

Advanced Experiments

Expert NMR made easy

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Agilent Technologies

“I know what I like... and I like what I know.”
Dr. J. Josephs, Sr.



Courtesy: L. Park

Outline

- Better resolution in less time – Non-Uniform Sampling
- Collecting only the right data – Band Selective Experiments
- Simplifying spectra – The Pureshift 1D Experiment
- Maximizing signal intensity for H/C experiments – CRISIS
- Using just the right amount of time – qNMR + Adaptive NMR
- Don't waste time on sample prep – Automated Solvent Suppression



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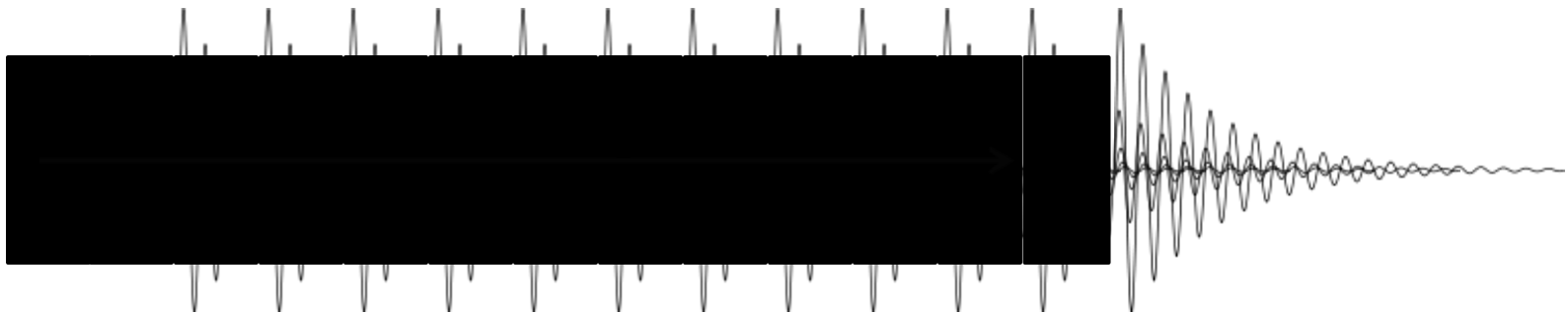
Conventional Sampling in 2D Spectroscopy

- t_1 period is incremented linearly (uniformly):

$$0, 1/\text{sw1}, 2/\text{sw2}, \dots, (\text{ni}-1)/\text{sw1}$$

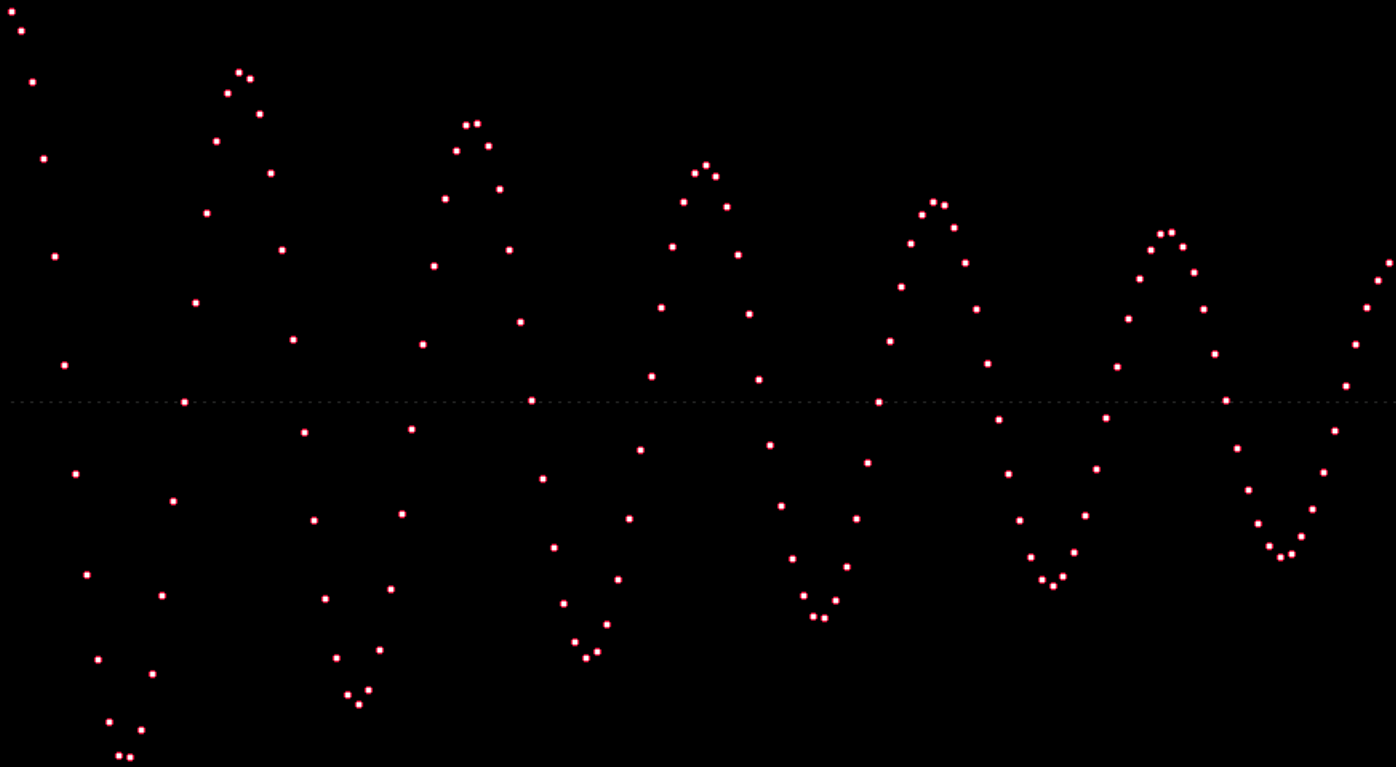
Where sw1 is spectral width in F_1 dimension and ni is total number of t_1 increments

Resolution in F_1 is given by maximum t_1 acquisition (evolution) time $(\text{ni}-1)/\text{sw1}$



Time Domain

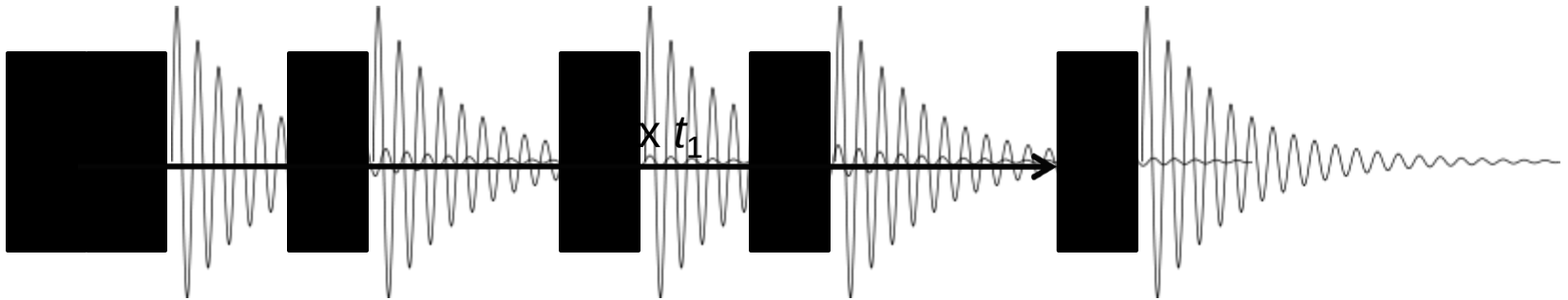
Full Time Domain Data



Uniform Sampling

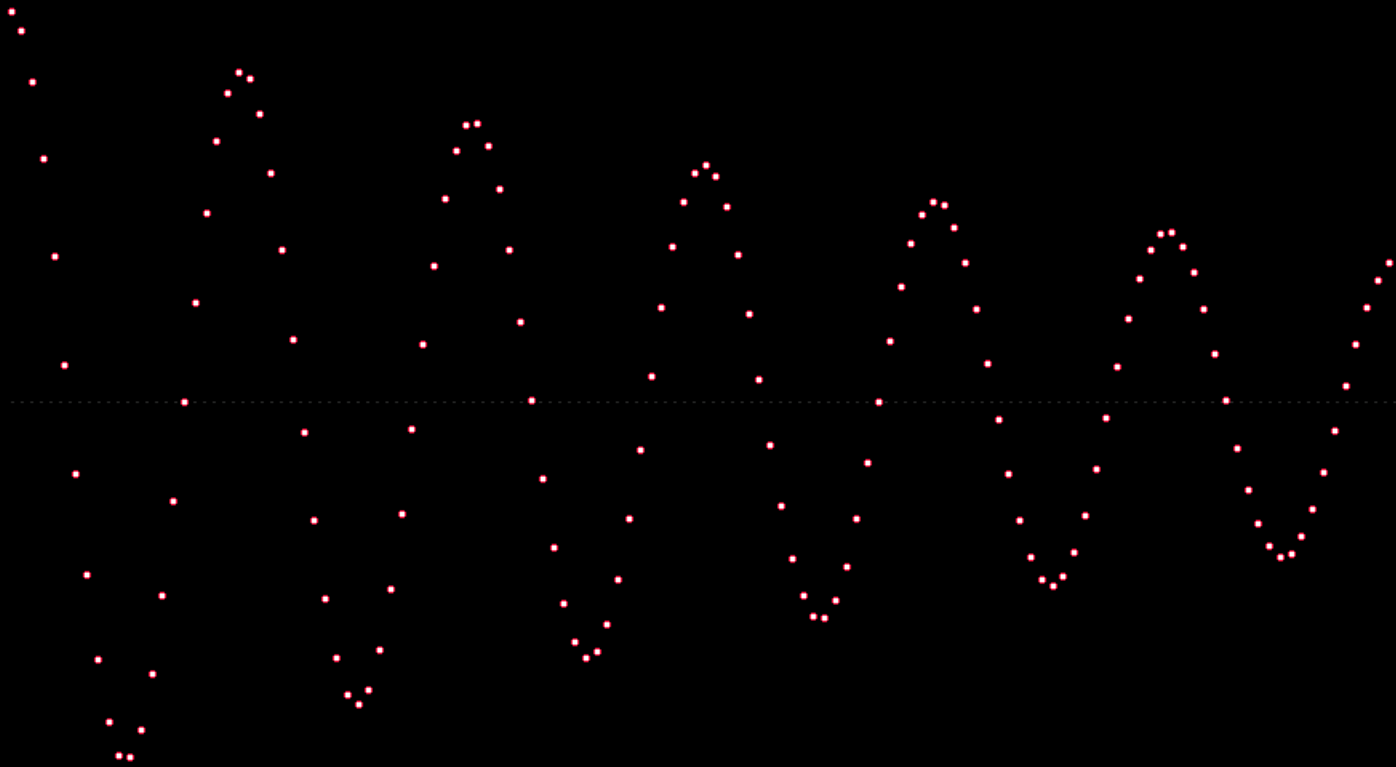
Do we need to sample every t_1 increment?

- No! This is done to satisfy the requirements of FT
- Non-Uniform Sampling (NUS) is the better way to collect data



Time Domain

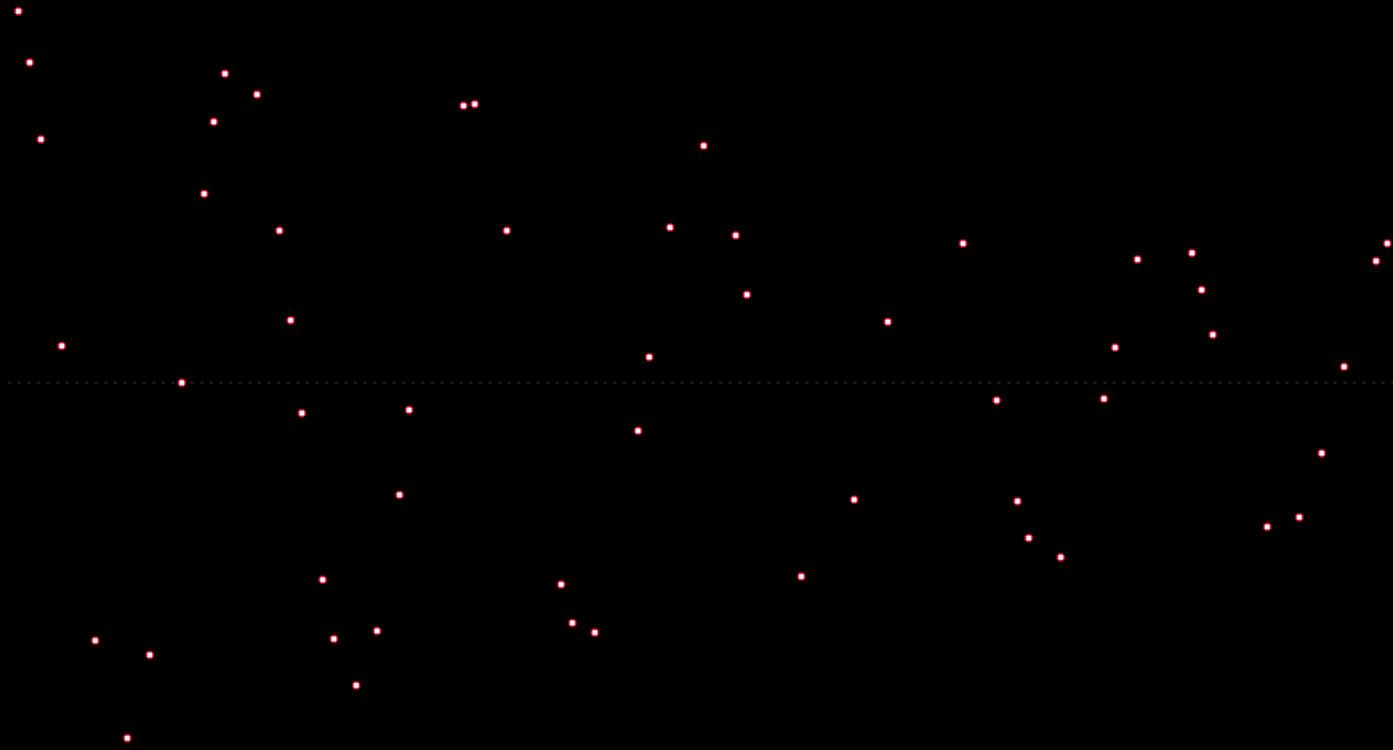
Full Time Domain Data



Uniform Sampling

Time Domain

NUS Time Domain Data

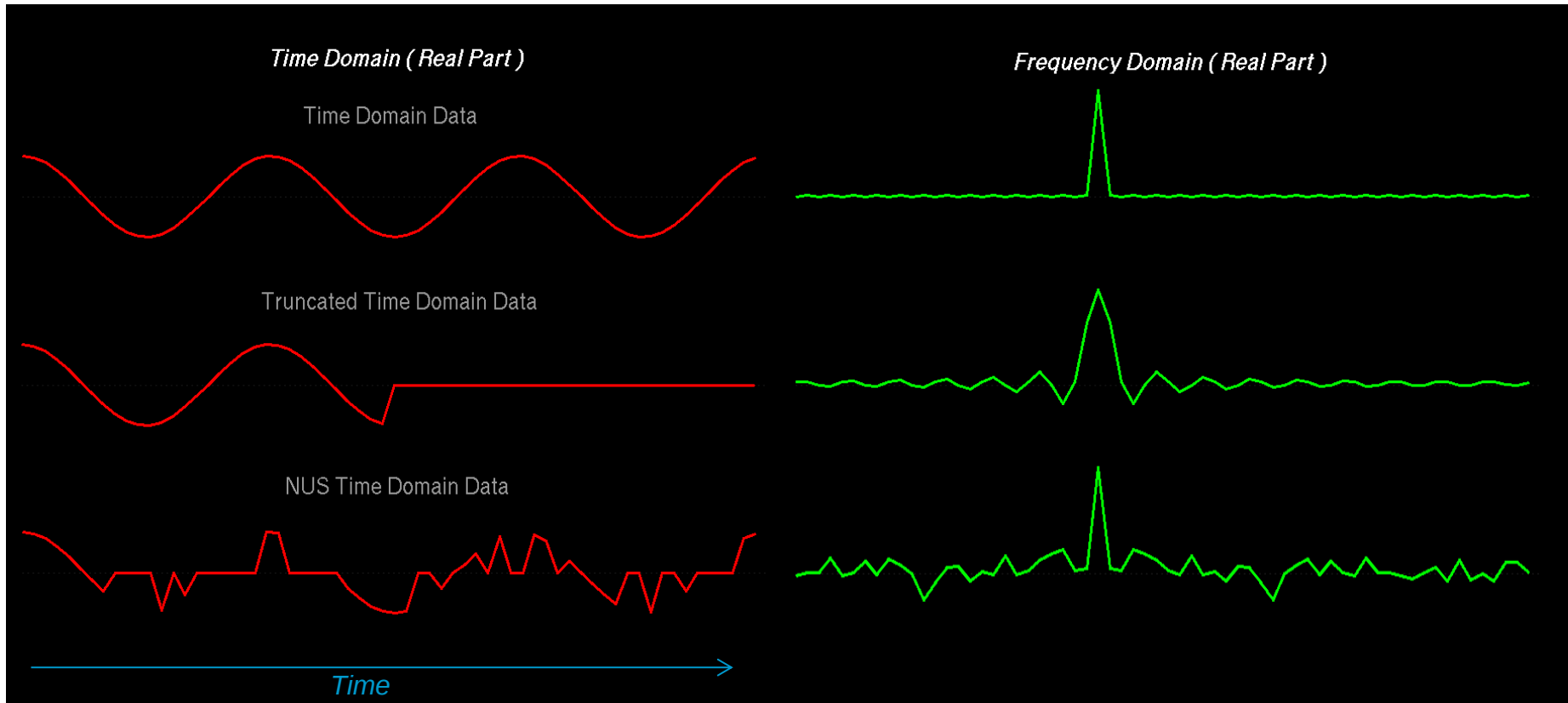


Non-Uniform Sampling: Skip a Fraction of the Points

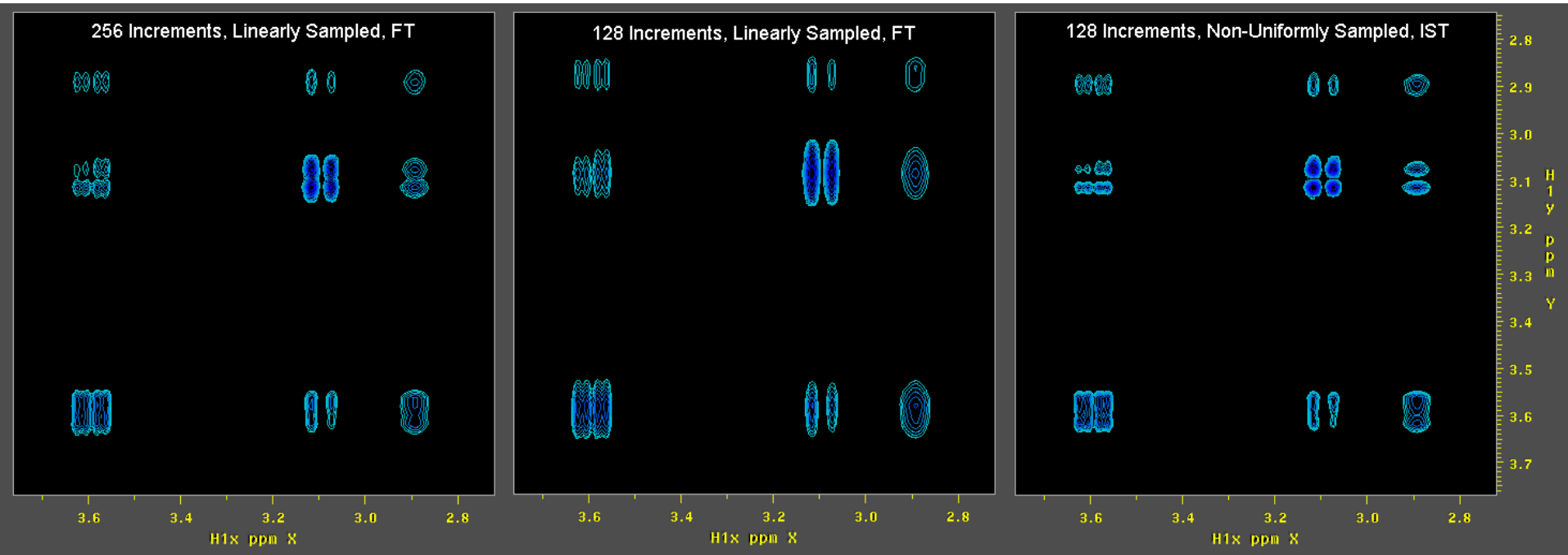
Linear versus Non-Uniform Sampling

Linear Sampling: Broad Line and Periodic Truncation Wiggles

Non-Uniform Sampling: Narrow Line and Random Noise-Like Artifacts



Example Results



*Conventional
Linearly-Sampled*

*Conventional – Half of Original
Measurement Time*

*NUS – Half of Original
Measurement Time*

Turning it on is just a mouse click

- NUS “Acquire” panel

The screenshot shows the 'Acquire' panel of a software interface. The panel has a sidebar on the left with a tree view containing: Defaults, Acquisition, Pulse Sequence, NUS (highlighted), PRESAT, WET, Parameters, Channels, Flags, Future Actions, and Overview. The main area of the panel has a top bar with buttons: Start, Acquire, Process, Show time, Go, Stop, Arrays, Sequence diagram, and Sequence help. Below the top bar, the 'Enable non-uniform sampling' checkbox is checked and highlighted with a red rectangle. Underneath this, there is a section titled 't1 sampling schedule' which contains two rows of controls. The first row is for 'No. increments in reconstructed data', showing a value of 96 in a text box and a dropdown menu, with a note '(Resolution: 209.4 Hz/pt; Max. evolution time: 0.00473 s)'. The second row is for 'Sampling density', showing a value of 50.00 in a text box and a dropdown menu set to 50, followed by a '%' symbol, with a note '(48 increments recorded; total experiment time 4.5 min)'. At the bottom of this section, there is an unchecked checkbox for 'Use advanced schedule options' and a button labeled 'Show schedule text file'.

Start Acquire Process Show time Go Stop Arrays Sequence diagram Sequence help

Defaults
Acquisition
Pulse Sequence
NUS
PRESAT
WET
Parameters
Channels
Flags
Future Actions
Overview

☒ Enable non-uniform sampling

t1 sampling schedule

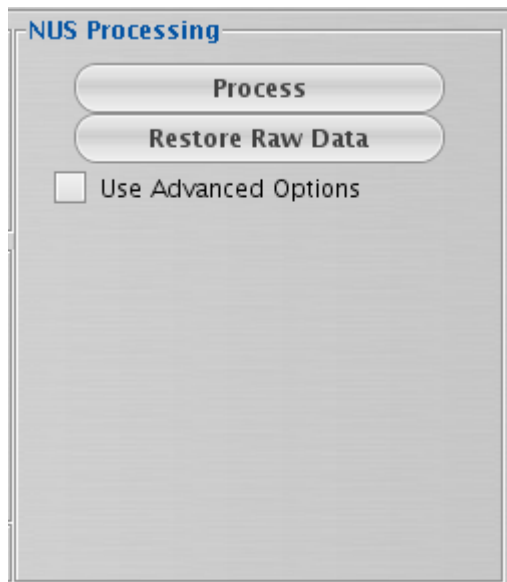
No. increments in reconstructed data 96 96 ▼
(Resolution: 209.4 Hz/pt; Max. evolution time: 0.00473 s)

Sampling density 50.00 50 ▼ %
(48 increments recorded; total experiment time 4.5 min)

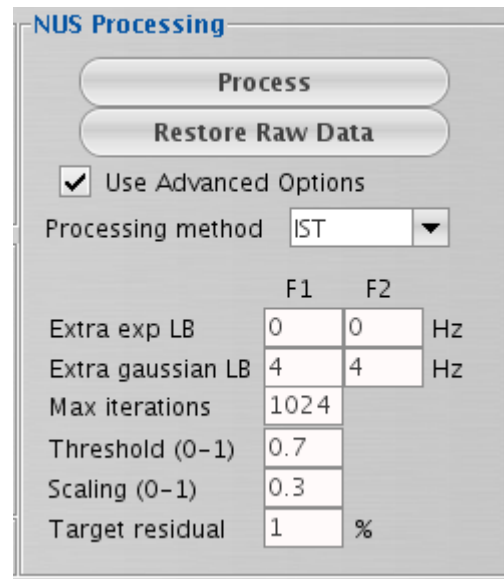
☐ Use advanced schedule options Show schedule text file

NUS Processing Options

- Data measured in StudyQ processed automatically
- Can reprocess NUS data with a single button click
- CLEAN takes a few seconds (typically less than 5)
- IST takes about a minute



The image shows a software dialog box titled "NUS Processing". It contains two buttons: "Process" and "Restore Raw Data". Below these buttons is a checkbox labeled "Use Advanced Options" which is currently unchecked.



The image shows the same "NUS Processing" dialog box, but with the "Use Advanced Options" checkbox checked. This reveals additional settings:

- Processing method:** A dropdown menu set to "IST".
- Extra exp LB:** Input field with "0", followed by "F1" and "Hz".
- Extra gaussian LB:** Input field with "4", followed by "F2" and "Hz".
- Max iterations:** Input field with "1024".
- Threshold (0-1):** Input field with "0.7".
- Scaling (0-1):** Input field with "0.3".
- Target residual:** Input field with "1", followed by a "%" symbol.

Non-Uniform Sampling: Summary

- NUS gives the same quality of data in less time

or

- Higher quality data (better resolution) in the same time

or

- A bit of both! 😊

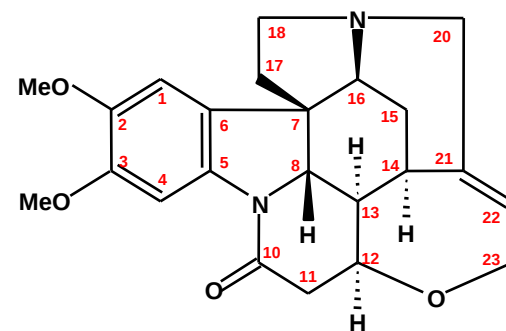
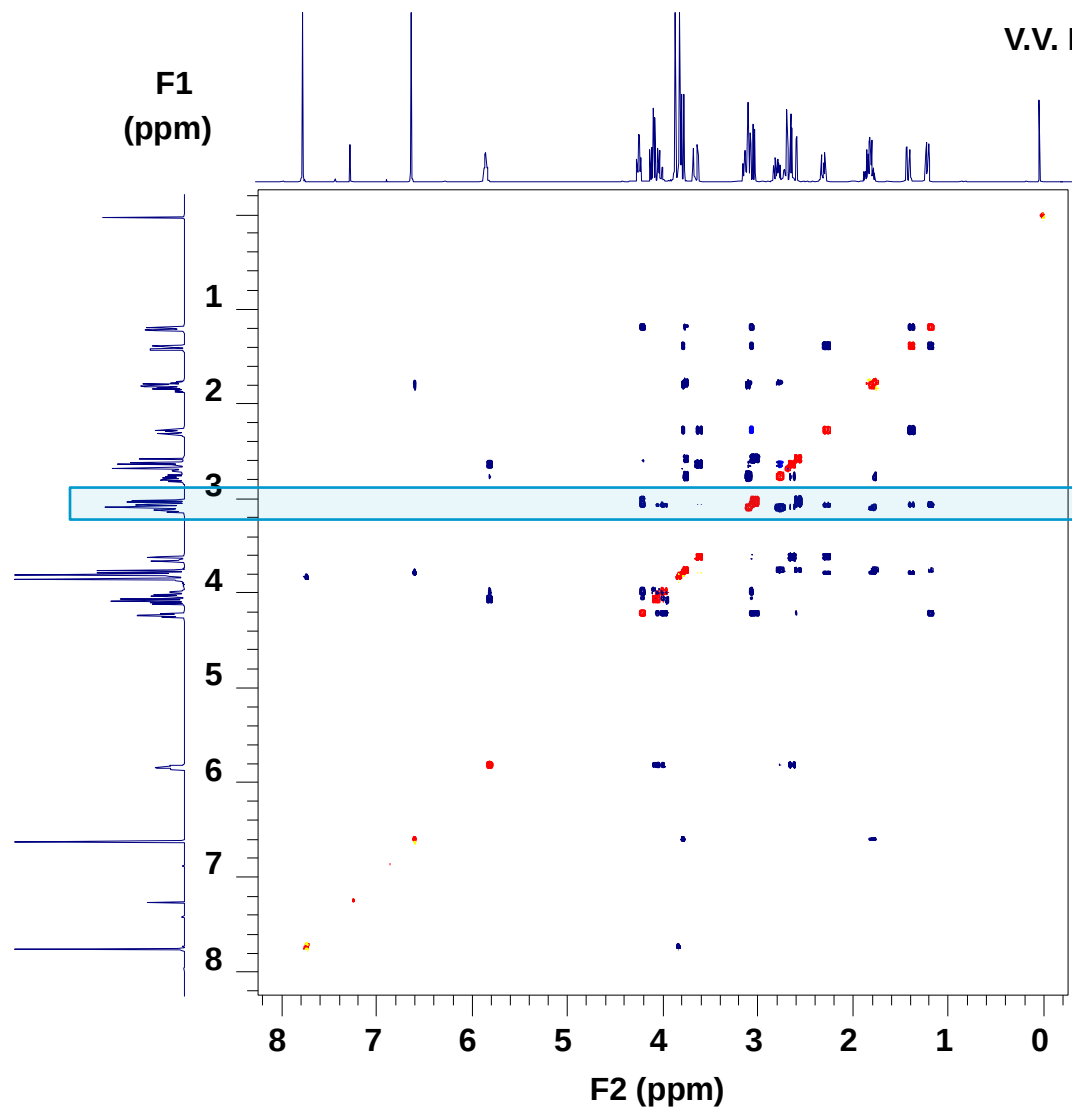


Outline

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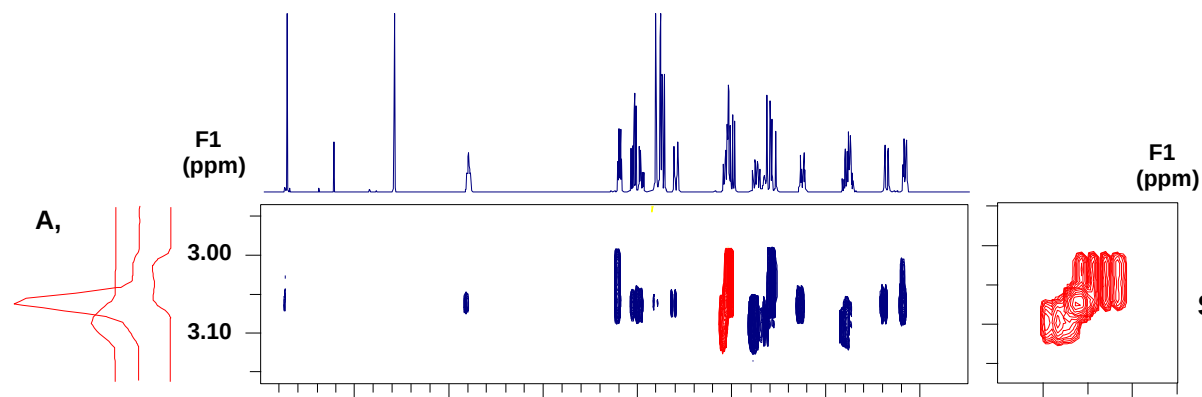
Band-selective 2D NOESY (bashdNOESY)

V.V. Krishnamurthy, *Magn. Reson. Chem.* 35, 9 (1997)



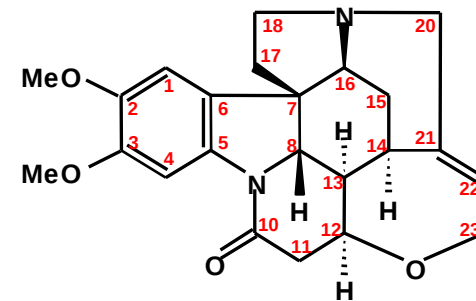
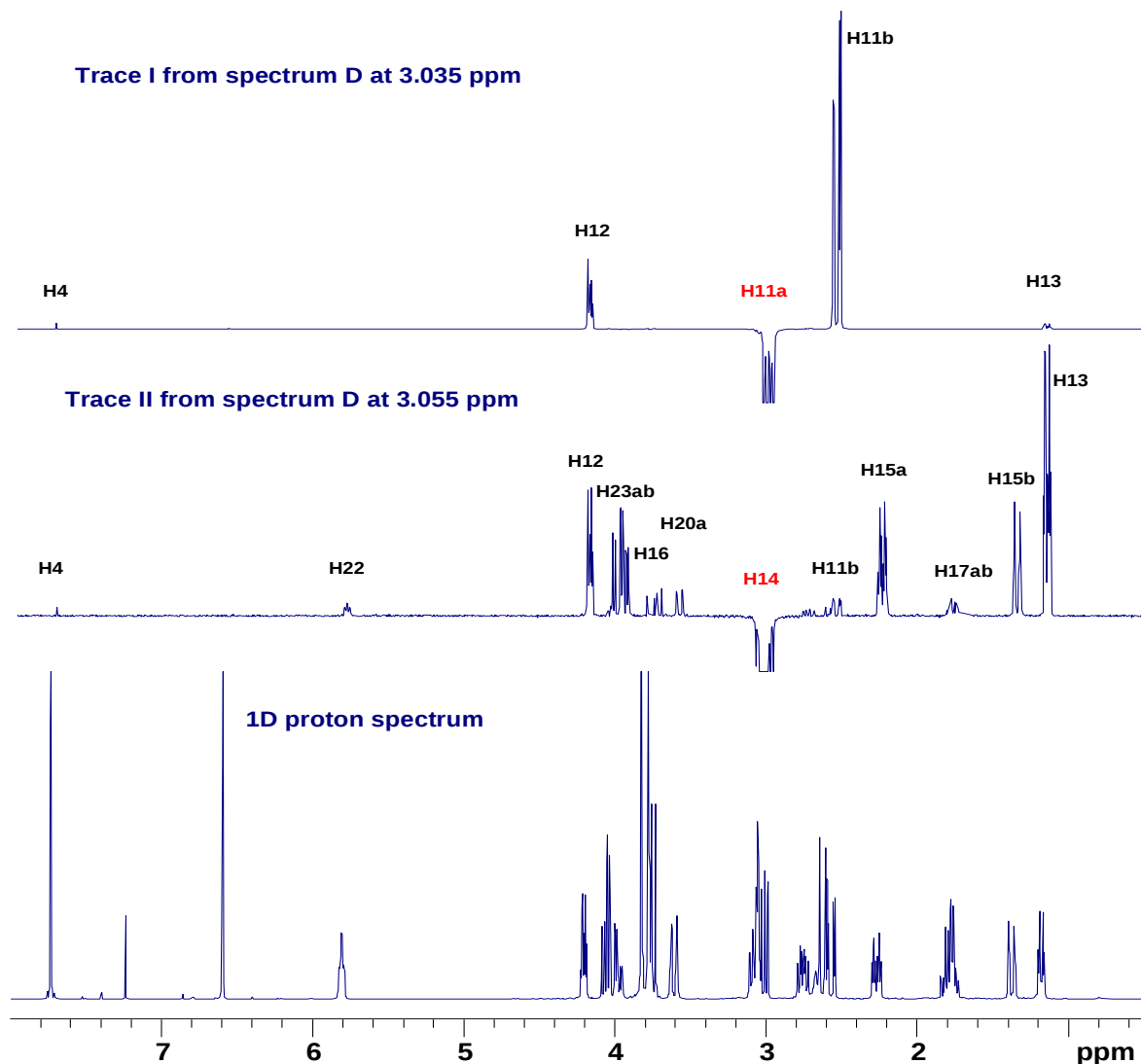
sw=sw1=4006, ni=480, at=0.256
exp. time = 440 min

Band-selective 2D NOESY (bashdNOESY)



2D NOESY (expansion)
sw1=4006, ni=480, at=0.256
exp. time = 440 min

Band-selective 2D NOESY (bashdNOESY)



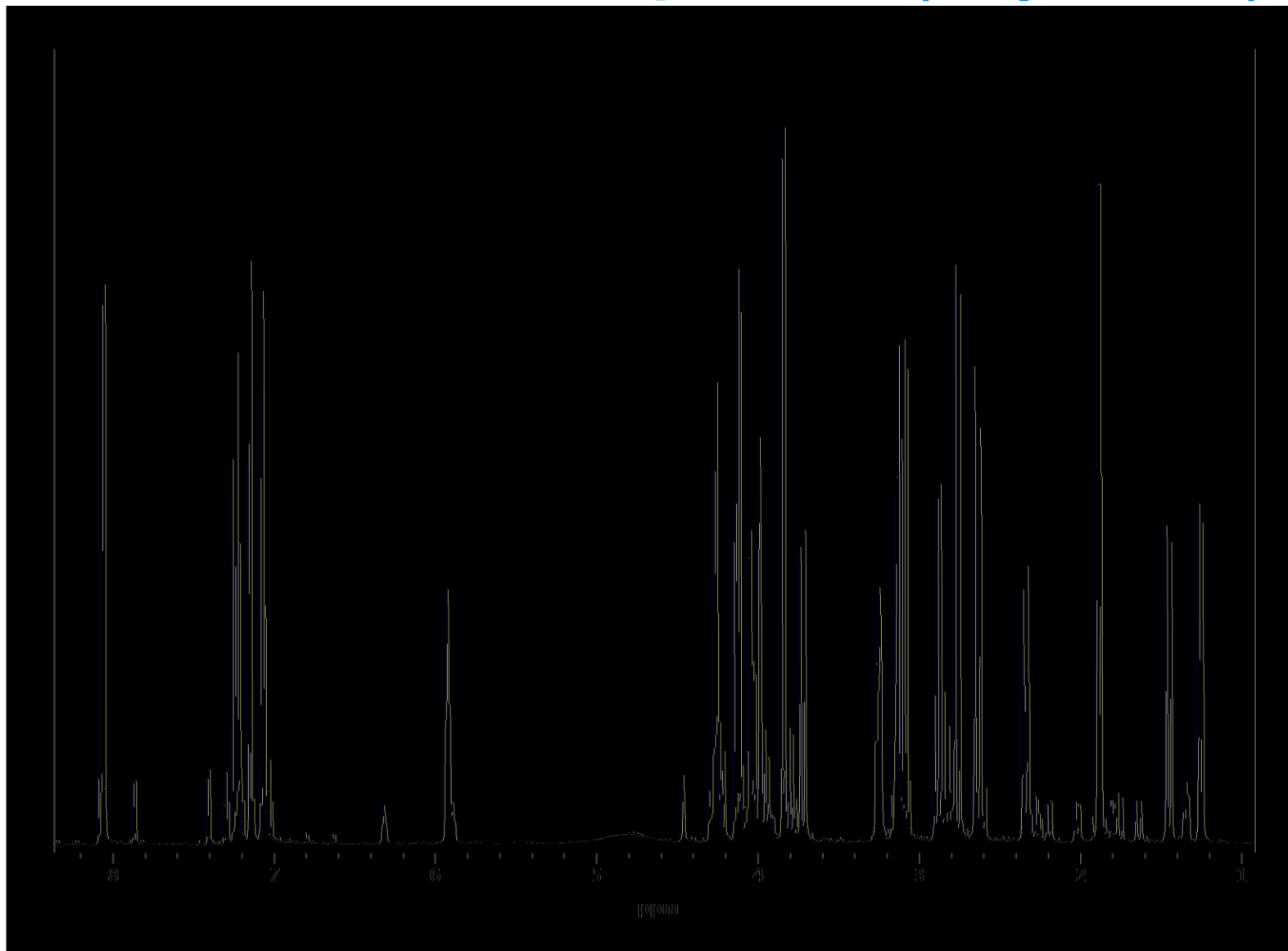
Band-Selective Experiments: Summary

- Reduce measurement time AND get better quality data by focusing on signals/regions of interest
- Setup done in a few mouse clicks
- Entire family of band-selective 1D and 2D experiments in VnmrJ

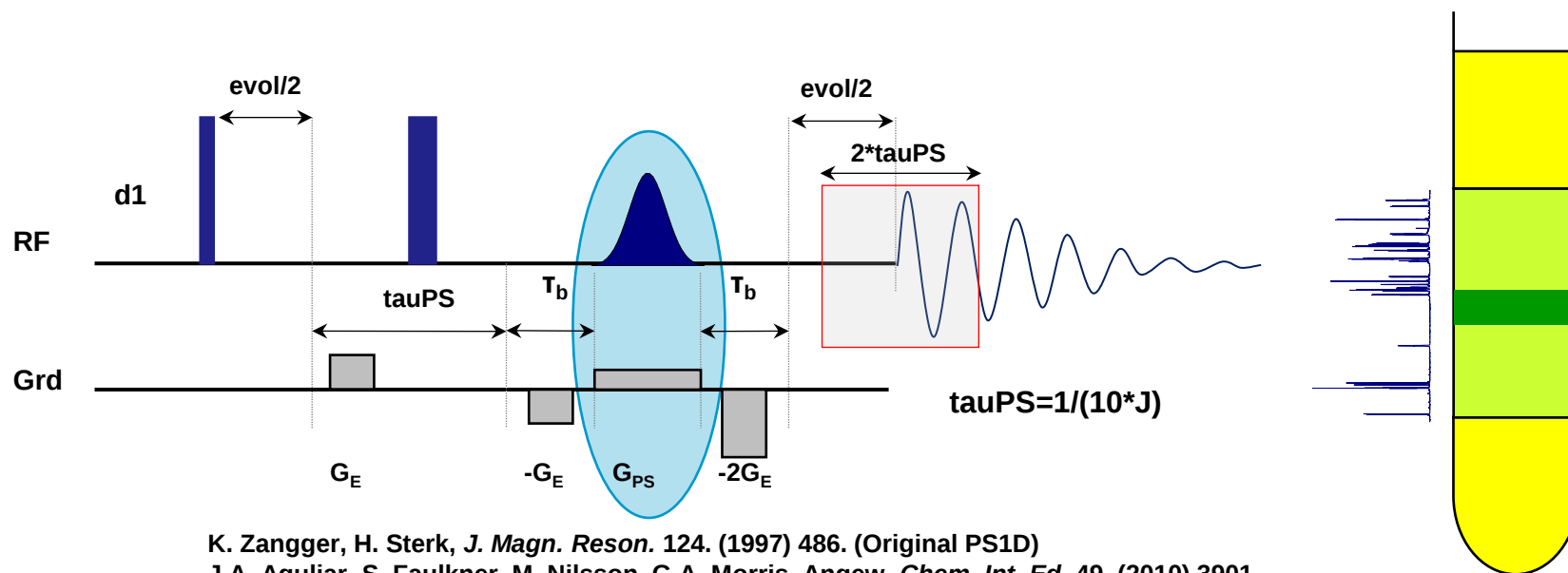
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A “Traditional” Proton Spectrum (Strychnine)



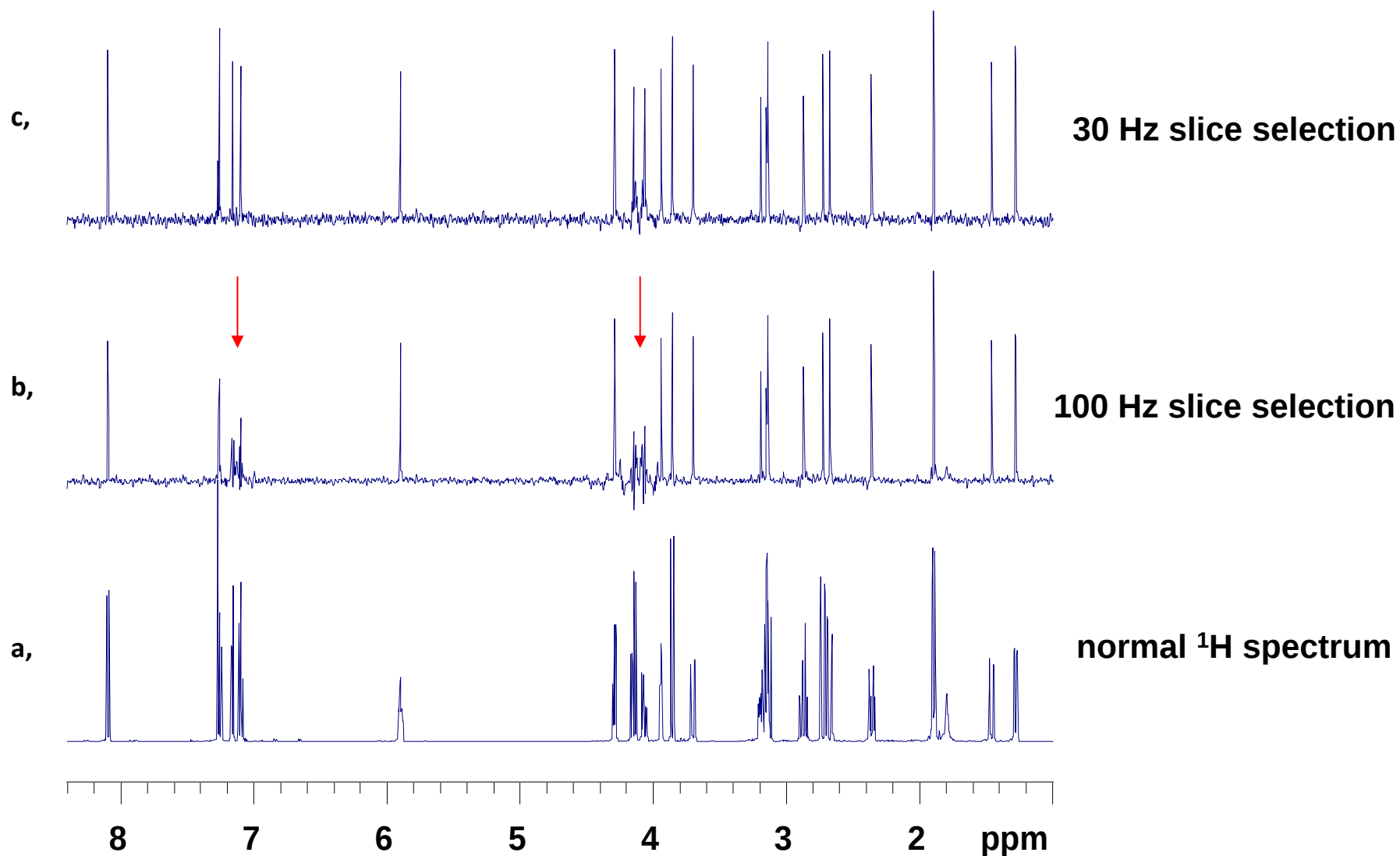
PS1D – ^1H Spectrum with Broadband Proton Decoupling



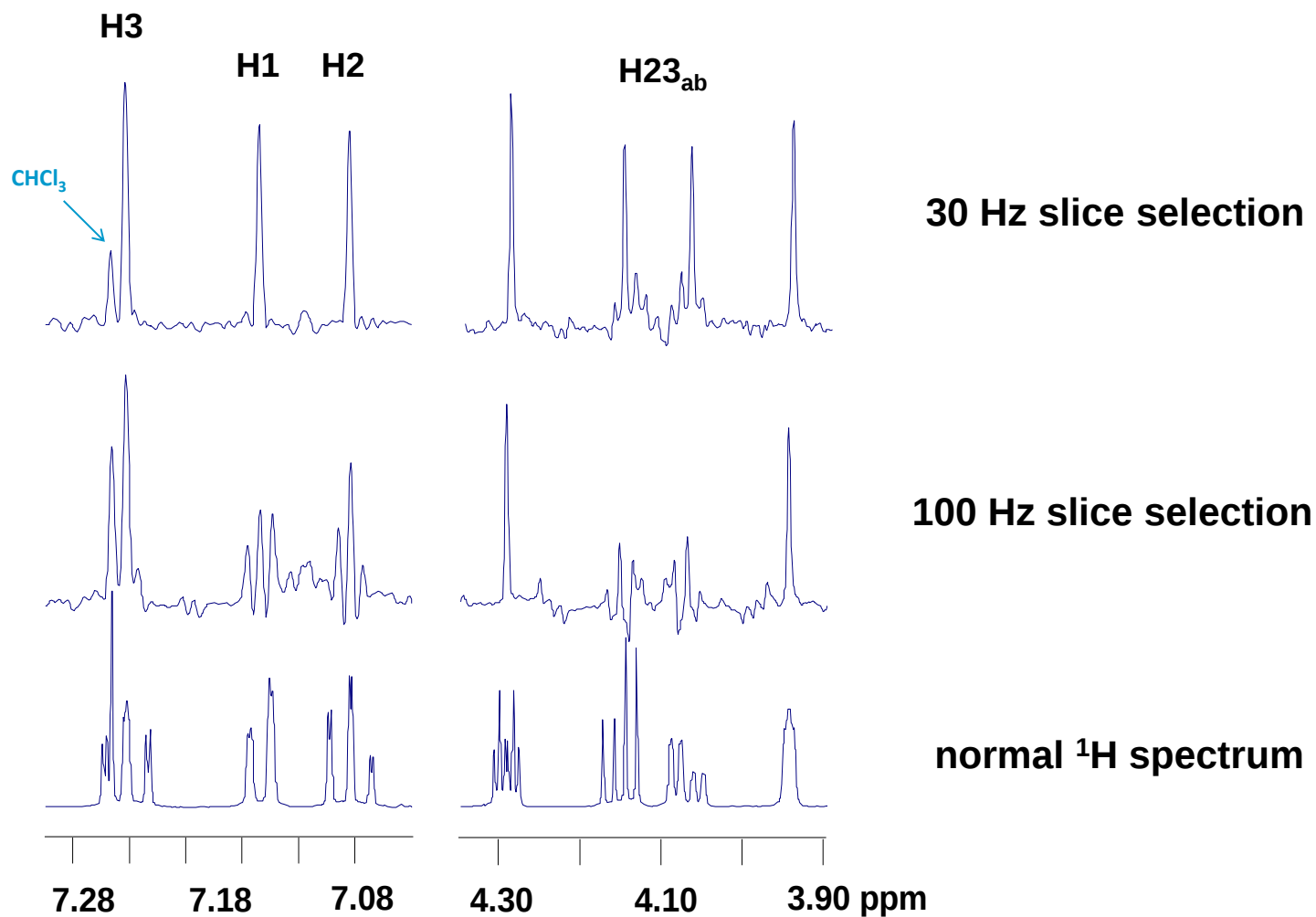
Two parameters to play with:

- Pure Shift Delay – determined by J
- Slice Selection Bandwidth – determined by separation between spins

PS1D – ^1H Spectrum with Broadband Proton Decoupling



PS1D – ^1H Spectrum with Broadband Proton Decoupling



PS1D: Summary

- Greatly simplifies proton spectra by removing multiplet structure due to homonuclear J-couplings
- Very easy to set up and run (three mouse clicks)



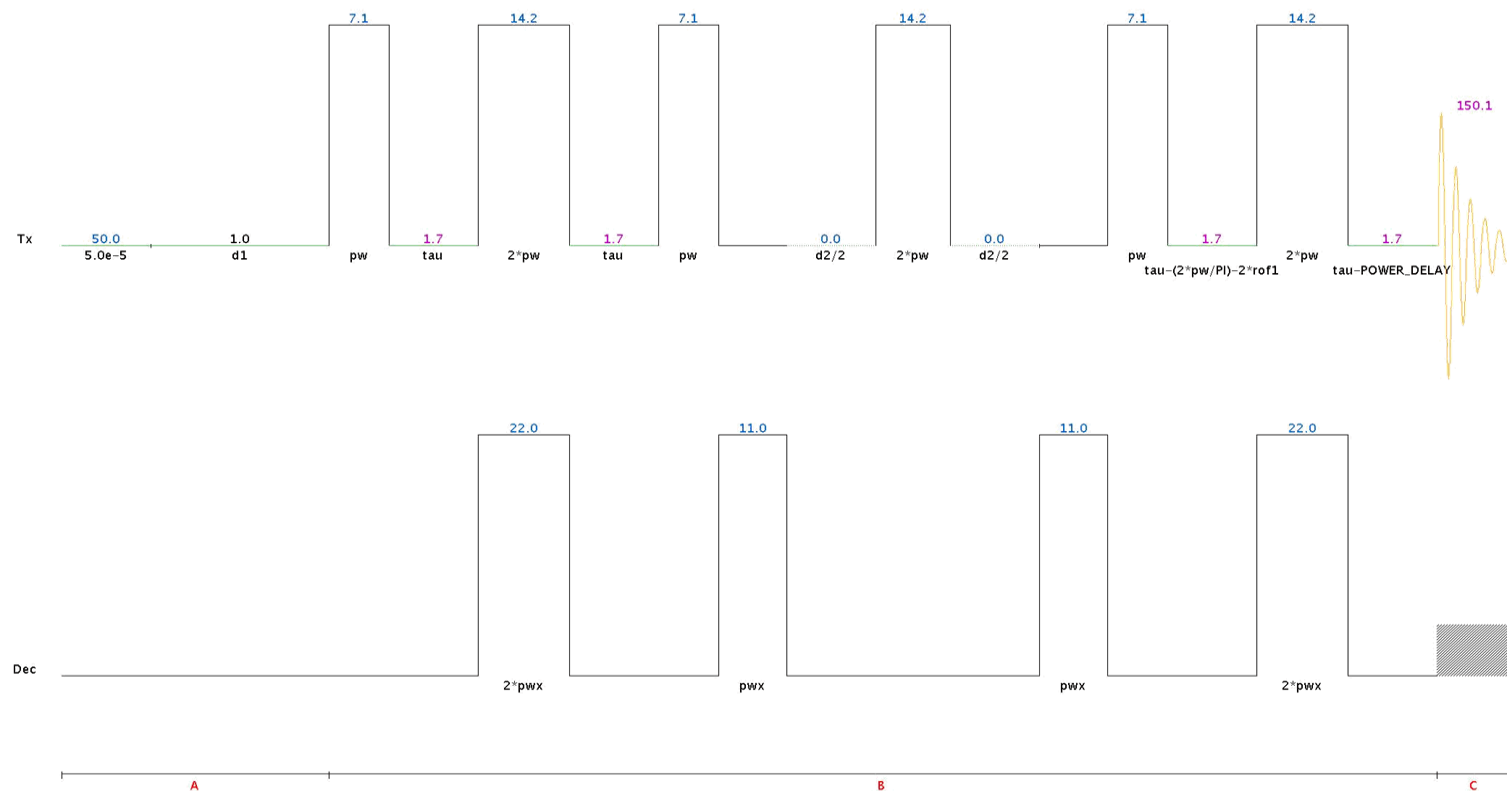
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CRISIS Experiments

The “original” HSQC experiment

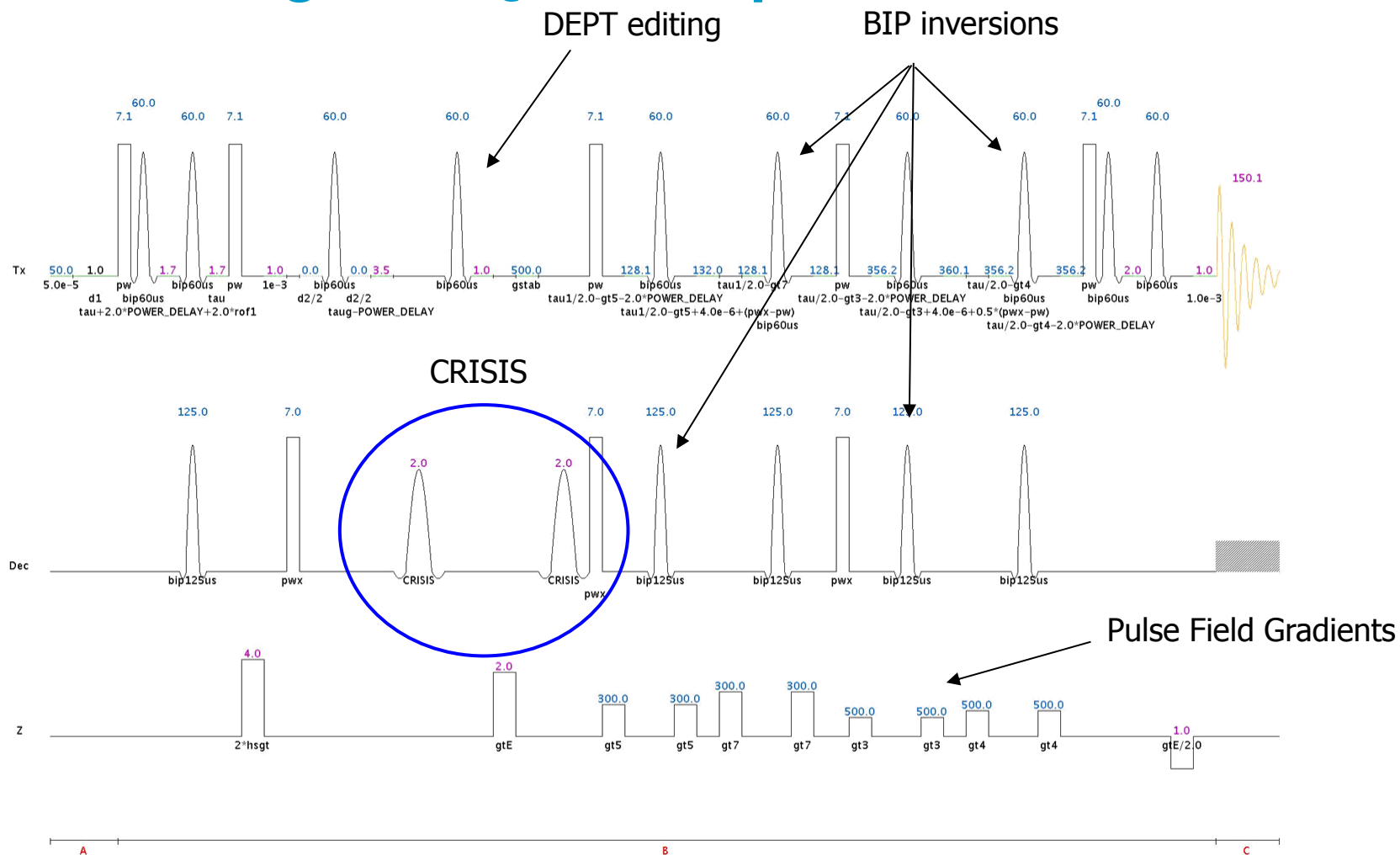


G. Bodenhausen, D. J. Ruben, *Chem. Phys. Lett.* **1980**, 69, 185;
 G. W. Vuister, R. Boelens, R. Kaptein, R. E. Hurd, B. John, P. C.M. van
 Zijl, *J. Am. Chem. Soc.* **1991**, 113, 9688;
 A. Bax, S. Pochapsky, *J. Magn. Reson.* **1992**, 99, 638.



CRISIS Experiments

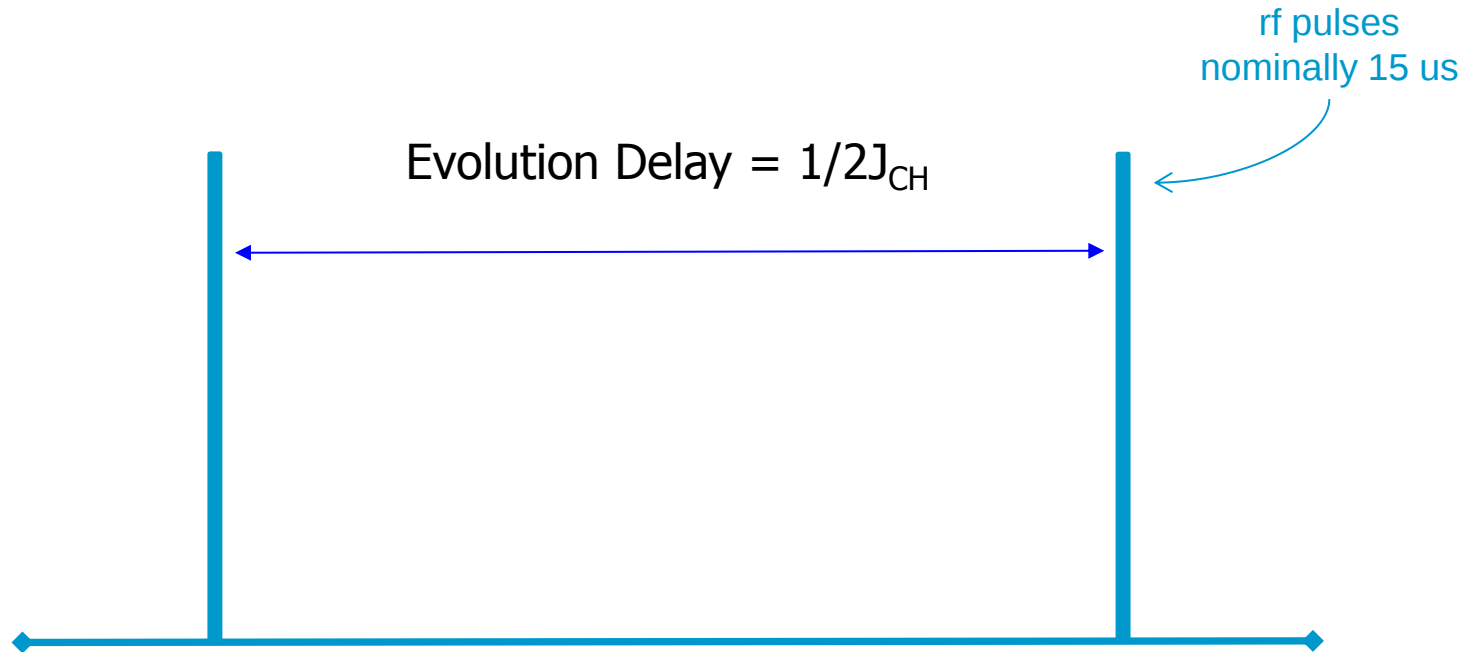
The modern gc2HSQCse sequence



R. Boyer, R. Johnson, K. Krishnamurthy, *J. Magn. Reson.* **2003**, 165-253.

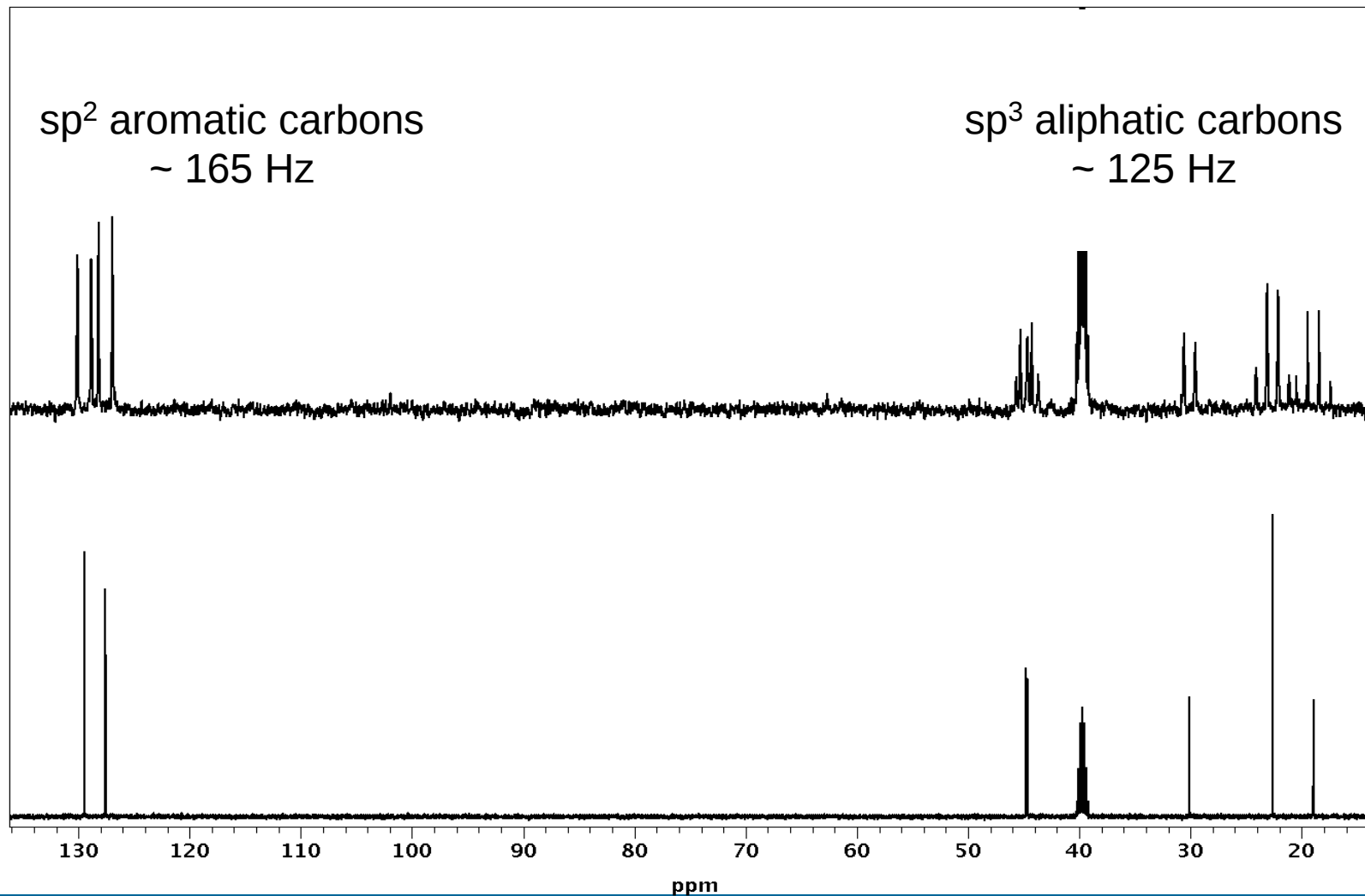
CRISIS Experiments

The CRISIS condition – how it works



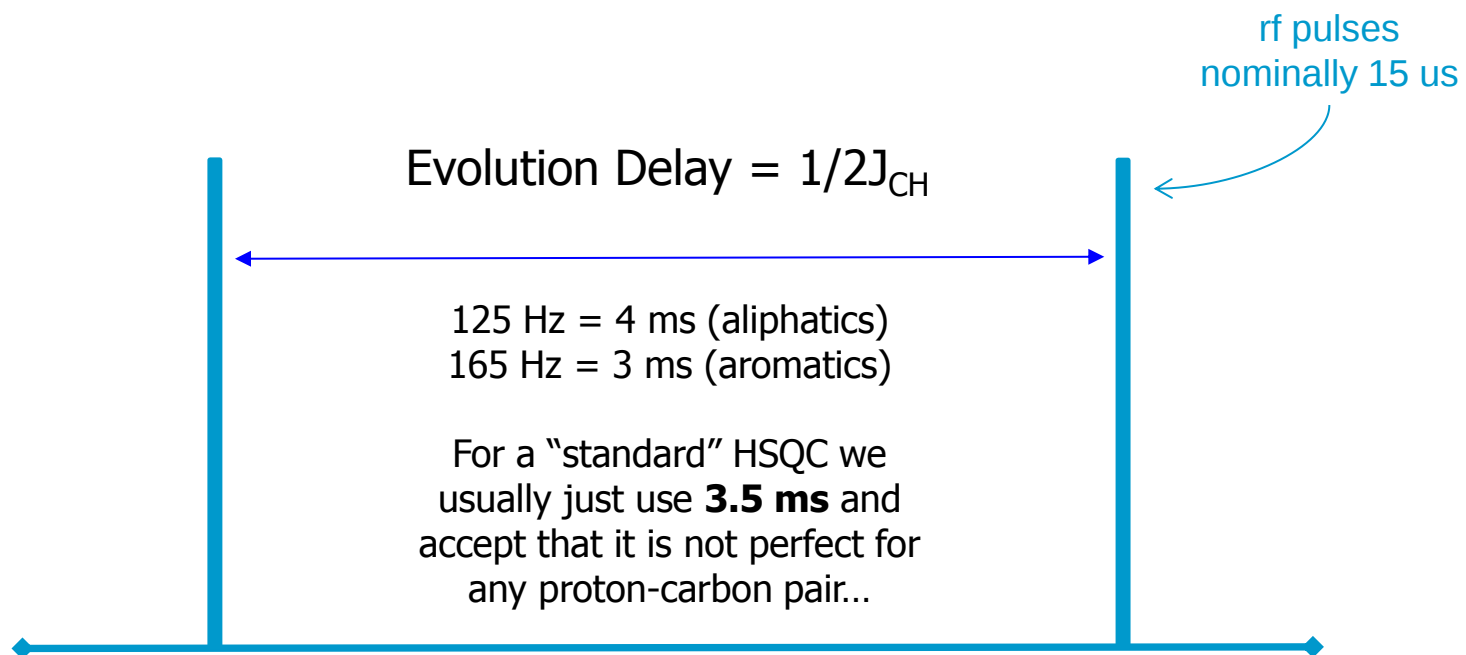
CRISIS Experiments

So, what is J_{CH} ?



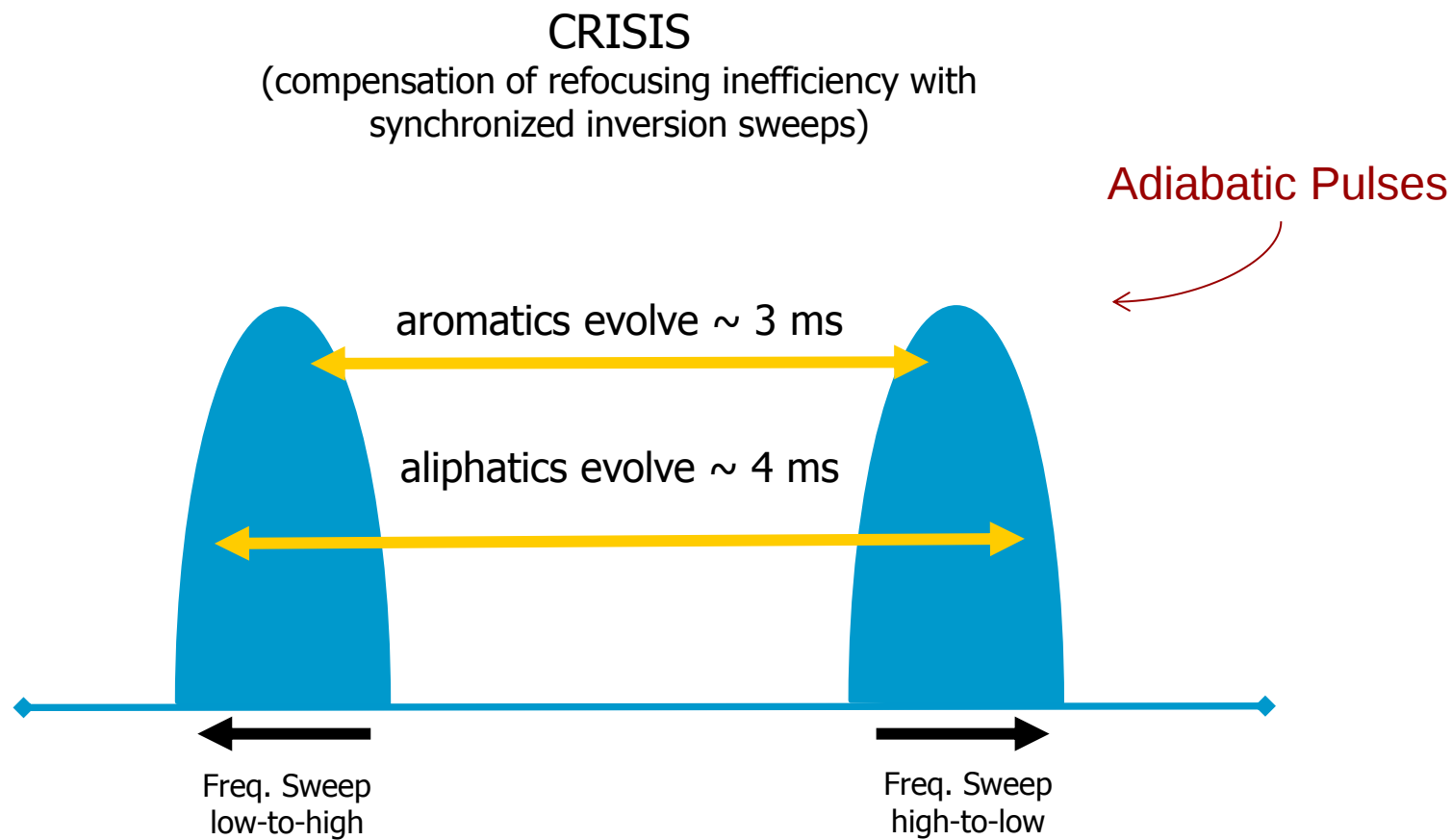
CRISIS Experiments

The CRISIS condition - how it works



CRISIS Experiments

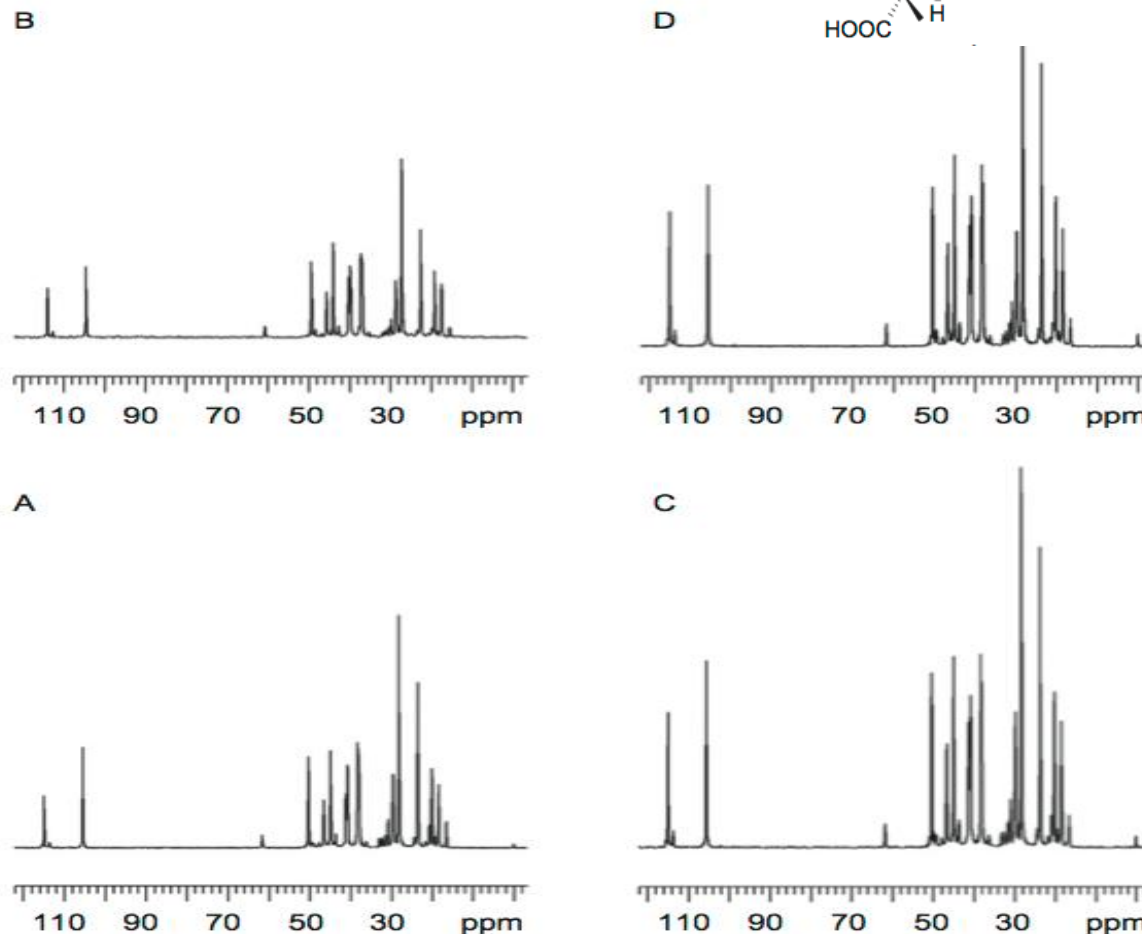
The CRISIS condition - how it works



CRISIS Experiments

Results

The use of the CRISIS enhanced sequence results in approximately a 50% increase in sensitivity. This means that data of the same quality can be collected **4 times faster** by using the correct sequence!



CRISIS Experiments: Summary

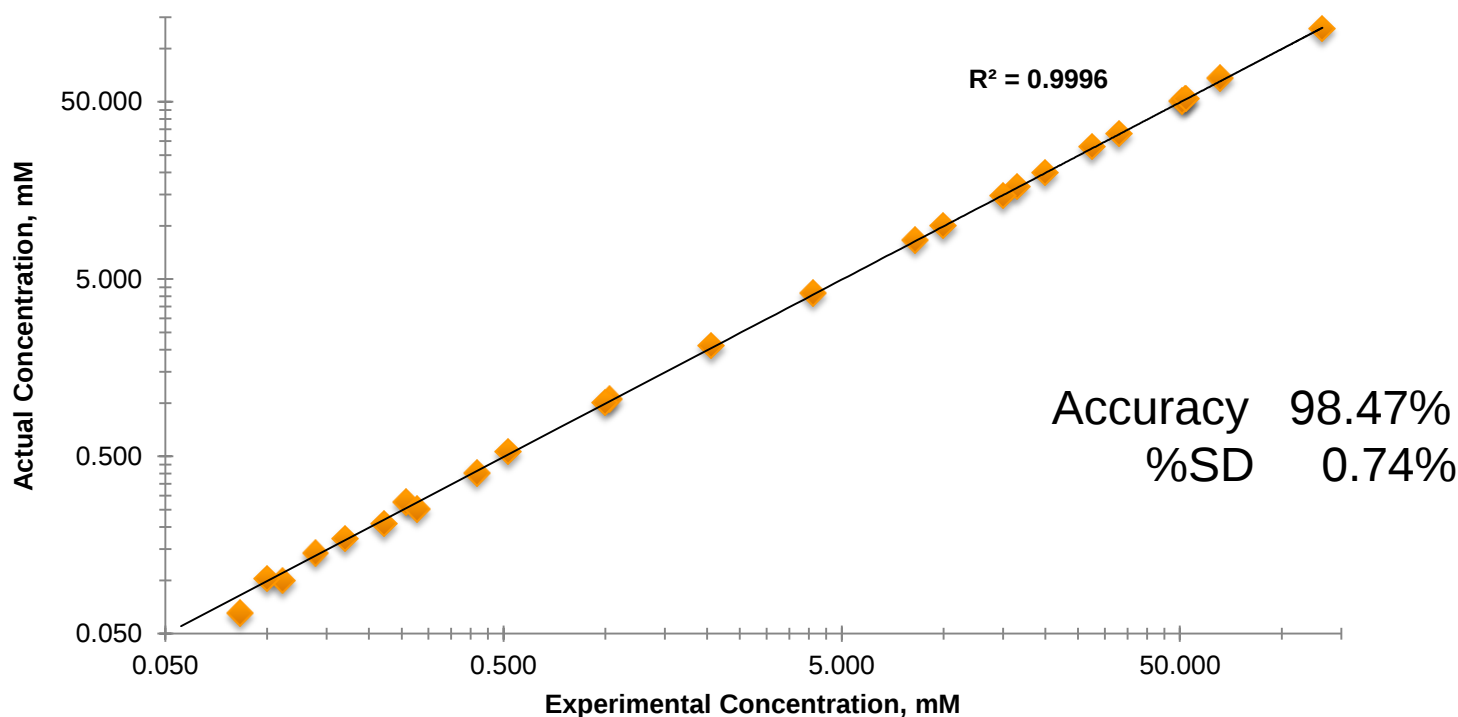
- Significantly greater sensitivity
- More tolerant of experiment “imperfections” (e.g. solvent effects on tuning and pulse calibrations)
- All CRISIS parameters are set up automatically – no additional steps required

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qNMR – Accuracy and Precision, made simple

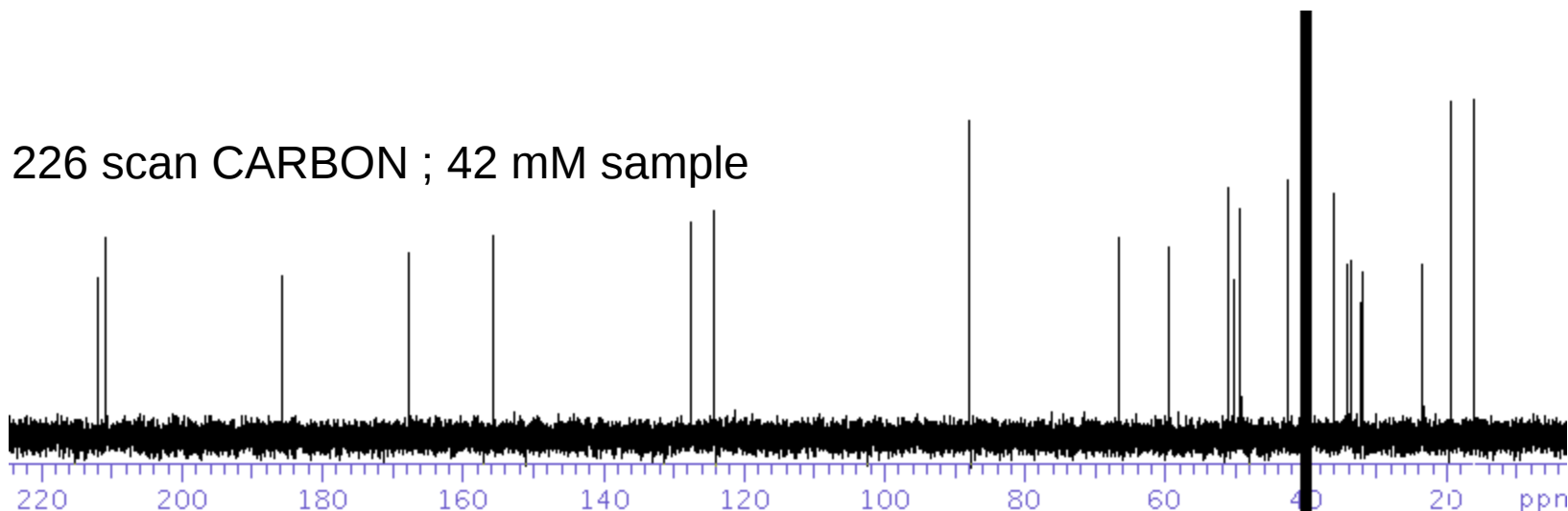
Linearity across systems, solvents,
analytes, and time



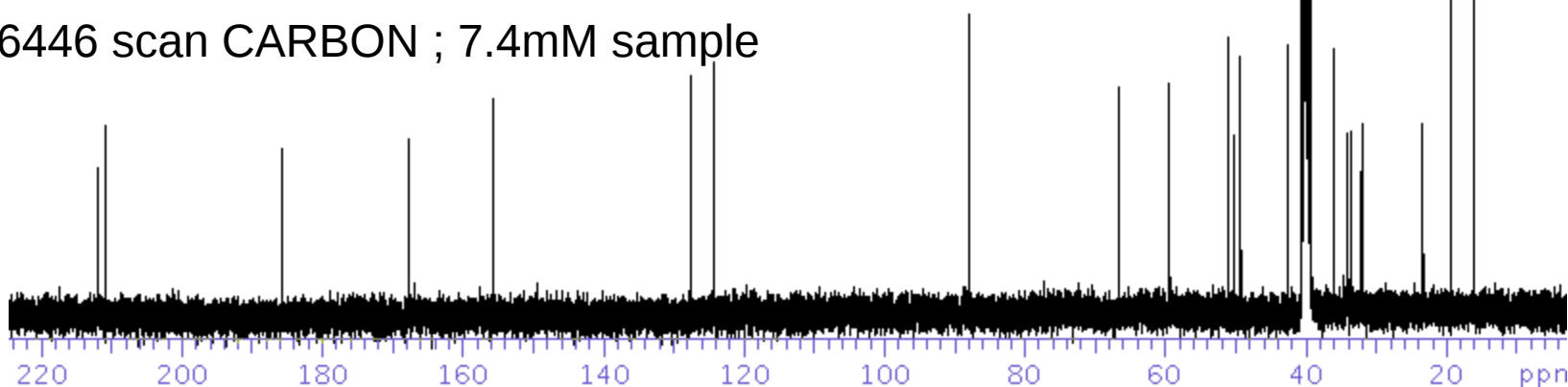
Adaptive NMR

Automatically adjusts experiment time to ensure quality results

226 scan CARBON ; 42 mM sample



6446 scan CARBON ; 7.4mM sample



qNMR and Adaptive NMR: Summary

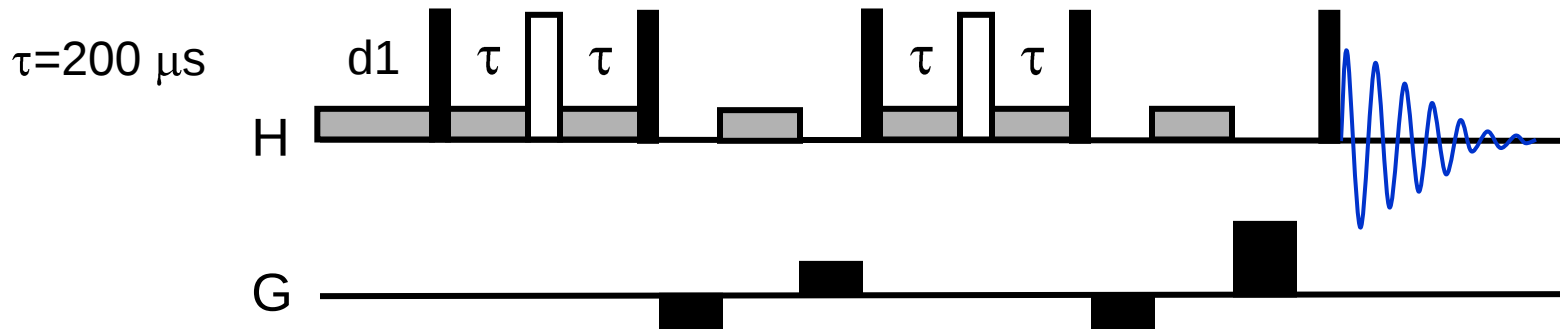
- Absolute quantitation is available for every sample.
- Adaptive NMR is transparent to the user, and it maximizes the efficient use of the available spectrometer time.

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PURGE-PRESATURATION

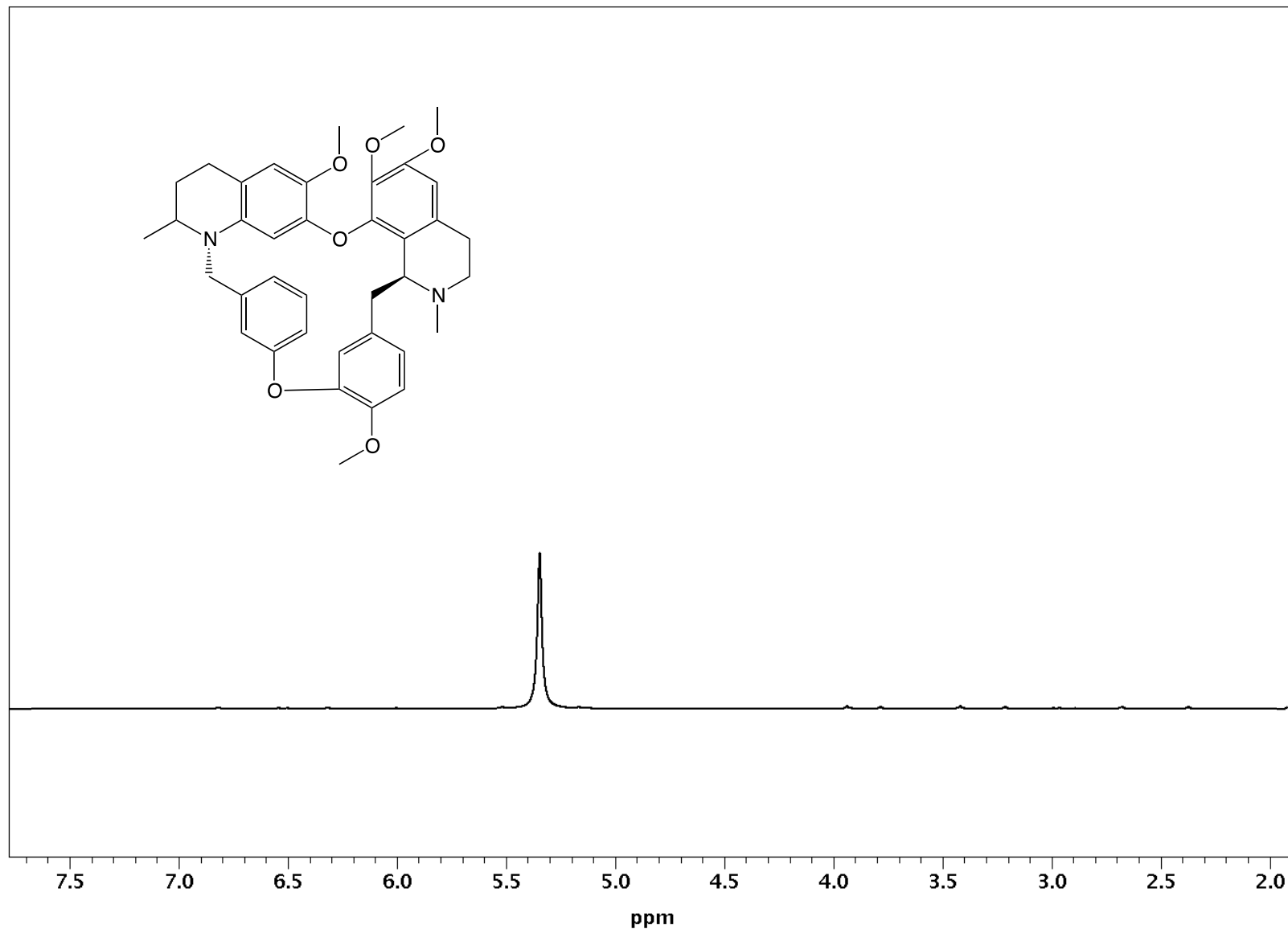


- Presaturation Utilizing Relaxation Gradients and Echoes (PURGE)
 - + One of the most efficient water suppression techniques
 - + Easy to setup
 - Exchangeable protons suffer

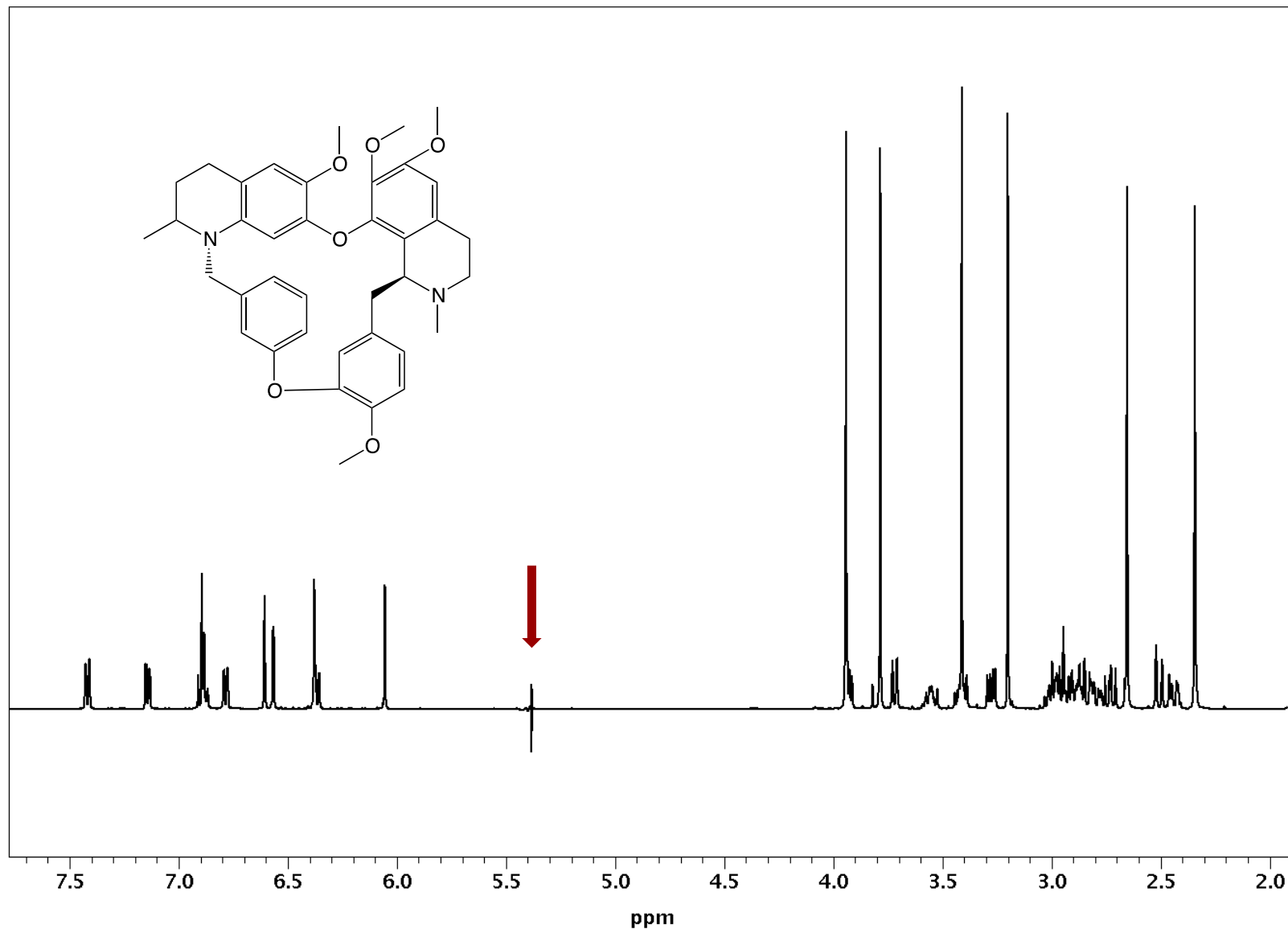
A.J. Simpson & S.A. Brown: *JMR* 175, 340-346 (2005).



Tetrandrine in 100% CH₂Cl₂

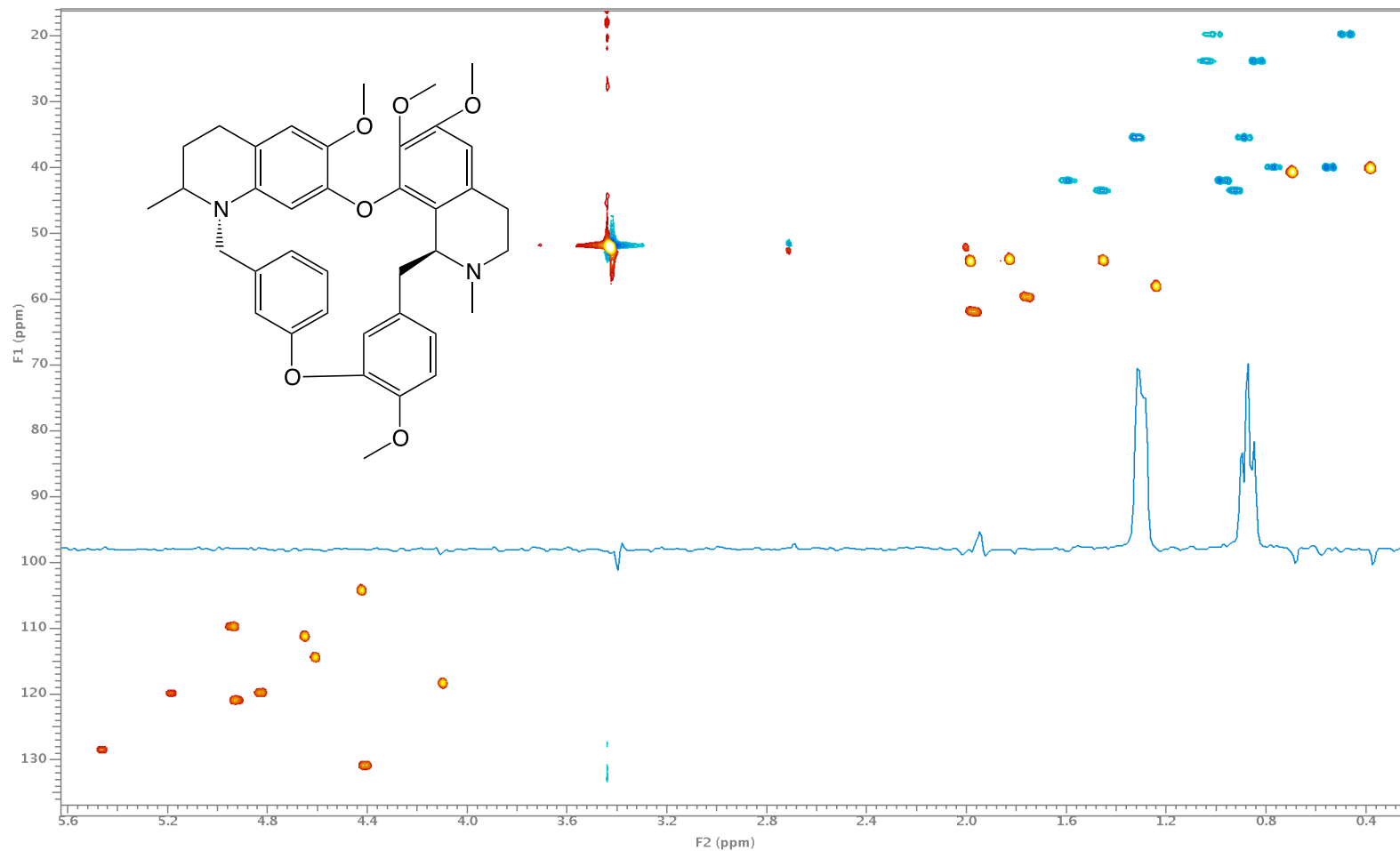


Tetrandrine in 100% CH₂Cl₂



Tetrandrine in 100% CH₂Cl₂

CRISIS HSQC



Solvent Suppression: Summary

- It's EASY!
- It is automatic.
- It is available, and useful.



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Thank You!



Acknowledgements:

Ron Crouch
Peter Sandor
Krish Krishnamurthy
Frank Delaglio
Lydia Park



Agilent Technologies

Questions?

Q&A now and later

Additional questions can be asked on our Spinsights NMR & MRI Community at <https://spinsights.chem.agilent.com>.

Welcome to the new Spinsights Community!

- **Help Yourself**, by searching for: software patches, manuals, bug lists, application notes, videos, newsletters, and more.
- **Help Each Other**, by answering or posing questions in our discussion forums.
- **Ask Agilent**, by using the **AskTAC** link.

MAIN SPACES

- Downloads & Resources
- Discussion Forum**
- AskTAC
- NMRPipe
- Help using Spinsights

SPINSIGHTS BLOGS

- Magnet cutaways - fun and educational
2 weeks ago in Spinsights Blogs
by Agilent BlogManager
- Spinsights News Archive Now Complete
1 week ago in Spinsights Blogs
by Rolf Kyburz

FEATURED SCIENCE

- Evaluation of the Use of Non-Uniform Sampling (ENC 2013 Poster)
1 month ago in Featured

Welcome to the Discussion Forum!

Here's a quick orientation:

- General questions and discussions regarding hardware, software, and troubleshooting belong in the **Discussion Forum**
- Questions, discussions, and posted materials pertaining to specific application families belong in the four Groups:
 - Small Molecule NMR**
 - Biomolecular NMR**
 - Solids NMR**
 - Imaging**
- Macros, pulse sequences, scripts, etc. belong in the **User Library**
- Discussions of facility administration, infrastructure, accounting, training, etc. belong in **Facility Managers**
- TAG ALL YOUR POSTS by adding keywords in the "tags" box at the bottom of your drafts
- Requests, complaints, and suggestions should be tagged with the keywords **requests**, **complaints**, or **suggestions**
- Placing your posts in your own blog or discussion area makes them nearly invisible; it's better to keep them public in **Discussion Forum**

TOPICAL DISCUSSION GROUPS

- Solids NMR**
This is the home for solids NMR discussions.
- Facility Managers**
This group is for facility managers, and is the best home for discussions of administration, accounting, training, etc.

SEARCH & ASK THE DISCUSSION FORUM

Type your question

Ask it

Note: registration is required to access the Spinsights online community.

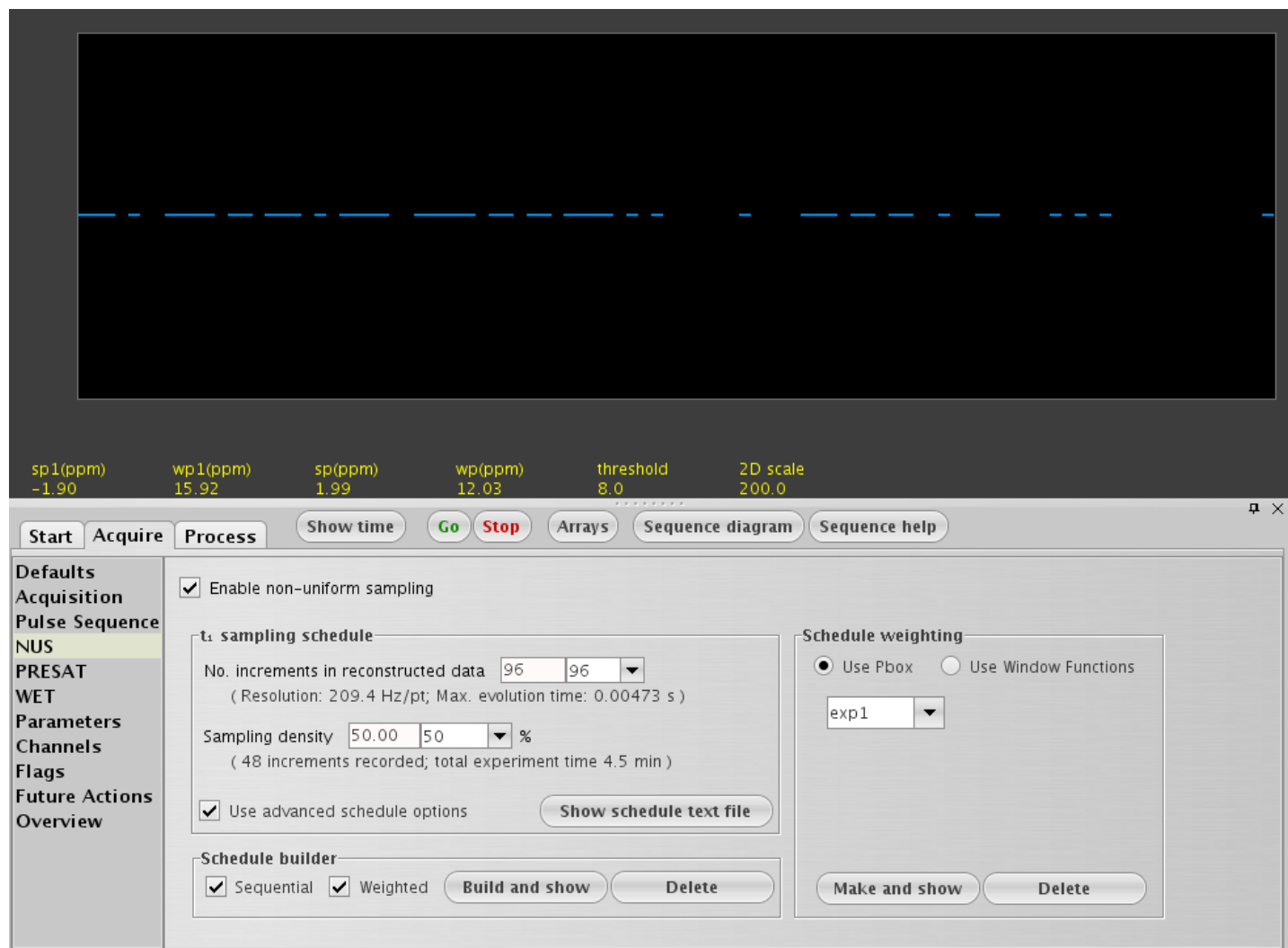
Non-Uniform Sampling Processing Techniques

- NUS data require special processing techniques to reconstruct the 2D spectrum:
 - Maximum Entropy (MaxEnt) reconstruction
 - Filter Diagonalization Method (FDM)
 - Multi-Dimensional Decomposition (MDD)
 - CLEAN (Kupce *et. al.*)
 - Iterative Soft Thresholding (IST)
 - And more...

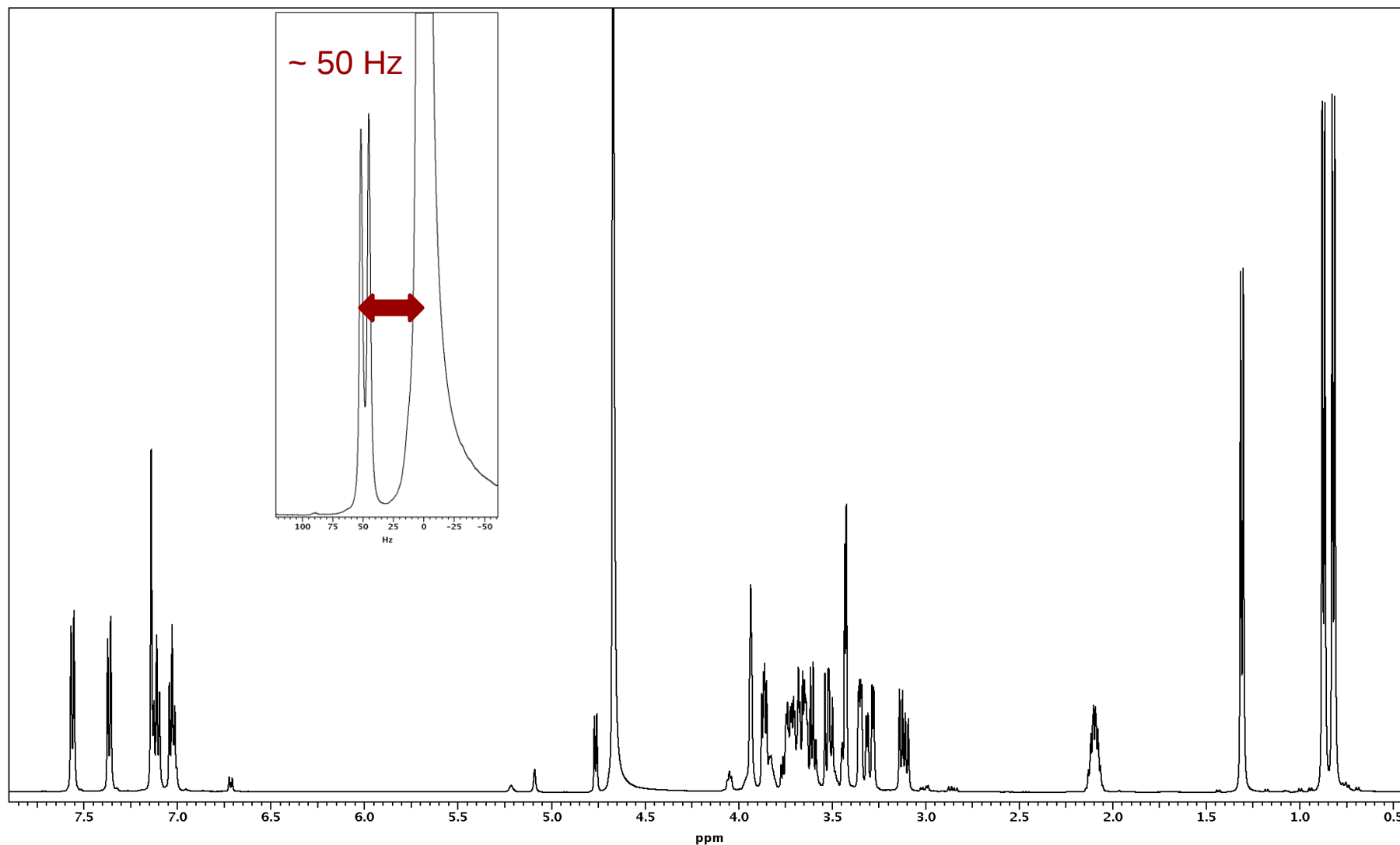


More Advanced Options

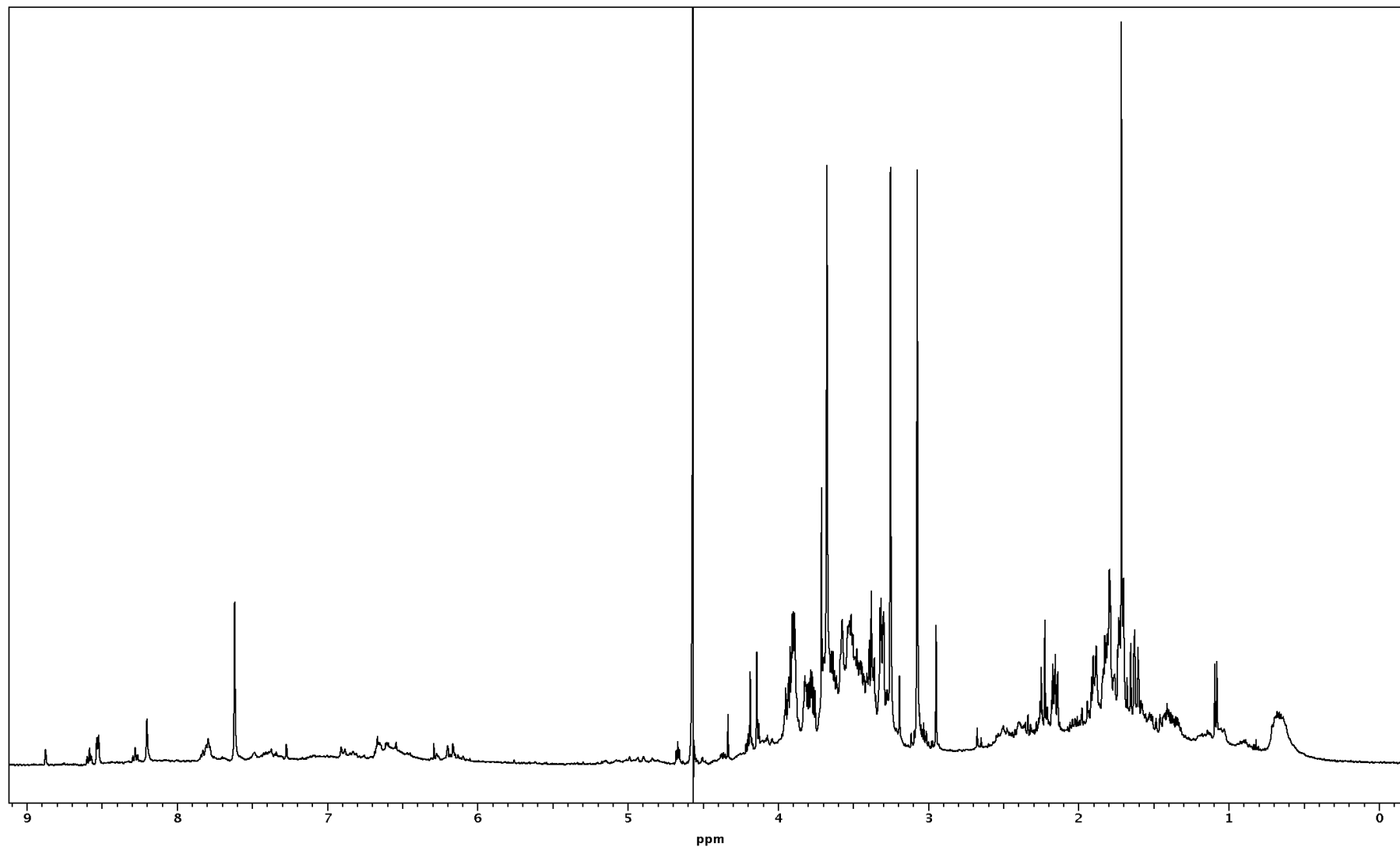
- Weighted or non-sequential sampling schedules
- Window functions or VnmrJ's Pbox used to define weighting of schedule
- Schedule displayed graphically showing the "skipped" points



Amino Acid/Saccharide Sample in 100% H₂O



Coffee – neat



PS1D – ^1H Spectrum with Broadband Proton Decoupling

Homonuclear Broadband-Decoupled NMR Spectra

Klaus Zangger and Heinz Sterk

Journal of Magnetic Resonance, Vol 124, Issue 2

February 1997, Pages 486-489



PS1D – ^1H Spectrum with Broadband Proton Decoupling

3.8 Hz separation

It's like having a 5 GHz spectrometer!

