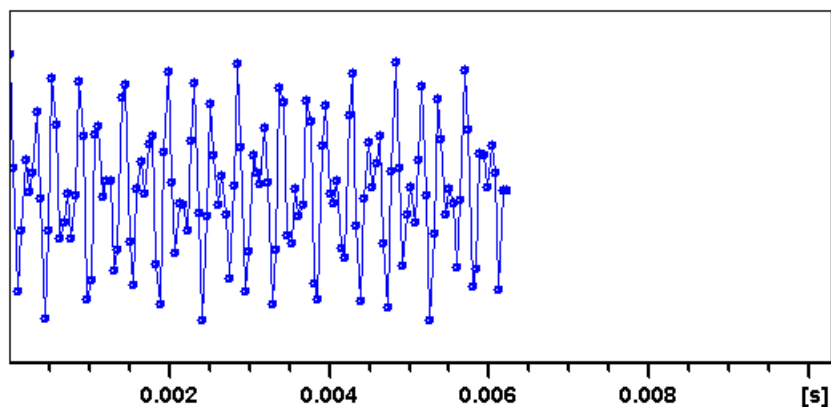
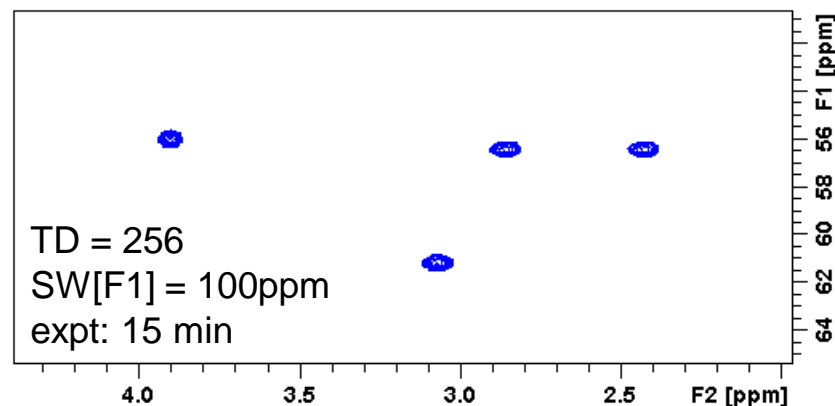
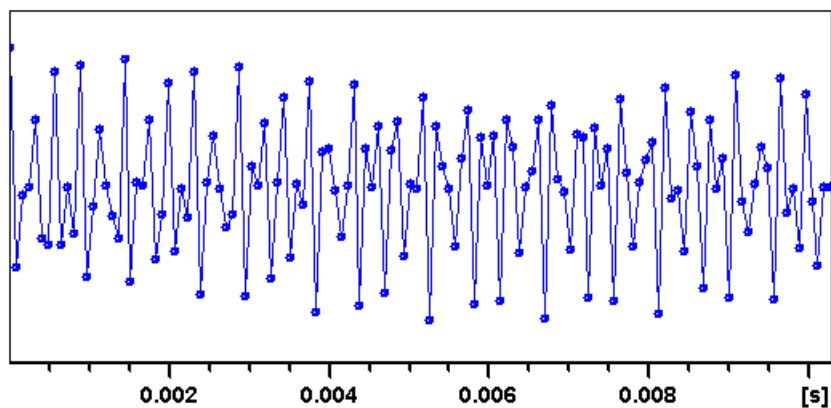


- If we're particularly interested in a few correlations in certain regions of our spectrum, we might be able to get that info faster, with higher resolution using selective experiments
- Built into Topspin:
 - Band-selective versions of 2D HSQC and HMBC
 - 1D selective versions of standard 2D homonuclear experiments
 - COSY
 - TOCSY
 - ROESY
 - NOESY

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



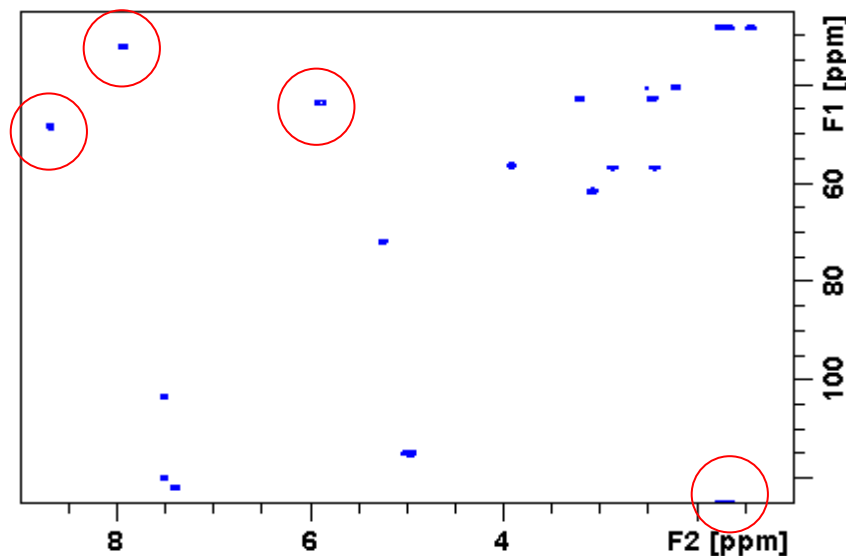
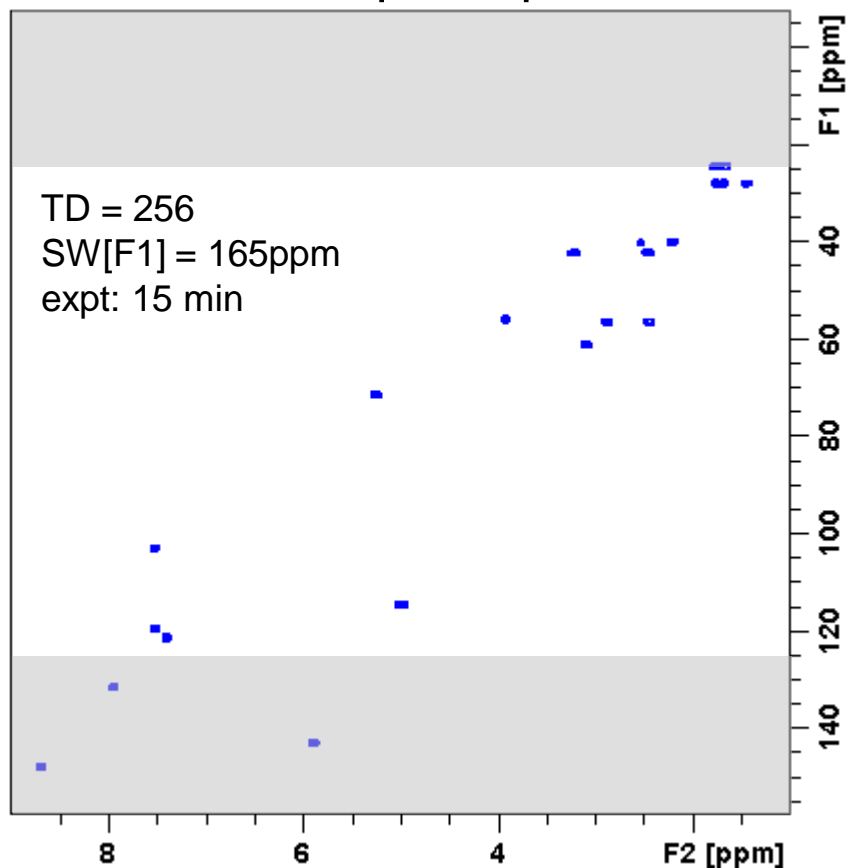
- We can increase the resolution of the indirect dimension of a 2D by reducing the sweep width



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



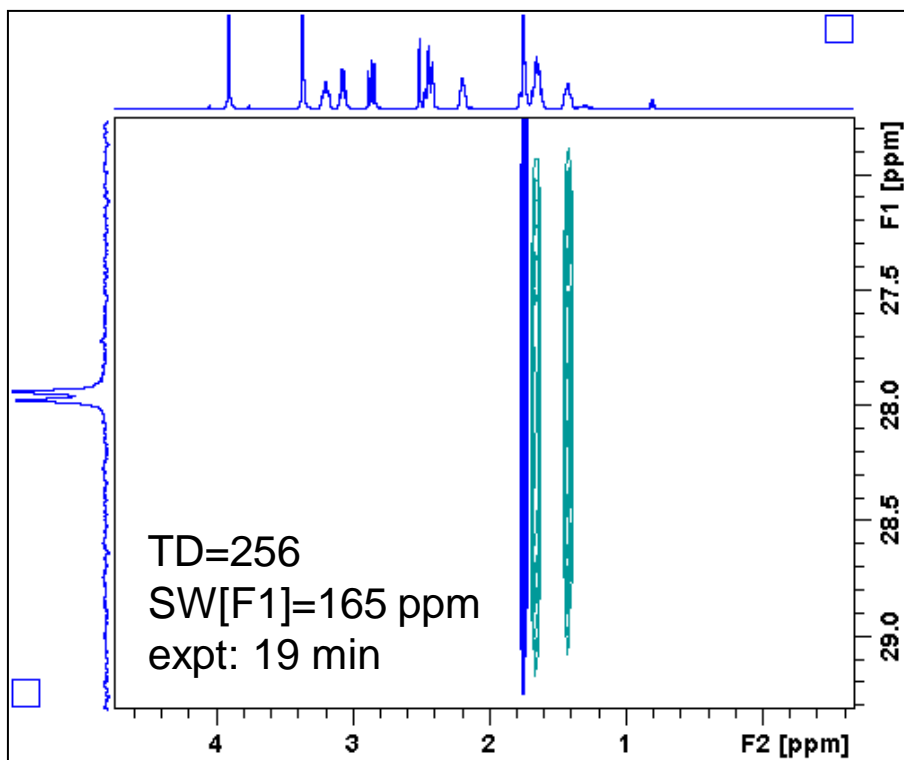
- What if we can prevent peaks outside our sweep width from folding or aliasing back into our spectrum?
 - Use band-selective excitation or refocusing pulses in the indirect dimension
 - We can focus on one region of the spectrum
 - Peaks outside the sweep width are never excited and therefore don't get in our way
- Topspin contains band-selective versions of
 - 2D ^1H - ^{13}C HSQC and 2D ^1H - ^{13}C HMBC

Band-selective 2D HSQC

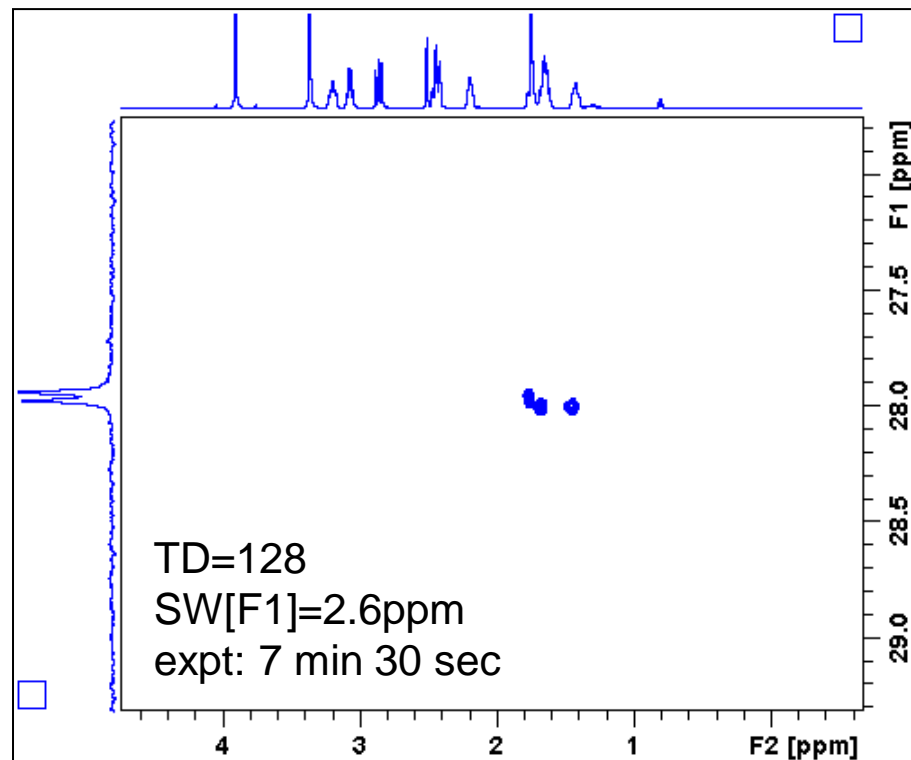


- Let's focus on the peaks at $\delta^{13}\text{C} = 28$ ppm

standard edited HSQC



band-selective HSQC

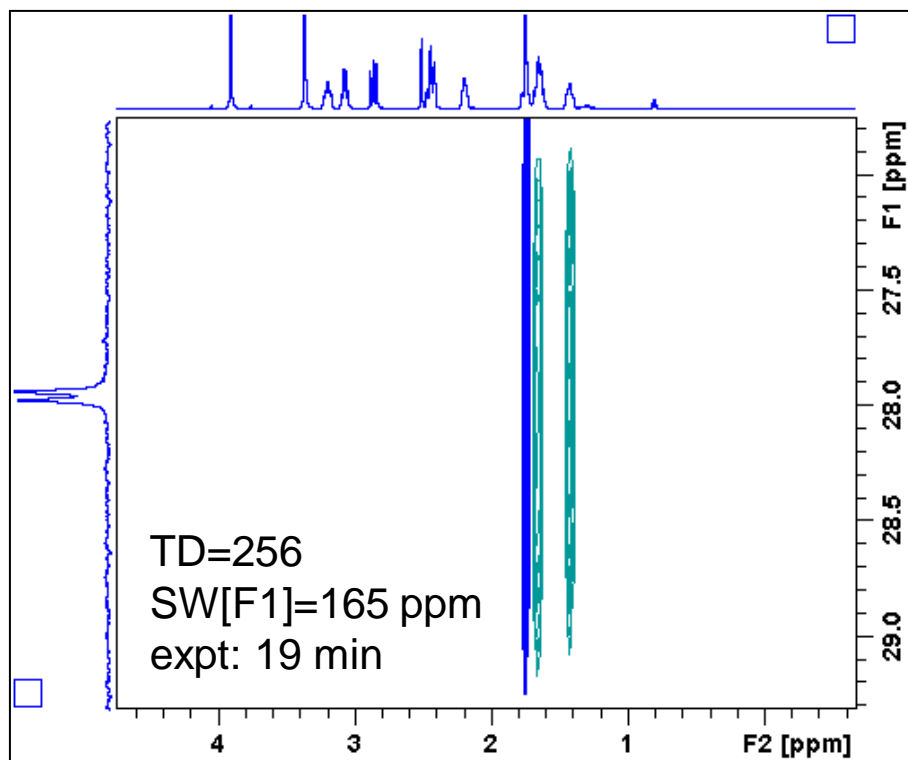


Band-selective 2D HSQC... combined with NUS

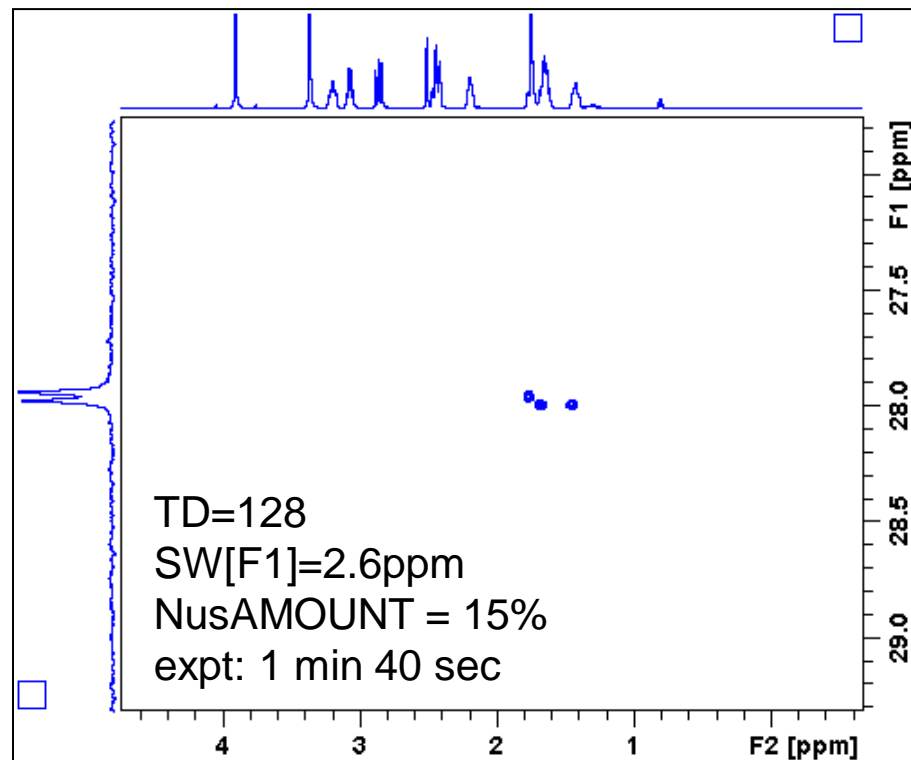


- Let's focus on the peaks at $\delta^{13}\text{C} = 28$ ppm

standard edited HSQC



band-selective HSQC, using NUS

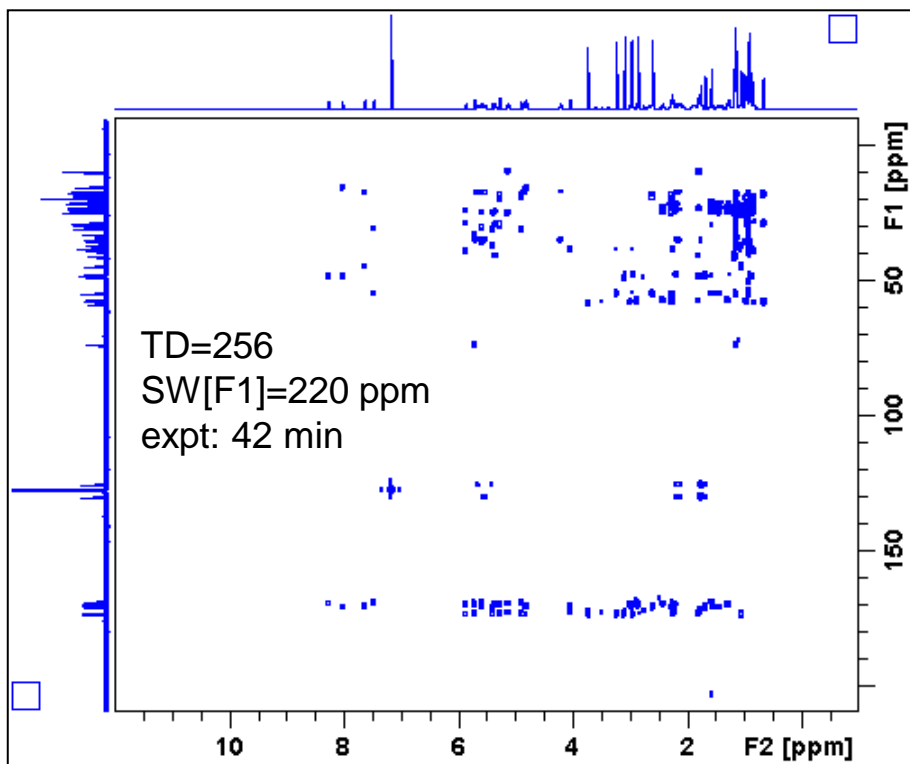


Band-selective 2D HMBC example

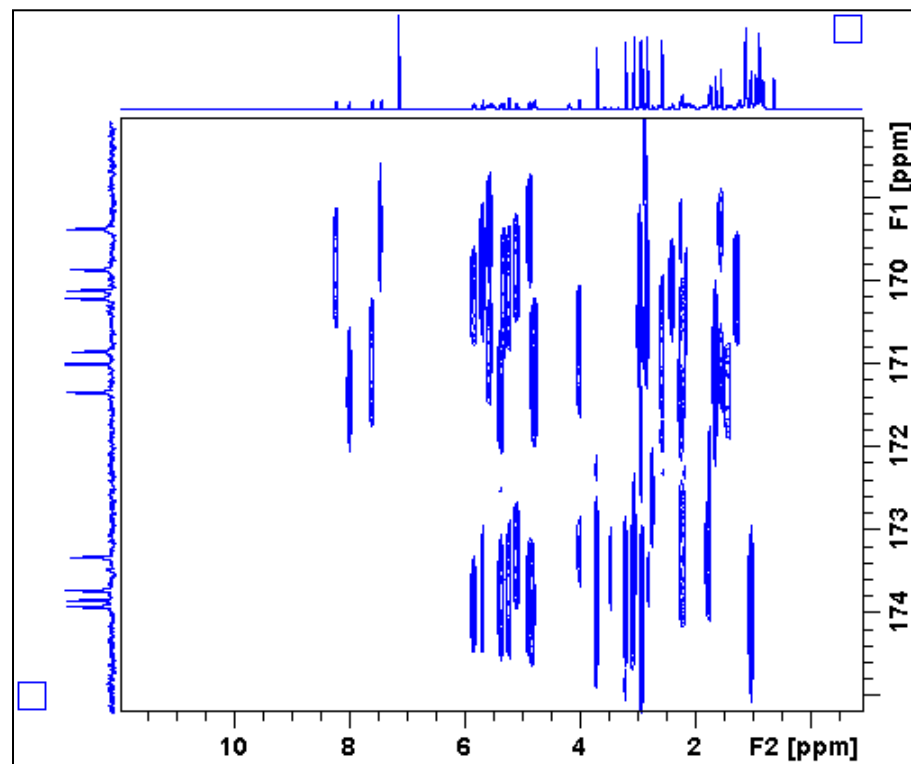


- 50mM cyclosporine in benzene-d6

standard HMBC



expansion of CO region

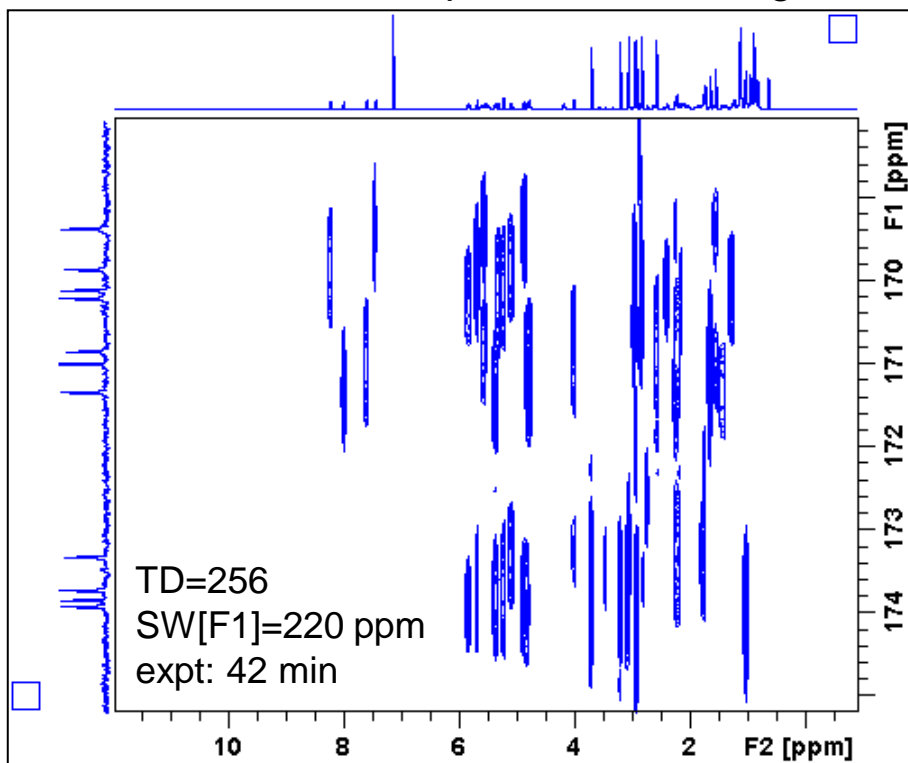


Band-selective 2D HMBC example

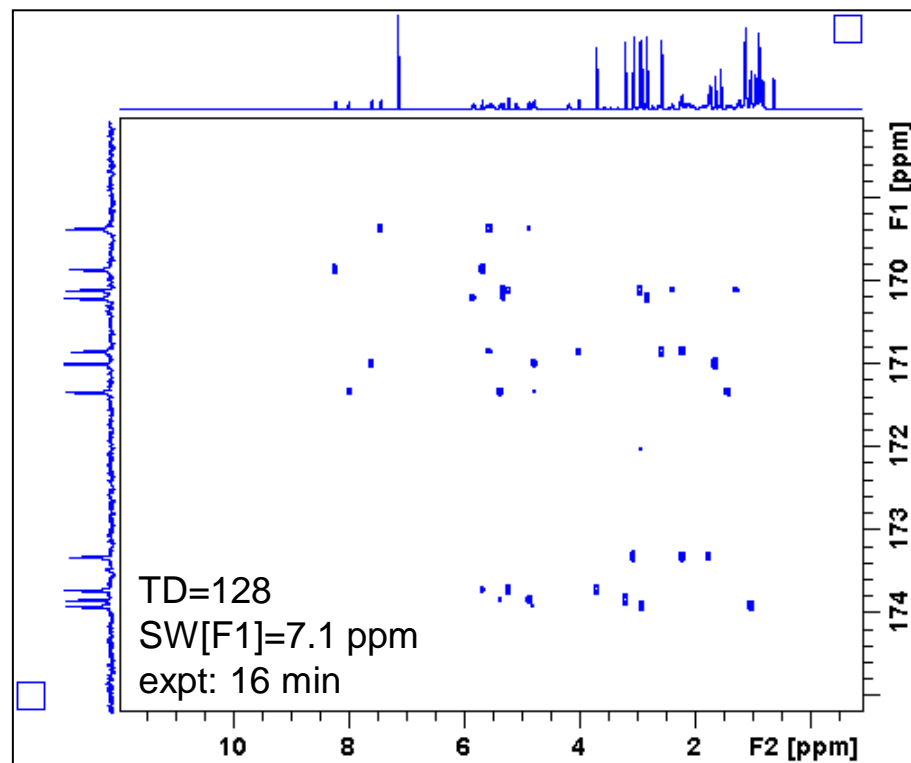


- 50mM cyclosporine in benzene-d6

standard HMBC - expansion of CO region



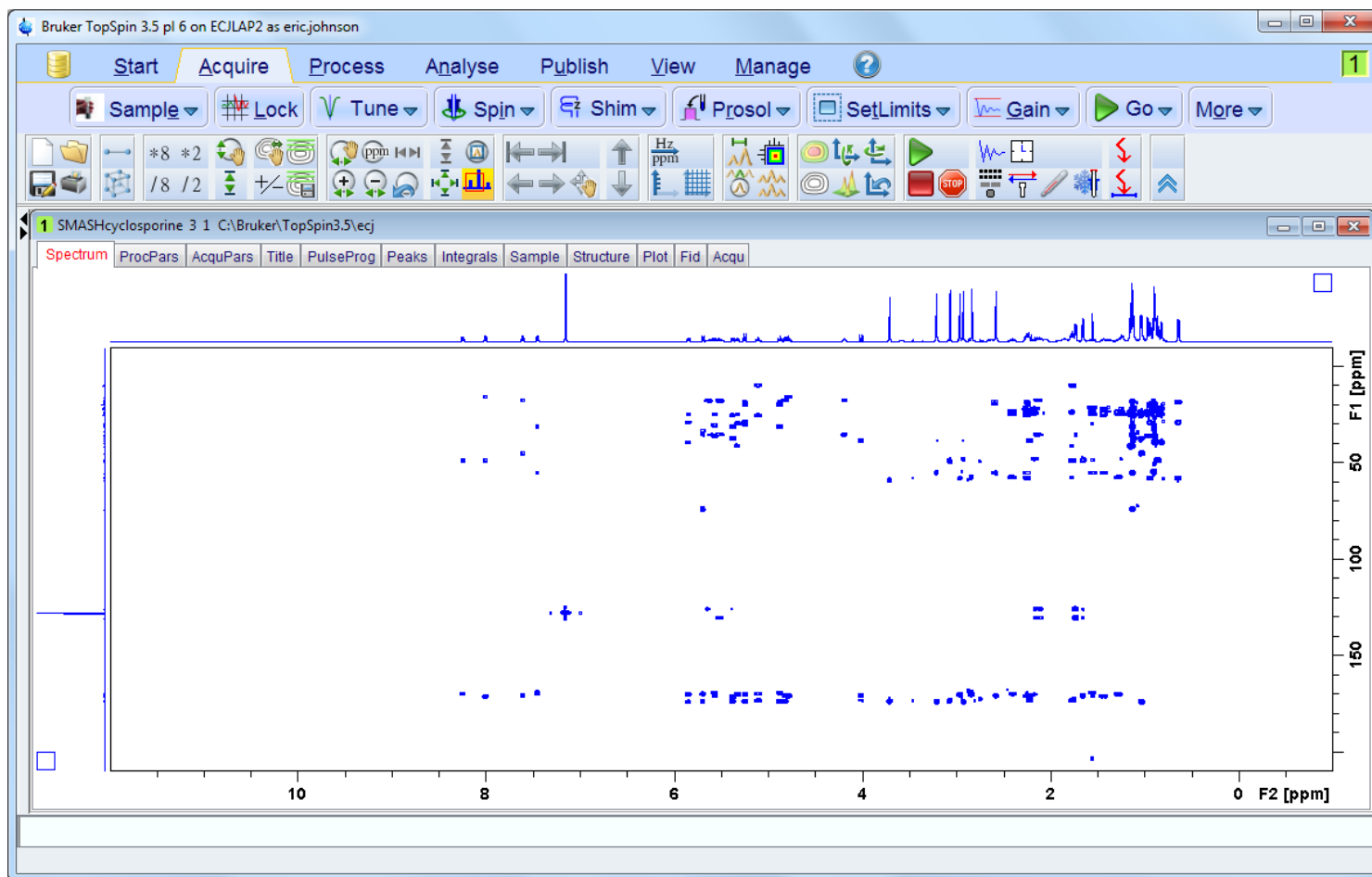
band-selective HMBC



Setting up the band-selective 2D experiments



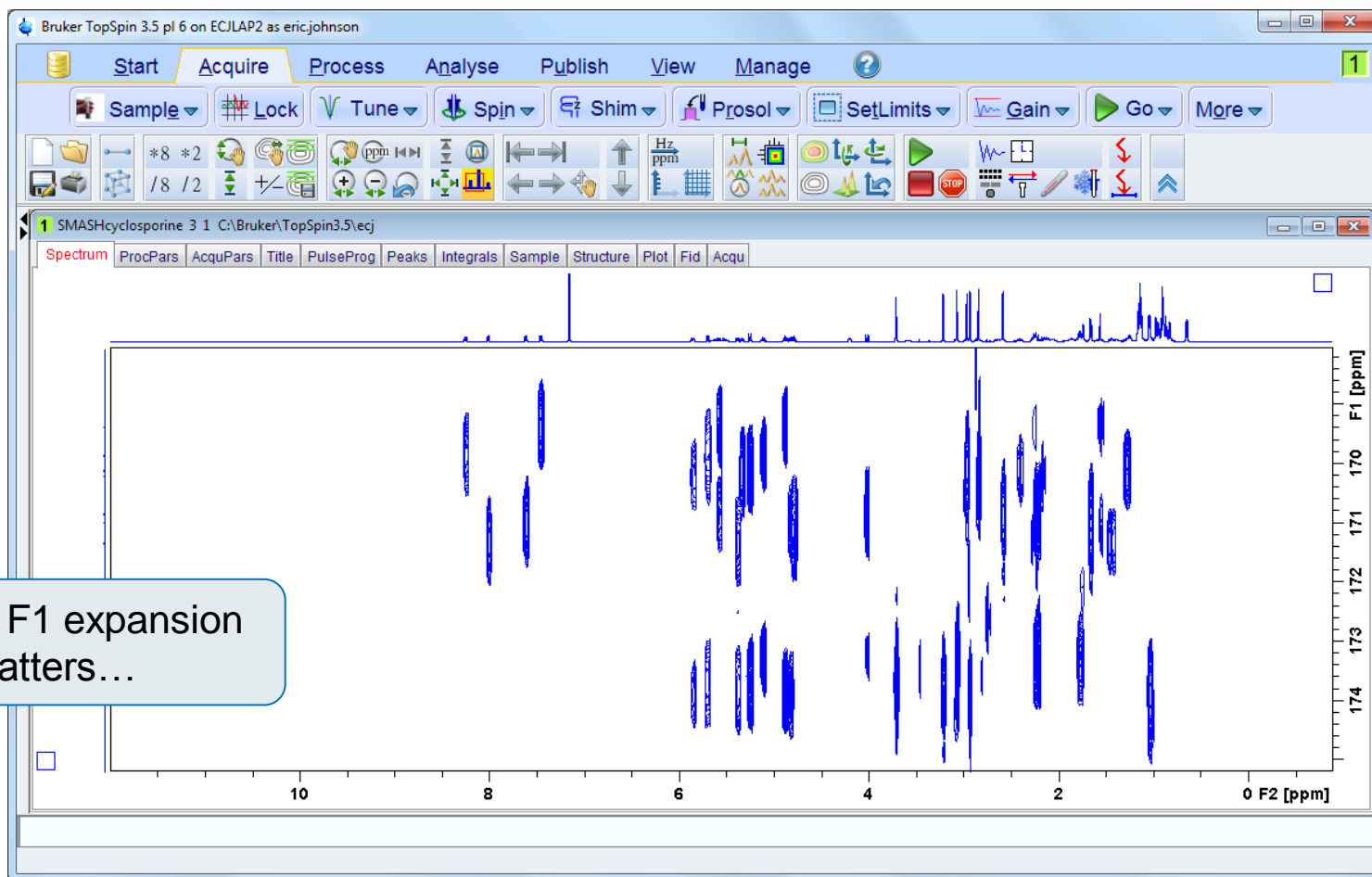
Method 1 – starting from a non-selective HSQC or HMBC



Setting up the band-selective 2D experiments



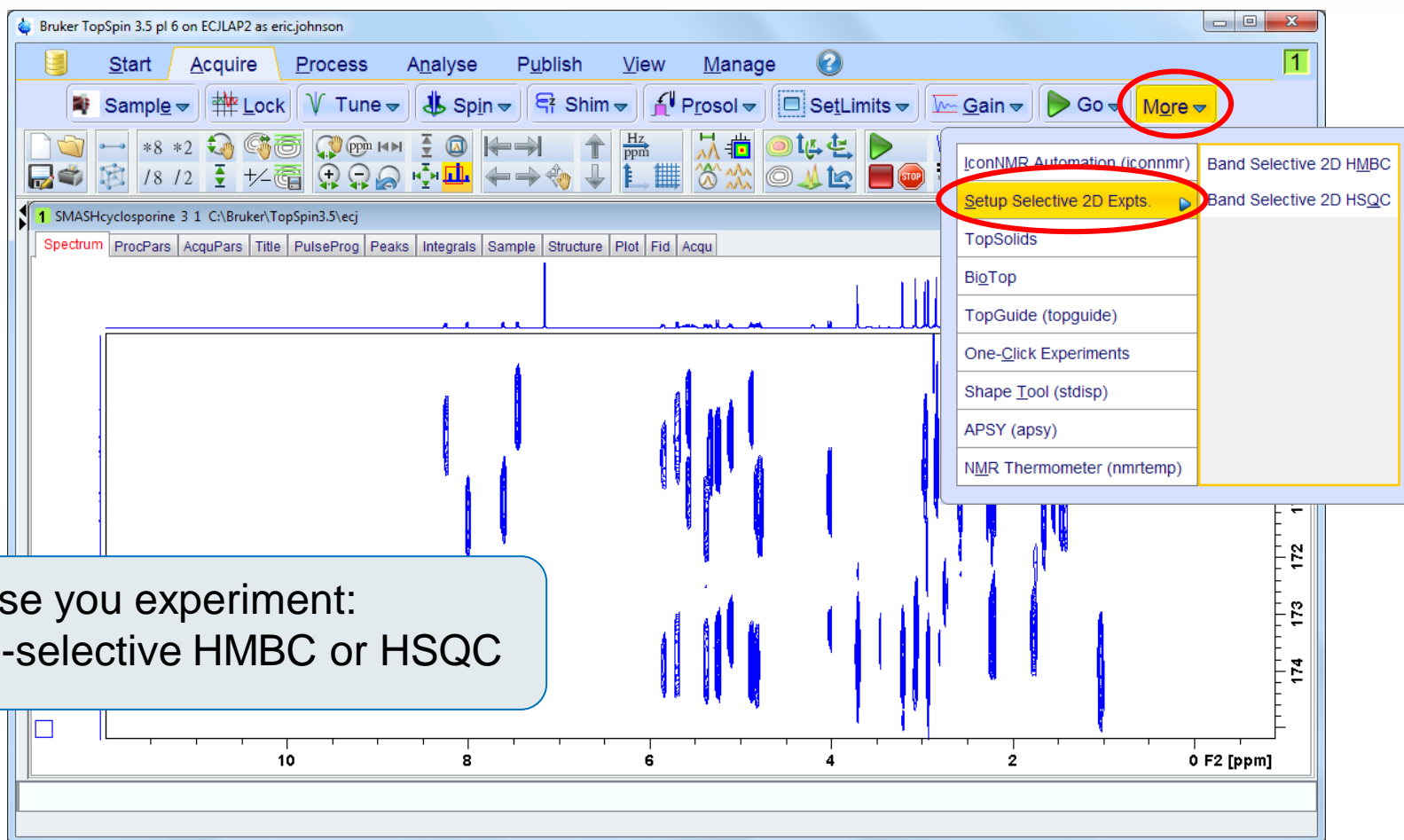
ii) zoom into the spectral region of interest



Setting up the band-selective 2D experiments

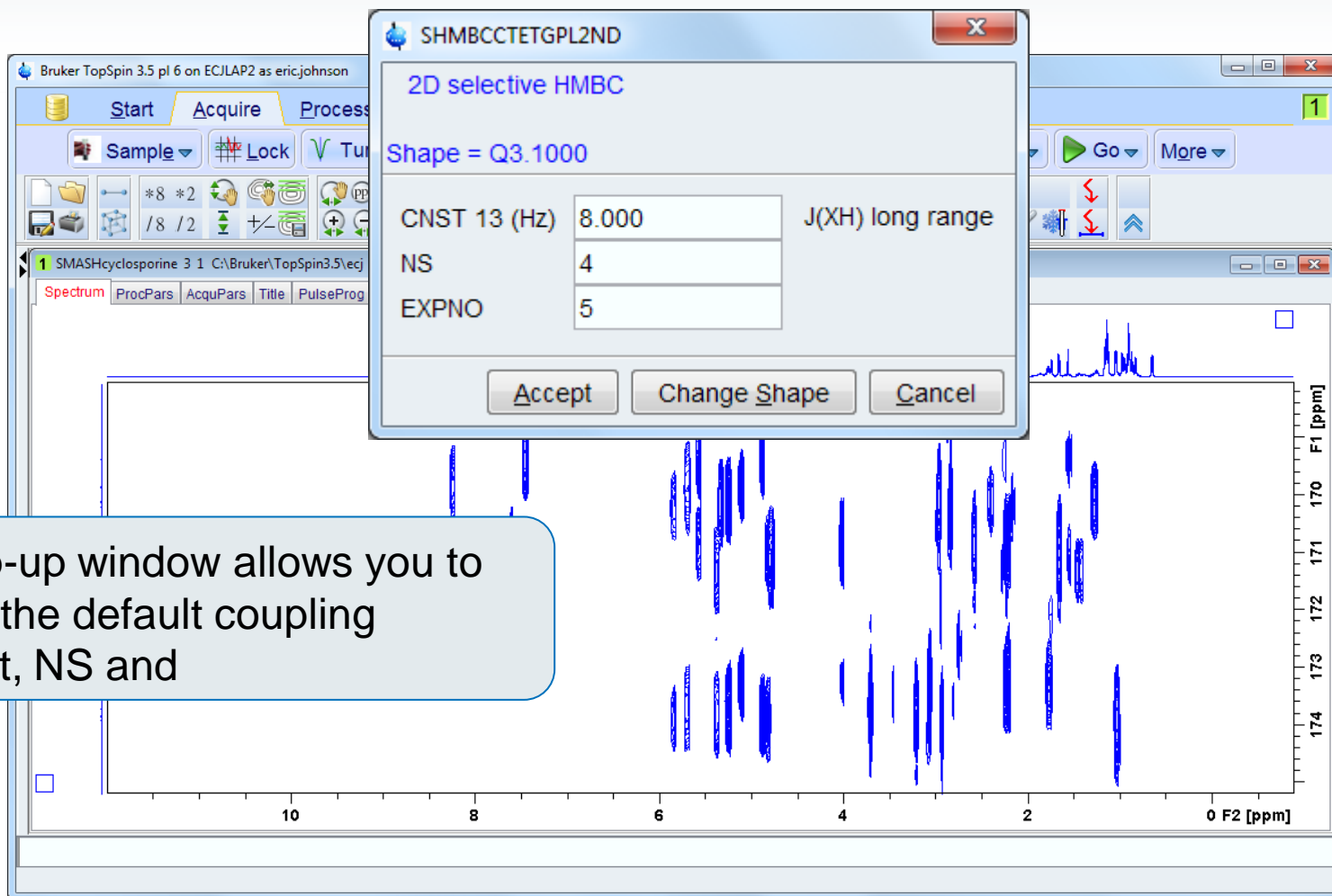


iii) choose “Setup Selective 2D Expts” under “More” in the “Acquire” flow-bar



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

The screenshot shows the Bruker software interface with a 2D NMR spectrum of SMASHcyclosporine. The x-axis is labeled from 10 to 6 ppm. A dialog box titled 'selinv' is overlaid on the right side of the screen, displaying acquisition parameters for a 2D selHMBC experiment.

Acquisition parameters for 2D selHMBC:
SHMBCCTETGPL2ND

Dataset created in EXPNO: 5

SW(F1) = 7.1393 ppm / 897.98 Hz
O2 = 171.6256 ppm / 21583.26 Hz
TD(F1) = 128
AQ(F1) = 0.0713 sec

shape pulse (SPNAM32): Q3_surbop.1
shape pulse duration (P 43): 5478.67 us
power level (SPW 32): 0.037093 W

Experiment time: 15 min 58 sec

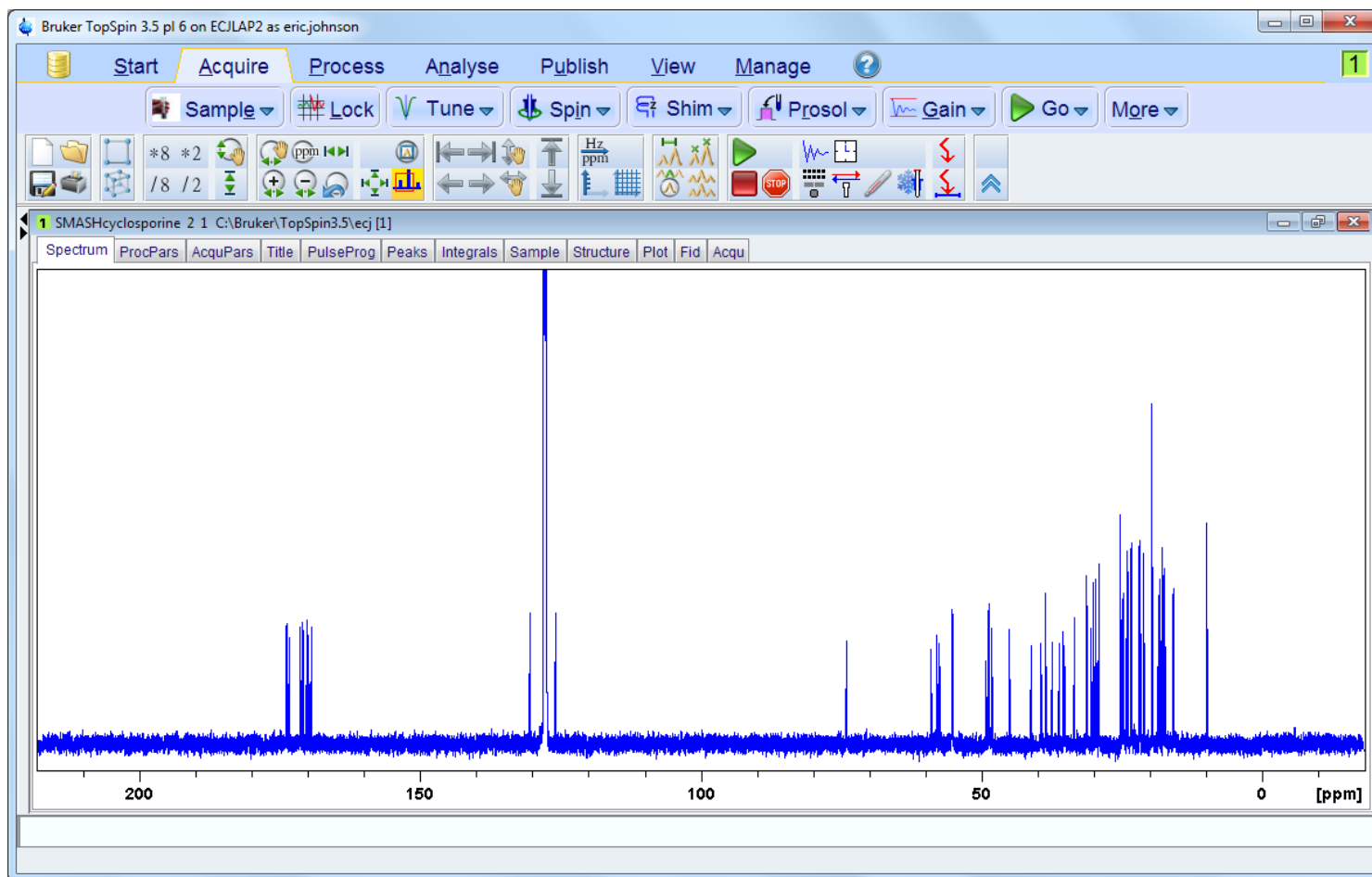
OK: starts acquisition
CANCEL: creates data sets only.

Buttons: OK, Cancel

Setting up the band-selective 2D experiments



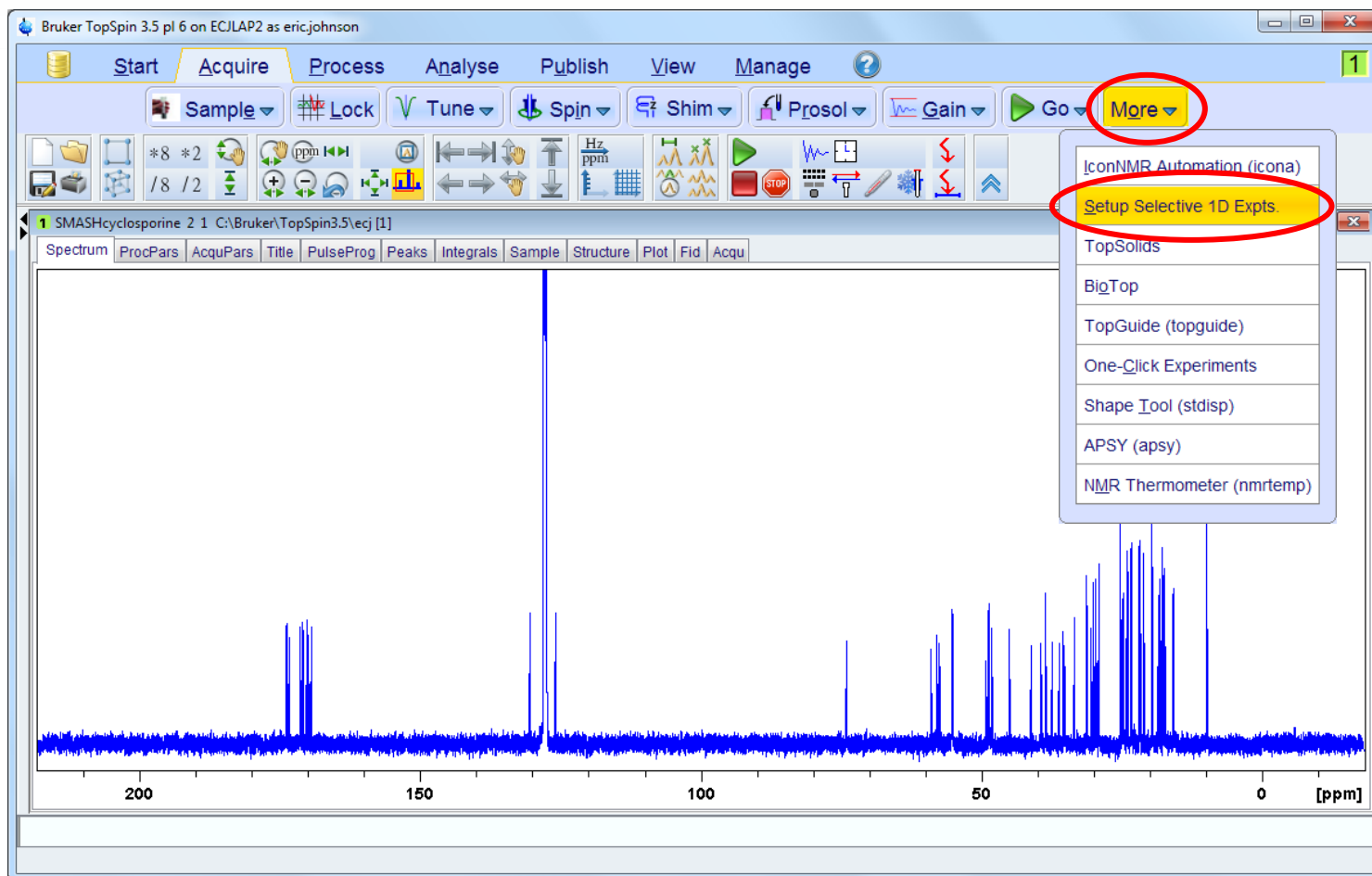
Method 2 – starting from a 1D ¹³C spectrum



Setting up the band-selective 2D experiments



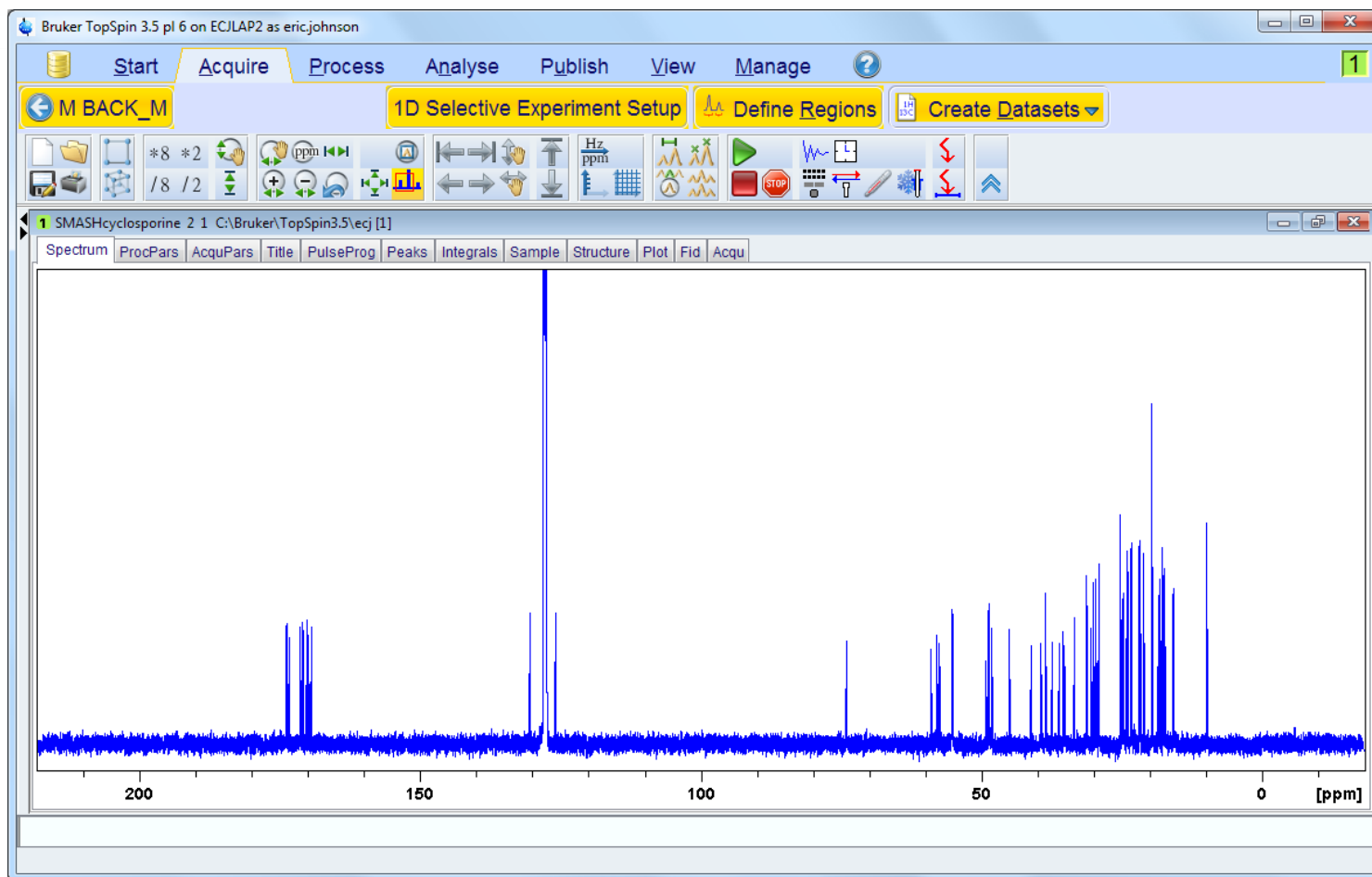
ii) choose “Setup Selective 1D Expts” under “More” in the “Acquire” flow-bar



Setting up the band-selective 2D experiments



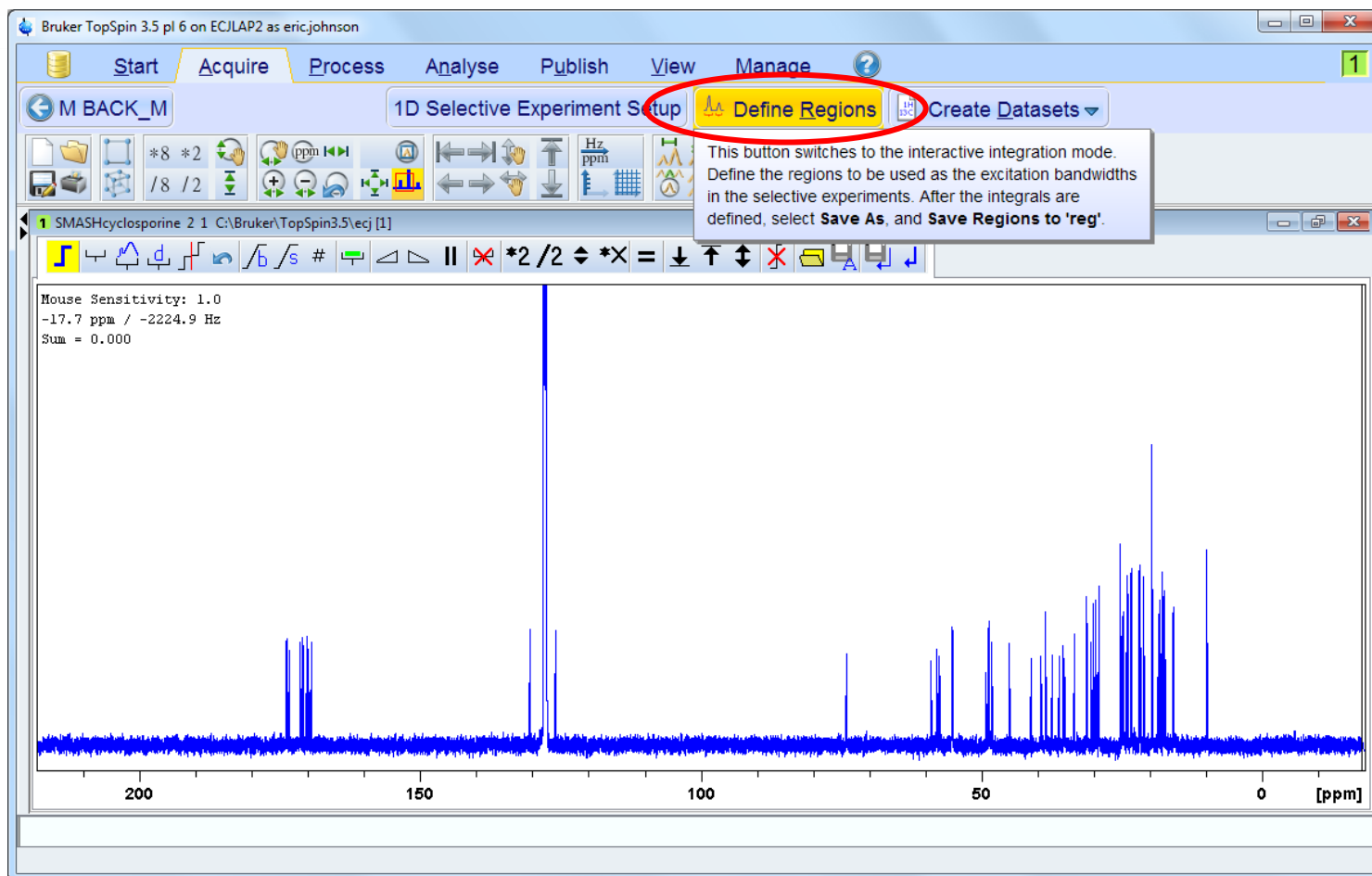
ii) choose “Setup Selective 1D Extps” under “More” in the “Acquire” flow-bar



Setting up the band-selective 2D experiments



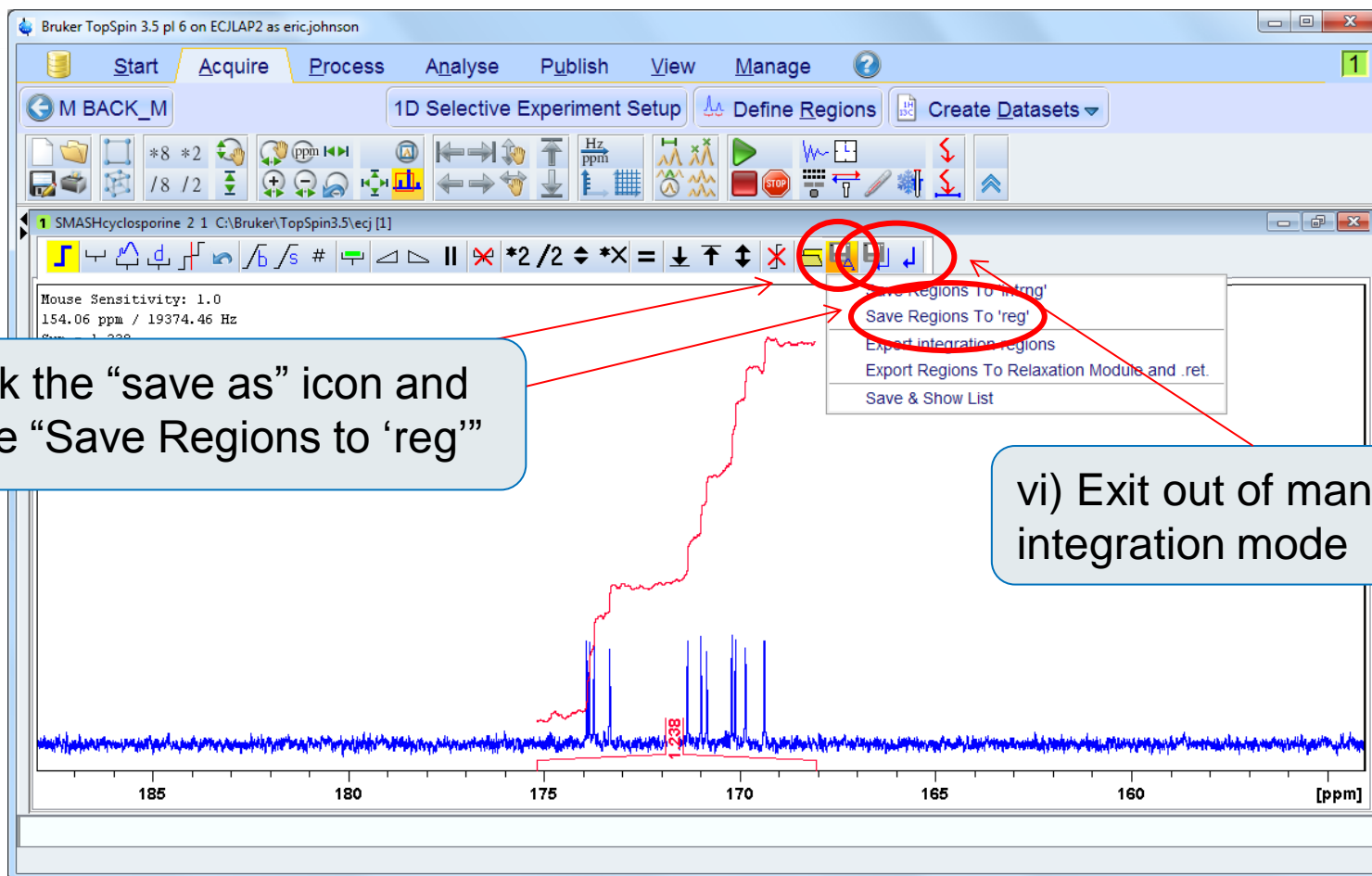
iii) choose “Define Regions” to start the manual integration mode



Setting up the band-selective 2D experiments



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



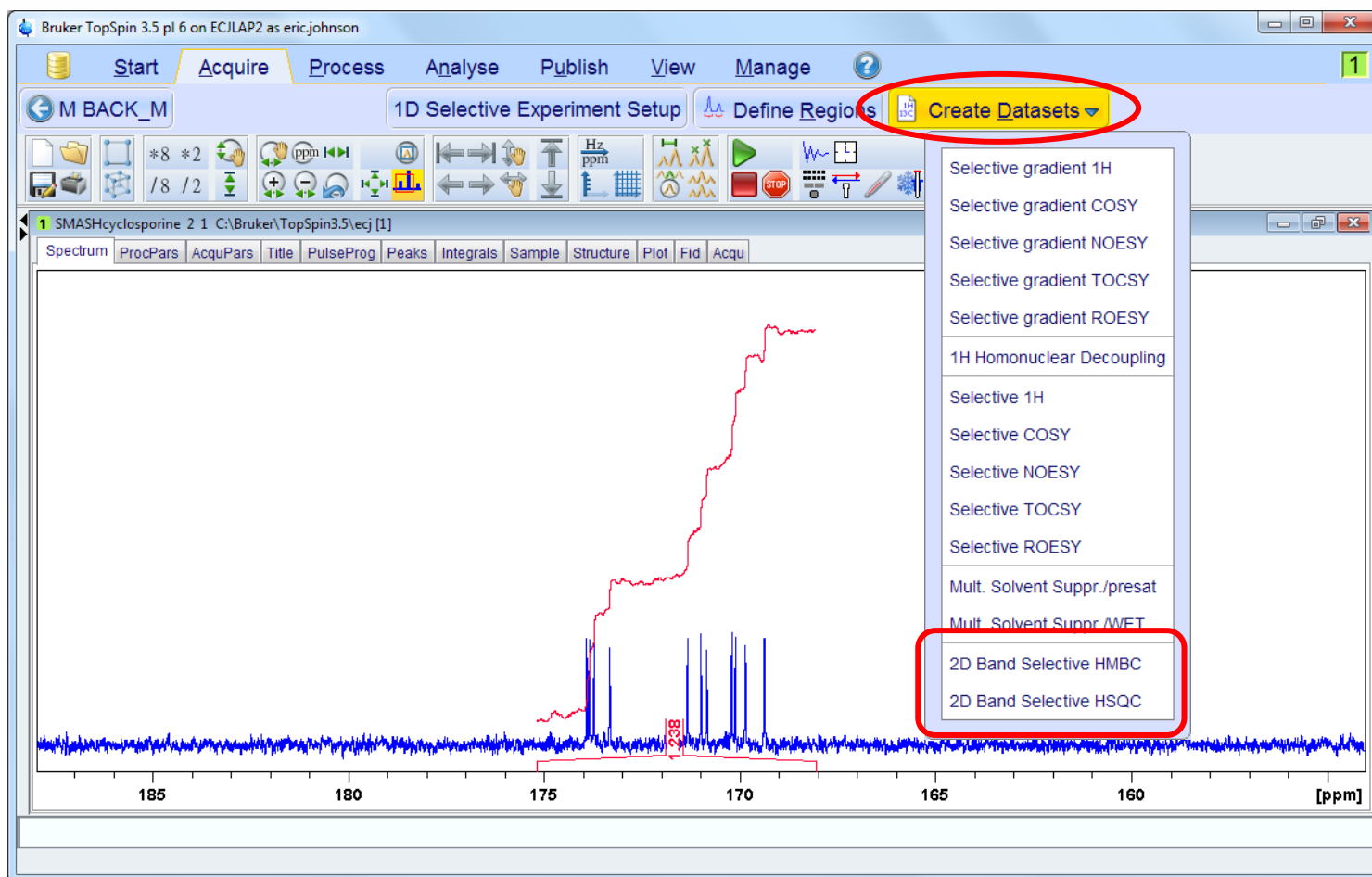
v) Click the “save as” icon and choose “Save Regions to ‘reg’”

vi) Exit out of manual integration mode

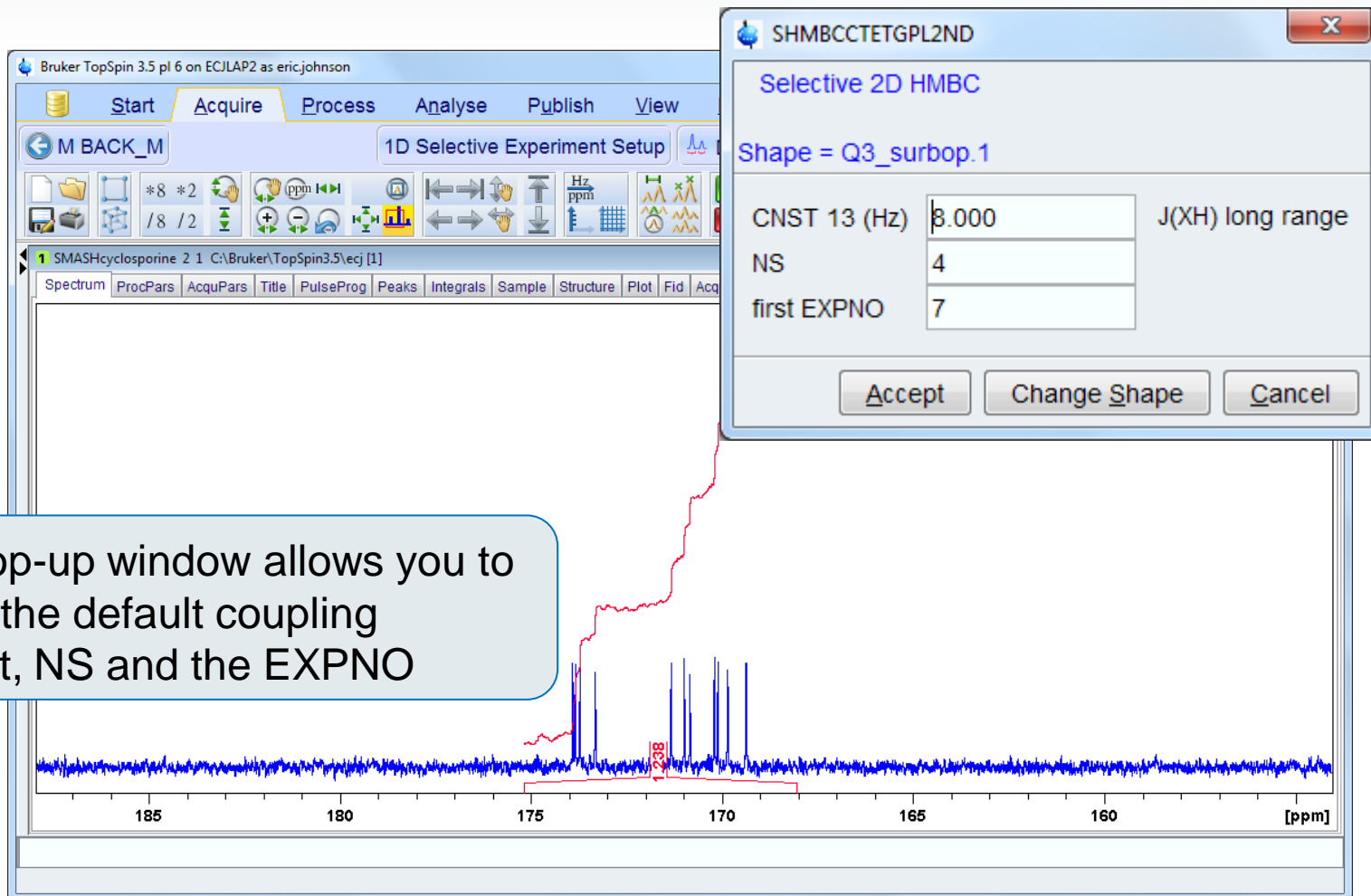
Setting up the band-selective 2D experiments



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

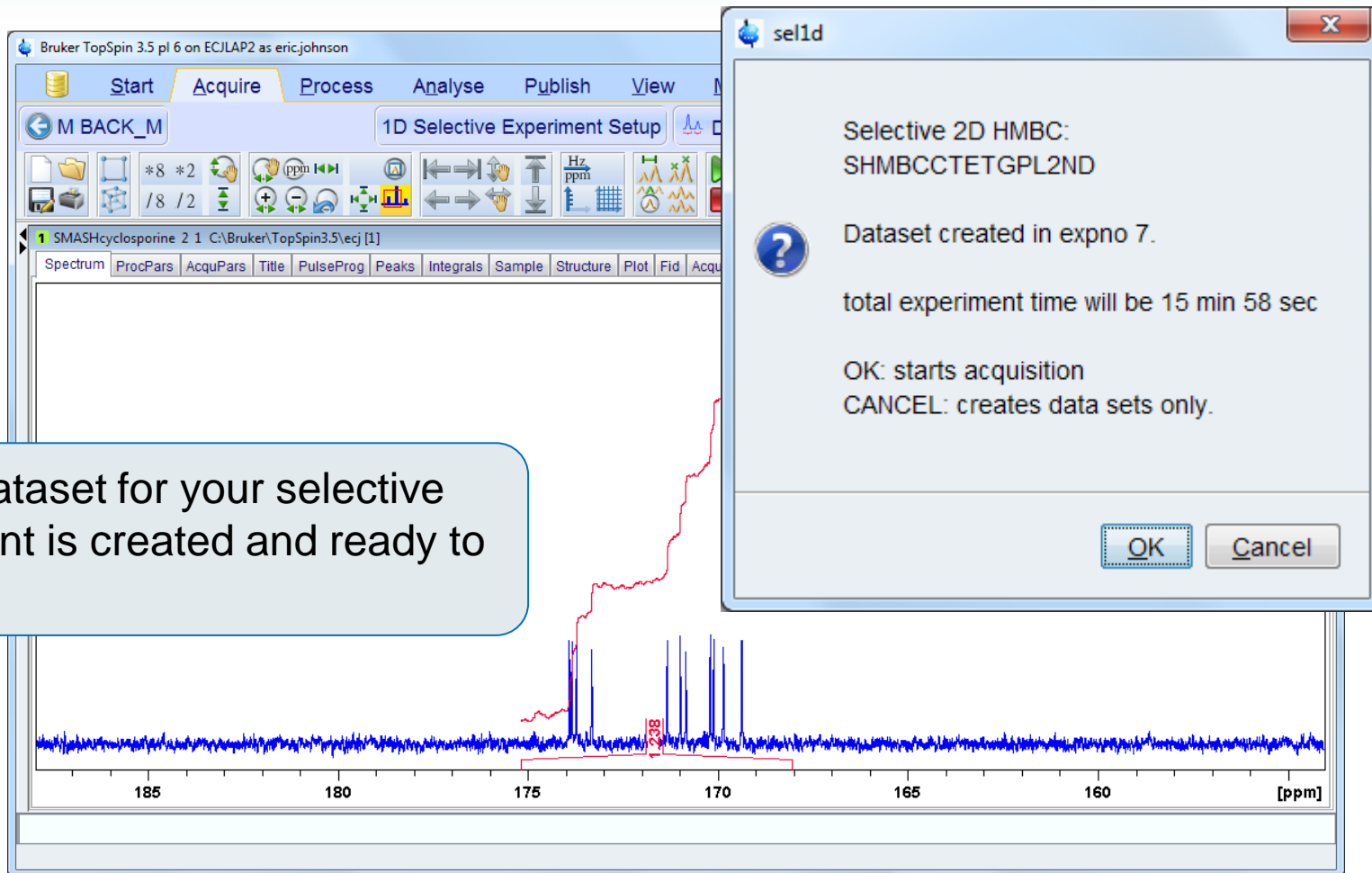


Setting up the band-selective 2D experiments



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



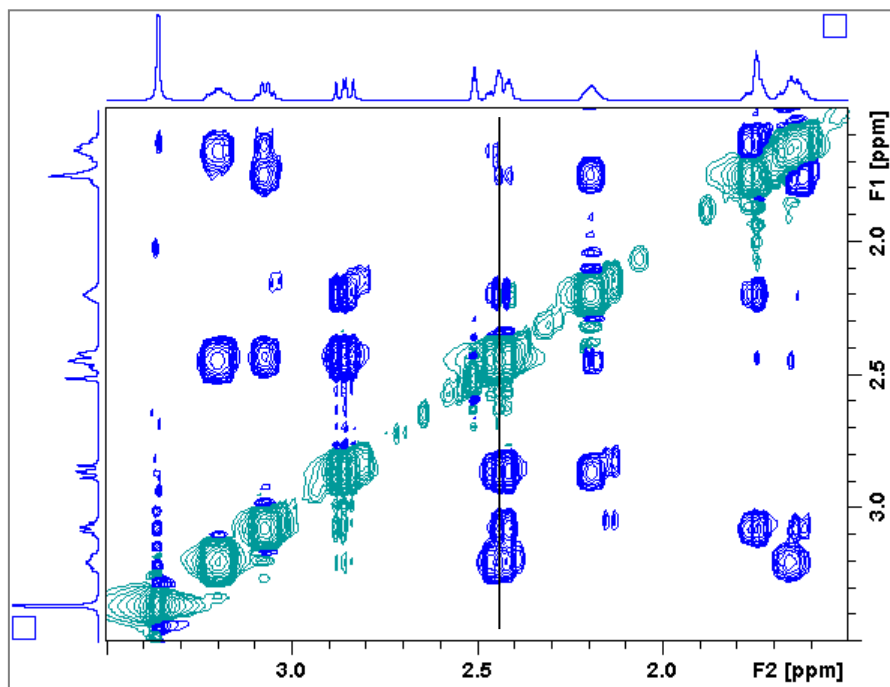
- Keep your SW[F1] greater than a couple ppm
 - as the SW decreases, the duration of the band-selective pulse increases
 - at a certain point, you'll start losing signal due to relaxation and diffusion
- Don't run "getprosol" on your selective dataset after it's been created with this interface
 - "getprosol" 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?



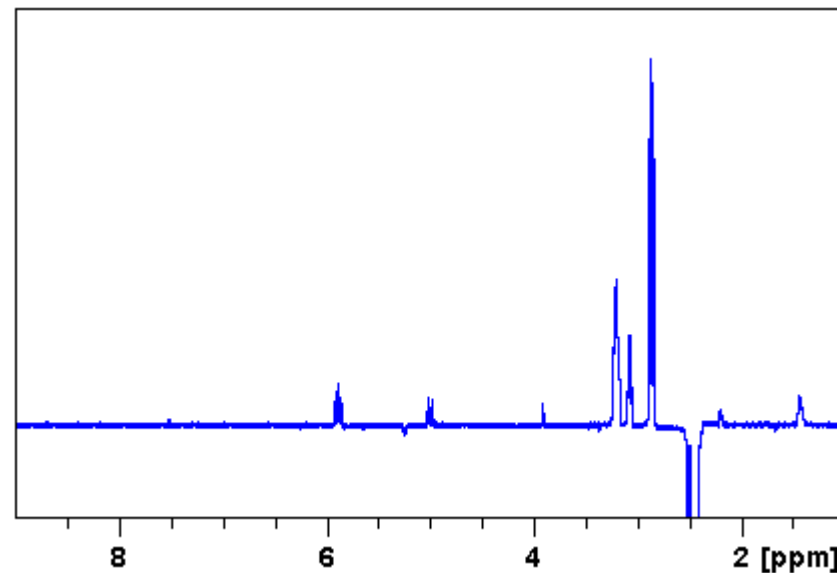
- When we're looking for a specific correlation
 - Much shorter acquisition time than full 2D

Expansion of 2D NOESY



Expt: 1 hr 36 min

1D selective NOESY

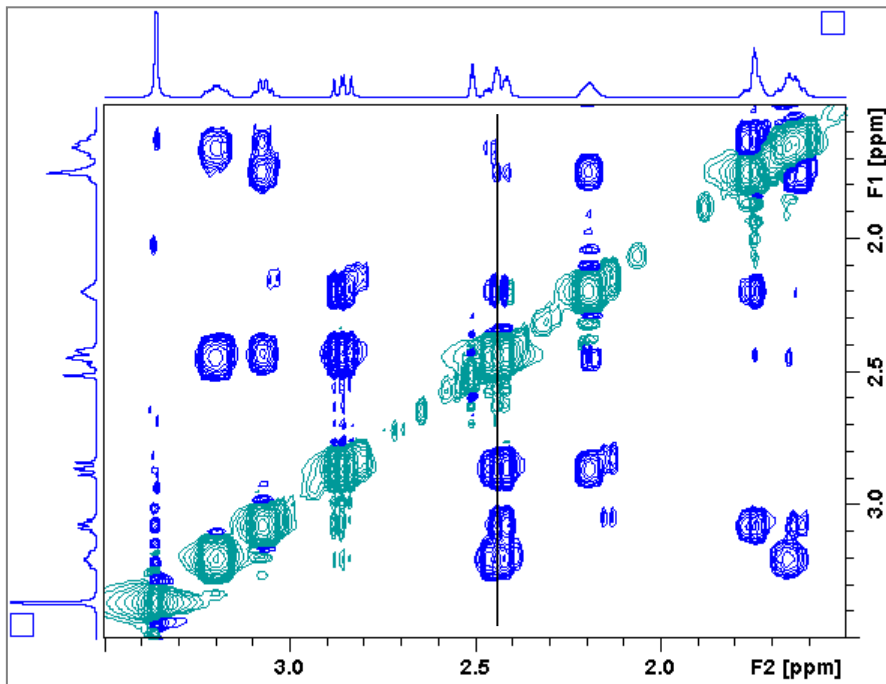


Expt: 1 min 50 sec

100mM quinine in DMSO-d6

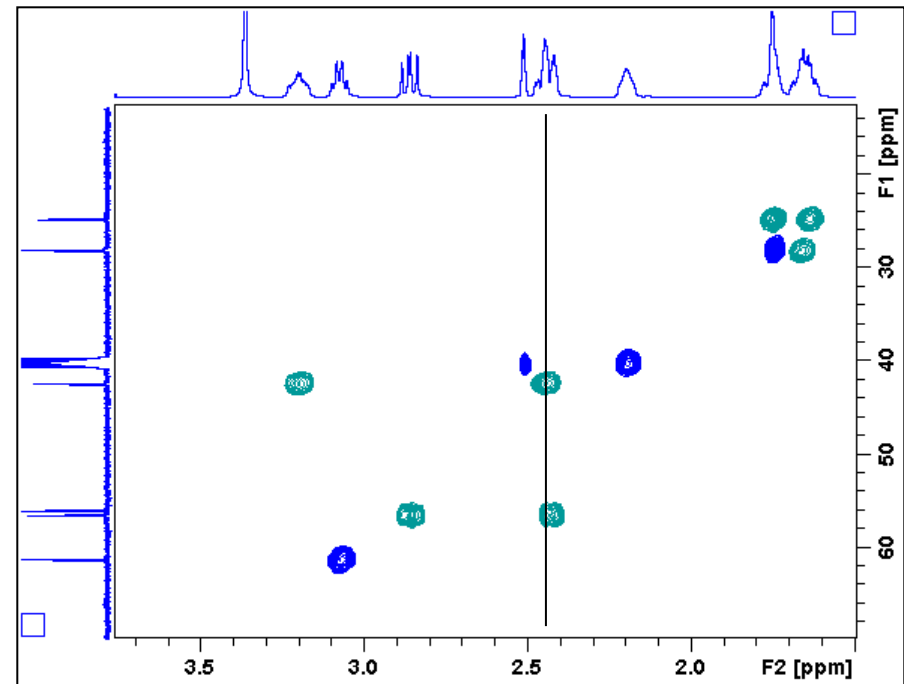
1D selective NOESY example

Expansion of 2D NOESY



Expt: 1 hr 36 min

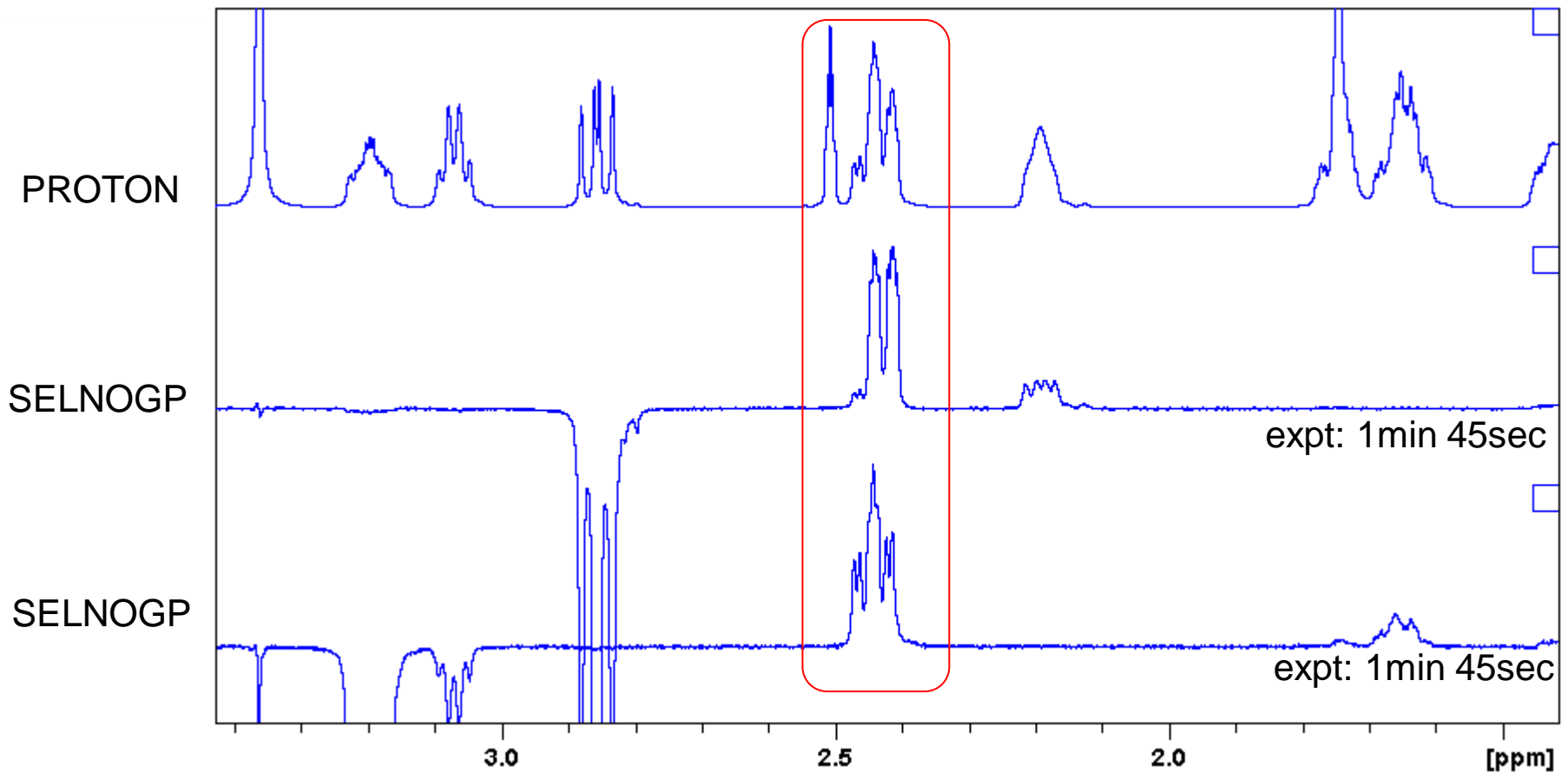
From the HSQC, we can clearly see that the peak at 2.5ppm has 2 overlapped multiplets



100mM quinine in DMSO-d6

1D selective NOESY example

- Much higher resolution correlations in much less time!

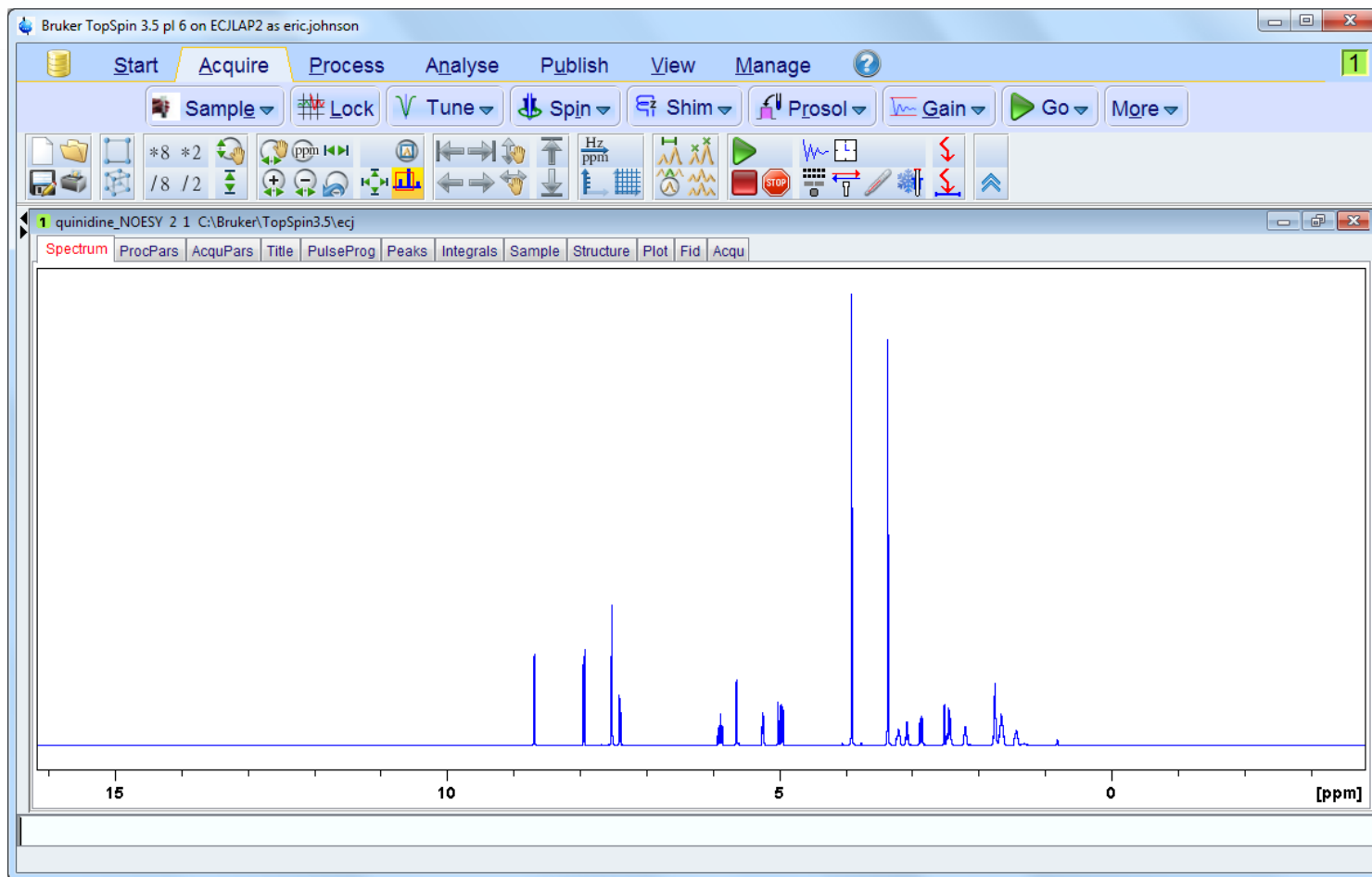


100mM quinine in DMSO-d6

Setting up the 1D selective experiments



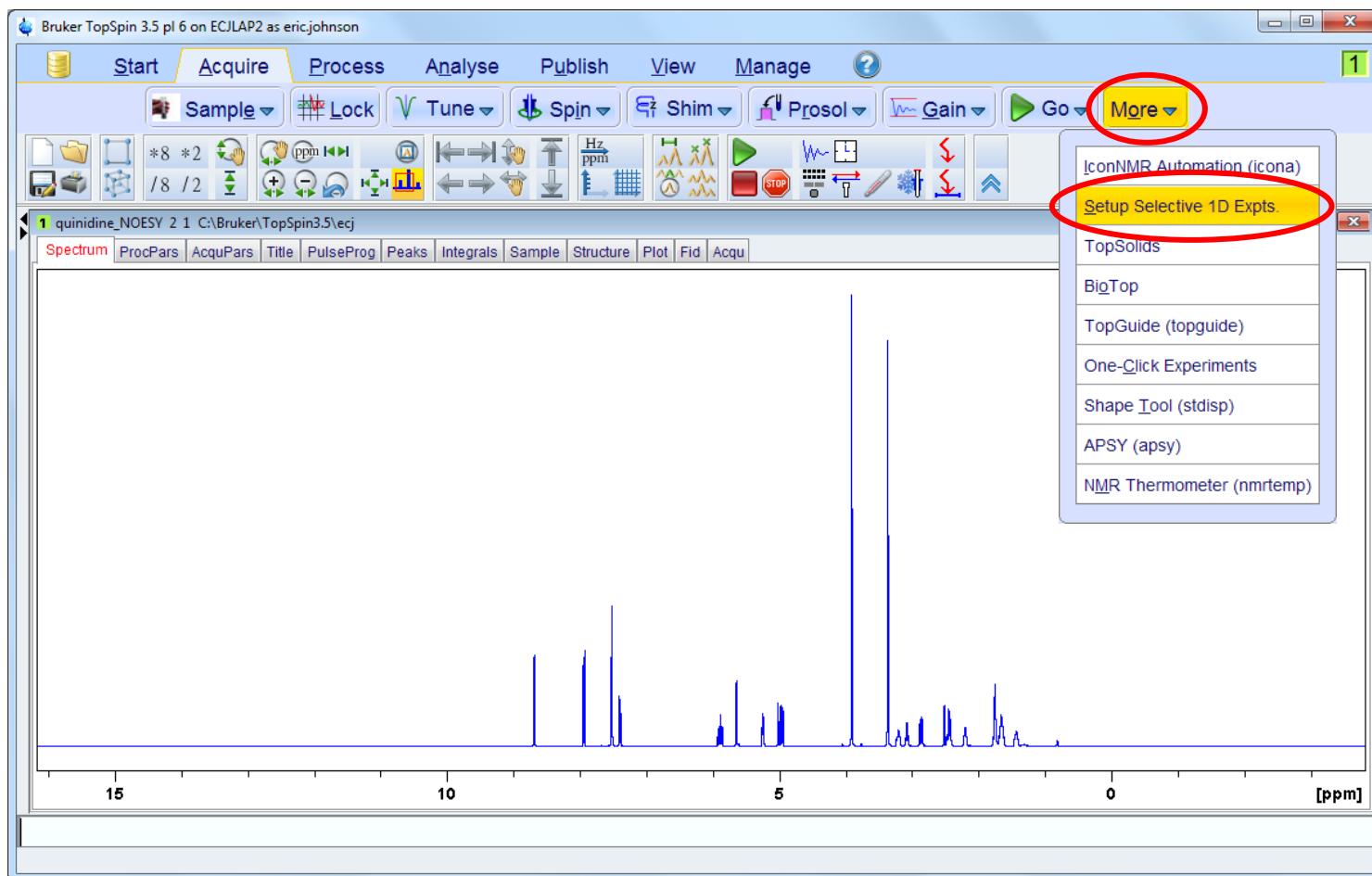
i) start from a 1D PROTON spectrum



Setting up the 1D selective experiments



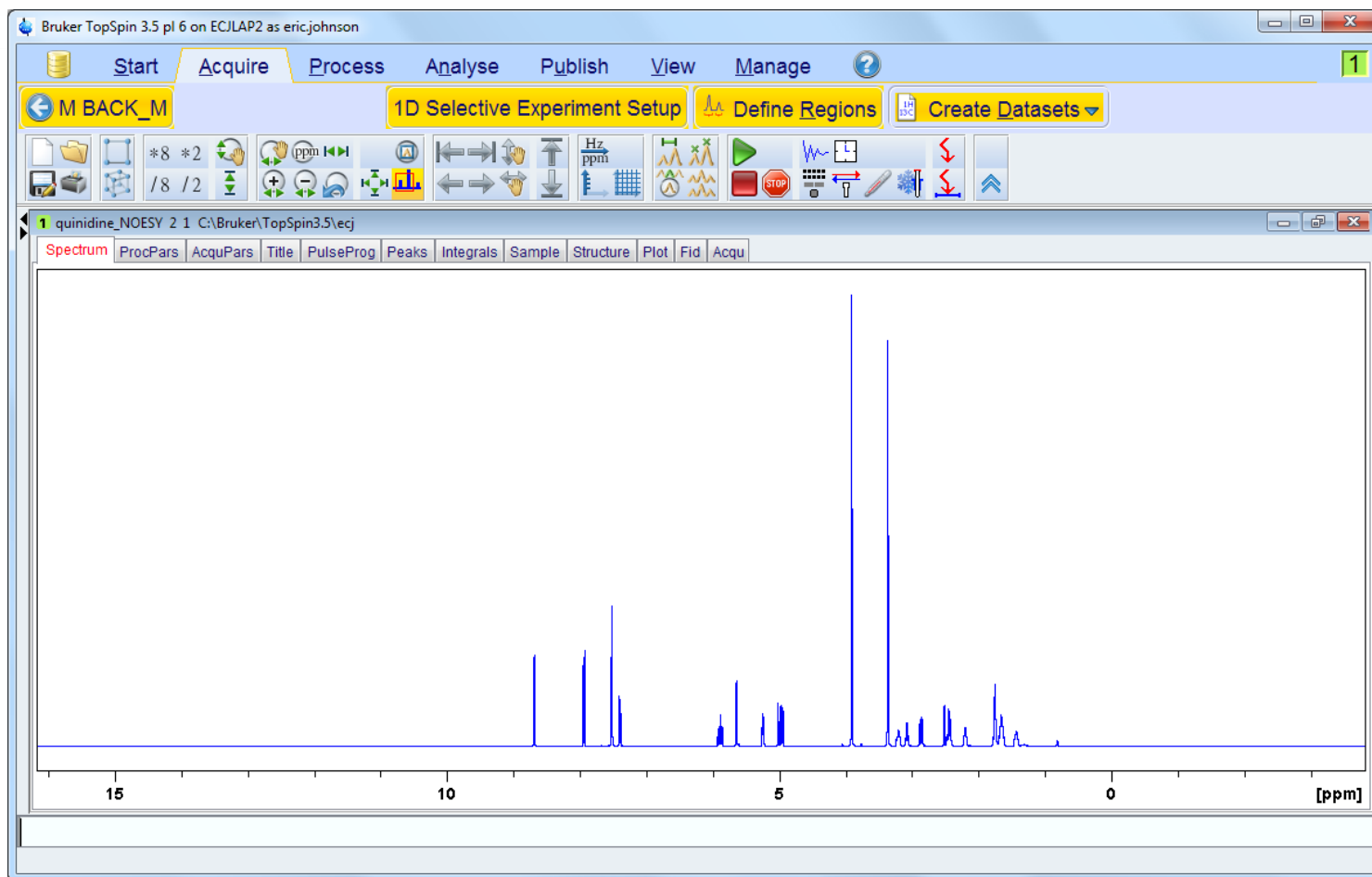
ii) choose “Setup Selective 1D Expts” under “More” in the “Acquire” flow-bar



Setting up the 1D selective experiments



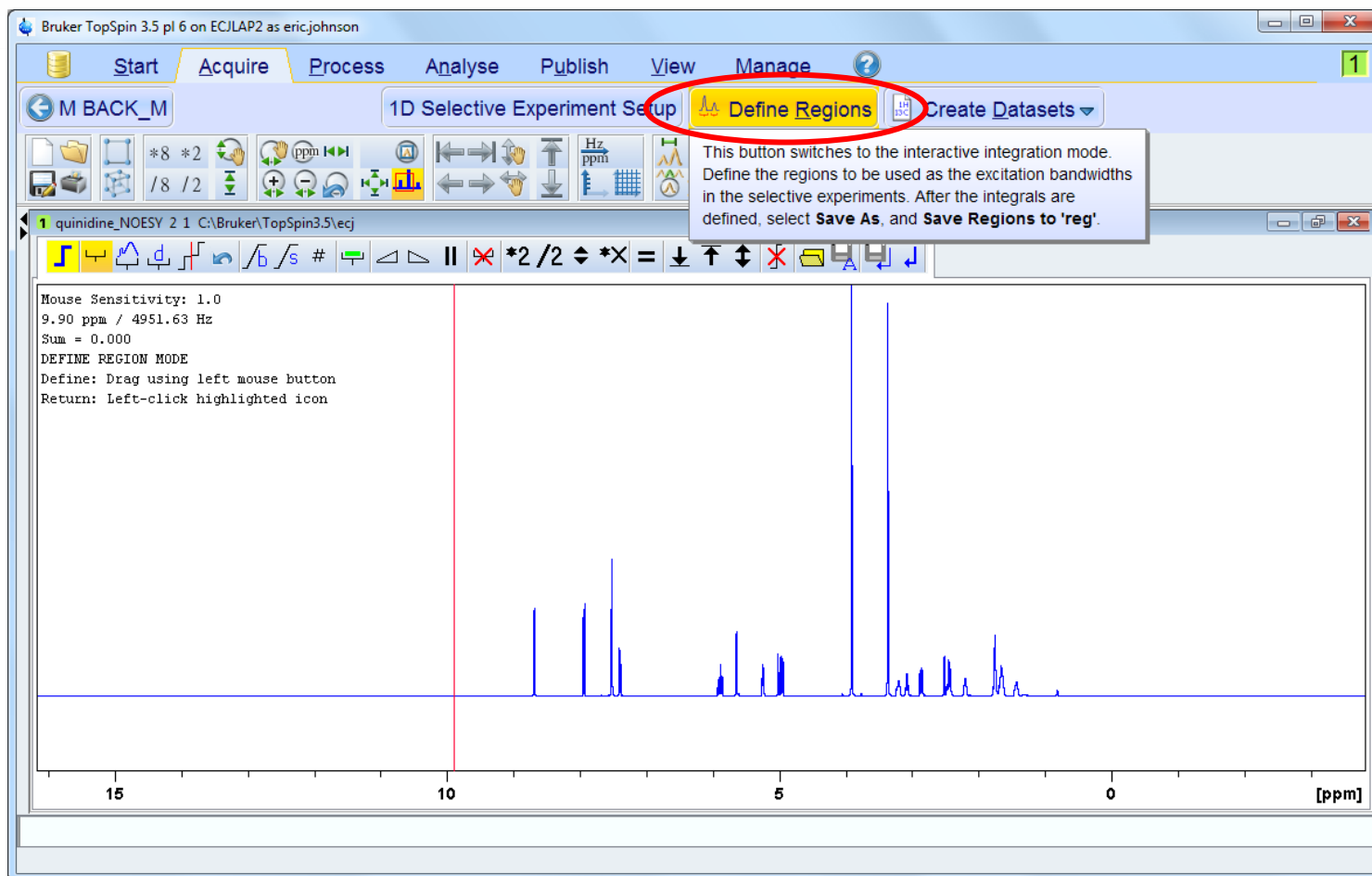
ii) choose “Setup Selective 1D Extps” under “More” in the “Acquire” flow-bar



Setting up the 1D selective experiments



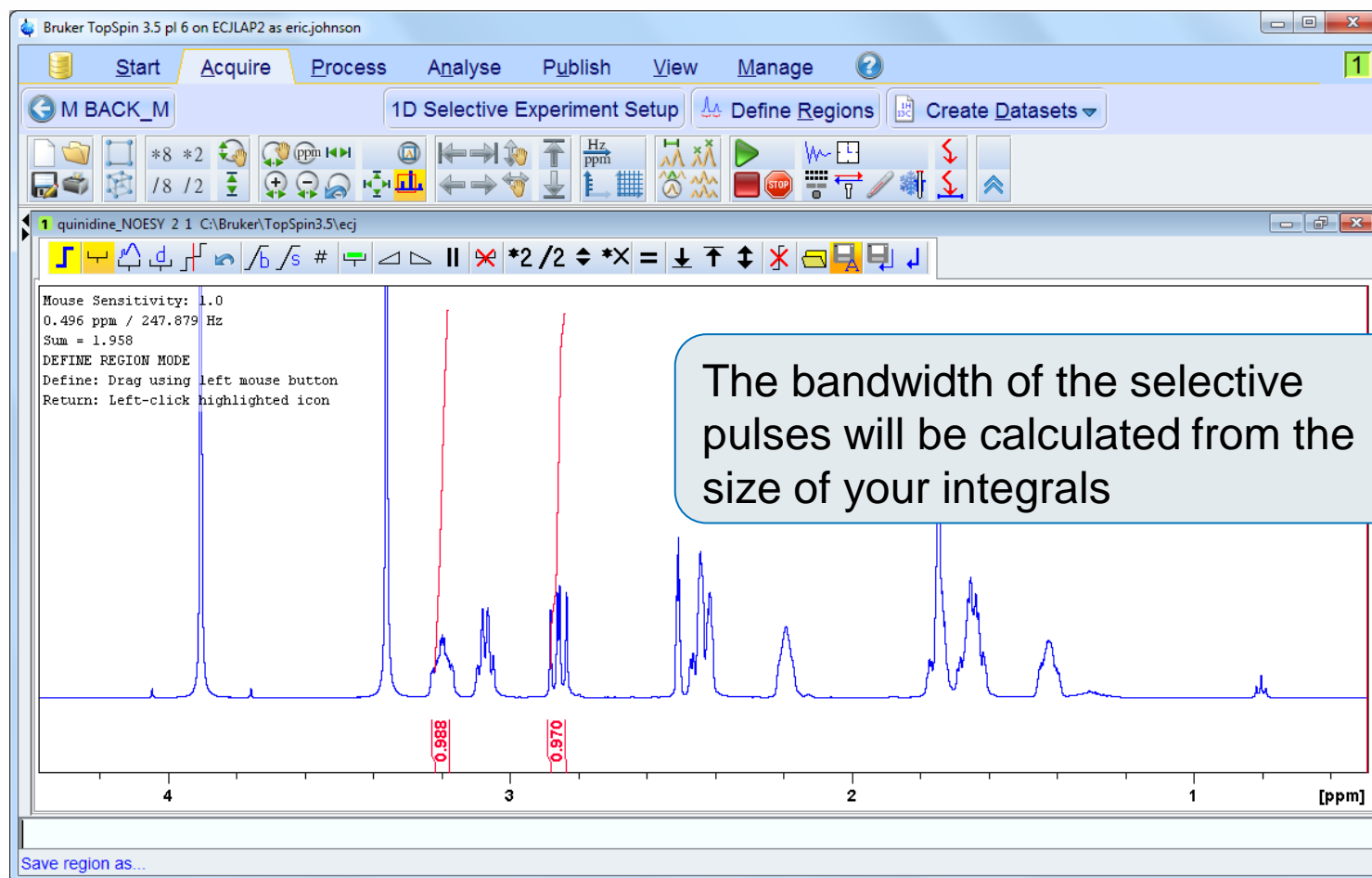
iii) choose “Define Regions” to start the manual integration mode



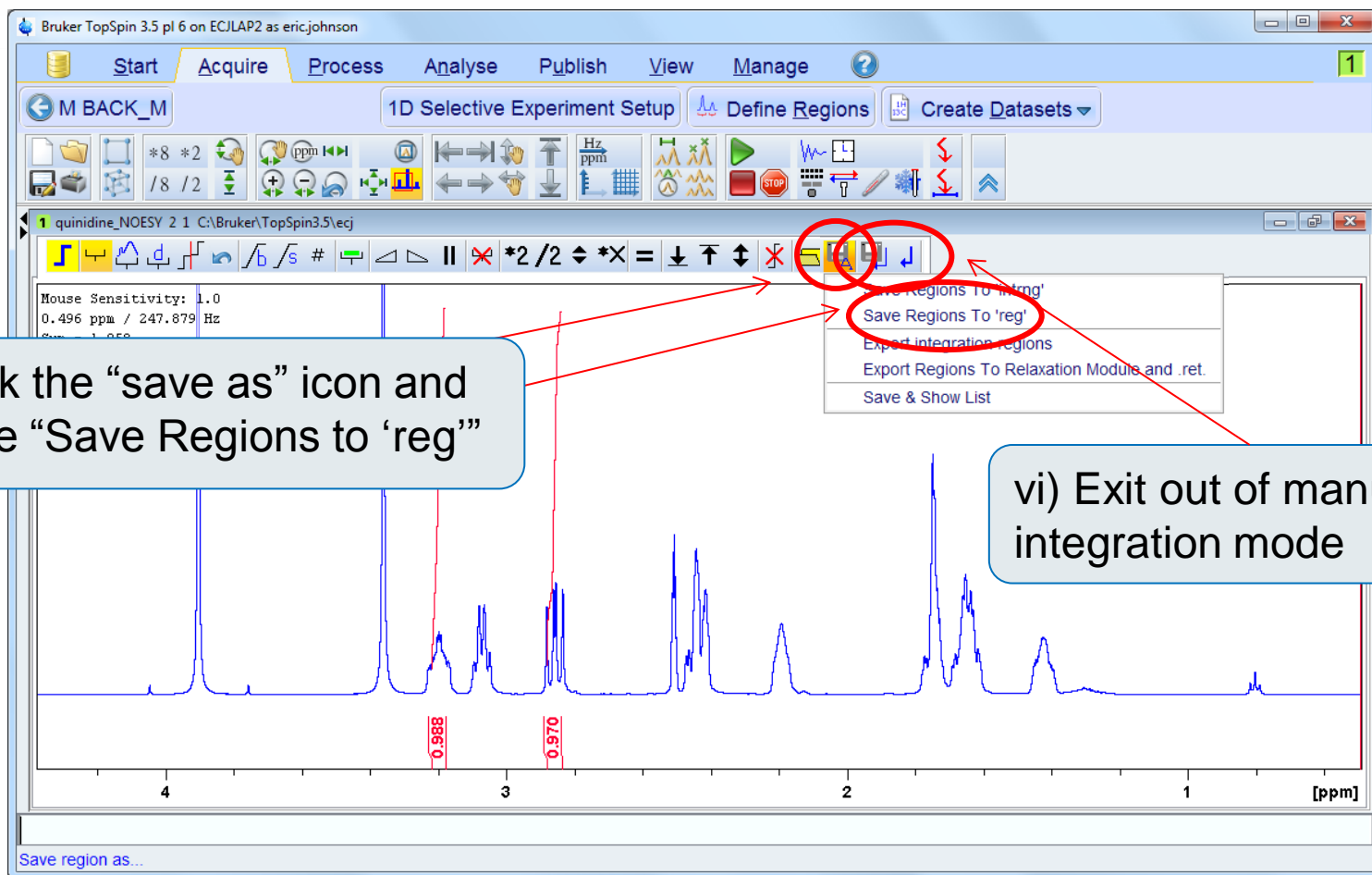
Setting up the 1D selective experiments



iv) integrate the peaks for selective excitation



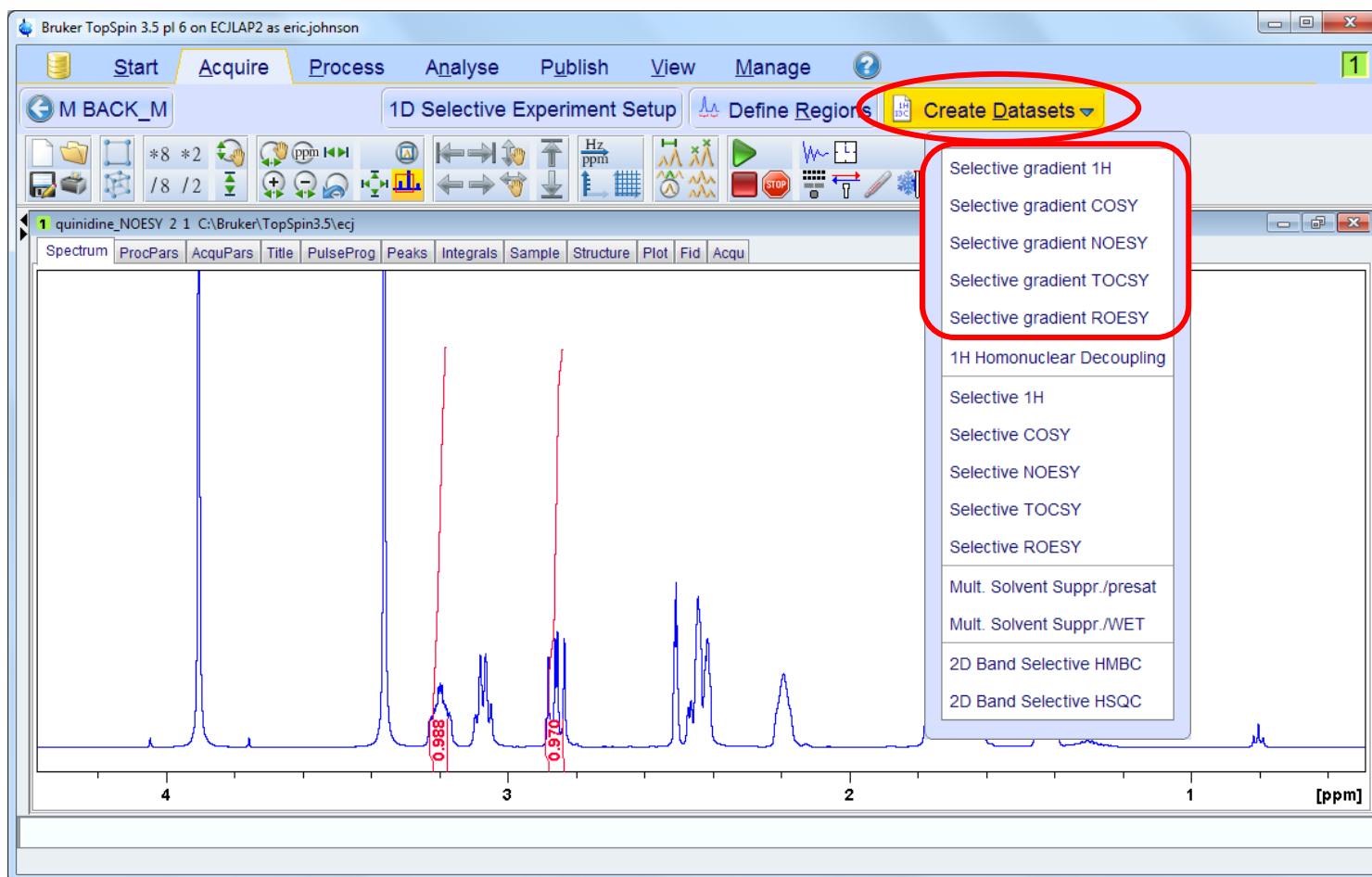
Setting up the 1D selective experiments



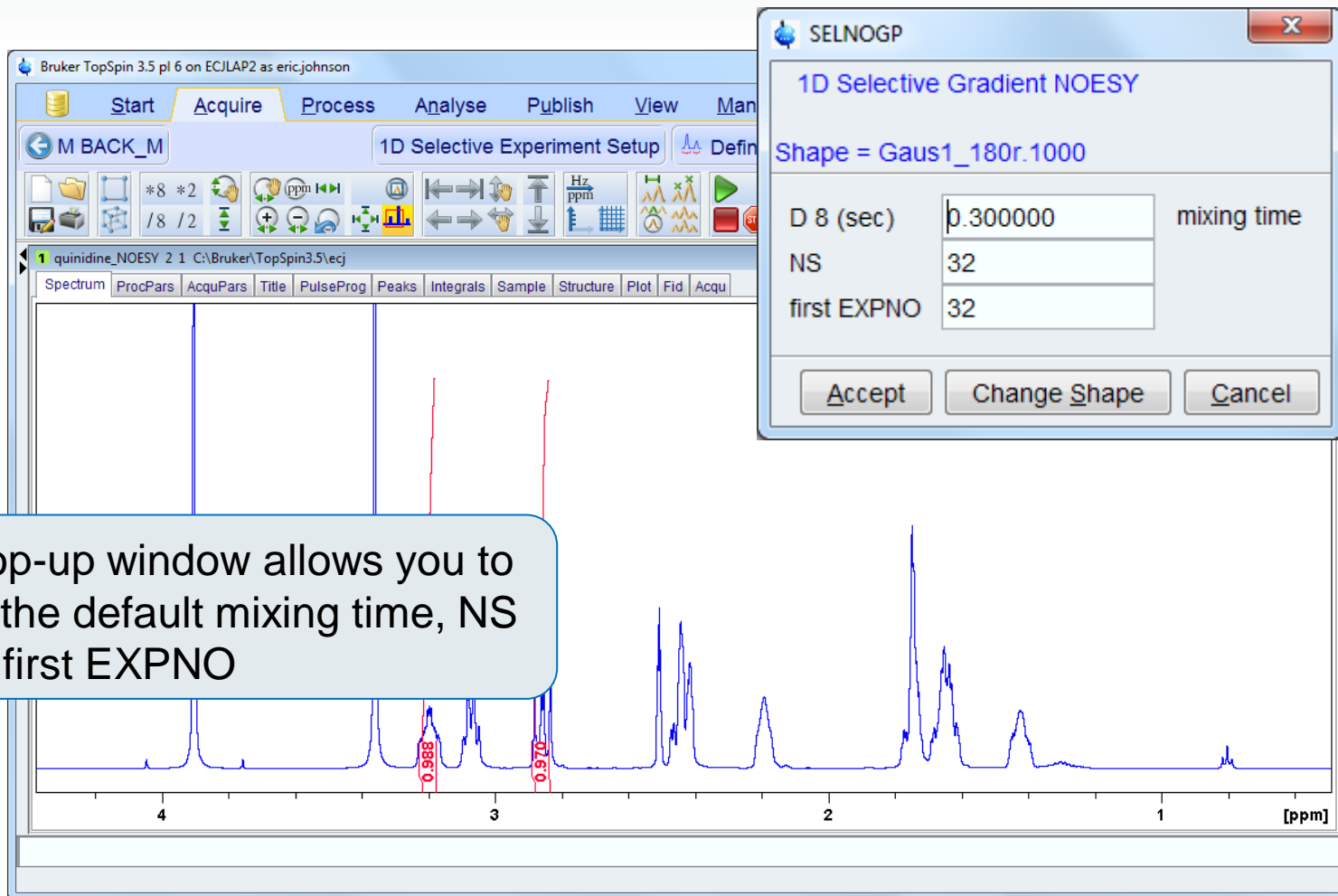
Setting up the 1D selective experiments



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

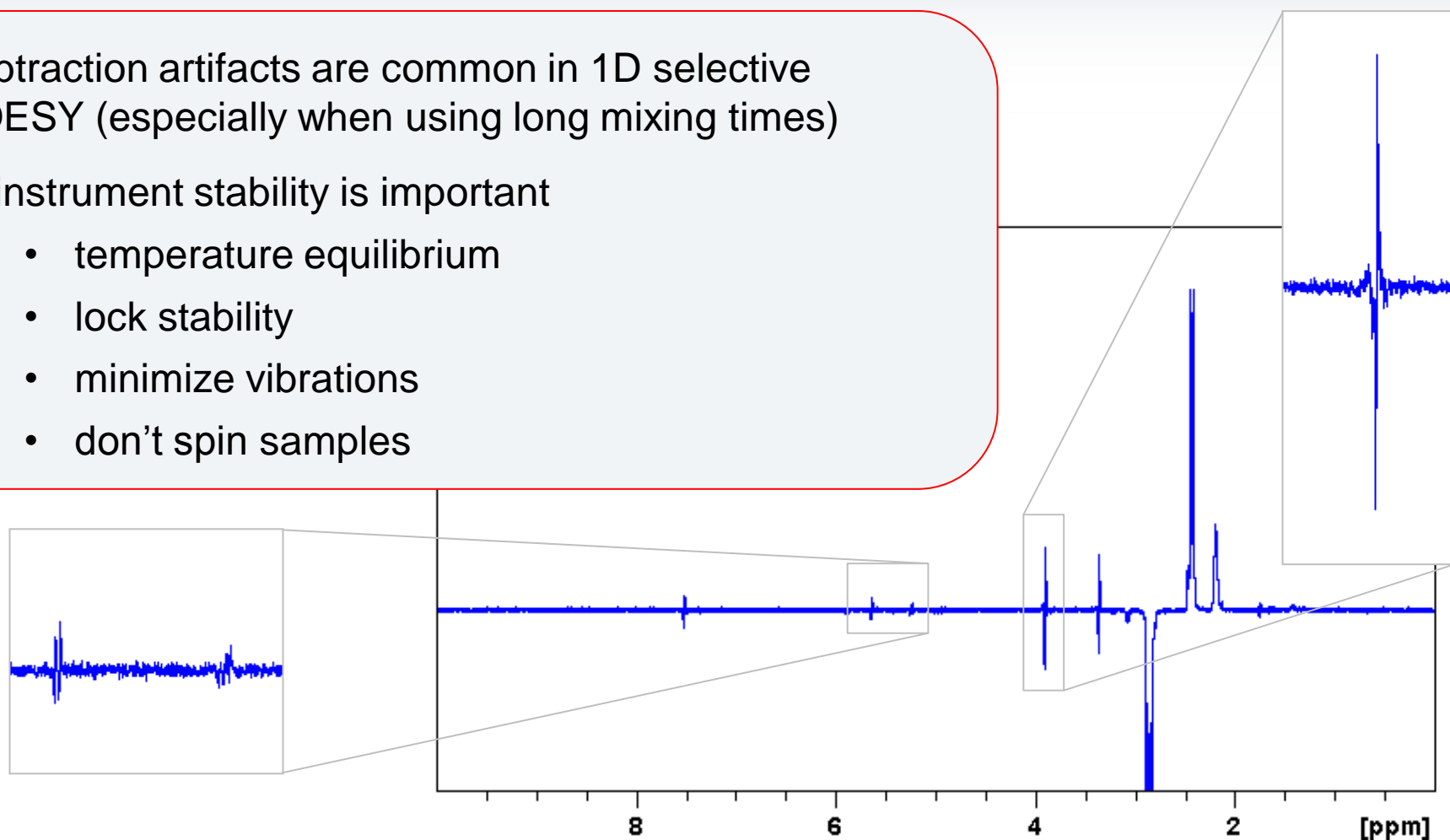
A separate selective experiment is setup for each integral you defined

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

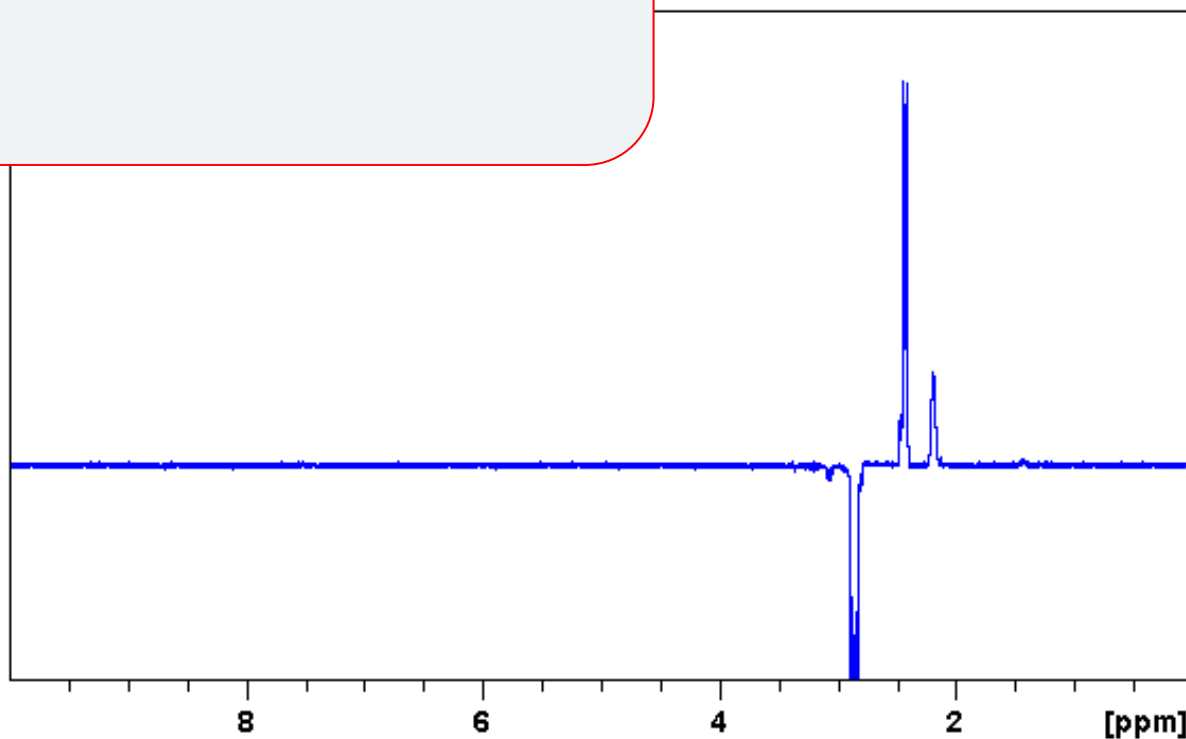


Tips on selective 1D's



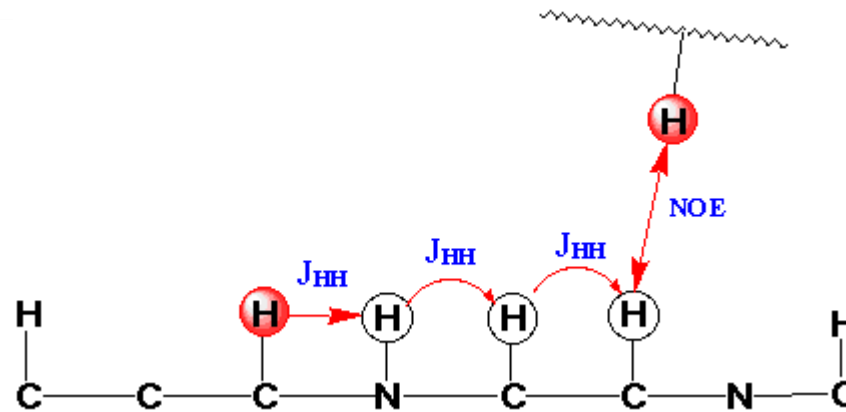
Subtraction artifacts are common in 1D selective NOESY (especially when using long mixing times)

- 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

stepNOESY example – stereochemical assignment in a highly overlapped spectrum



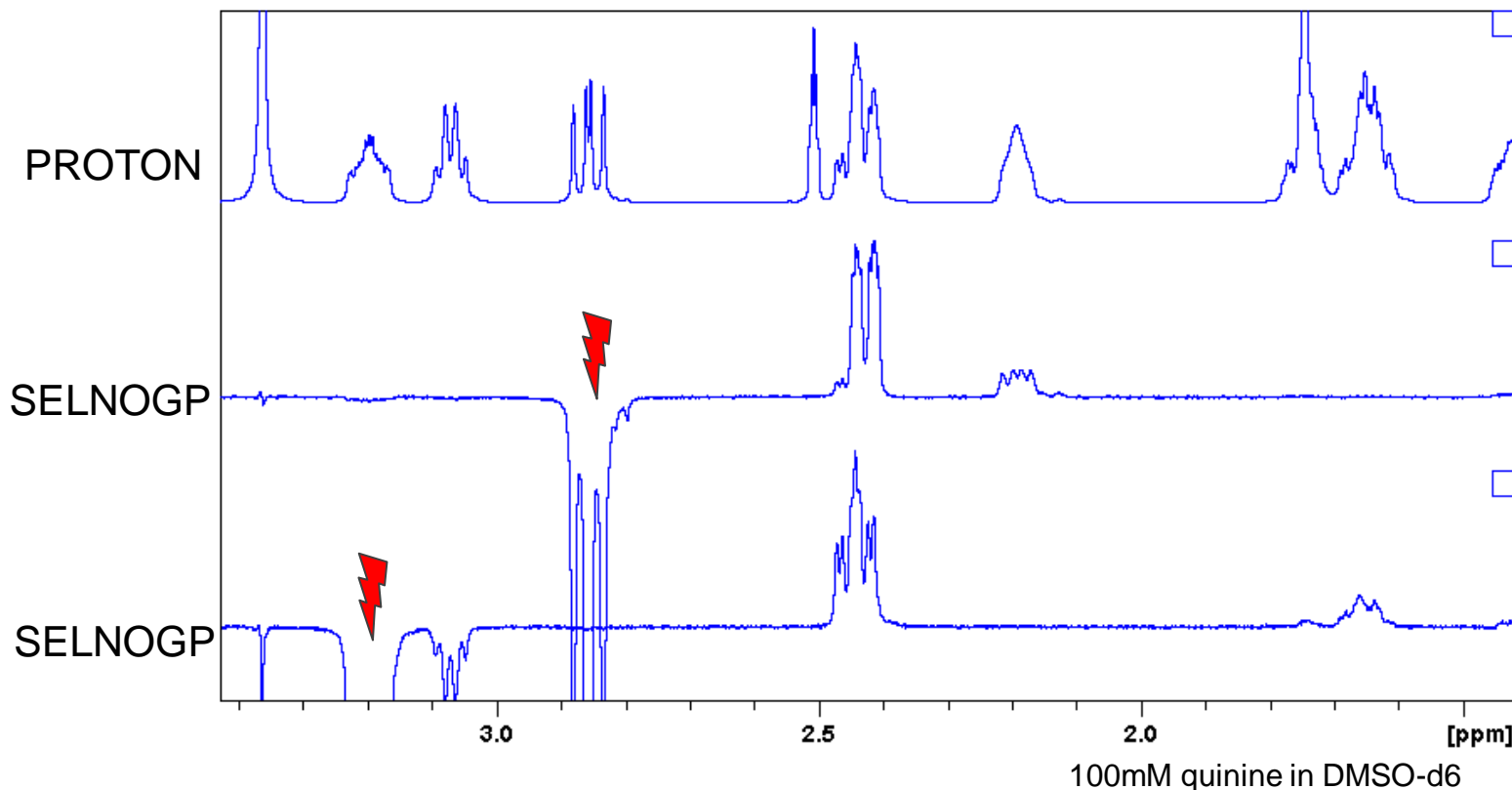
Example from the original presentation has been removed until those results are published...

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



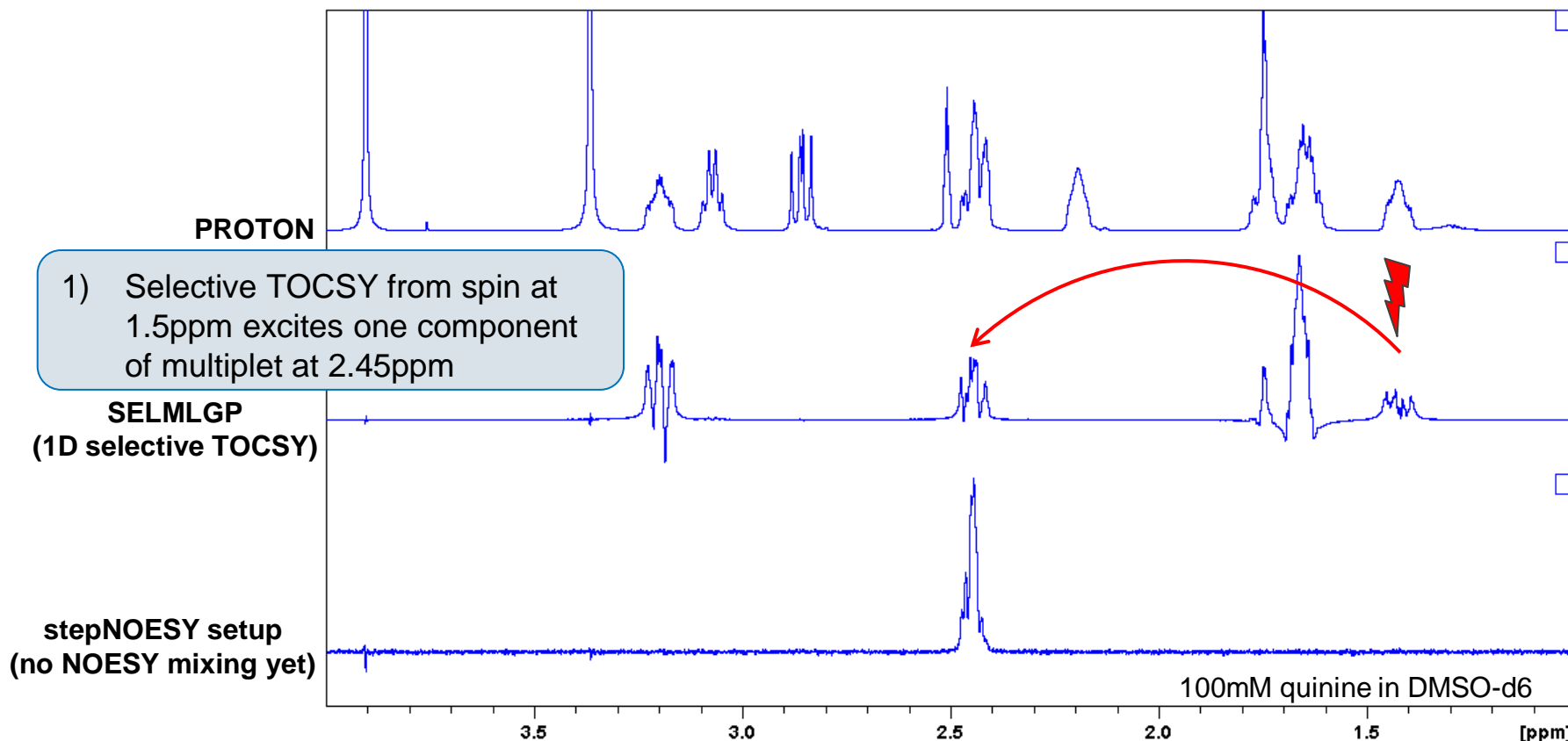
stepNOESY – 1D selective NOESY from overlapped peaks



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

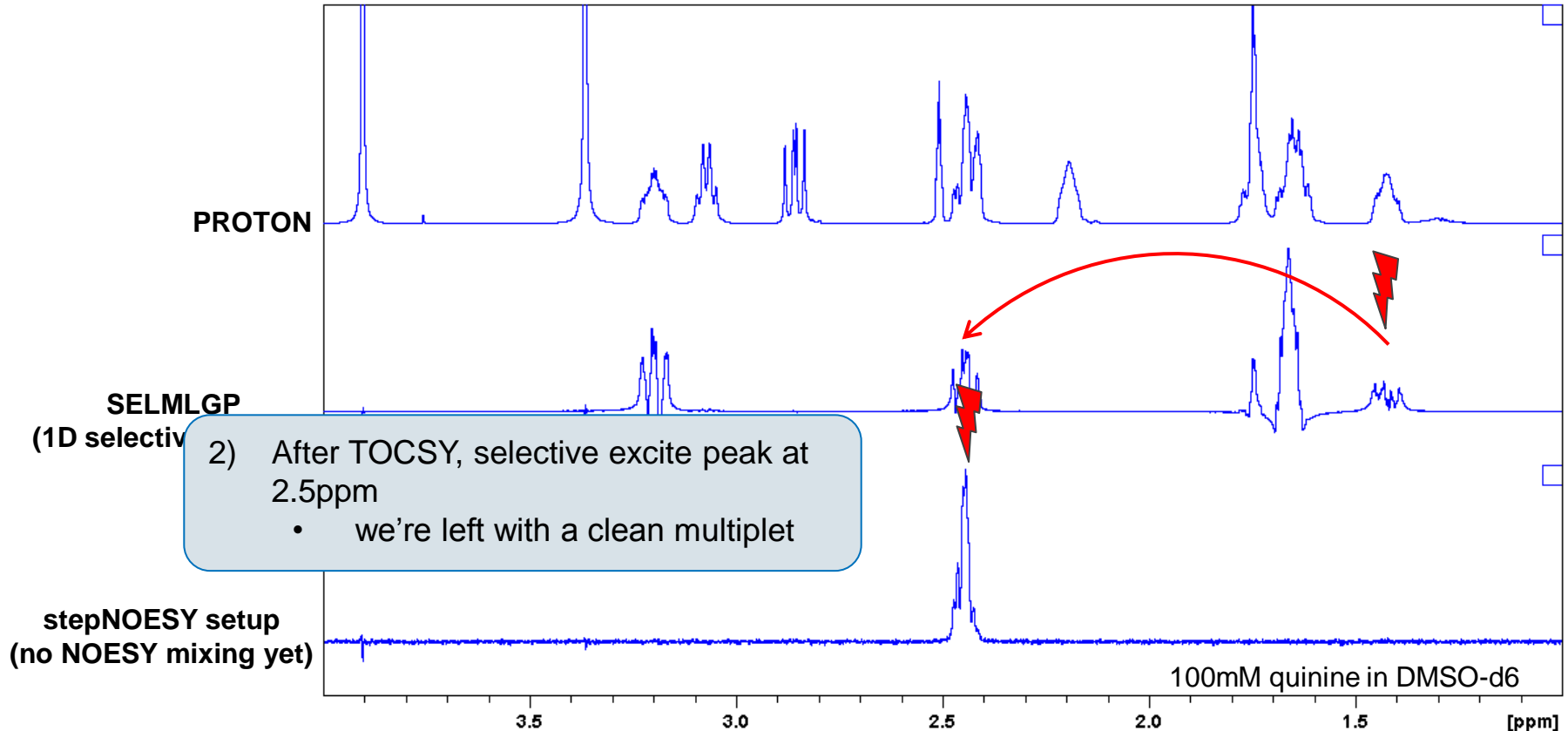


stepNOESY – 1D selective NOESY from overlapped peaks

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

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

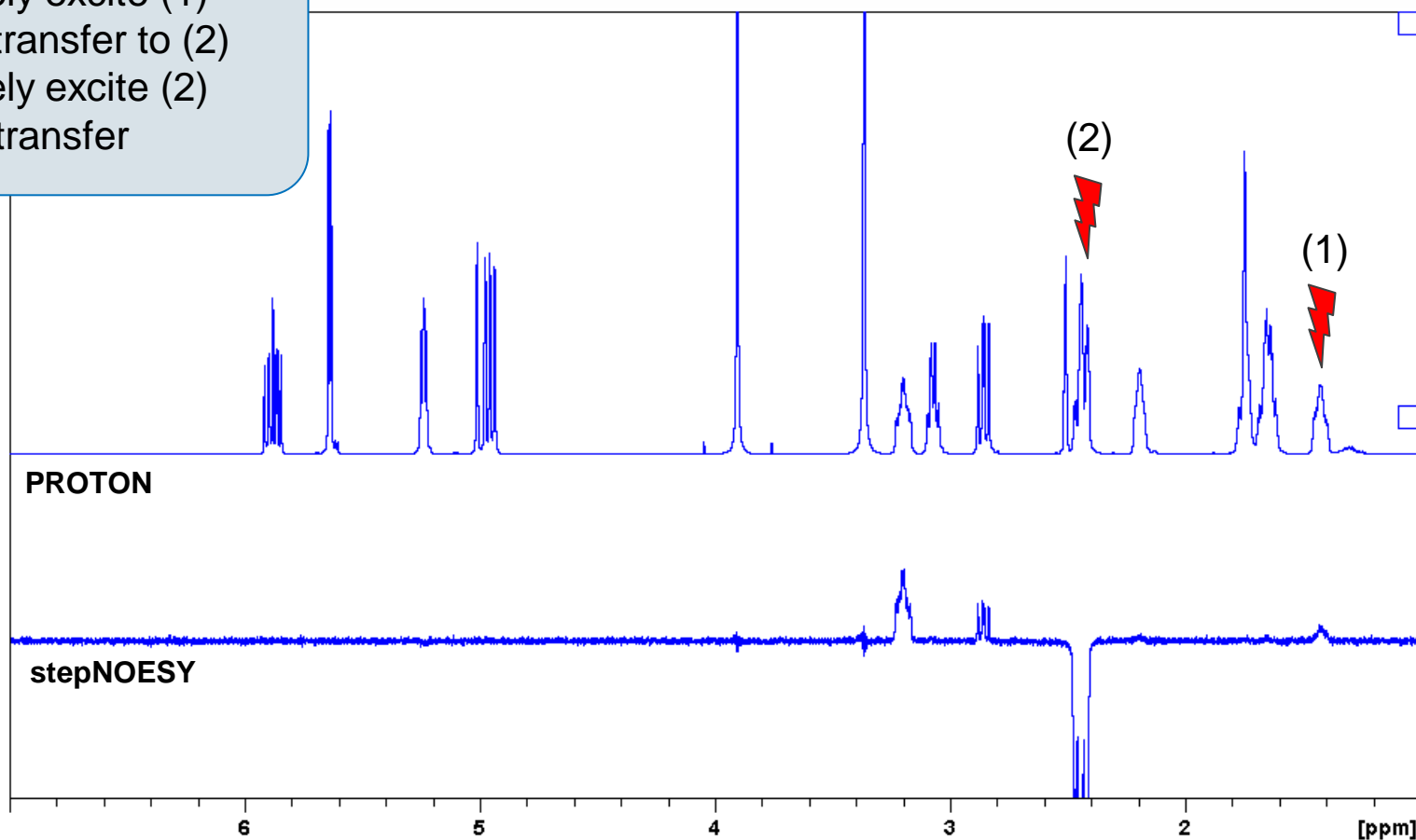
We can do a TOCSY transfer to them from another spin



stepNOESY – 1D selective NOESY from overlapped peaks



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

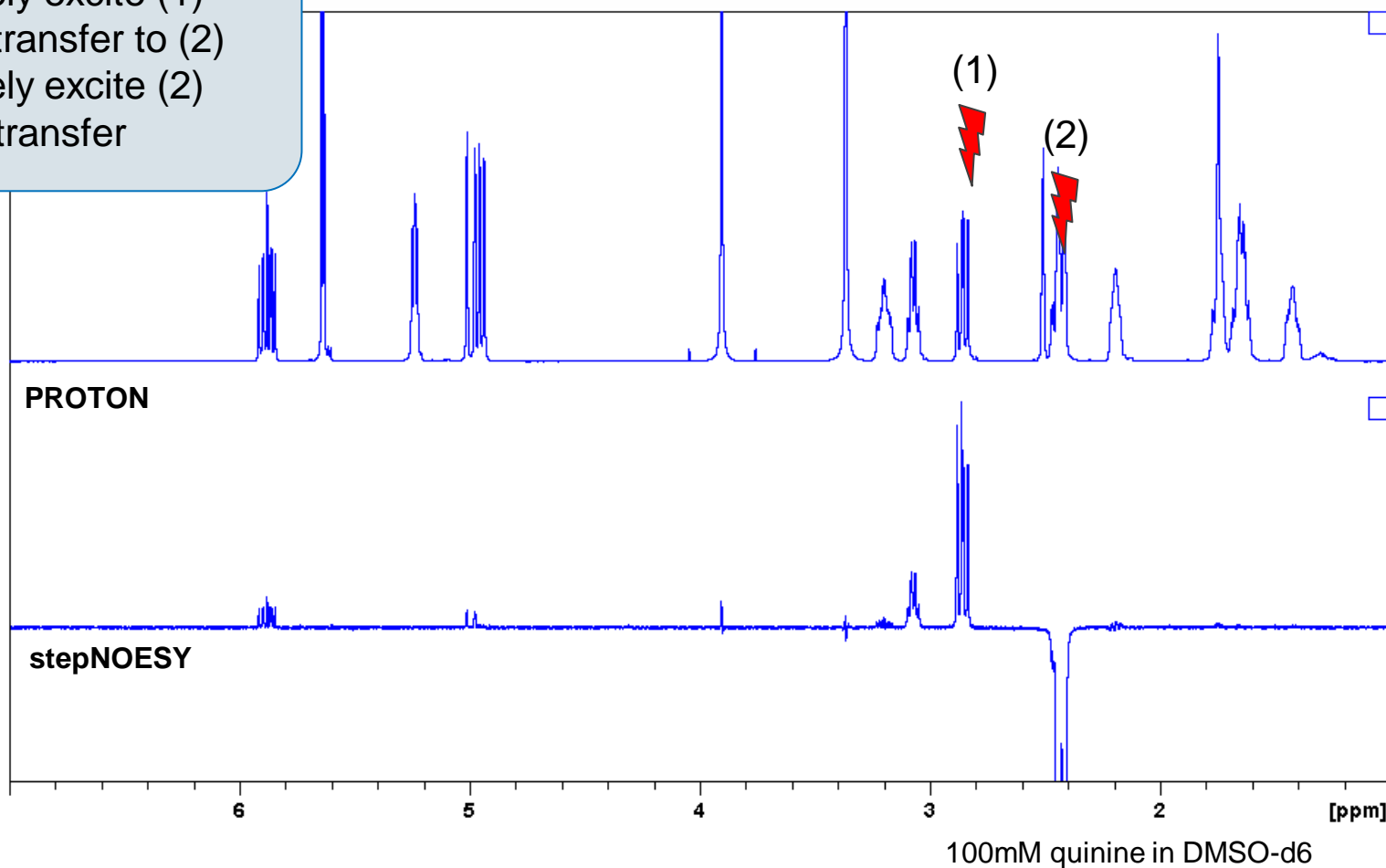


100mM quinine in DMSO-d6

stepNOESY – 1D selective NOESY from overlapped peaks



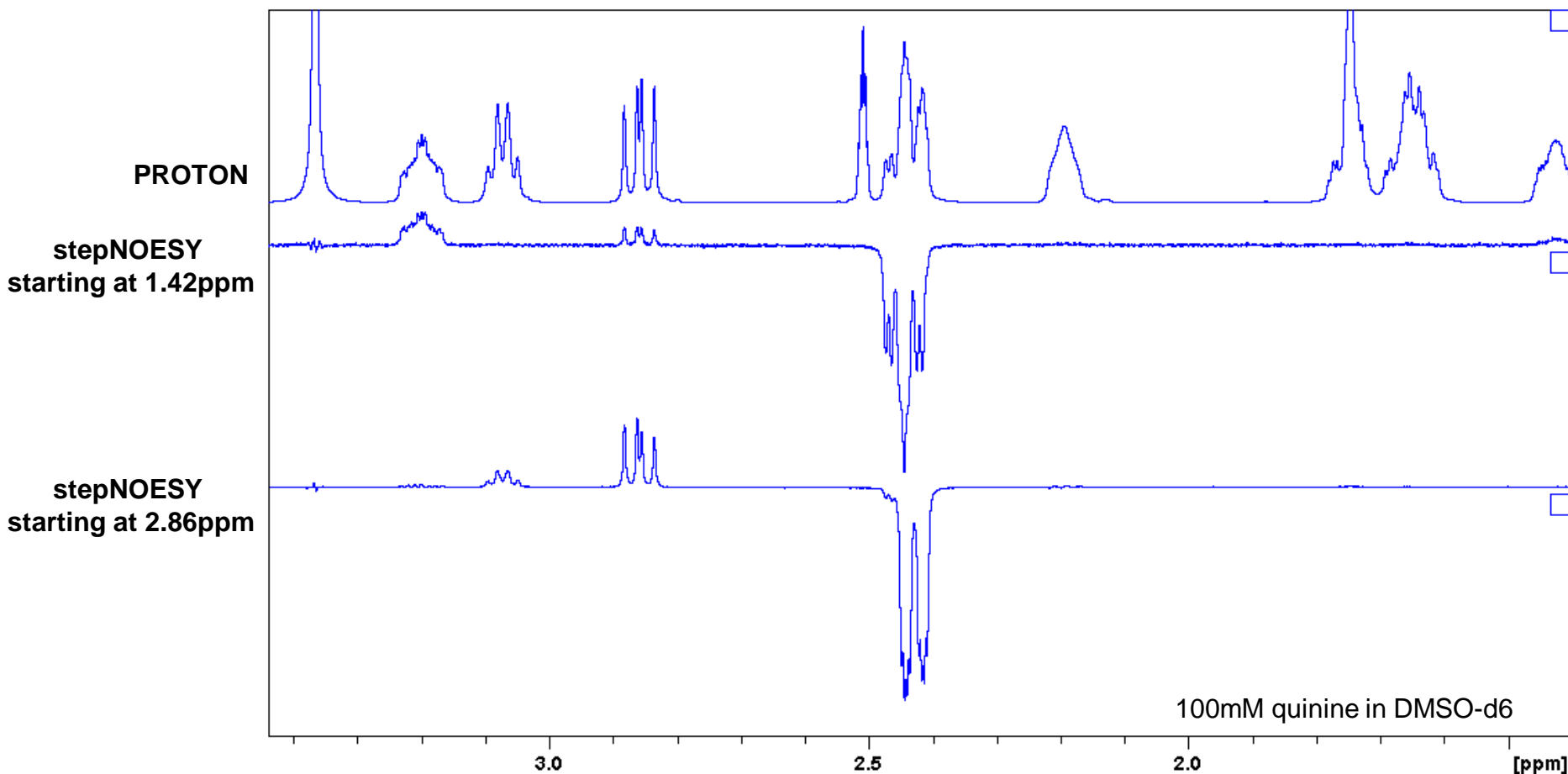
1. Selectively excite (1)
2. TOCSY transfer to (2)
3. Selectively excite (2)
4. NOESY transfer



stepNOESY – 1D selective NOESY from overlapped peaks



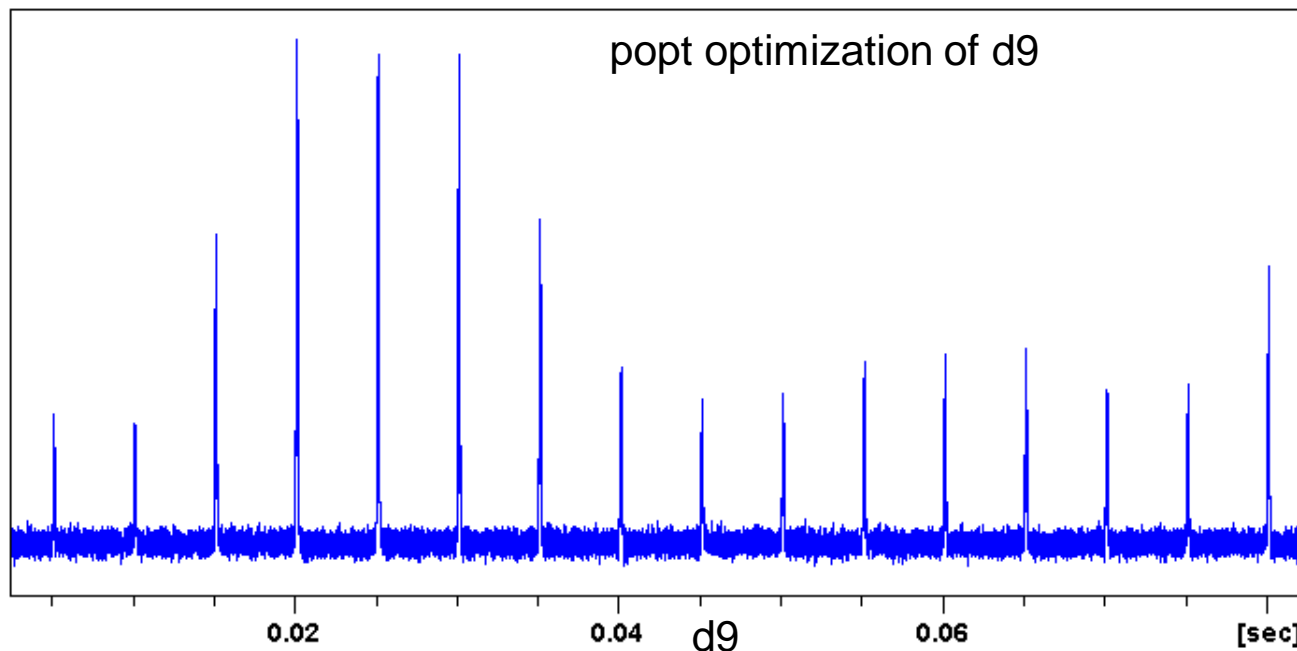
- Showing how cleanly the stepNOESY is exciting each component of the multiplet at 2.45ppm



stepNOESY – optimizing parameters



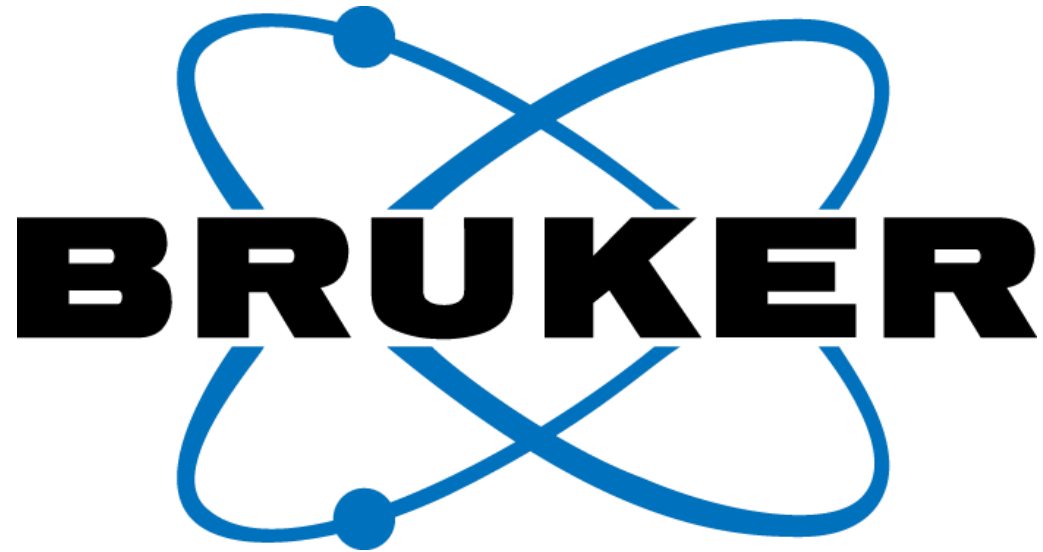
- The stepNOESY is much less sensitive than the standard selective NOESY
 - Signal losses during both TOCSY and NOESY parts
- When running the TOCSY, we usually keep the mixing time relatively long to transfer magnetization to the whole spin system
- He can optimize the mixing time “d9” to maximize the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