An Overview of NMR Applications

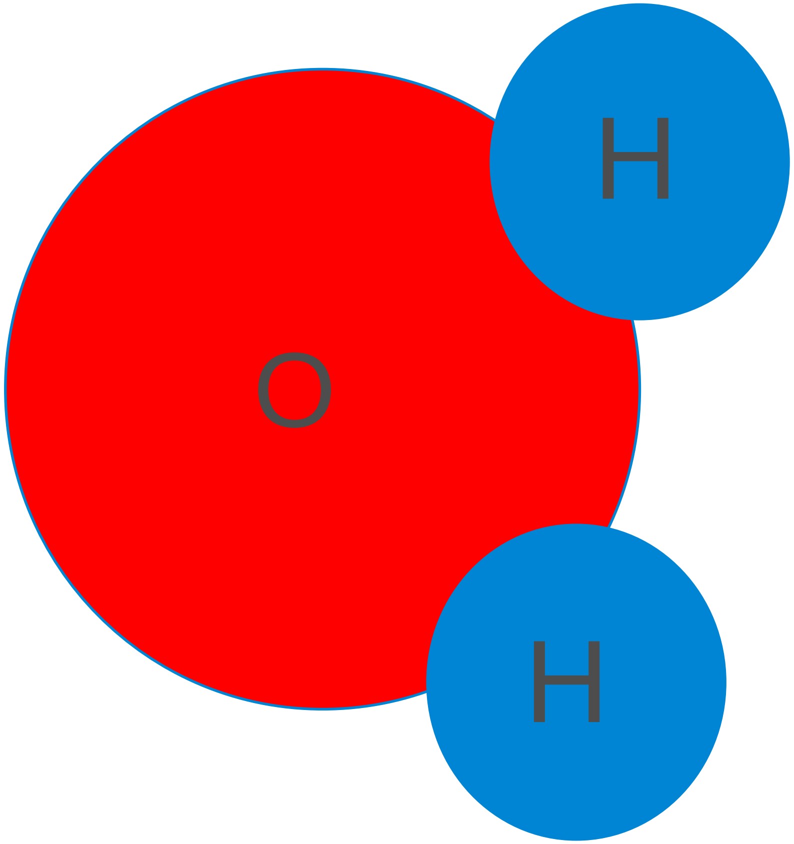
Introduction to classic applications for small molecule liquids NMR

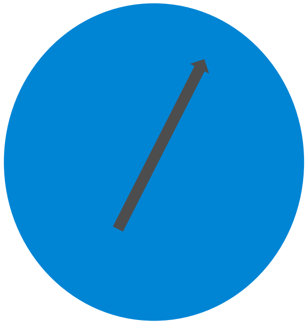
Ron Crouch

Brief History of NMR

- 1924: Pauli predicts the possibility of nuclear spin

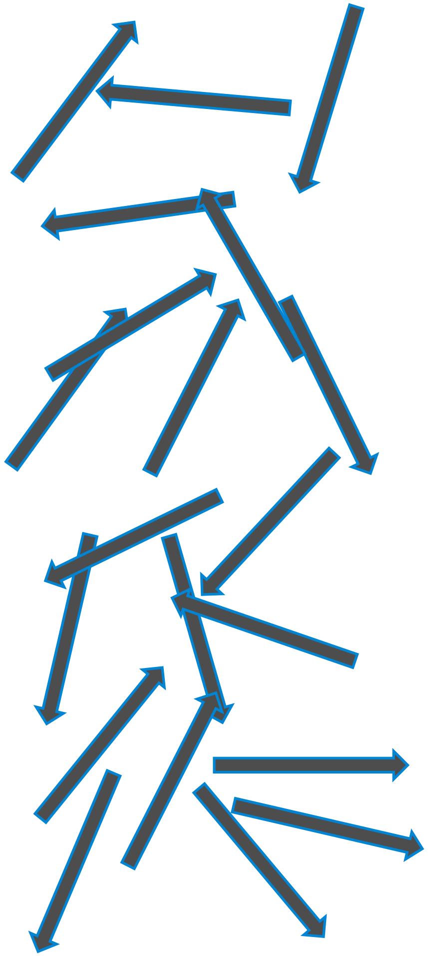
The Water Molecule, H_2O



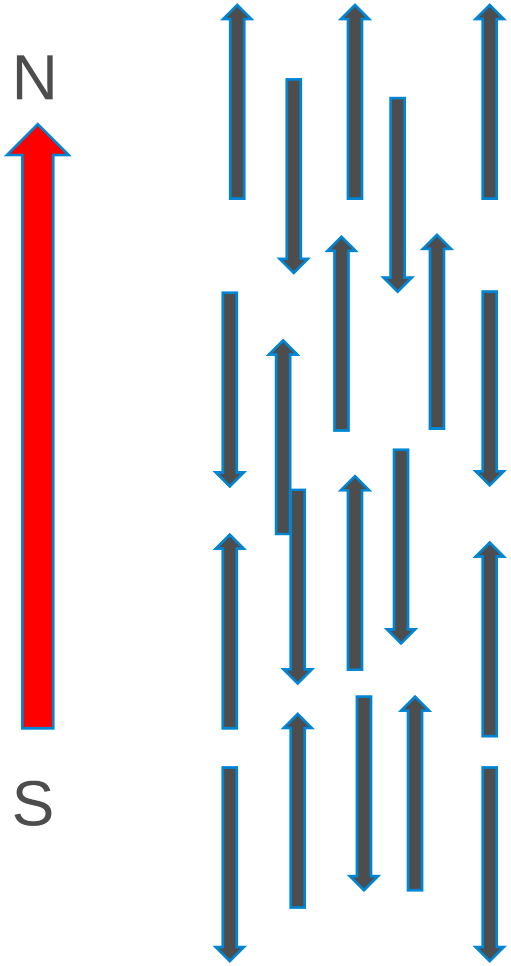


Nuclear Spin

What happens inside a magnet…

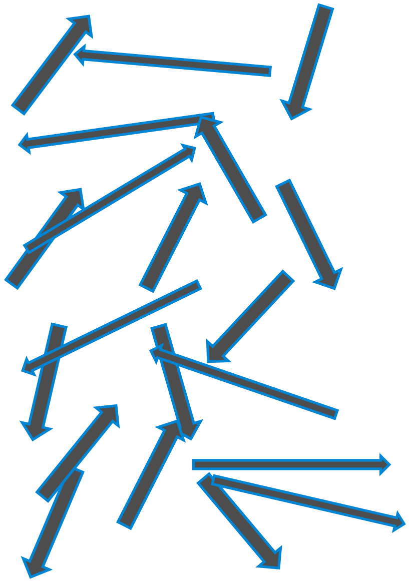


No Magnetic Field

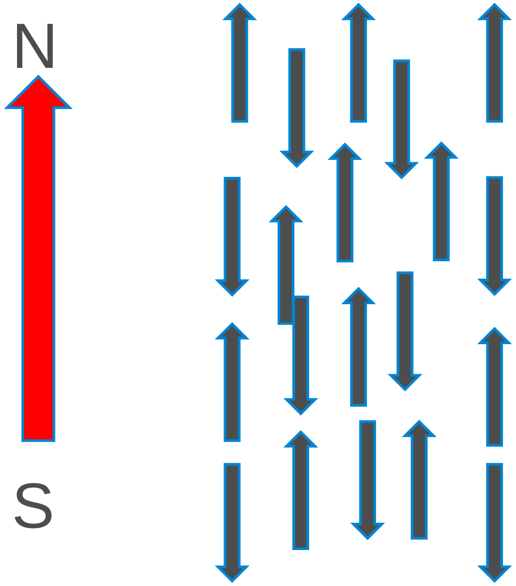


Strong Magnetic Field

What happens inside the magnet…



No Magnetic Field

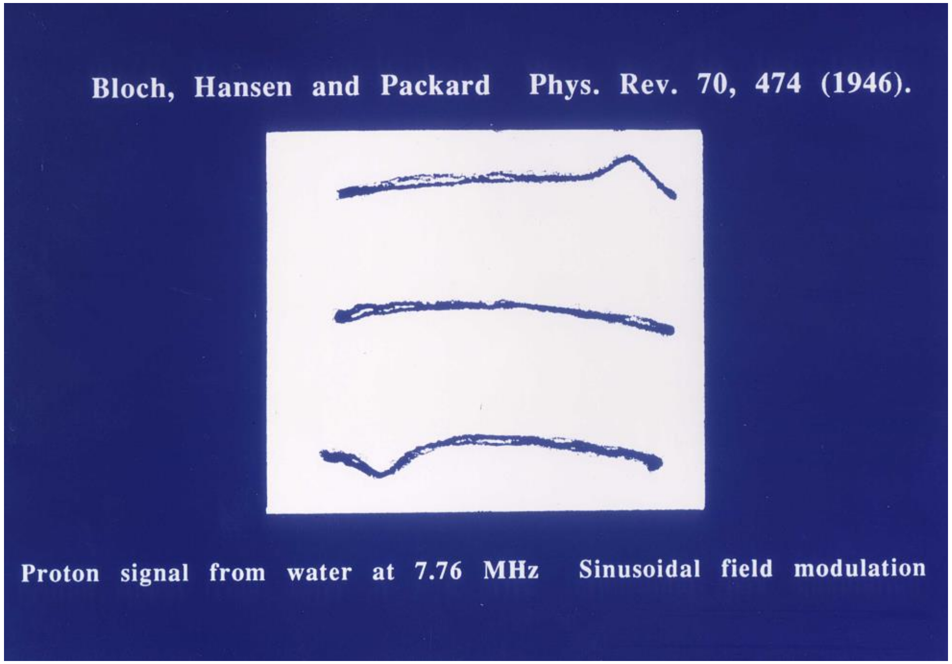


Strong Magnetic Field

- If we have 2,000,000 spins in there then 1,000,001 will be aligned with the magnetic field and 999,999 will be against it.
- We are really lucky because hydrogen is a very important element for organic chemistry and biochemistry and it is also the most sensitive one for NMR!

- If we somehow “kick” the spins then their orientation is reversed.
- But they do not like the kick and return back where they were, giving us the energy they absorbed.
- We supply the kick in the form of a radiofrequency pulse lasting a few micro-seconds.
- We measure the energy they give us back and thus record the spectrum.
- We are lucky that NMR was discovered when the technology for radio communications was already there!

The First Spectra…



Bloch, Hansen and Packard Phys. Rev. 70, 474 (1946).

Proton signal from water at 7.76 MHz Sinusoidal field modulation

Spectrum of water, Felix Bloch, Stanford, 1946

NMR was hailed as an excellent method to measure the strength of magnetic fields.

An invaluable tool for Physics!

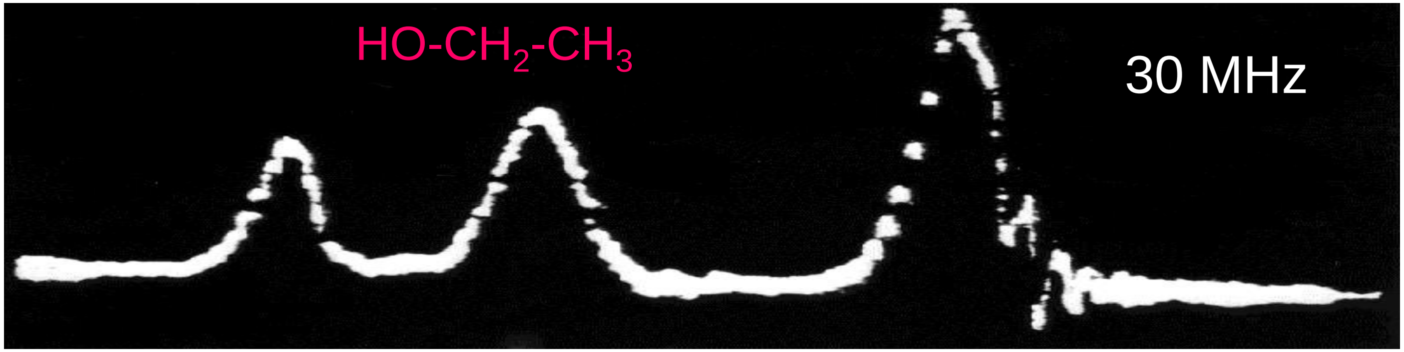
A Disaster for Physics…

In 1950 Proctor attempted to measure the NMR spectrum of nitrogen in ammonium nitrate (NH_4NO_3).

To his horror he discovered two signals instead of one!

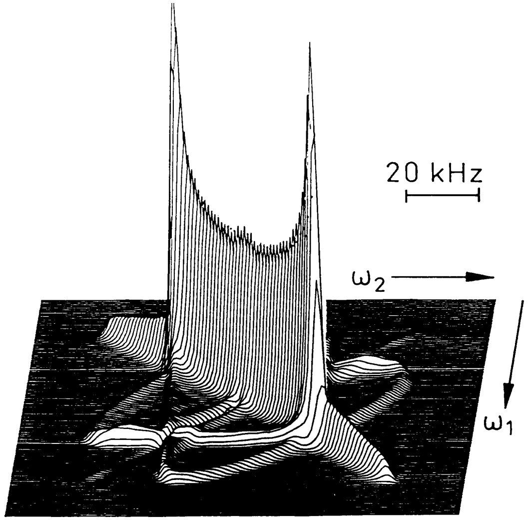
He described it as an "annoying chemical affect which could terribly impede our progress in trying to measure the magnitude of nuclear magnetic moments"

And so chemical shift was born…

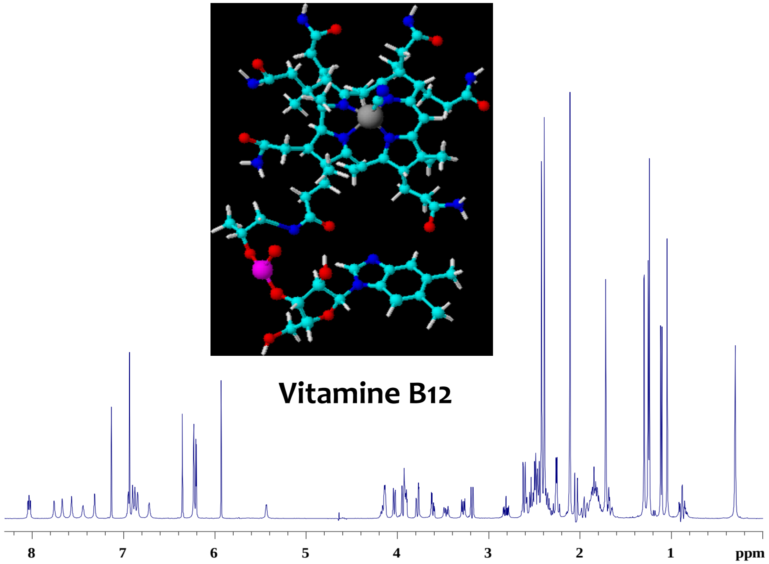


1951

Where are we now…



Solids state NMR
physics



Vitamine B12

Liquid state NMR
chemistry

NMR imaging
medicine

The Overview

NMR has an incredibly Broad span of applications.

Tools exist which focus on small molecules and complementary set of tools for large molecules.

Dynamics and motion have their own sets of NMR Applications.

Because of the breadth, today we will focus on the tool kit for classic small molecule Applications.

Will attempt to present information in a fashion that is useful to both beginning and experienced NMR Scientists!!

Omissions!

Too Many to list !!!

CRAFT – Spectrum to Spreadsheet – Automatically!!

Dynamic Interactions.. Future Webinar!

Solids NMR

Bio

Relative Sensitivity of ^{1}H/^{13}C and ^{15}N...

Product of relative natural abundance times the cube of the differences in frequency.
(a simplification for doing the math in your head ☺)

^{1}H is 100% abundance and highest frequency so it is most sensitive.

^{13}C is 1.1% abundance and frequency is 1/4th ^{1}H.

So.. ^{13}C is (4*4*4)/0.011 or ~5,800 less sensitive than ^{1}H!!

^{15}N is 0.36% abundance and frequency is ~1/9.88th ^{1}H.

So… ^{15}N is (9.88*9.88*9.88)/0.0036 or ~268,000 less sensitive than ^{1}H.

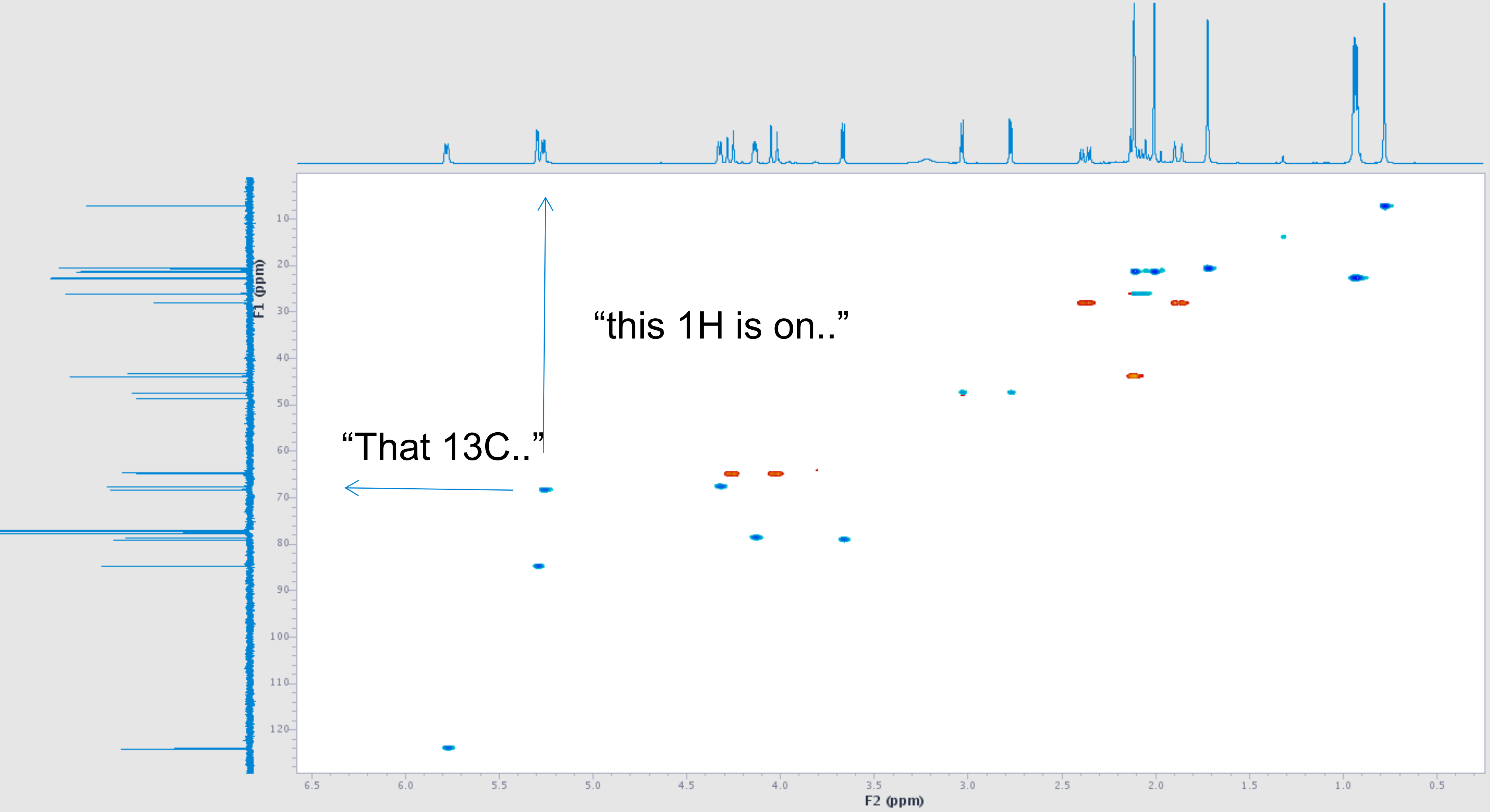
“Indirect Detection”

The idea of observing rare and difficult NMR nuclei like ^{13}C and ^{15}N by 2-dimensional methods at the high abundance and frequency of ^{1}H is called Indirect Detection.

Indirect detection revolutionized NMR! This pretty much began in the 1990s and since then nearly every day new useful experiments appear.

Not only is raw sensitivity greatly increased, we also gain an additional dimension to simplify a complex spectrum!

Simple Example of 2 dimensional (2D) NMR



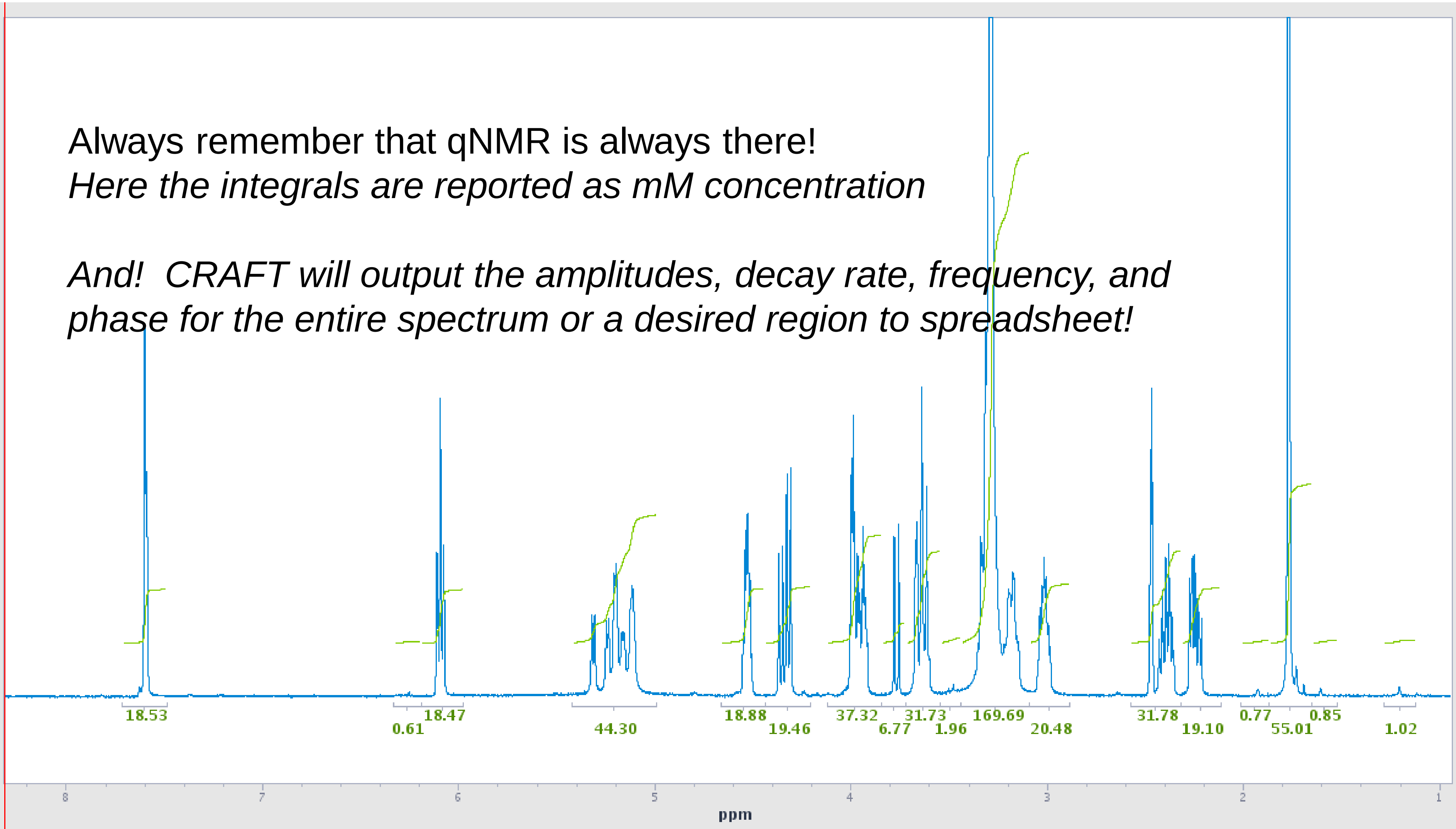
“this 1H is on..”

“That 13C..”

Always remember that qNMR is always there!

Here the integrals are reported as mM concentration

And! CRAFT will output the amplitudes, decay rate, frequency, and phase for the entire spectrum or a desired region to spreadsheet!



Classes of Pulse Sequences – The Basics

Simple Editing – APT/DEPT

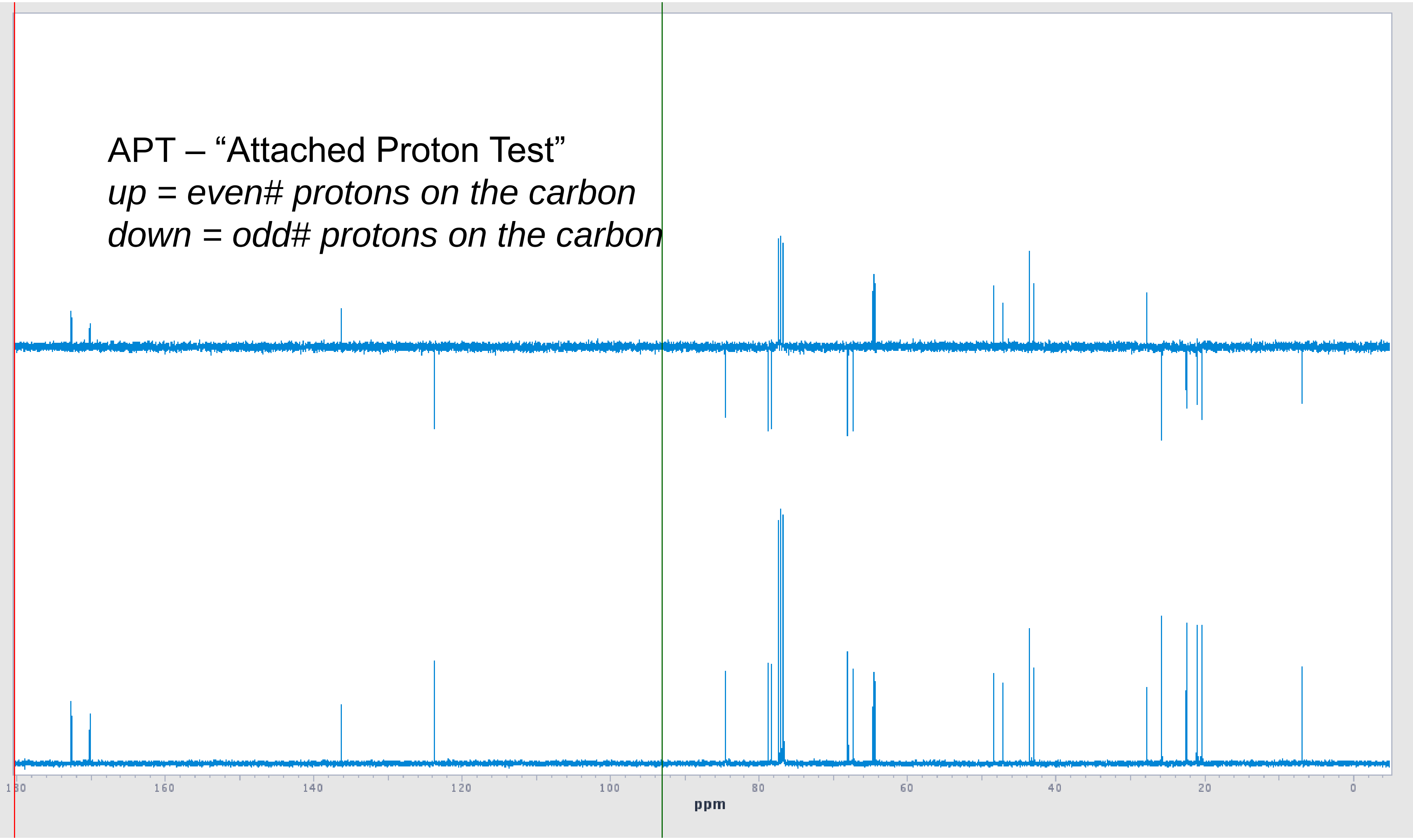
Frequency-symmetric 2Dcorrelations between spins of the same nuclei. COSY, TOCSY, NOESY, ROESY. Can contain both through bond or through space correlation information.

Direct one-bond 2D correlations between two (or more) different nuclei. HETCOR, HSQC, HMQC.

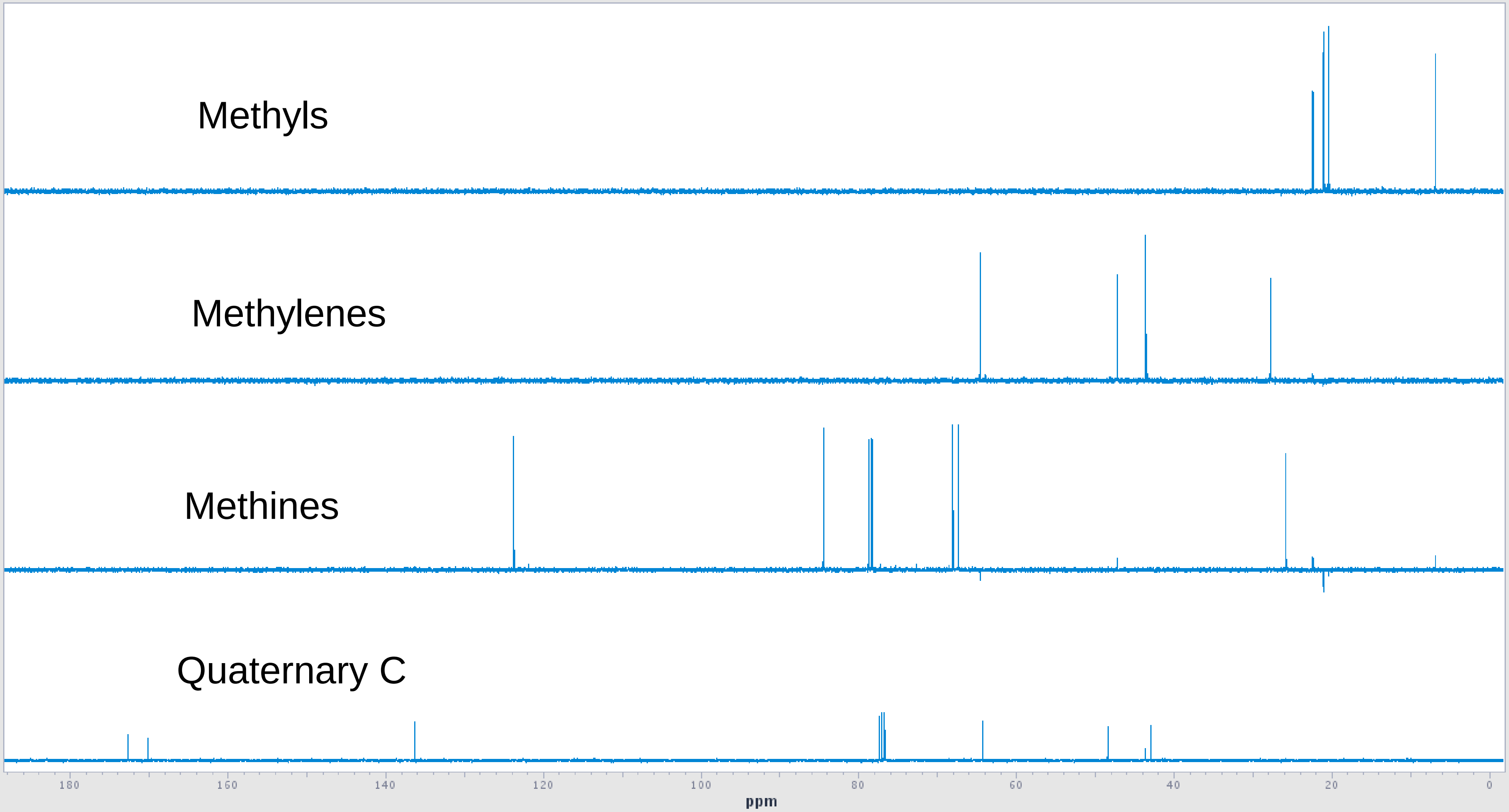
APT – “Attached Proton Test”

up = even# protons on the carbon

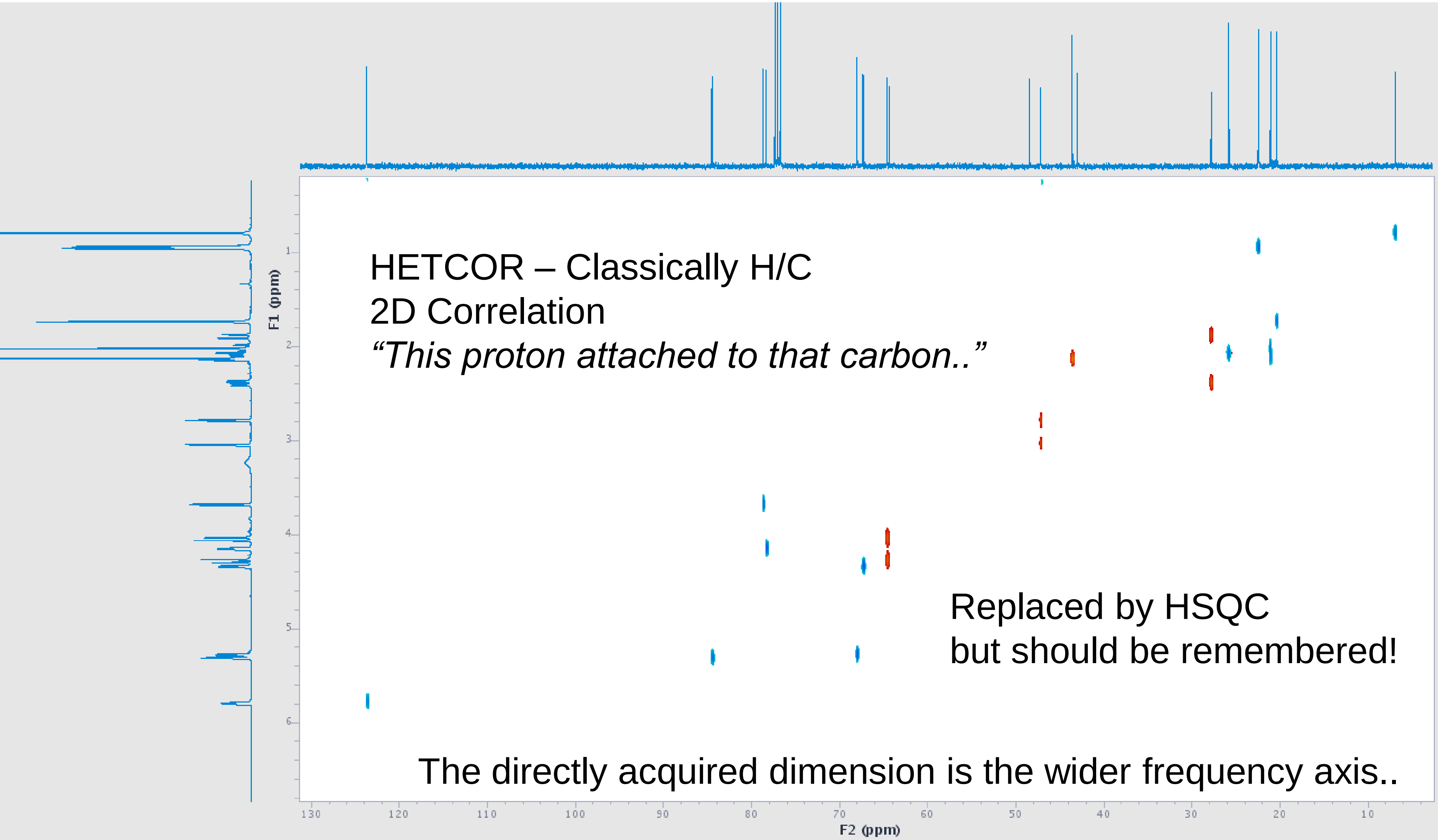
down = odd# protons on the carbon



Fully Edited DEPT - Results in series of spectra by type C/CH/CH_2/CH_3 !!



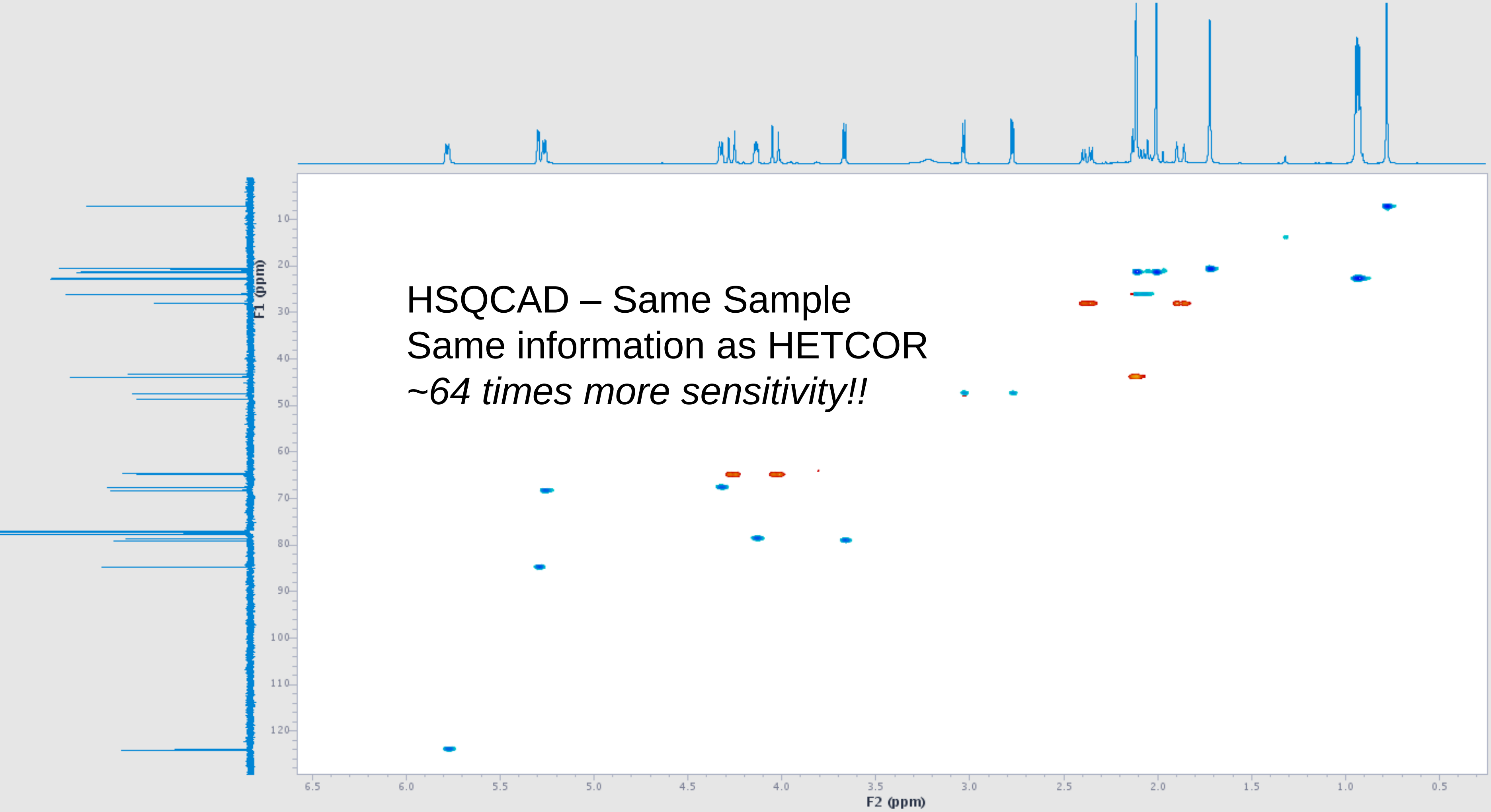
More Book keeping information easily Available!!



HETCOR – Classically H/C
2D Correlation
“This proton attached to that carbon..”

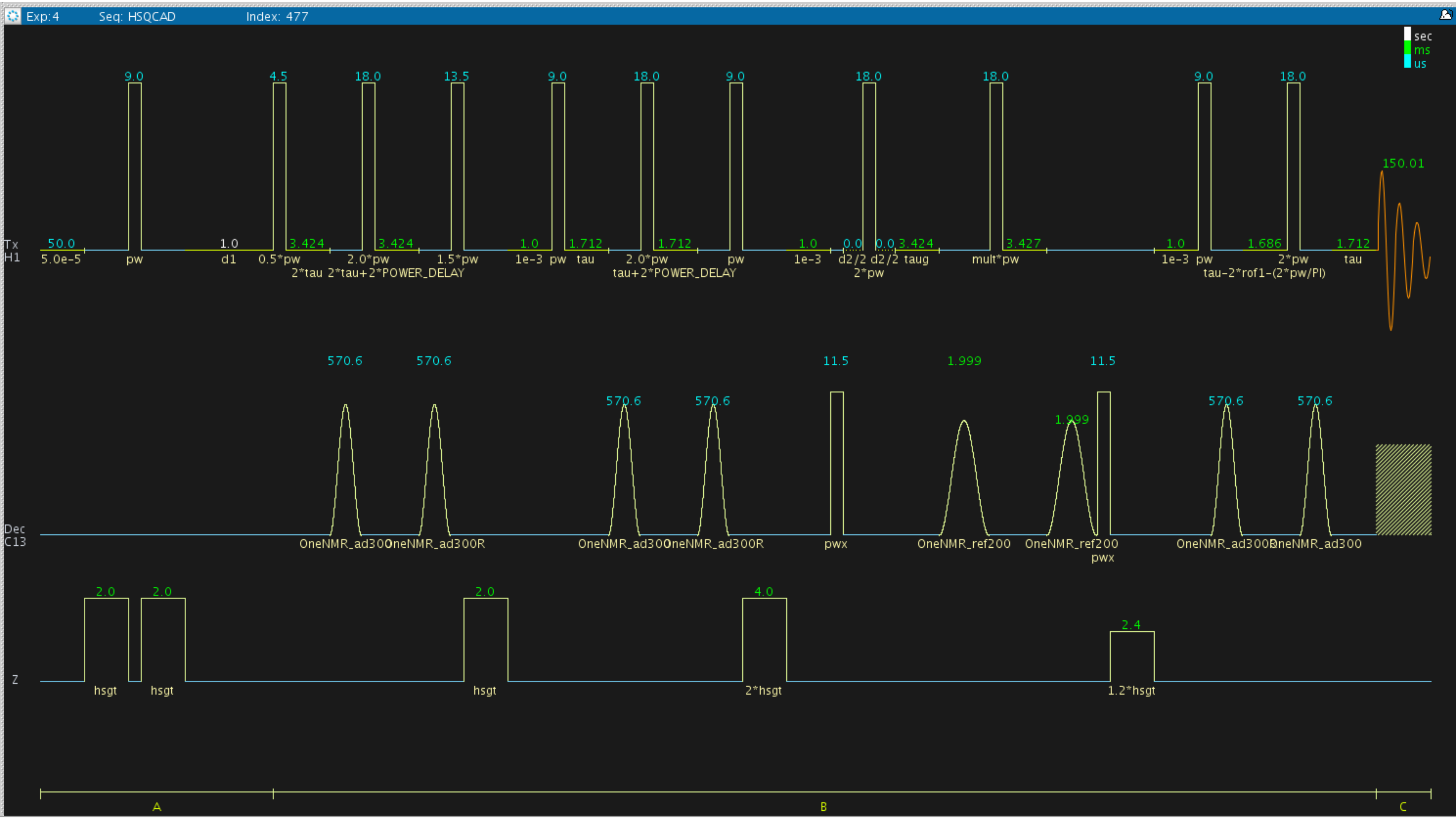
Replaced by HSQC
but should be remembered!

The directly acquired dimension is the wider frequency axis..

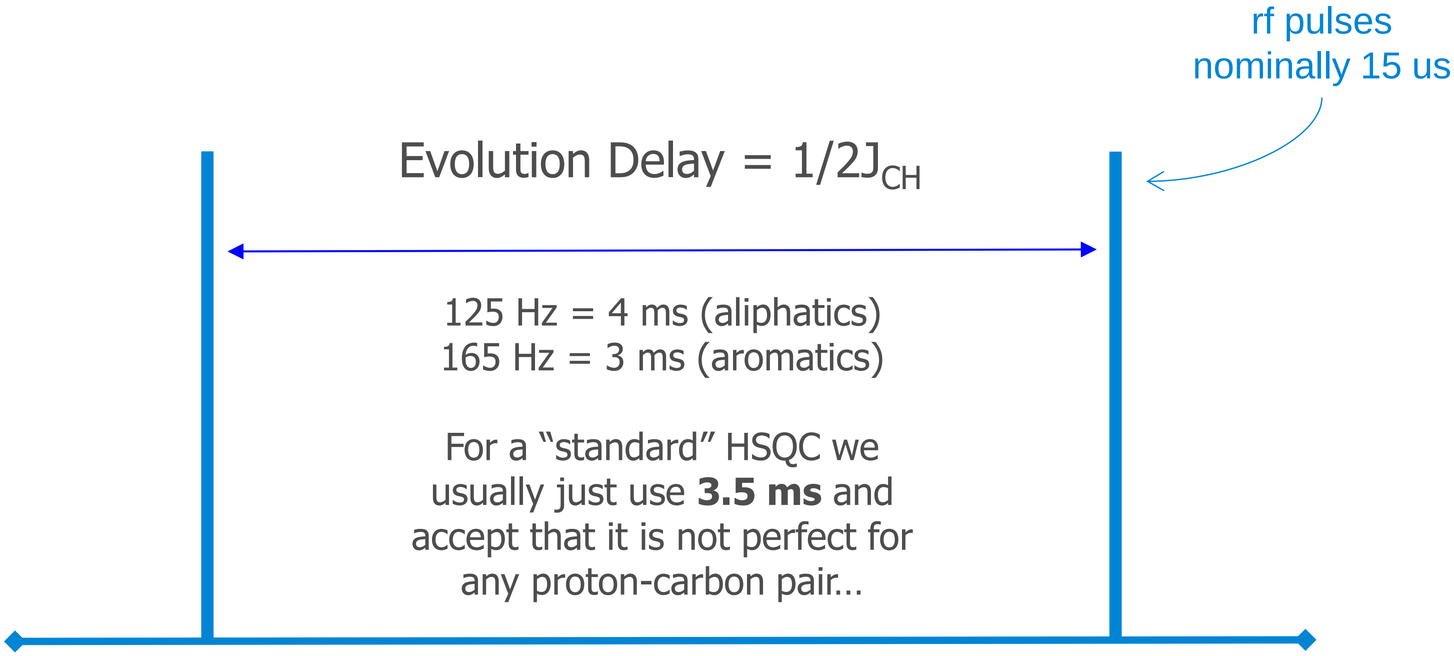


HSQCAD – Same Sample
Same information as HETCOR
~64 times more sensitivity!!

The HSQCAD Pulse Sequence – *Very special Actually good Quantitation!!!*



CRISIS Experiments
The CRISIS condition - how it works



rf pulses
nominally 15 us

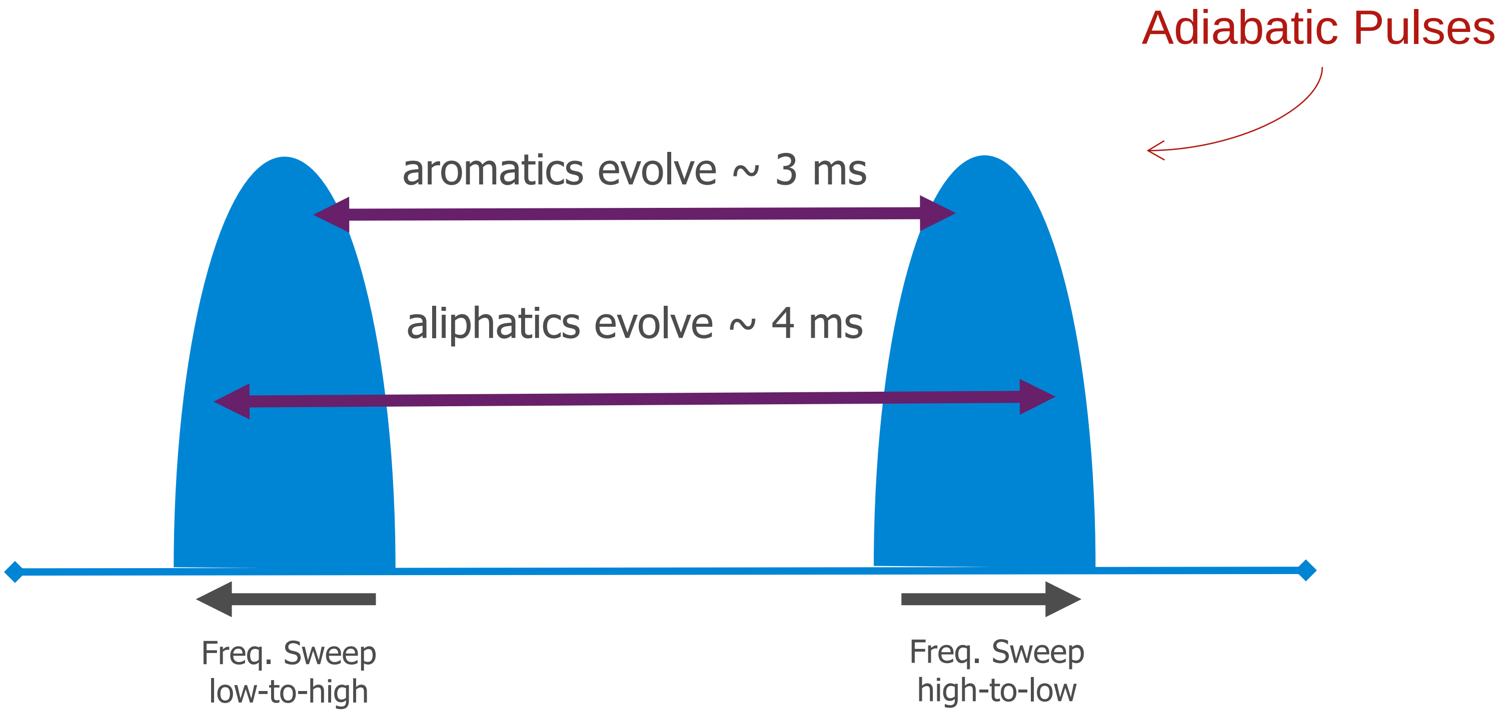
Evolution Delay = $1/2J_{CH}$

125 Hz = 4 ms (aliphatics)
165 Hz = 3 ms (aromatics)

For a “standard” HSQC we usually just use **3.5 ms** and accept that it is not perfect for any proton-carbon pair…

CRISIS Experiments
The CRISIS condition - how it works

CRISIS
(compensation of refocusing inefficiency with synchronized inversion sweeps)



The Problem of Resolution…

In 2D NMR the number of experiments you choose divided into the frequency width for the dimension is the resolution.

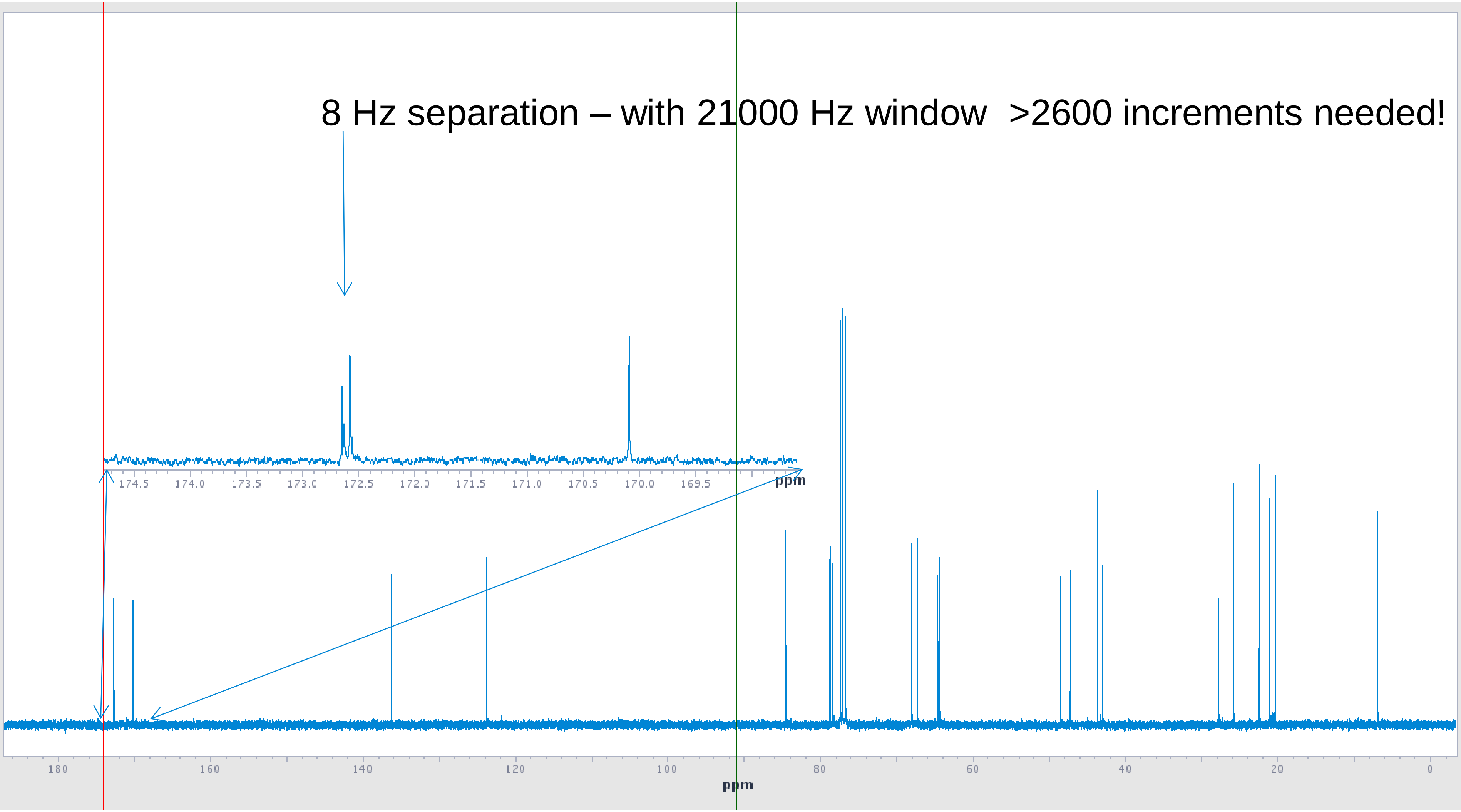
EG A 21,000 Hz window sampled by 200 increments = 21,000/200 or 105Hz.

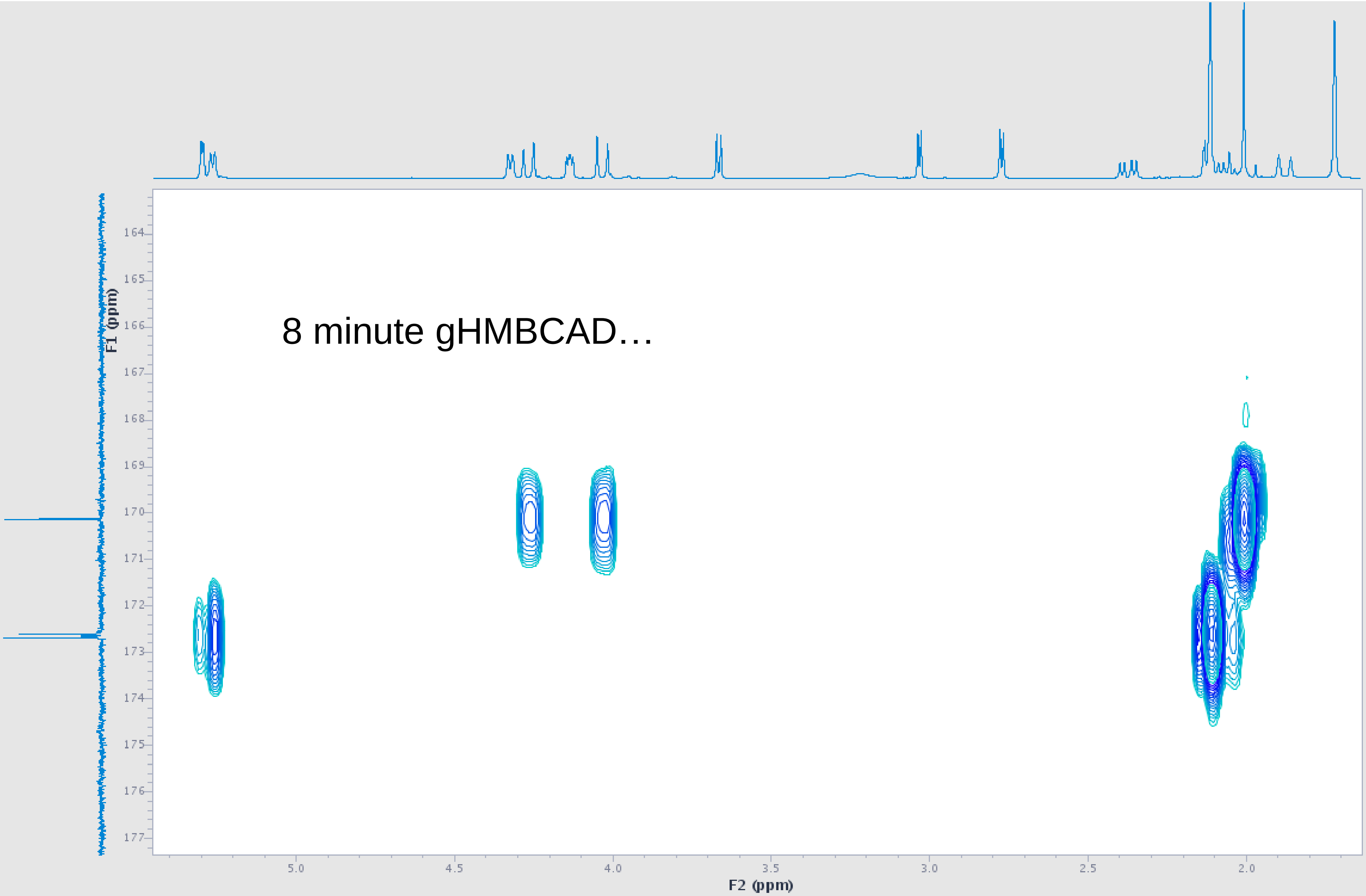
In a 400 MHz NMR that 105hz is a bit over 1ppm resolution for 13C.

So!! As needs for digital resolution increase the number of increments must follow directly… A bit about NUS later..

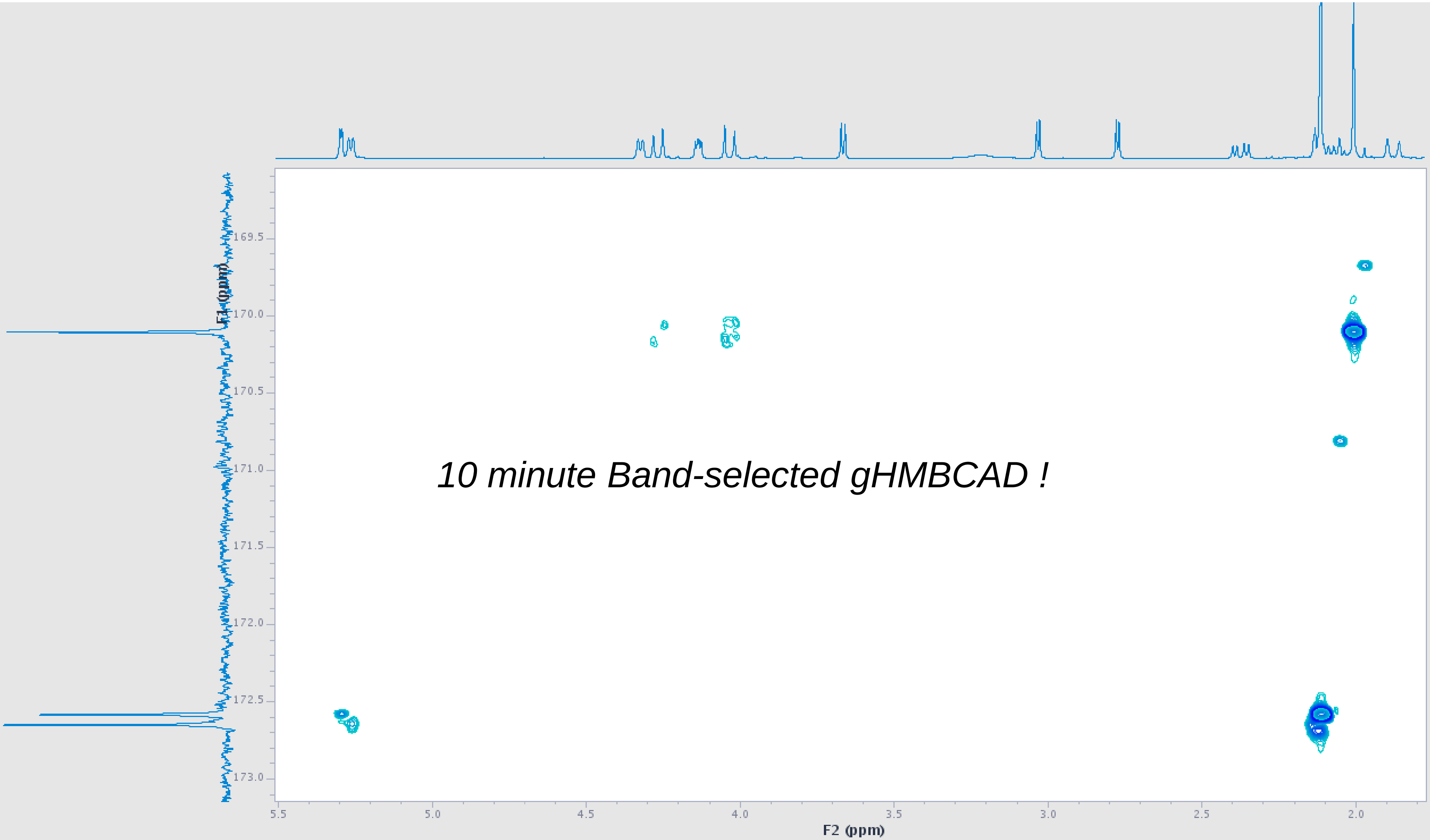
Band-selected pulses can allow huge time saving by focusing precisely where we look!!

8 Hz separation – with 21000 Hz window >2600 increments needed!

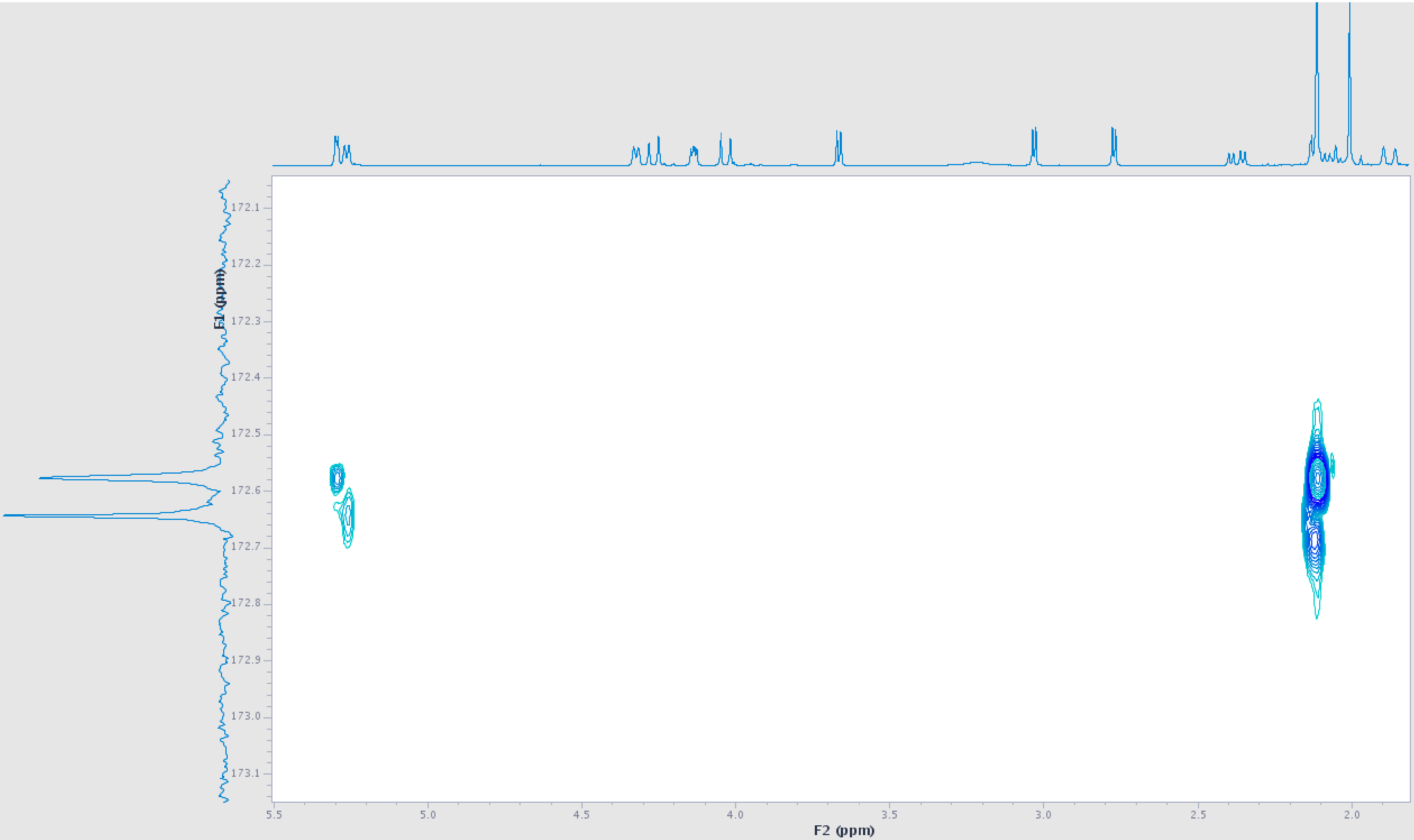




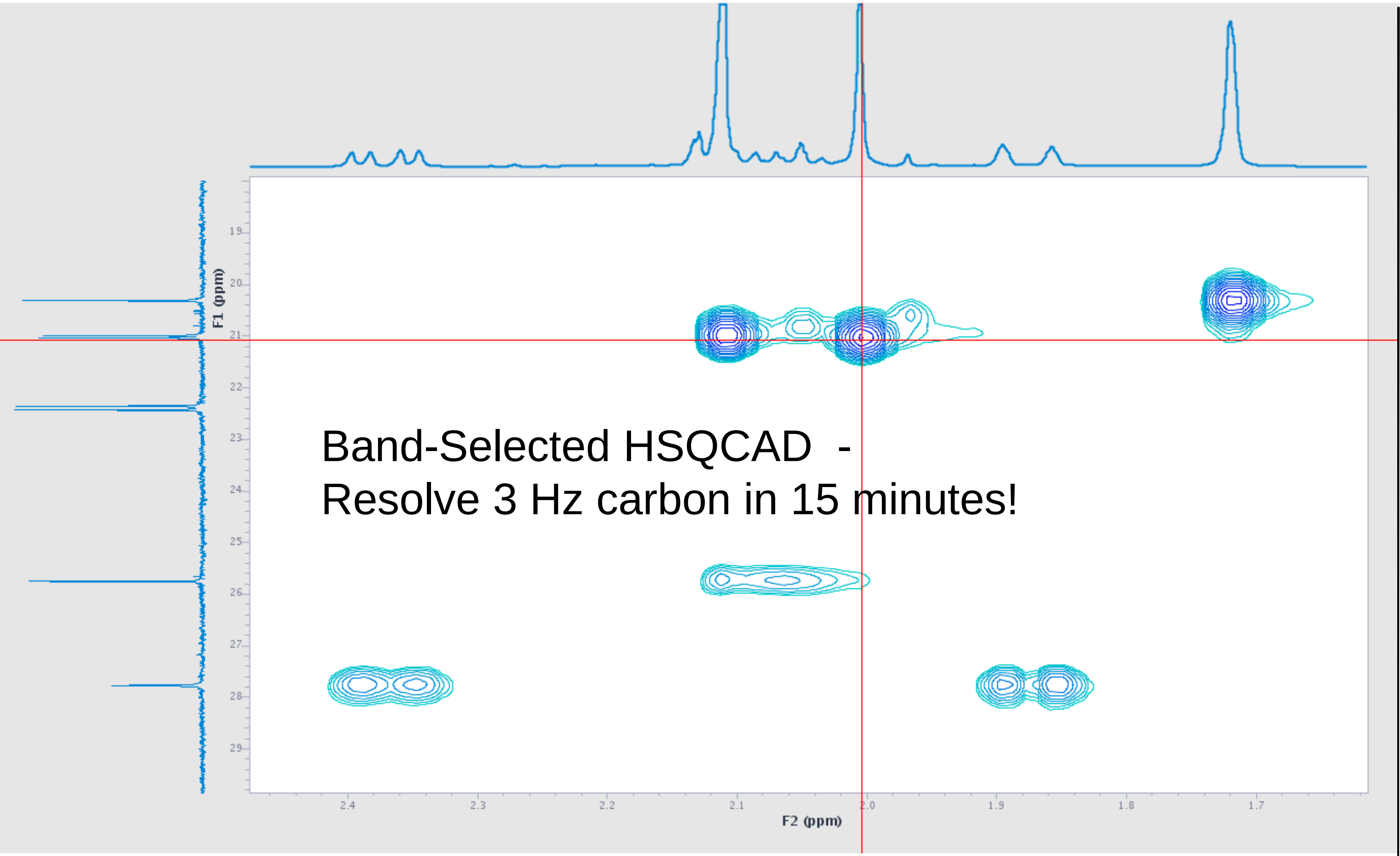
8 minute gHMBCAD…



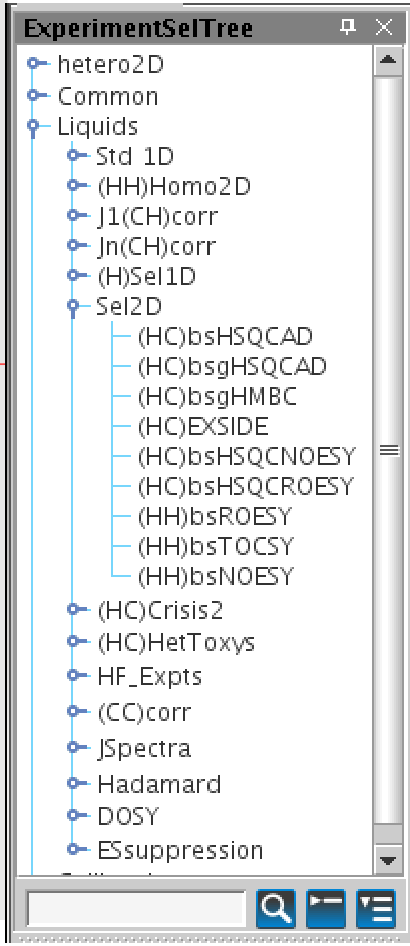
10 minute Band-selected gHMBCAD !

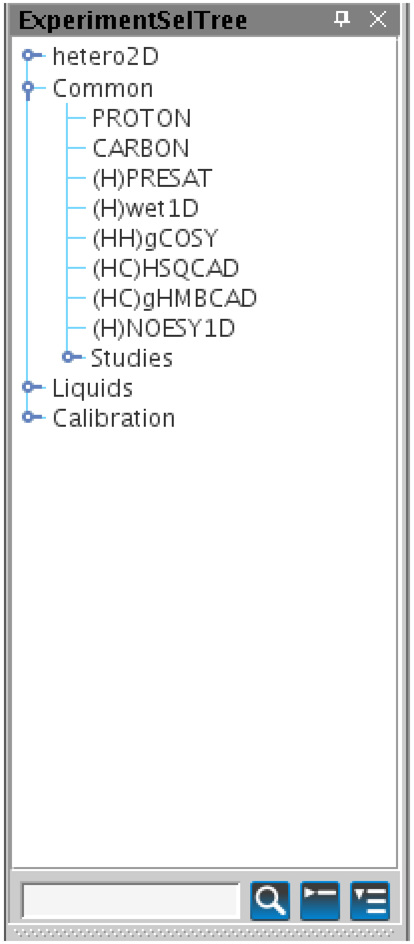

F1 (ppm)
172.1
172.2
172.3
172.4
172.5
172.6
172.7
172.8
172.9
173.0
173.1
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
F2 (ppm)

Band Selection is a suite of experiments..

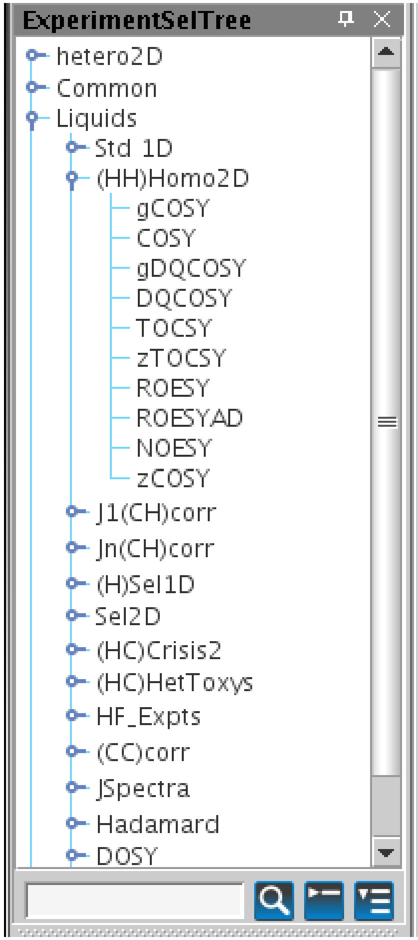


Band-Selected HSQCAD -
Resolve 3 Hz carbon in 15 minutes!

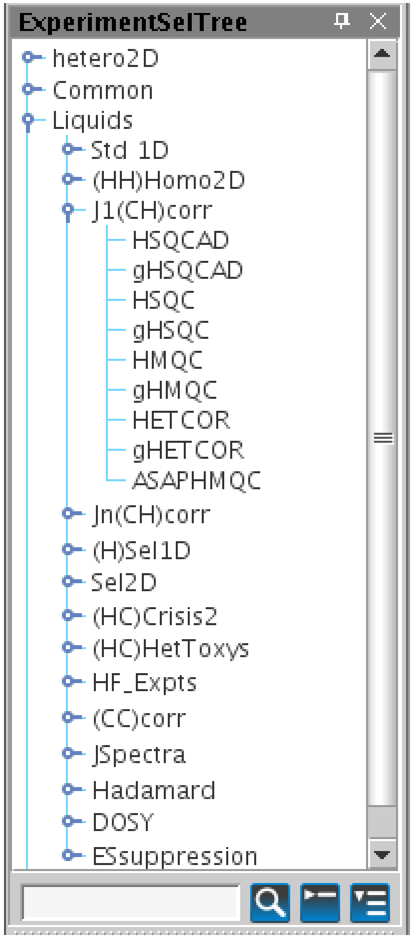



ExperimentSelTree

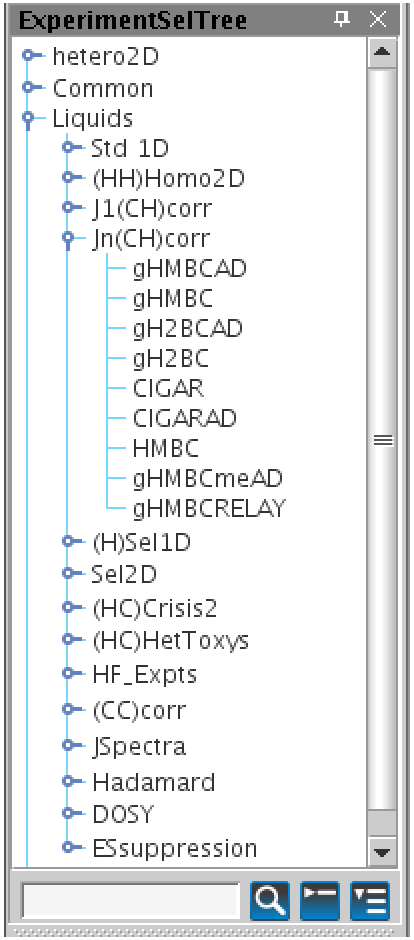
- hetero2D
- Common
  - PROTON
  - CARBON
  - (H)PRESAT
  - (H)wet1D
  - (HH)gCOSY
  - (HC)HSQCAD
  - (HC)gHMBCAD
  - (H)NOESY1D
  - Studies
- Liquids
- Calibration


ExperimentSelTree

- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
    - gCOSY
    - COSY
    - gDQCOSY
    - DQCOSY
    - TOCSY
    - zTOCSY
    - ROESY
    - ROESYAD
    - NOESY
    - zCOSY
  - J1(CH)corr
  - Jn(CH)corr
  - (H)Sel1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
  - HF_Expts
  - (CC)corr
  - JSpectra
  - Hadamard
  - DOSY

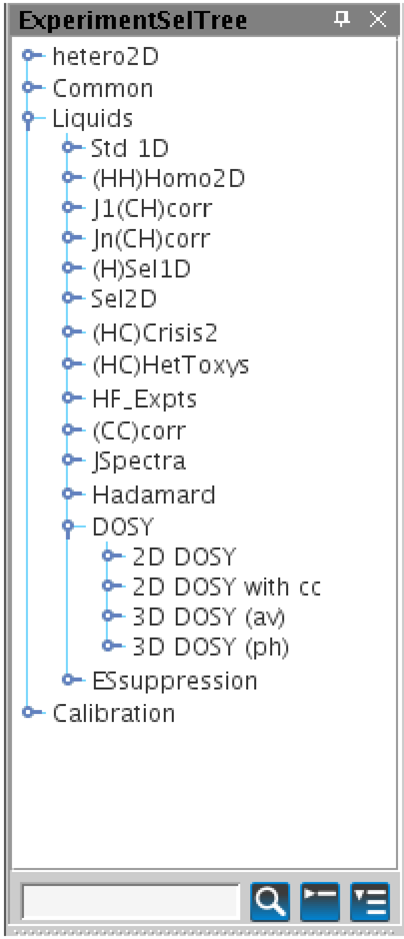

ExperimentSelTree

- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
  - J1(CH)corr
    - HSQCAD
    - gHSQCAD
    - HSQC
    - gHSQC
    - HMQC
    - gHMQC
    - HETCOR
    - gHETCOR
    - ASAPHMQC
  - Jn(CH)corr
  - (H)Sel1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
  - HF_Expts
  - (CC)corr
  - JSpectra
  - Hadamard
  - DOSY
  - ESsuppression


ExperimentSelTree

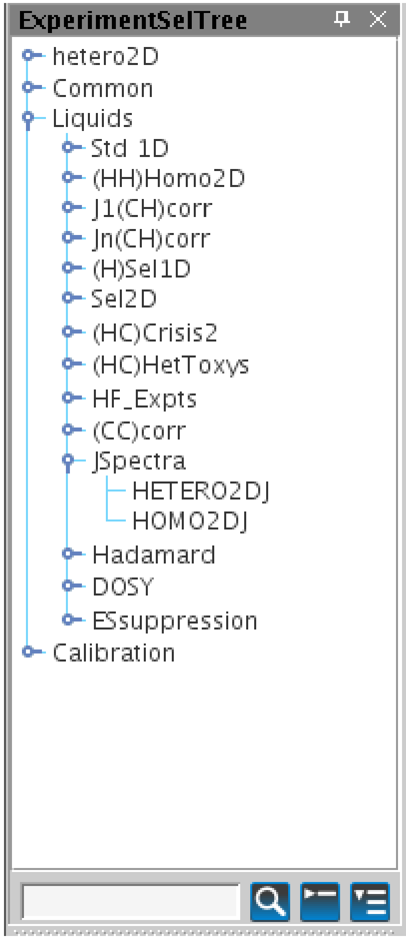
- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
  - J1(CH)corr
  - Jn(CH)corr
    - gHMBCAD
    - gHMBC
    - gH2BCAD
    - gH2BC
    - CIGAR
    - CIGARAD
    - HMBC
    - gHMBCmeAD
    - gHMBCRELAY
  - (H)Sel1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
  - HF_Expts
  - (CC)corr
  - JSpectra
  - Hadamard
  - DOSY
  - ESsuppression

Experiments are Organized by type and are even searchable!
You can add your own and show/organize/group as desired.



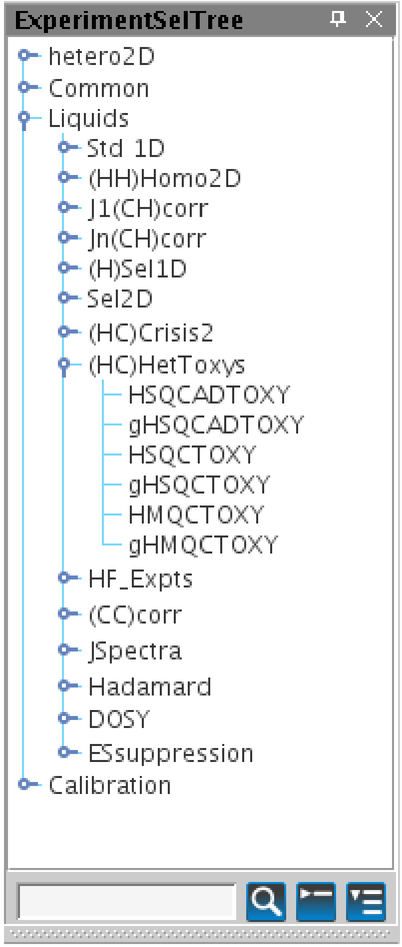
ExperimentSelTree

- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
  - J1(CH)corr
  - Jn(CH)corr
  - (H)Sel1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
  - HF_Expts
  - (CC)corr
  - JSpectra
  - Hadamard
  - DOSY
    - 2D DOSY
    - 2D DOSY with cc
    - 3D DOSY (av)
    - 3D DOSY (ph)
  - ESsuppression
- Calibration



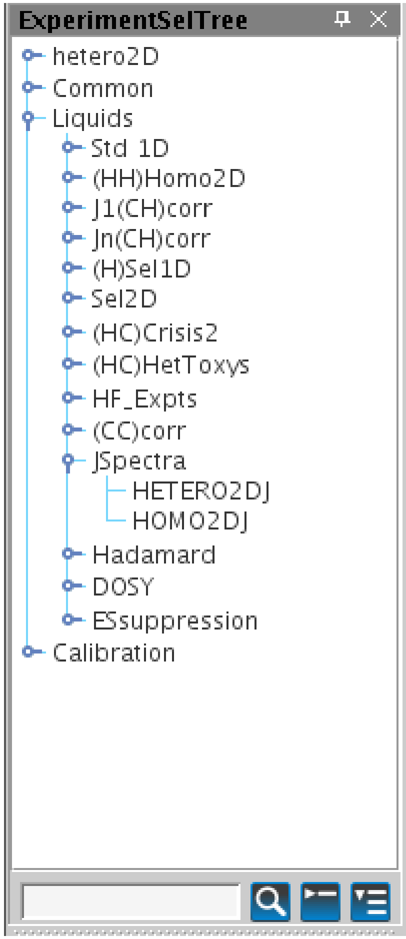
ExperimentSelTree

- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
  - J1(CH)corr
  - Jn(CH)corr
  - (H)Sel1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
  - HF_Expts
  - (CC)corr
  - JSpectra
    - HETERO2DJ
    - HOMO2DJ
  - Hadamard
  - DOSY
  - ESsuppression
- Calibration



ExperimentSelTree

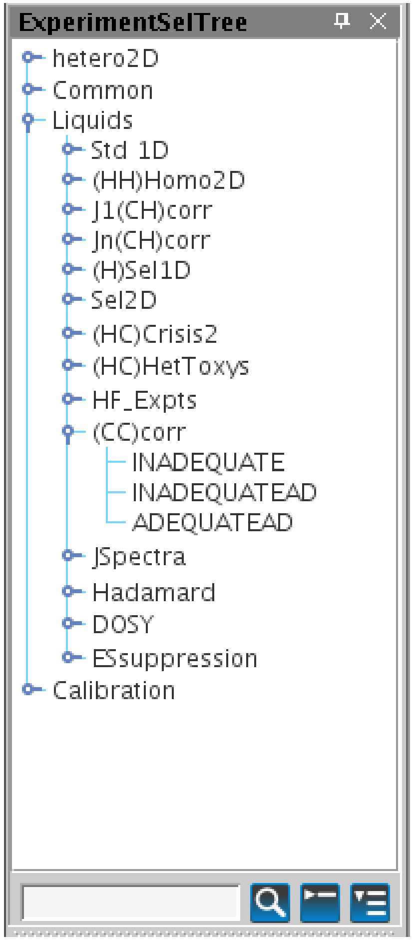
- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
  - J1(CH)corr
  - Jn(CH)corr
  - (H)Sel1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
    - HSQCADTOXY
    - gHSQCADTOXY
    - HSQCTOXY
    - gHSQCTOXY
    - HMQCTOXY
    - gHMQCTOXY
  - HF_Expts
  - (CC)corr
  - JSpectra
  - Hadamard
  - DOSY
  - ESsuppression
- Calibration



ExperimentSelTree

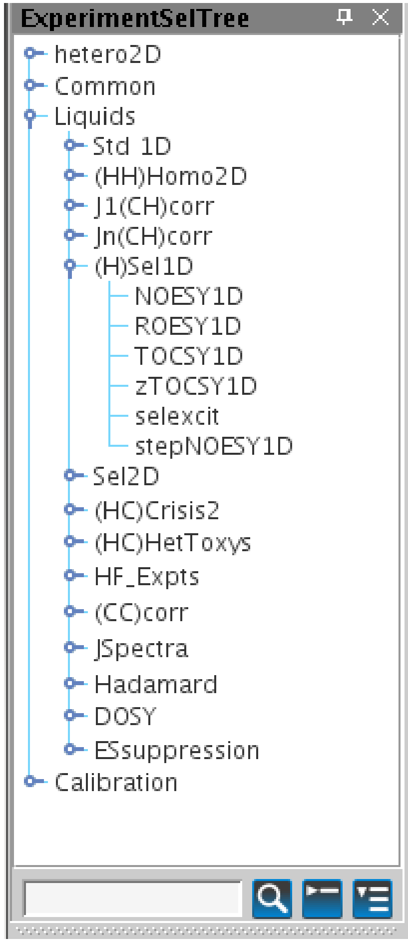
- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
  - J1(CH)corr
  - Jn(CH)corr
  - (H)Sel1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
  - HF_Expts
  - (CC)corr
  - JSpectra
    - HETERO2DJ
    - HOMO2DJ
  - Hadamard
  - DOSY
  - ESsuppression
- Calibration

Exotic …. Everyday…. Calibrations… All available with a click in or out of automation



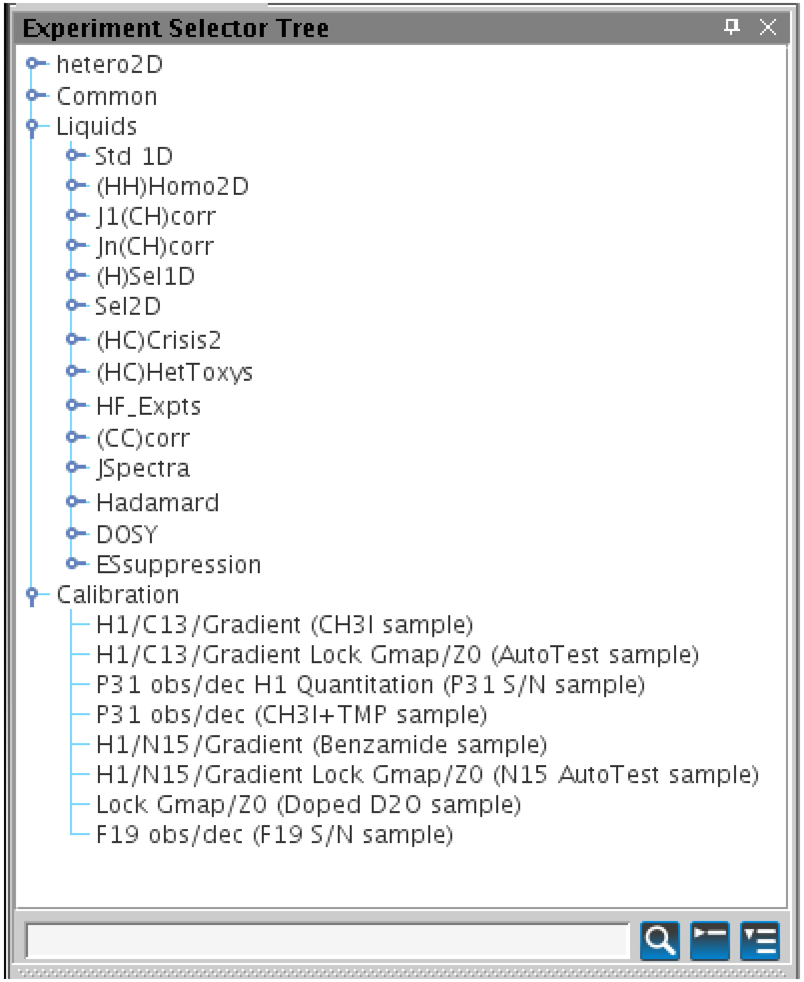
ExperimentSelTree

- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
  - J1(CH)corr
  - Jn(CH)corr
  - (H)Sel1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
  - HF_Expts
  - (CC)corr
    - INADEQUATE
    - INADEQUATEAD
    - ADEQUATEAD
  - JSpectra
  - Hadamard
  - DOSY
  - ESsuppression
- Calibration



ExperimentSelTree

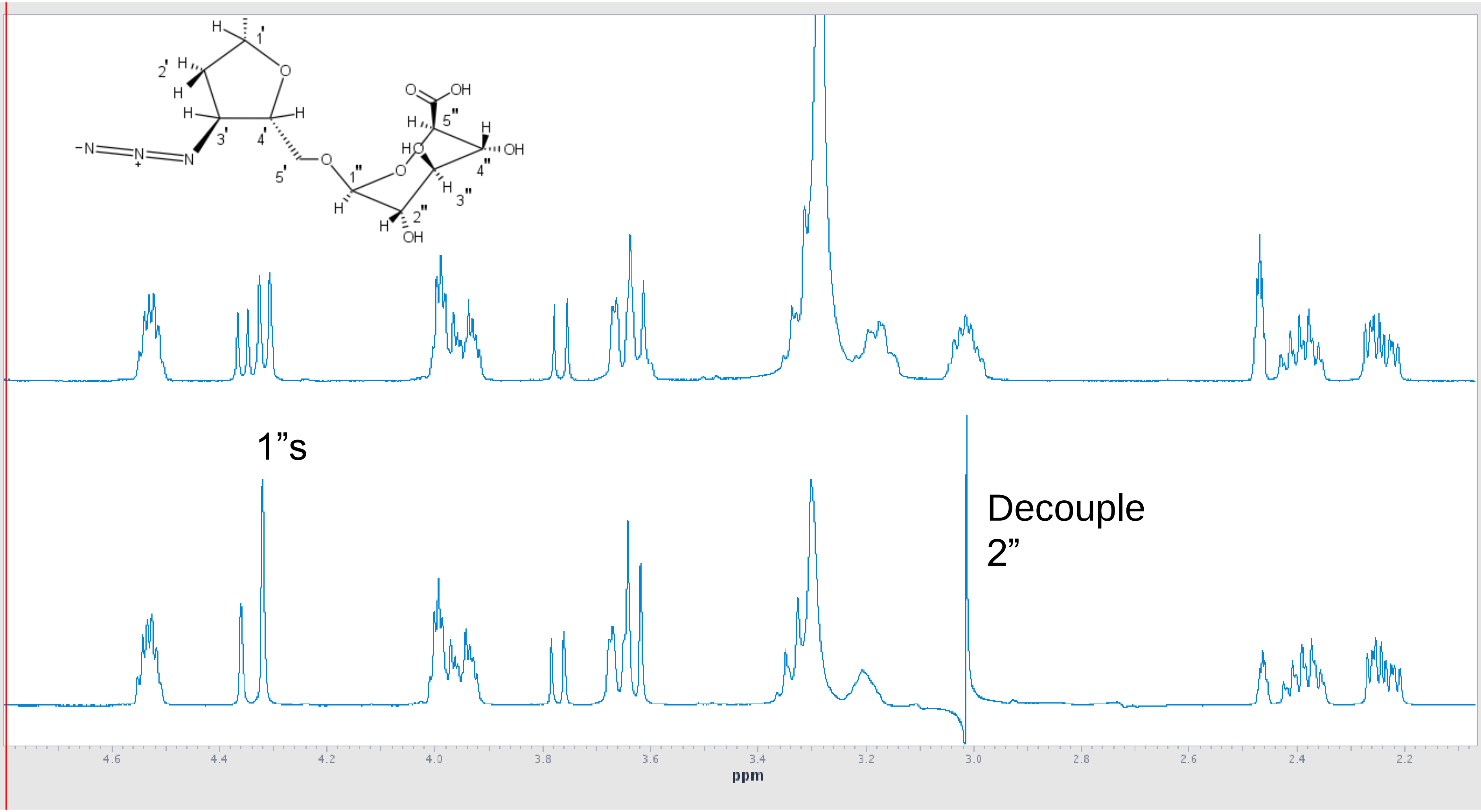
- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
  - J1(CH)corr
  - Jn(CH)corr
  - (H)Sel1D
    - NOESY1D
    - ROESY1D
    - TOCSY1D
    - zTOCSY1D
    - selexcit
    - stepNOESY1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
  - HF_Expts
  - (CC)corr
  - JSpectra
  - Hadamard
  - DOSY
  - ESsuppression
- Calibration



Experiment Selector Tree

- hetero2D
- Common
- Liquids
  - Std 1D
  - (HH)Homo2D
  - J1(CH)corr
  - Jn(CH)corr
  - (H)Sel1D
  - Sel2D
  - (HC)Crisis2
  - (HC)HetToxys
  - HF_Expts
  - (CC)corr
  - JSpectra
  - Hadamard
  - DOSY
  - ESsuppression
- Calibration
  - H1/C13/Gradient (CH3I sample)
  - H1/C13/Gradient Lock Gmap/Z0 (AutoTest sample)
  - P31 obs/dec H1 Quantitation (P31 S/N sample)
  - P31 obs/dec (CH3I+TMP sample)
  - H1/N15/Gradient (Benzamide sample)
  - H1/N15/Gradient Lock Gmap/Z0 (N15 AutoTest sample)
  - Lock Gmap/Z0 (Doped D2O sample)
  - F19 obs/dec (F19 S/N sample)

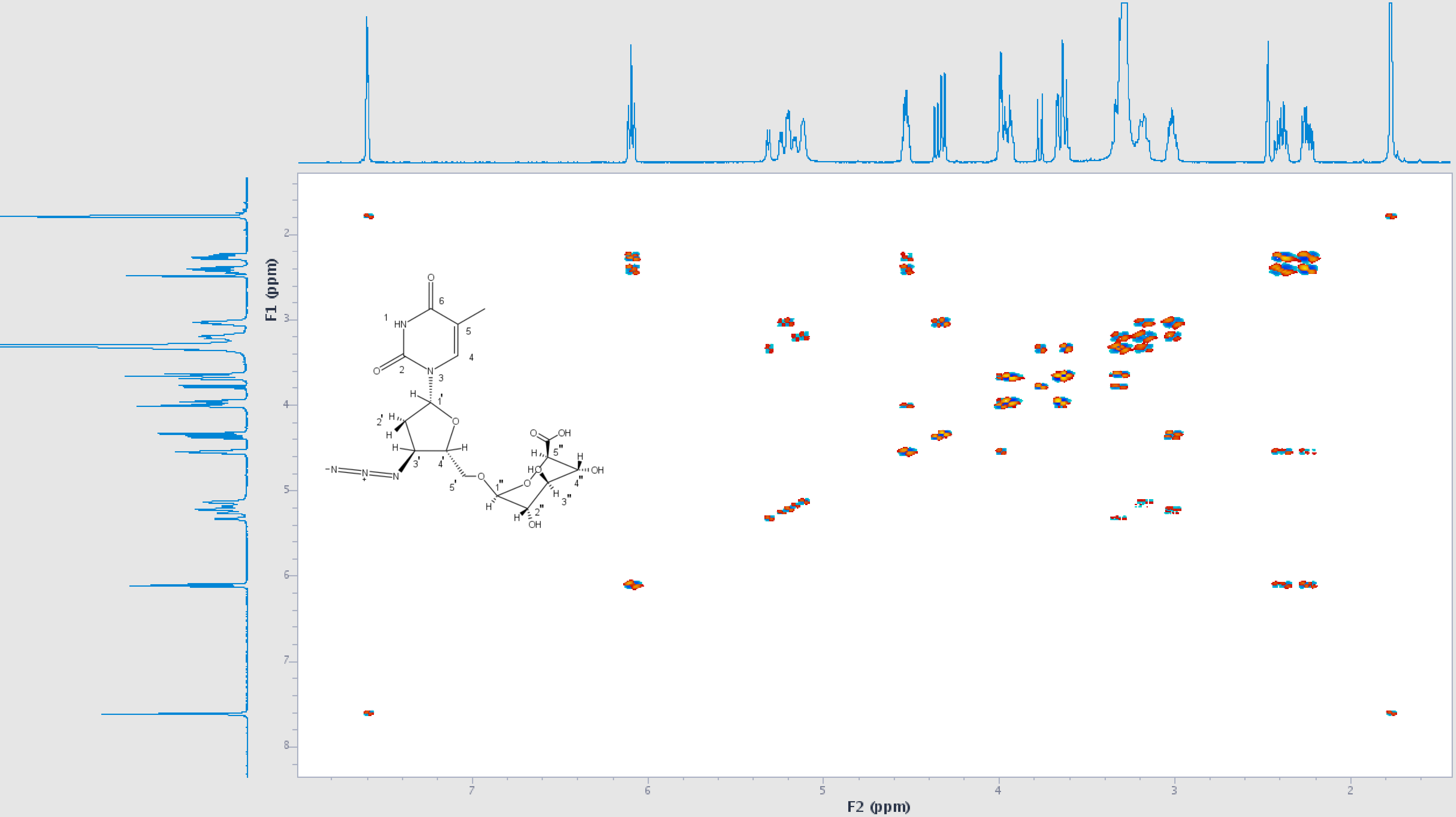
Homonuclear Decoupling replaced by 2Ds but remains very useful.
Can be applied during *any 2D experiment*!!



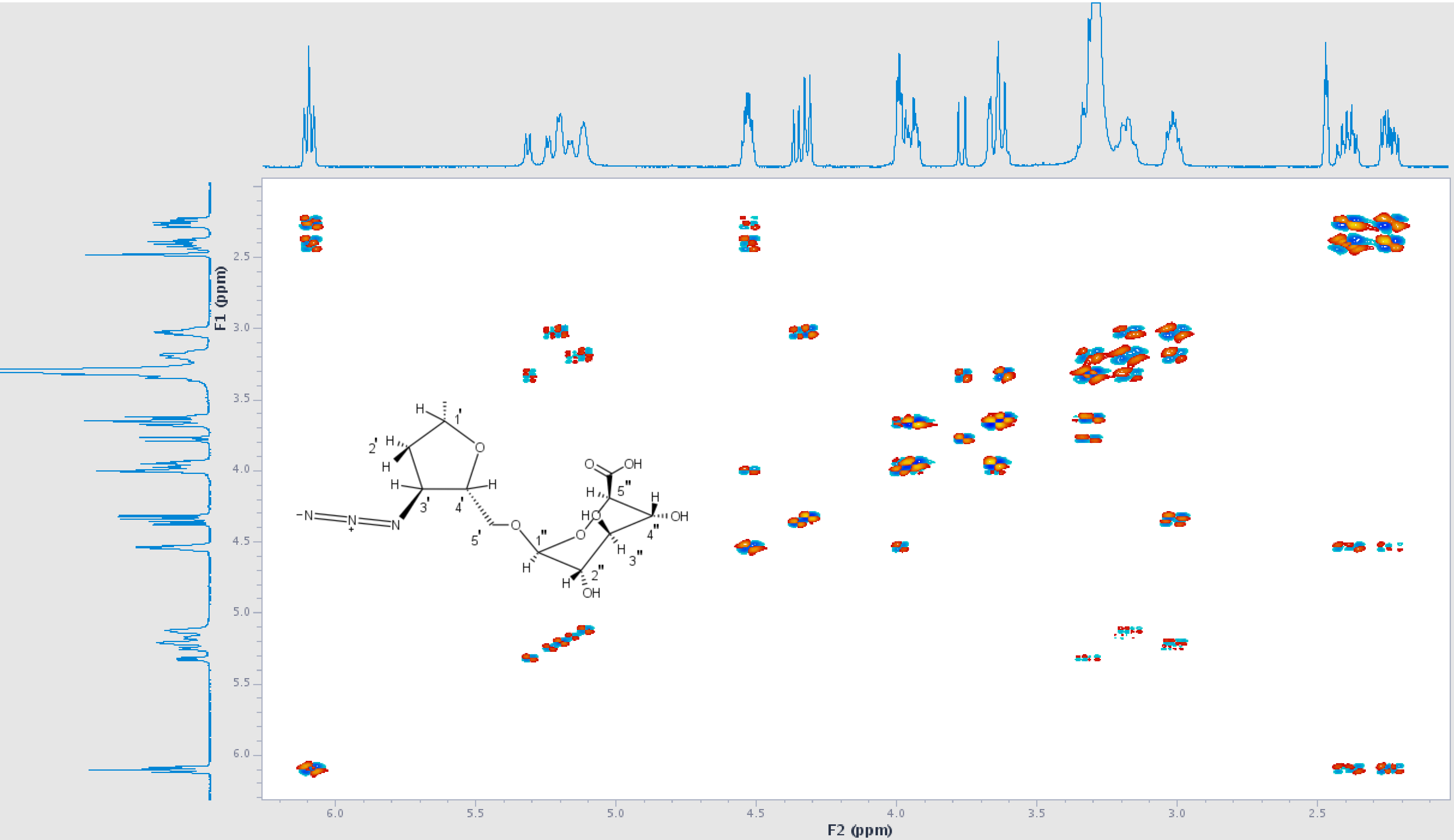
1”s

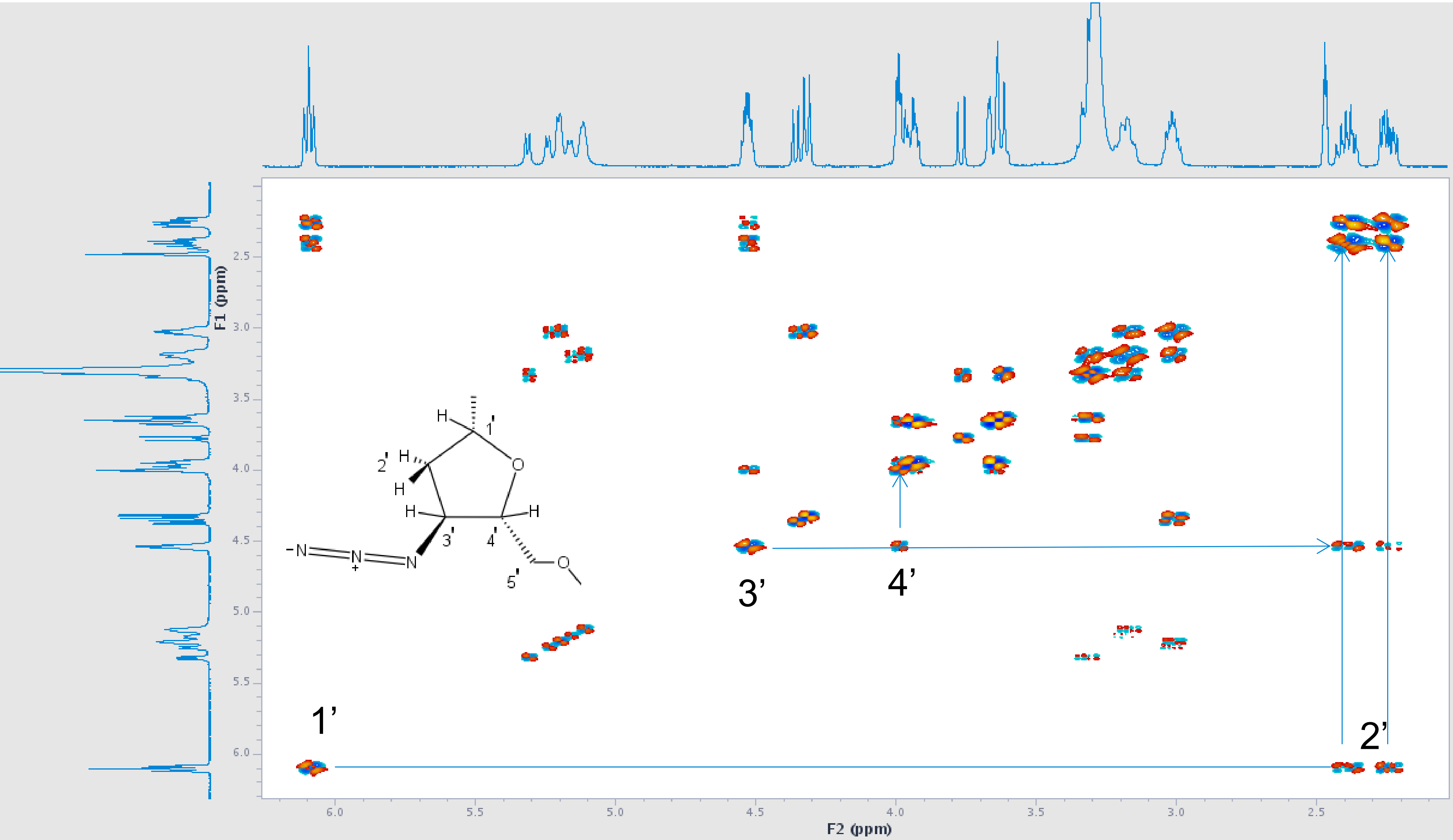
Decouple 2”

Consider the gDQCOSY for GAZT

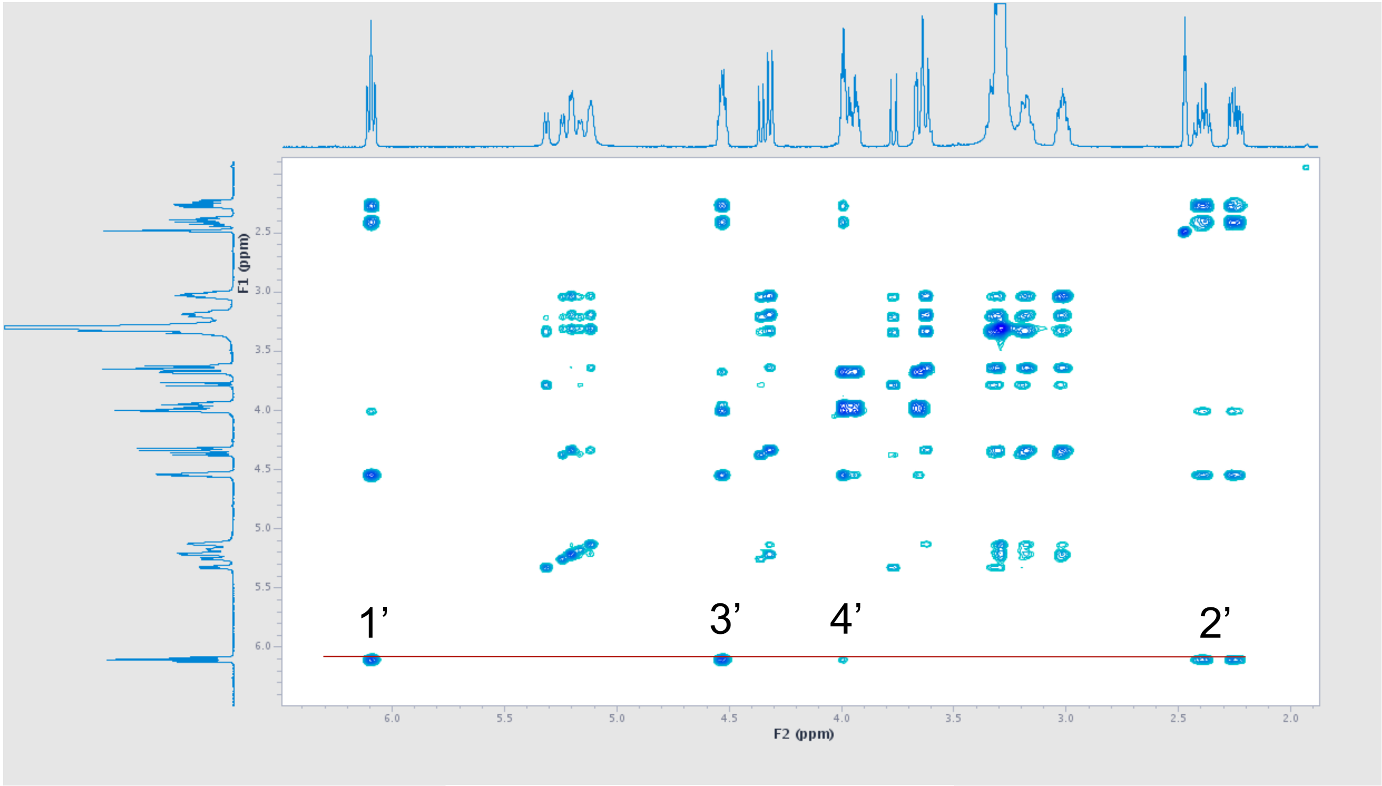


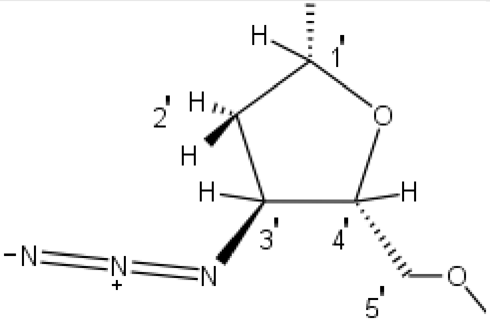
GAZT – Sugars Only….



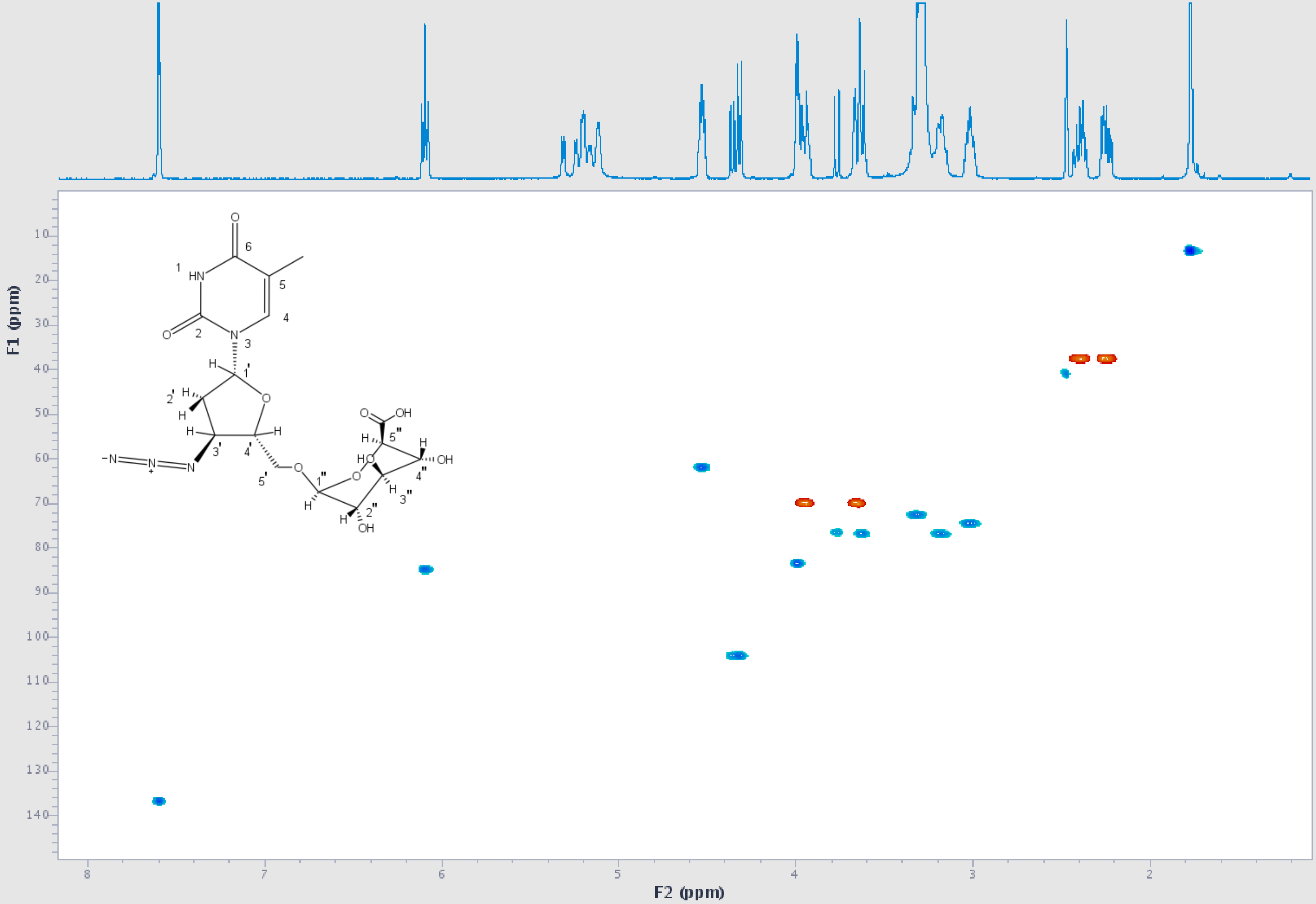

1'
2'
3'
4'
5'
H
O
N
N
N
+
F1 (ppm)
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
F2 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5

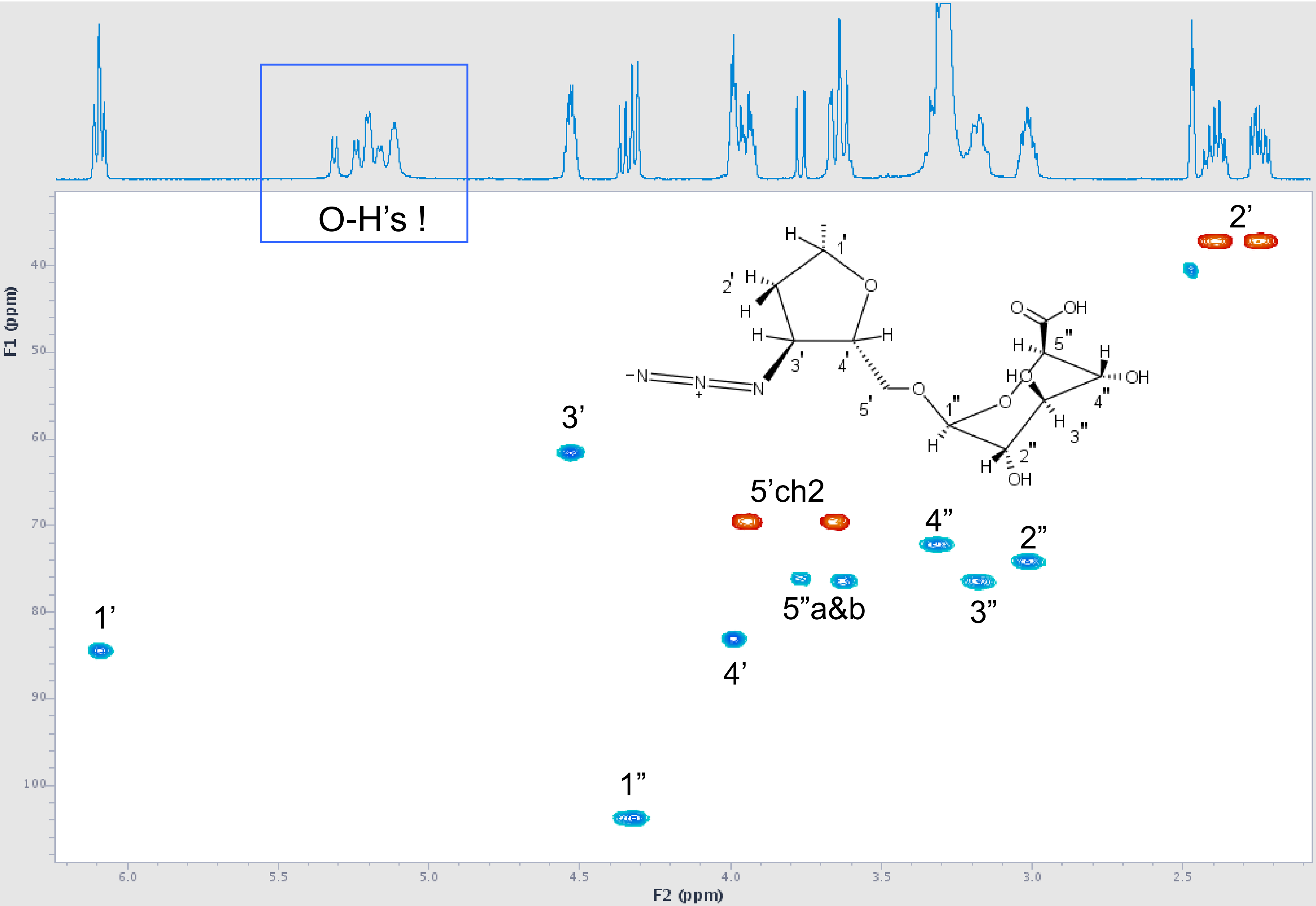
A 2D TOCSY of GAZT – Complementary to COSY – Easily considered trace by trace!!



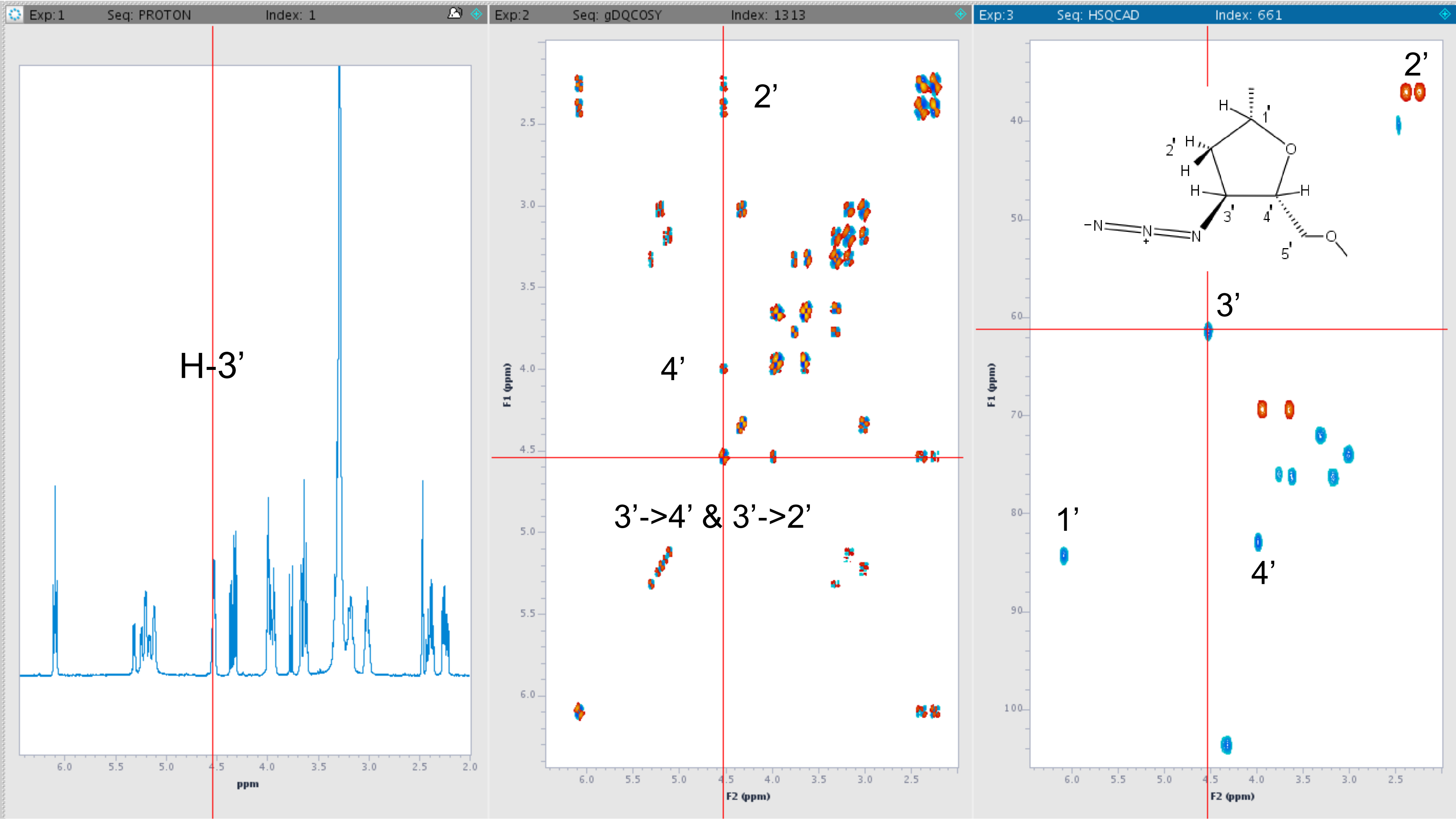


HSQCAD - GAZT


F1 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
F2 (ppm)
8
7
6
5
4
3
2
O
6
1
HN
5
4
O
2
N
3
H
1'
2'
H
H
O
H
H
3'
4'
5'
O
1"
O
2"
3"
4"
5"
OH
HO
H
N
N
N
+
−


O-H's !
2'
3'
5'ch2
4"
2"
5"a&b
3"
1'
4'
1"
F1 (ppm)
40
50
60
70
80
90
100
F2 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
N
N
N
O
OH
HO
OH
OH
O
O
O
H
1'
2'
3'
4'
5'
1"
2"
3"
4"
5"

Analysis the data in a “concerted fashion” ! *Chocolate Chip Cookie Principal*

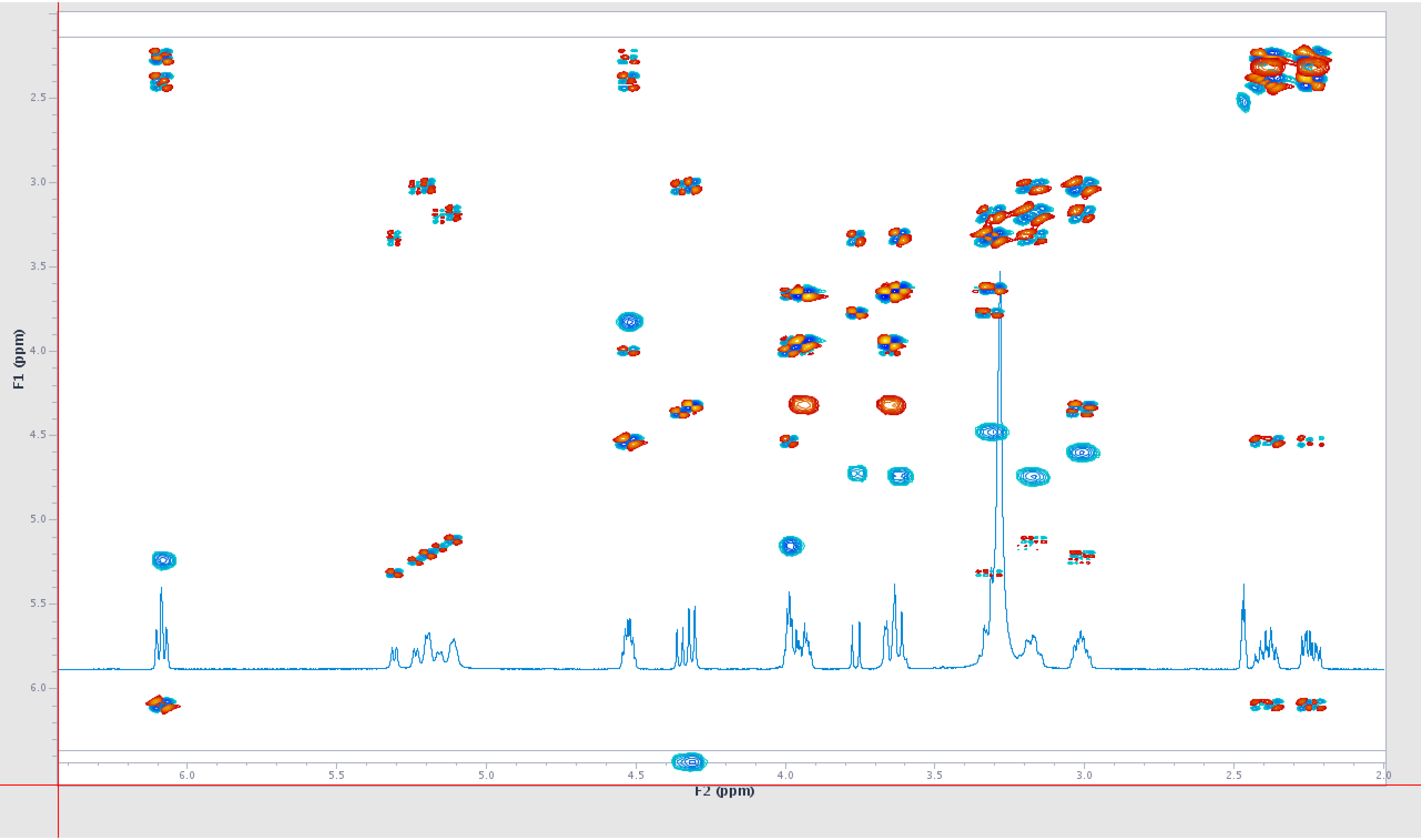


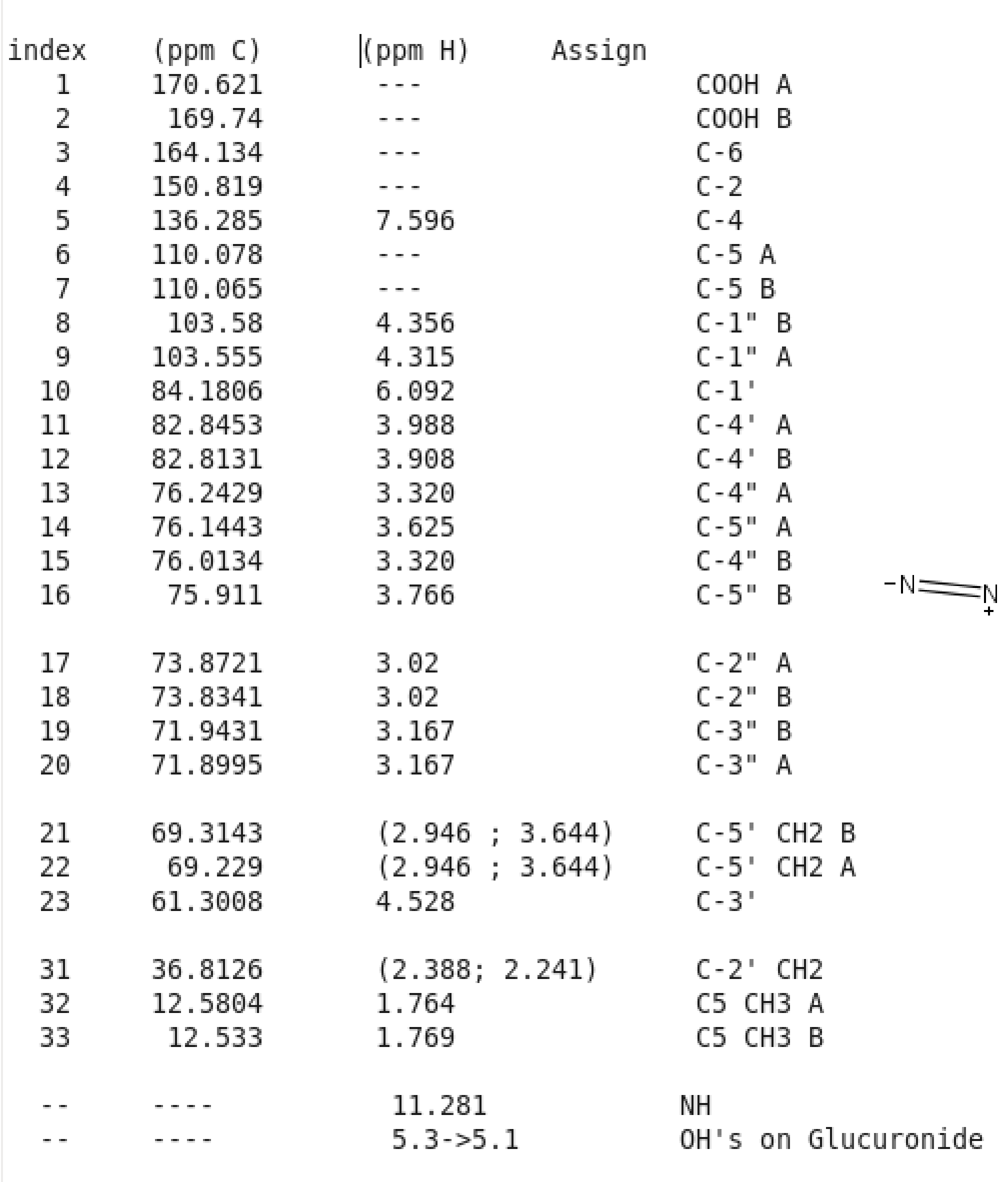
Exp:1 Seq: PROTON Index: 1

Exp:2 Seq: gDQCOSY Index: 1313

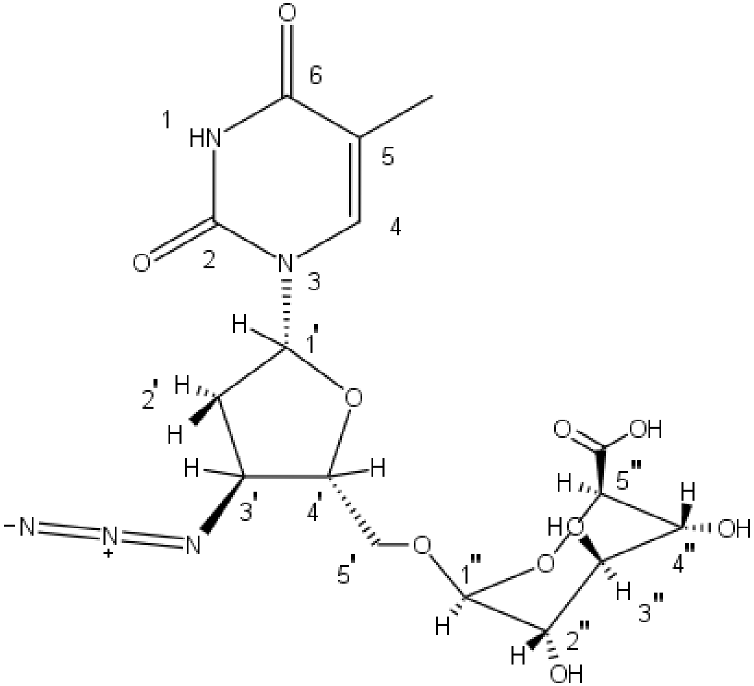
Exp:3 Seq: HSQCAD Index: 661

Alternate way to visualize multiple 2D's at the same time…



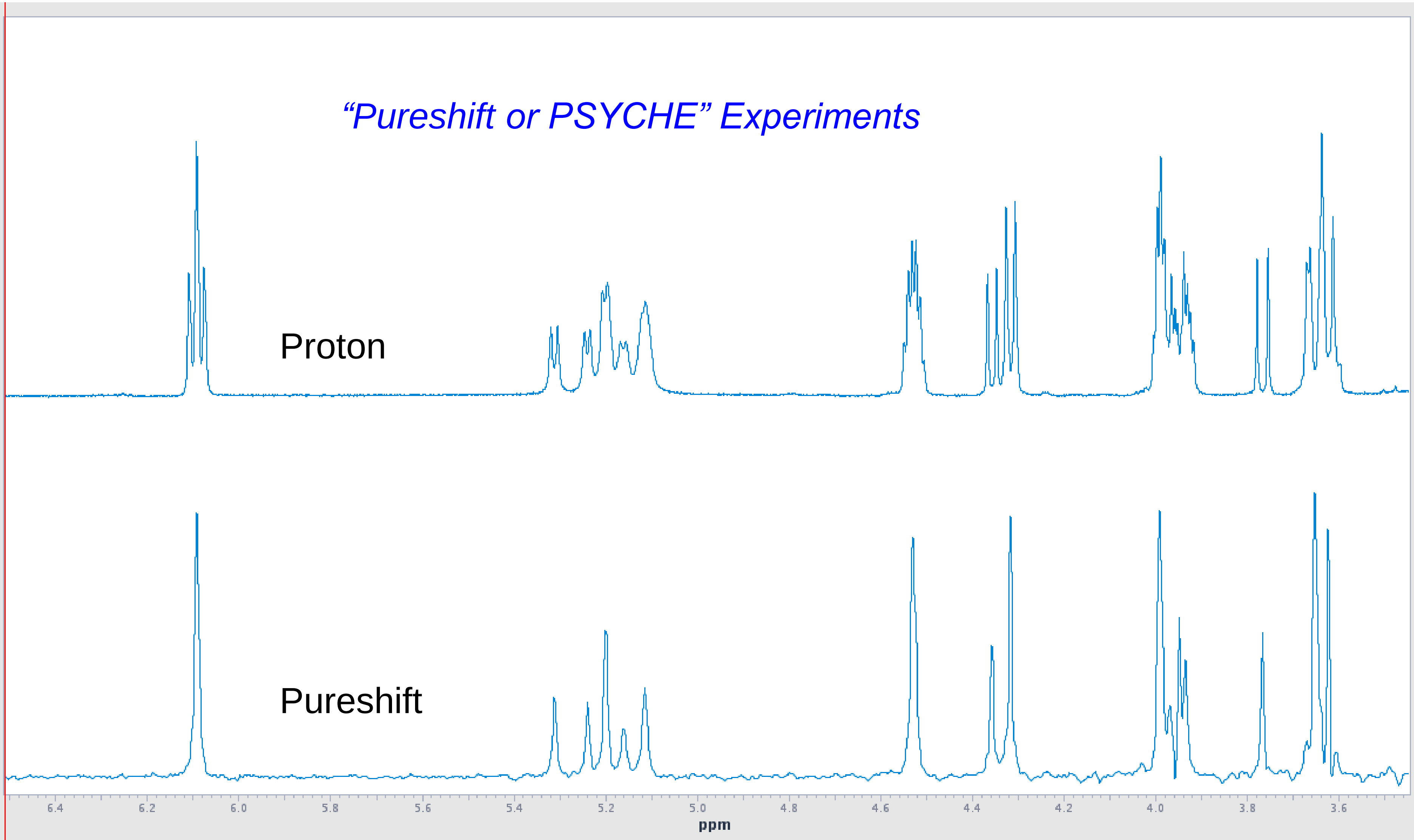


```
index      (ppm C)        |(ppm H)      Assign
   1       170.621         ---                   COOH A
   2        169.74         ---                   COOH B
   3       164.134         ---                   C-6
   4       150.819         ---                   C-2
   5       136.285         7.596                 C-4
   6       110.078         ---                   C-5 A
   7       110.065         ---                   C-5 B
   8        103.58         4.356                 C-1" B
   9       103.555         4.315                 C-1" A
  10       84.1806         6.092                 C-1'
  11       82.8453         3.988                 C-4' A
  12       82.8131         3.908                 C-4' B
  13       76.2429         3.320                 C-4" A
  14       76.1443         3.625                 C-5" A
  15       76.0134         3.320                 C-4" B
  16        75.911         3.766                 C-5" B

  17       73.8721         3.02                  C-2" A
  18       73.8341         3.02                  C-2" B
  19       71.9431         3.167                 C-3" B
  20       71.8995         3.167                 C-3" A

  21       69.3143         (2.946 ; 3.644)       C-5' CH2 B
  22        69.229         (2.946 ; 3.644)       C-5' CH2 A
  23       61.3008         4.528                 C-3'

  31       36.8126         (2.388; 2.241)        C-2' CH2
  32       12.5804         1.764                 C5 CH3 A
  33        12.533         1.769                 C5 CH3 B

  --       ----              11.281            NH
  --       ----              5.3->5.1          OH's on Glucuronide
```



Manual Assignment time?
less than 3 minutes!

Today we can simply acquire a proton directly with chemical shifts only!!



“Pureshift or PSYCHE” Experiments

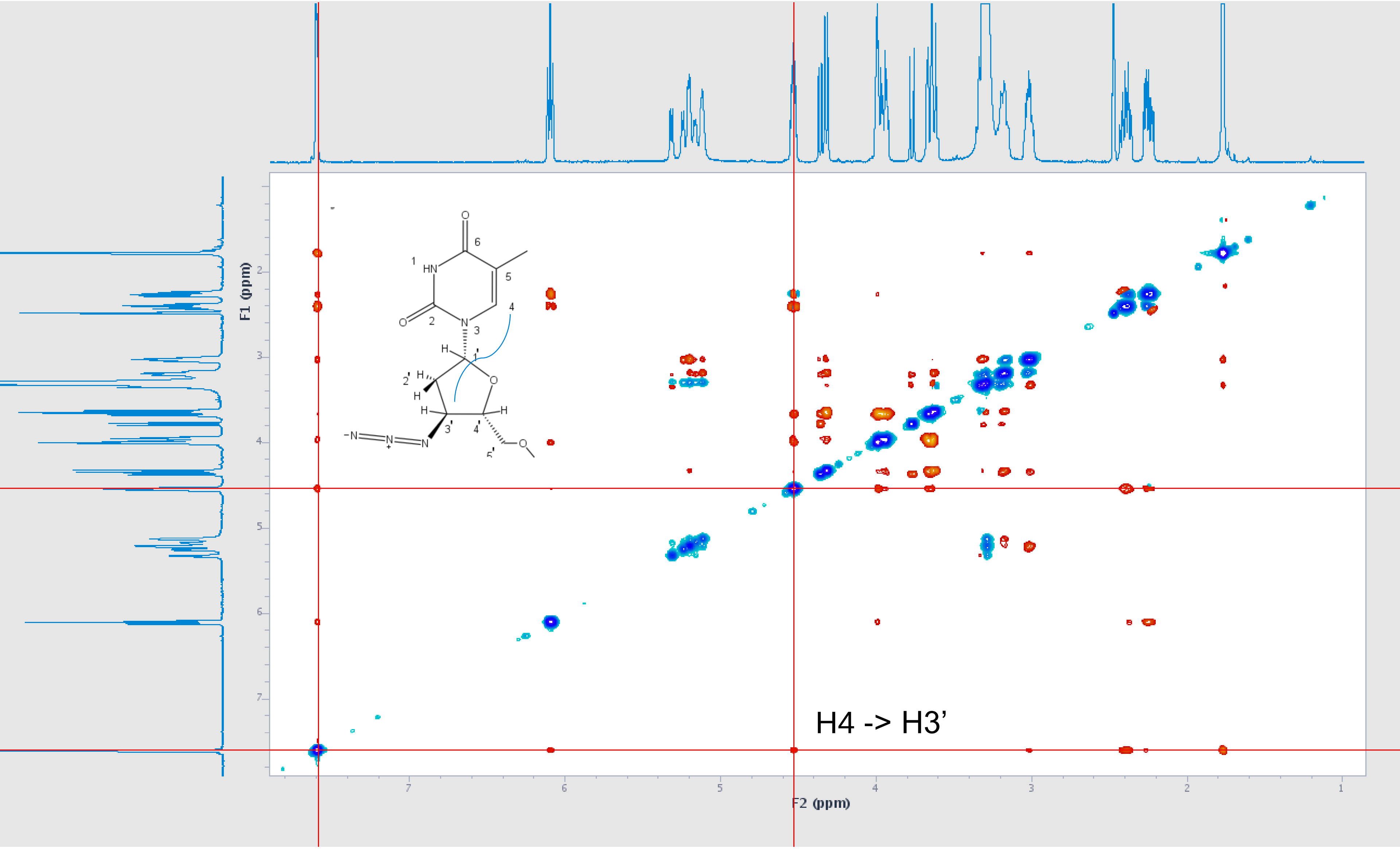
Classes of Pulse Sequences – Assisted Connectivity

Band-Selected homo-nuclear experiments such NOESY1D, ROESY1D, TOCSY1D, and their “Band selected 2D” companions.

Direct H/X correlations combined with 1H/1H connectivity such as HSQCTOCSY and H2BC.

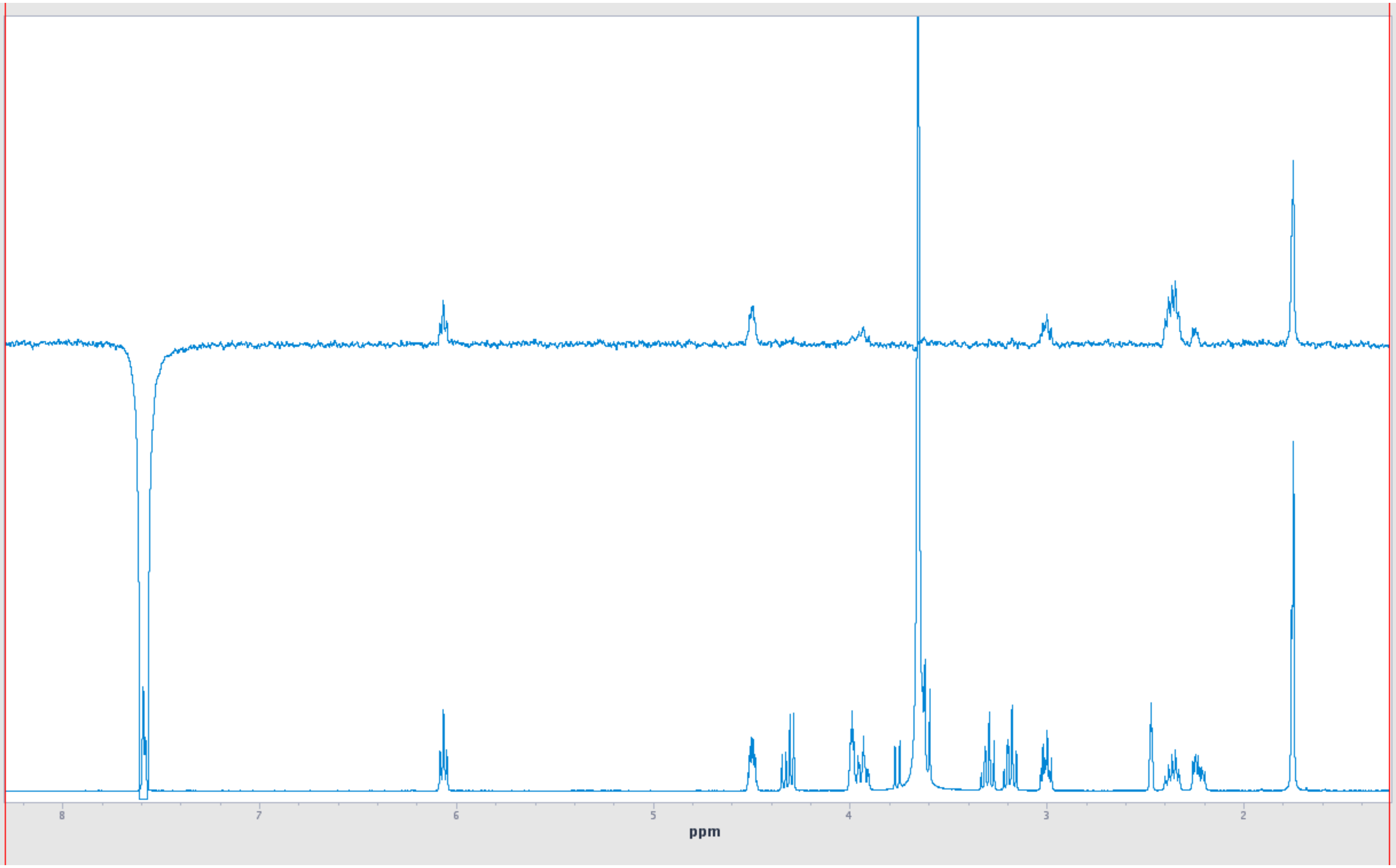
A cornerstone for drawing Chemical bonds = The HMBC family with the many useful variants.

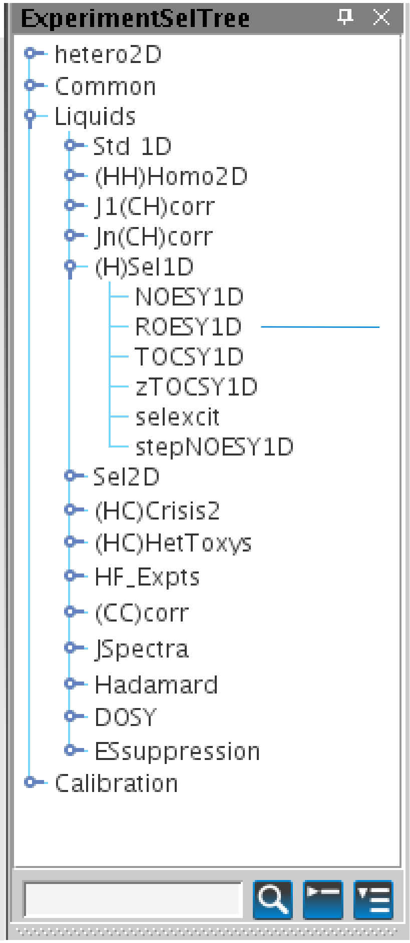
ROESY-AD - Through Space -



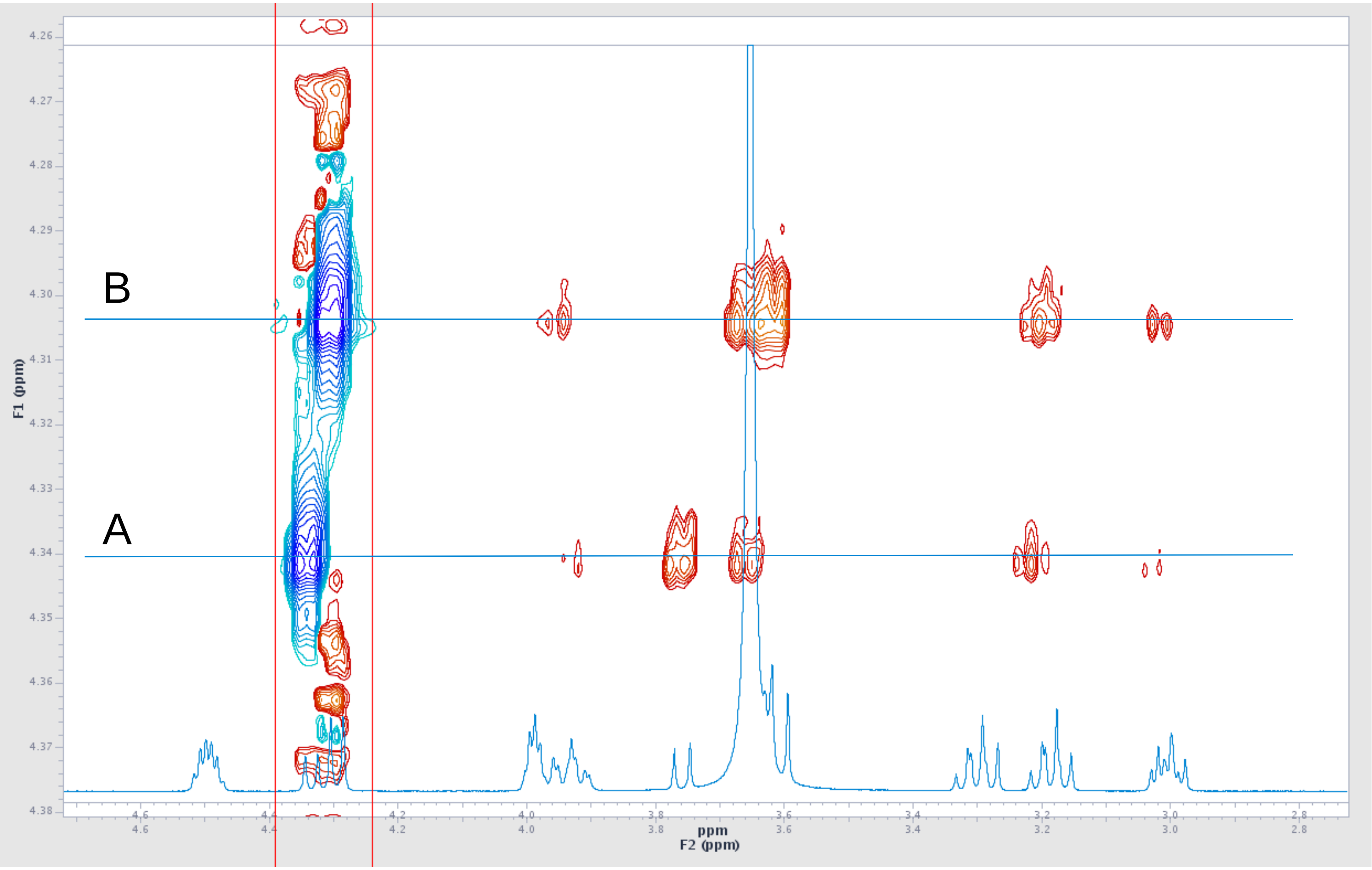
H4 -> H3’

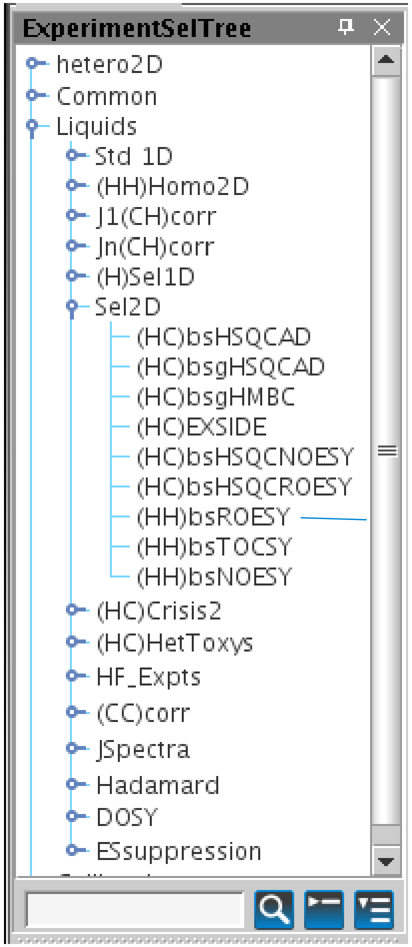
Often a simple 1D ROESY or NOESY alternative to a full 2D experiment.



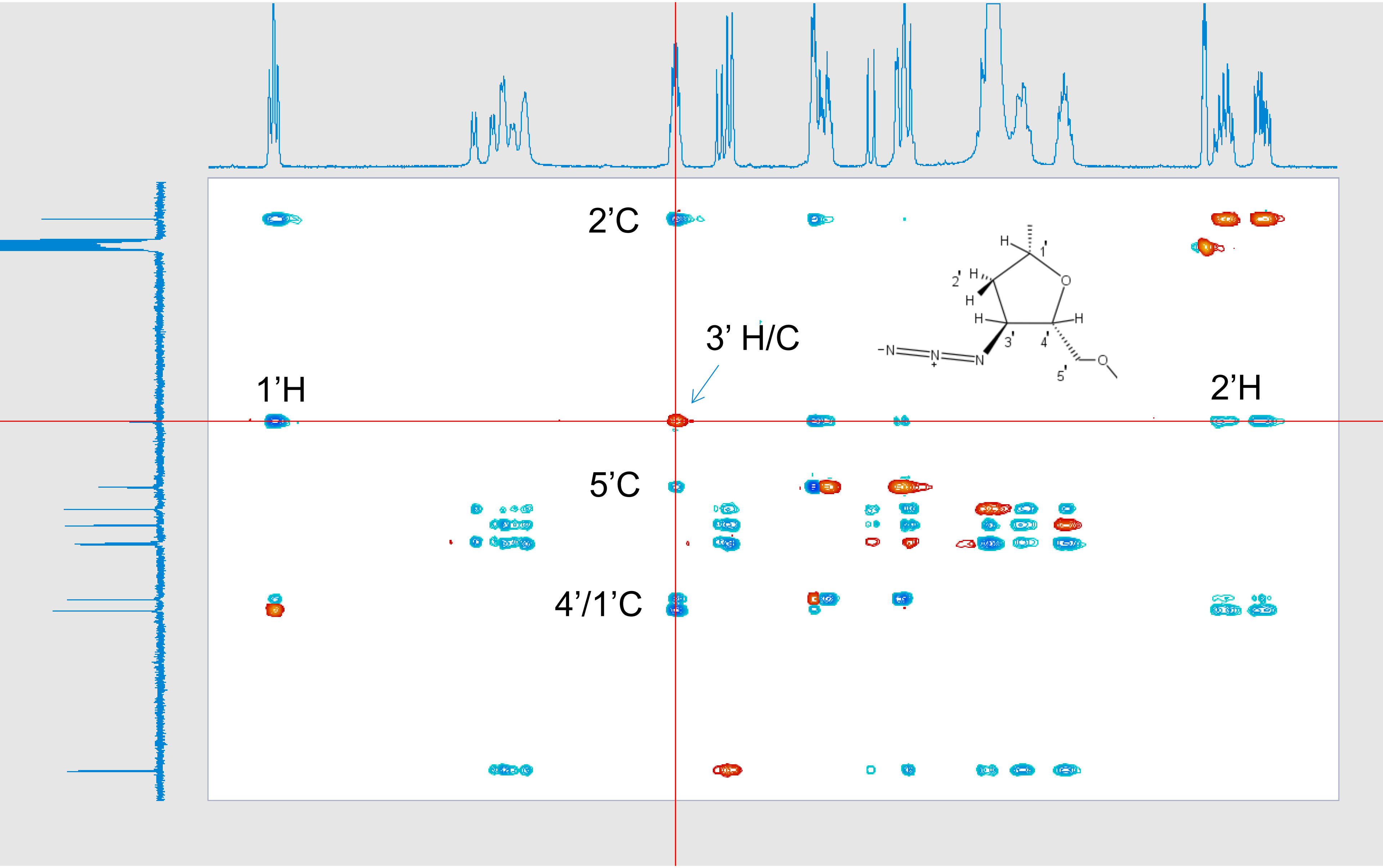


A nice suite of 2D Band-selected “homonuclear” experiments are provided!!



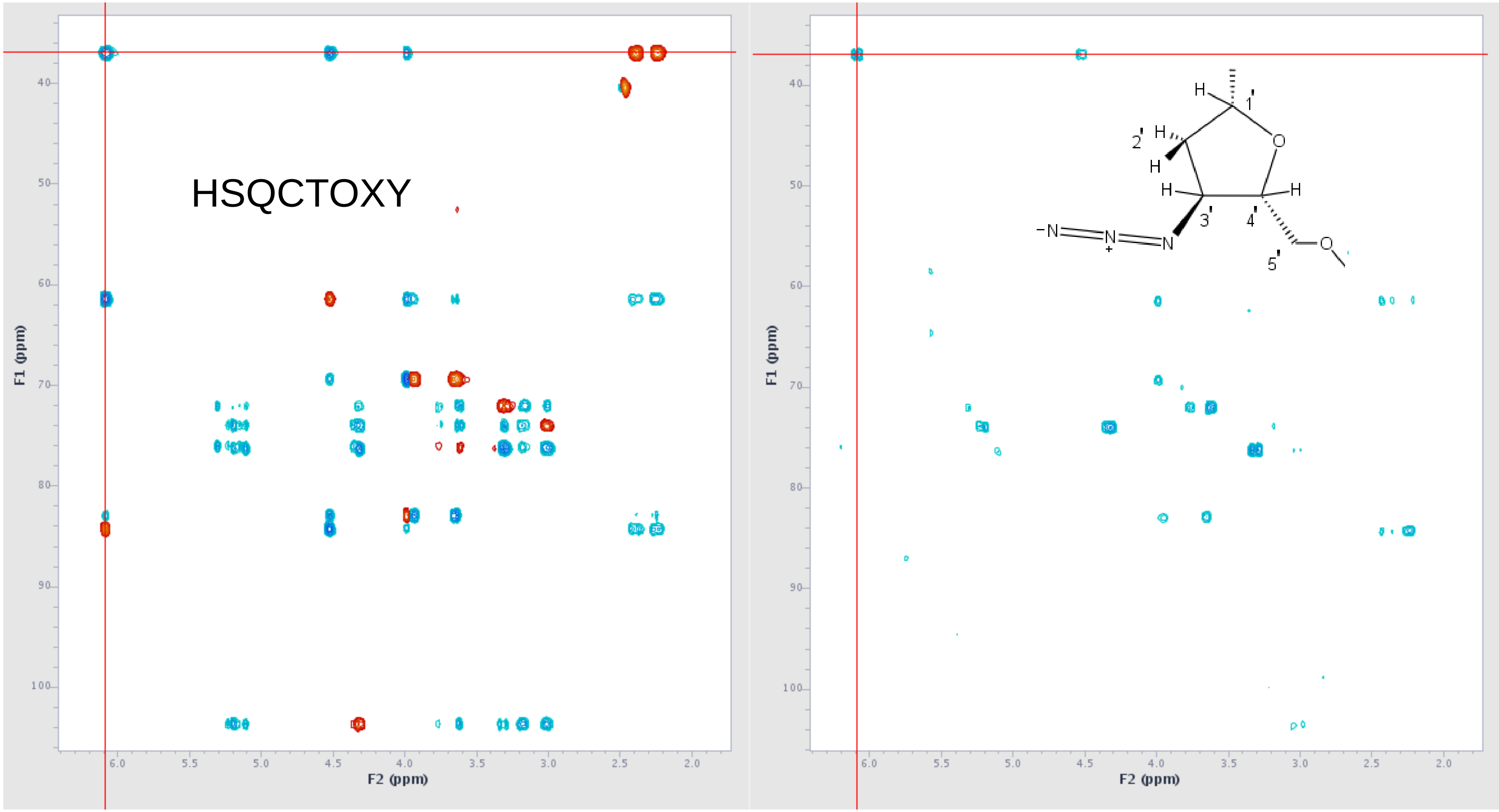


HSQCTOXY – Show 1H-1H connectivity with the resolution of 13C!!!

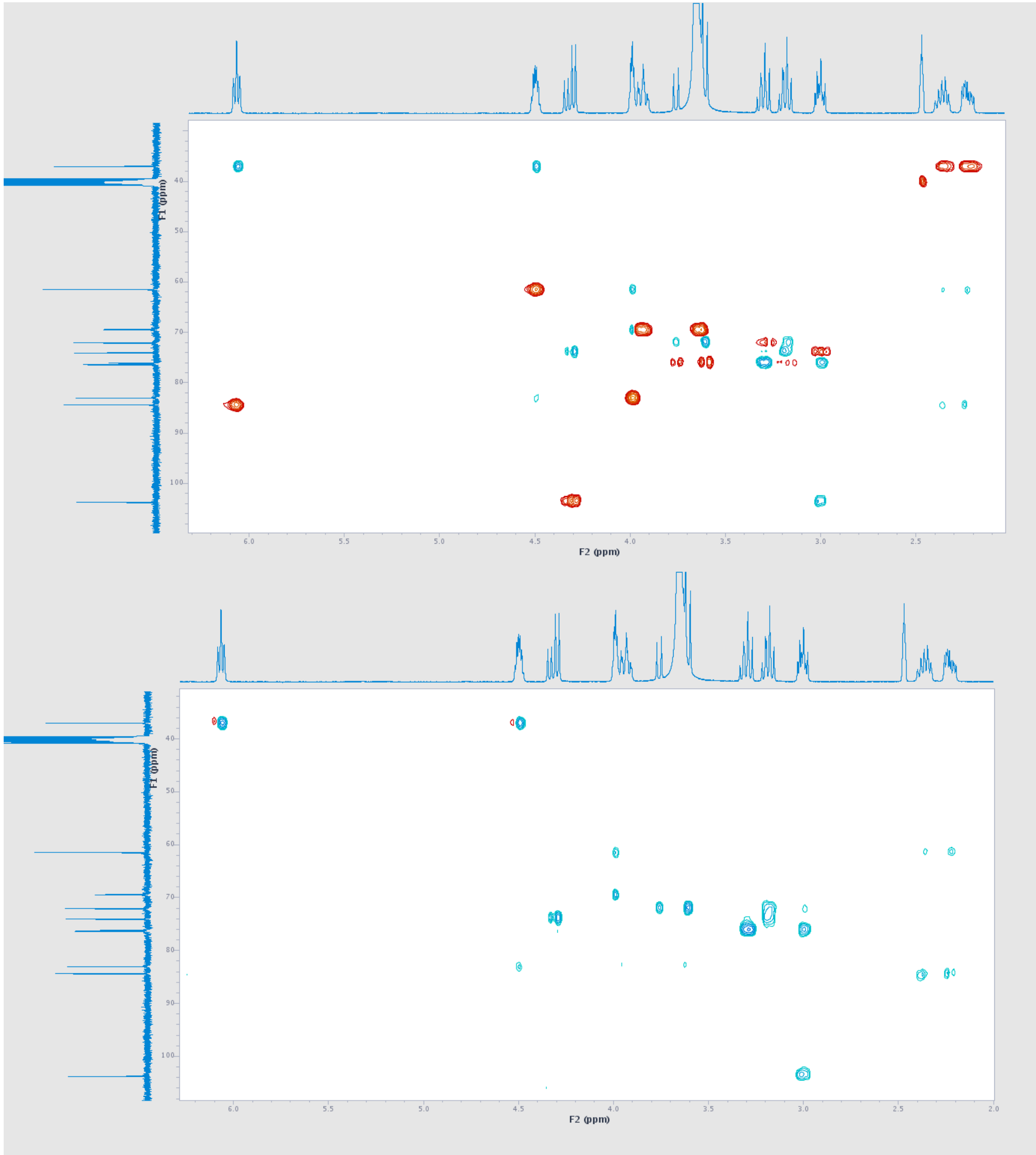


HSQCTOXY versus H2BC for 2-bond 1H-1H connectivity

Cursor on 1’->2’ connectivity…



HSQCTOXY – shorten Mix

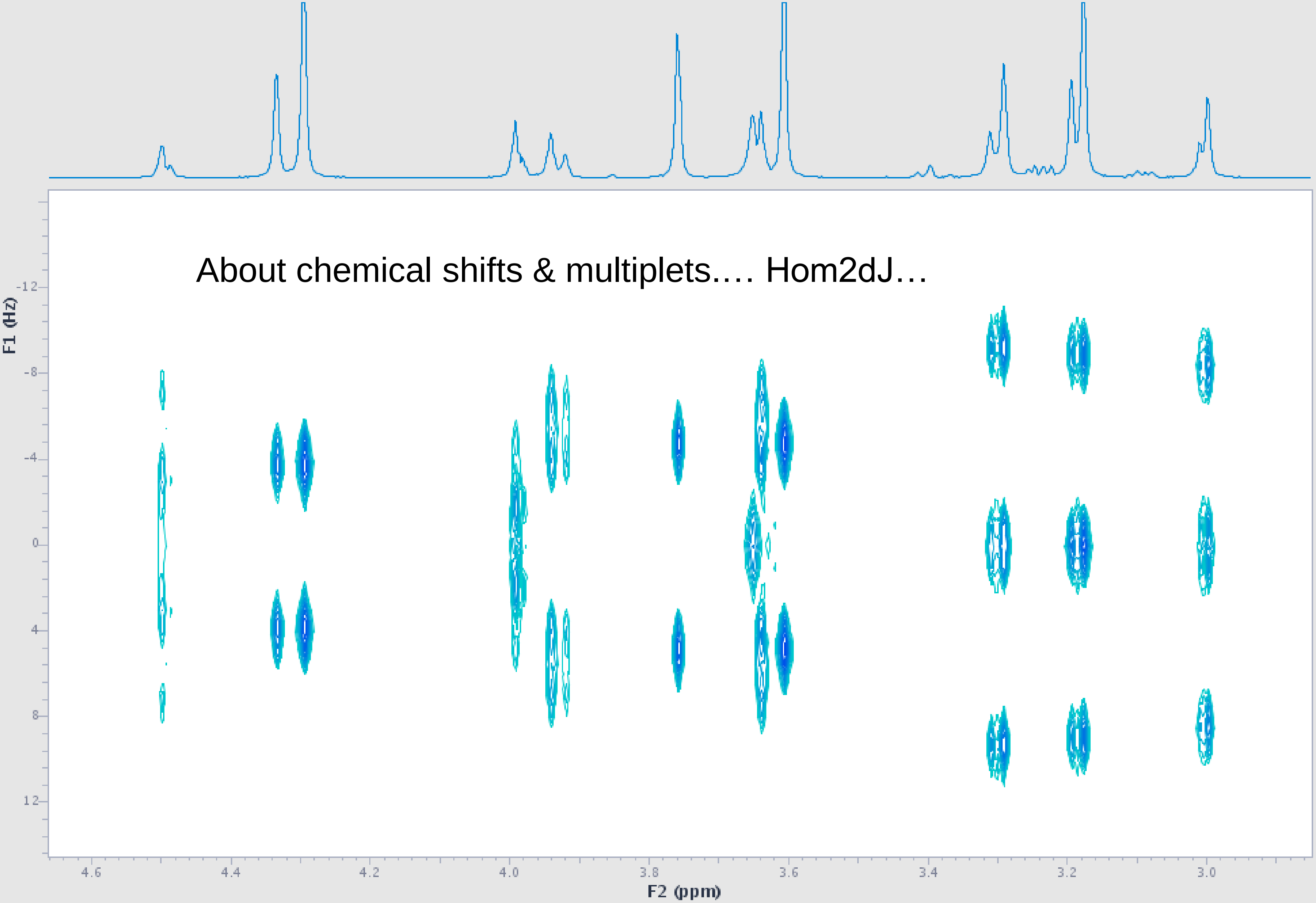


mixT=0.018
mult=2

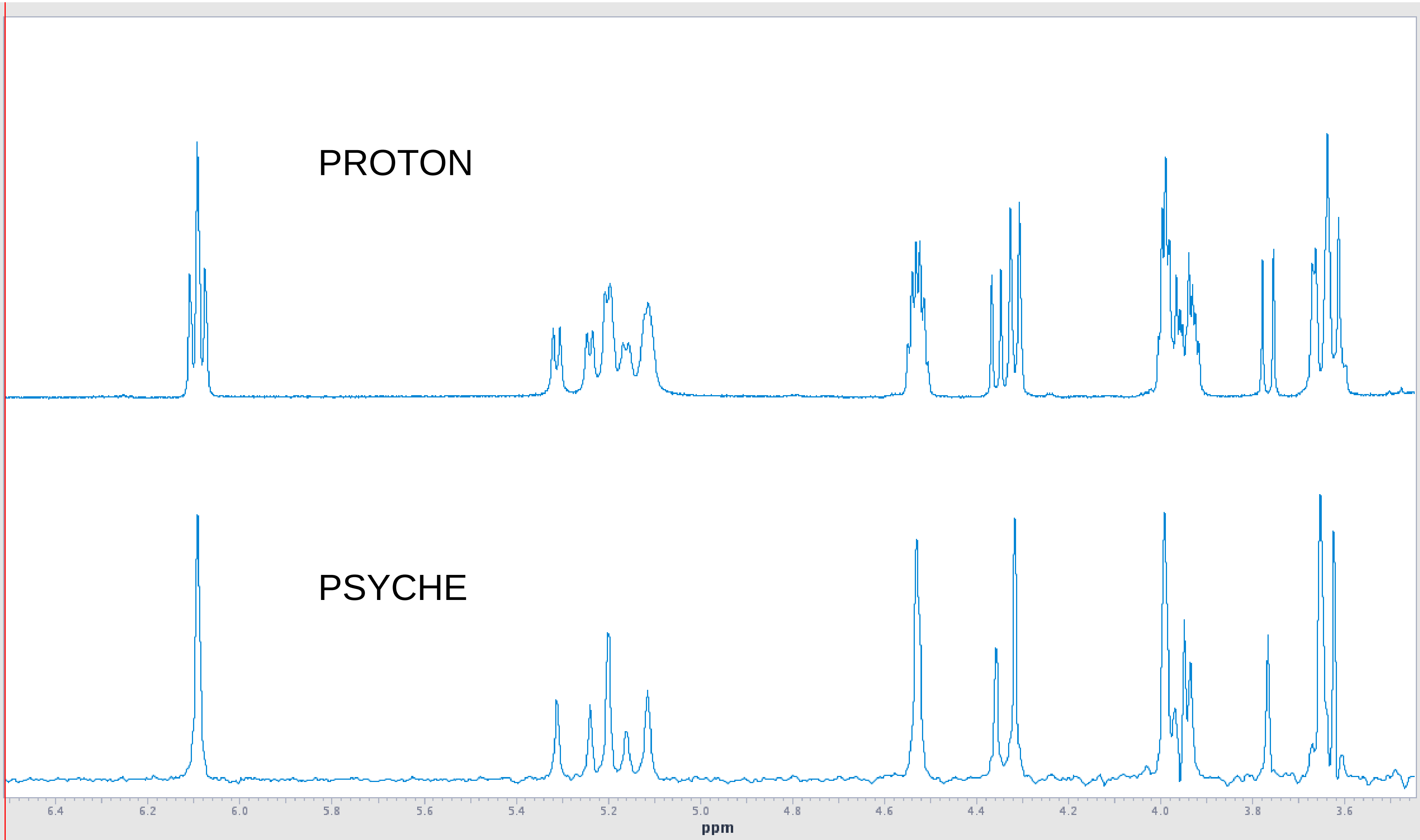
mixT=0.018
mult=1
dm='nnn'

Just like H2BC..
but more robust!

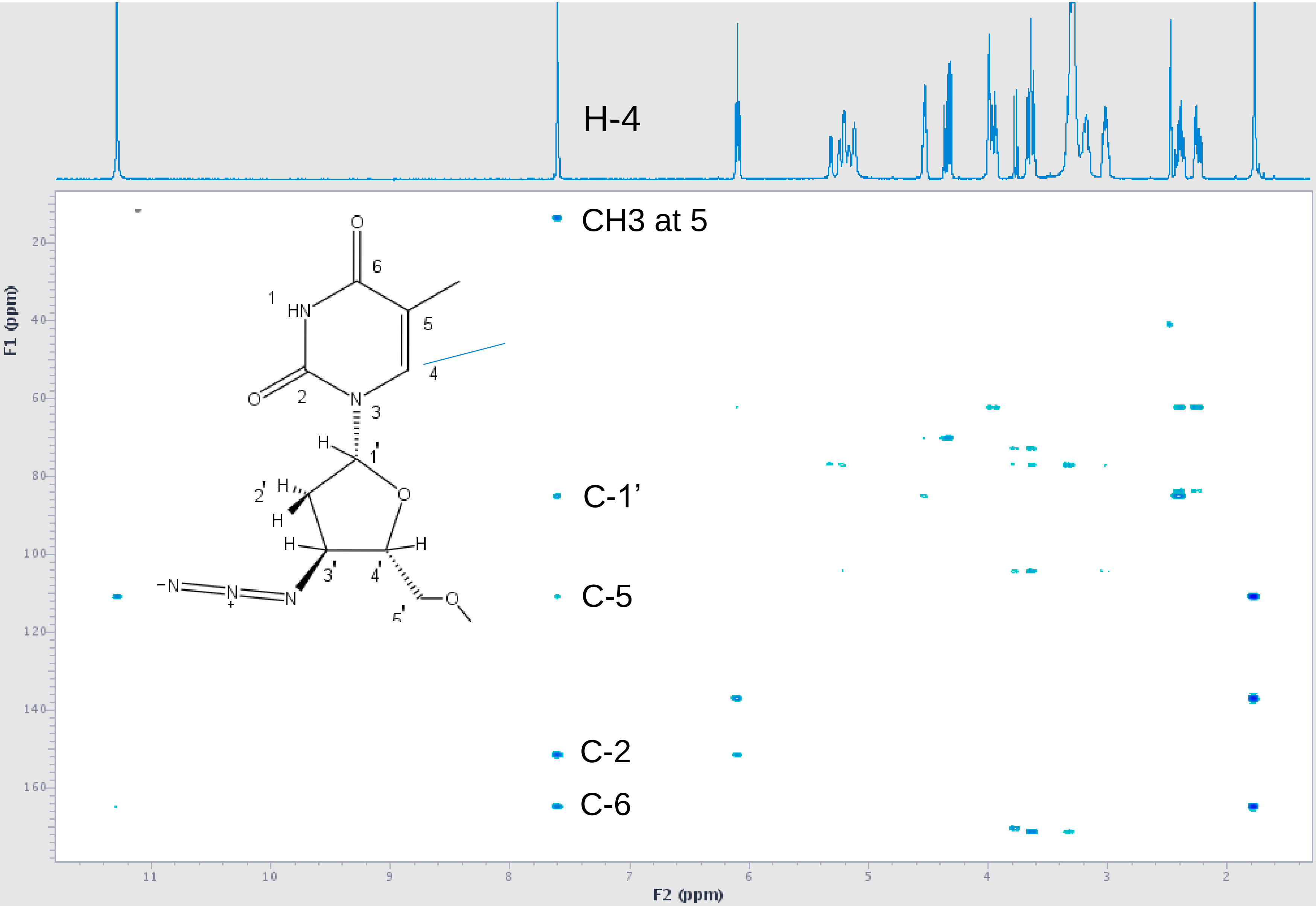
About chemical shifts & multiplets…. Hom2dJ…

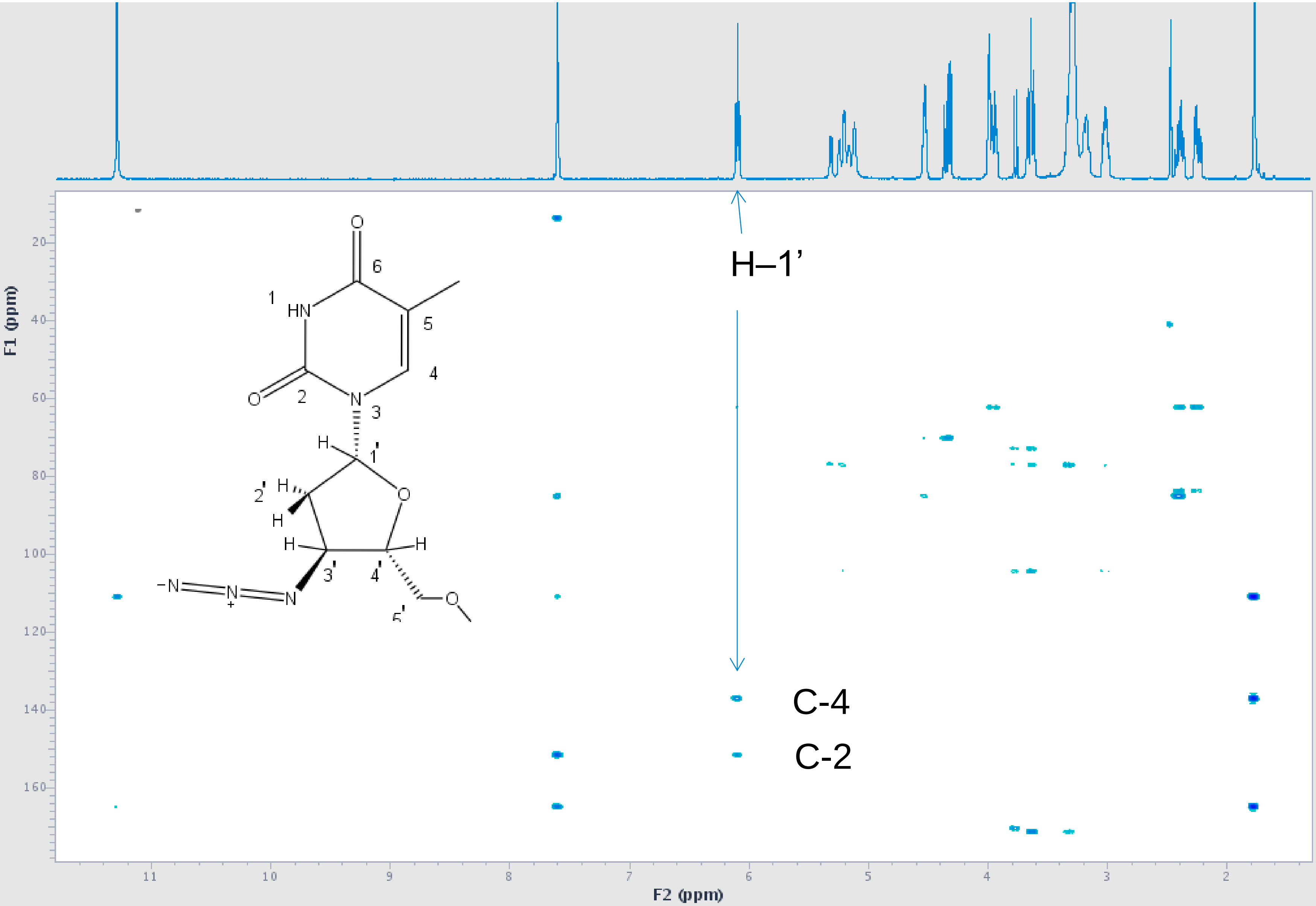


PSYCHE – improved PureShift 1D…(qNMR!!)



gHMBCAD – Long-range H/X connectivity – *Consider Just Thymidine –H4!*



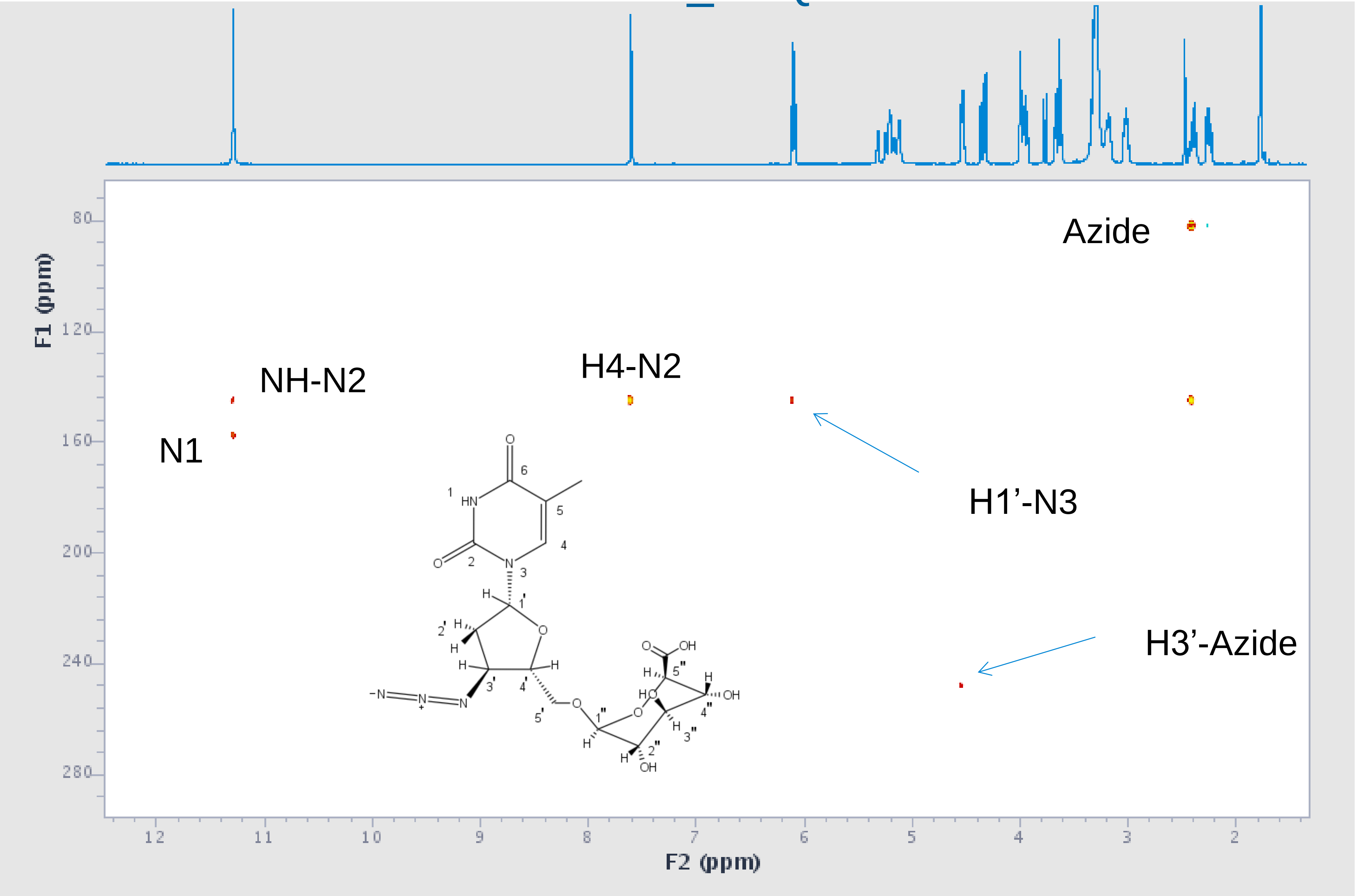

F1 (ppm)
20
40
60
80
100
120
140
160
F2 (ppm)
11
10
9
8
7
6
5
4
3
2
O
6
1
HN
5
4
O
2
N
3
H
1'
2'
H
O
H
H
H
3'
4'
−N
N
+
N
5'
O

H–1'

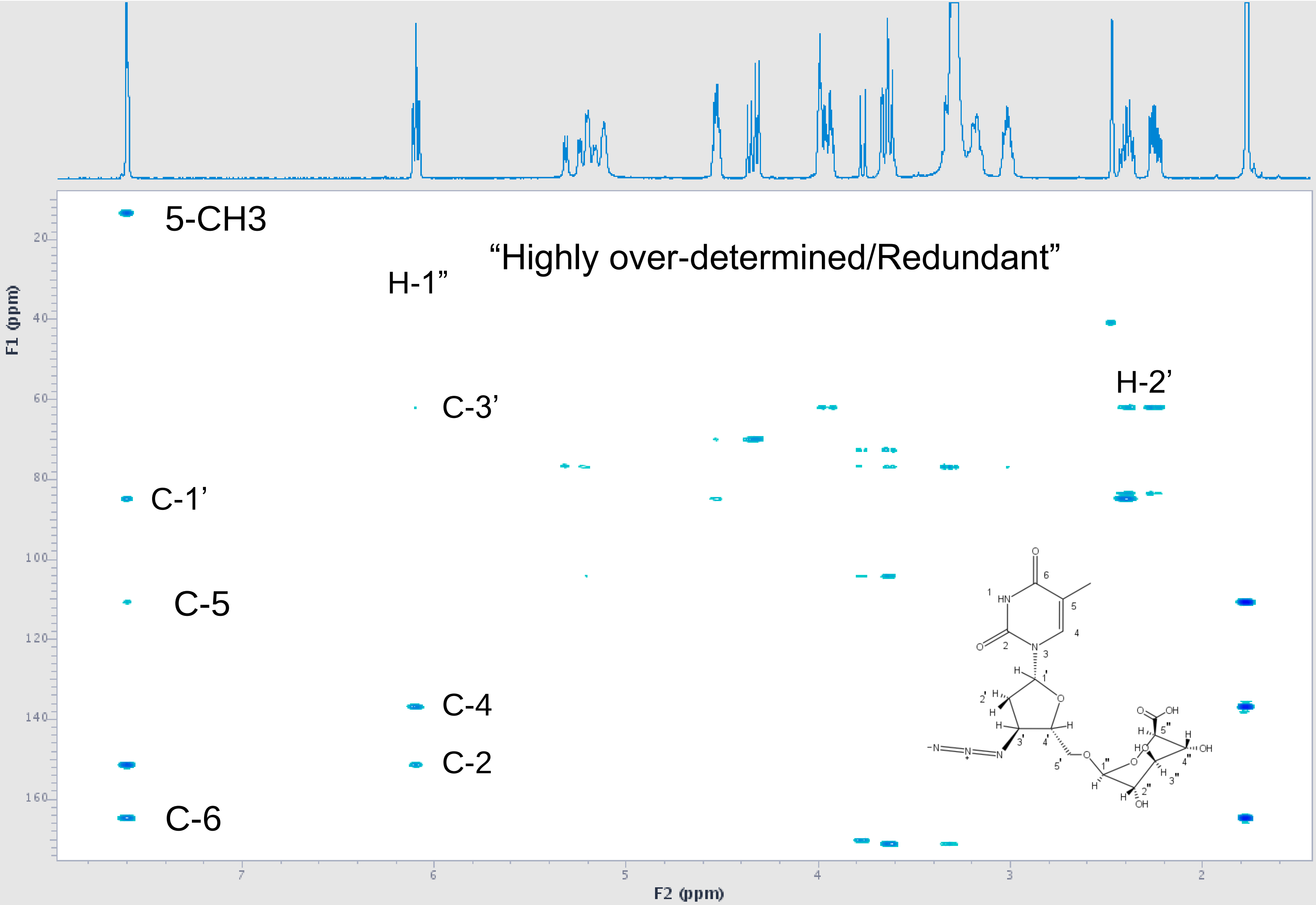
C-4

C-2

GAZT H-N LR_HSQMBC

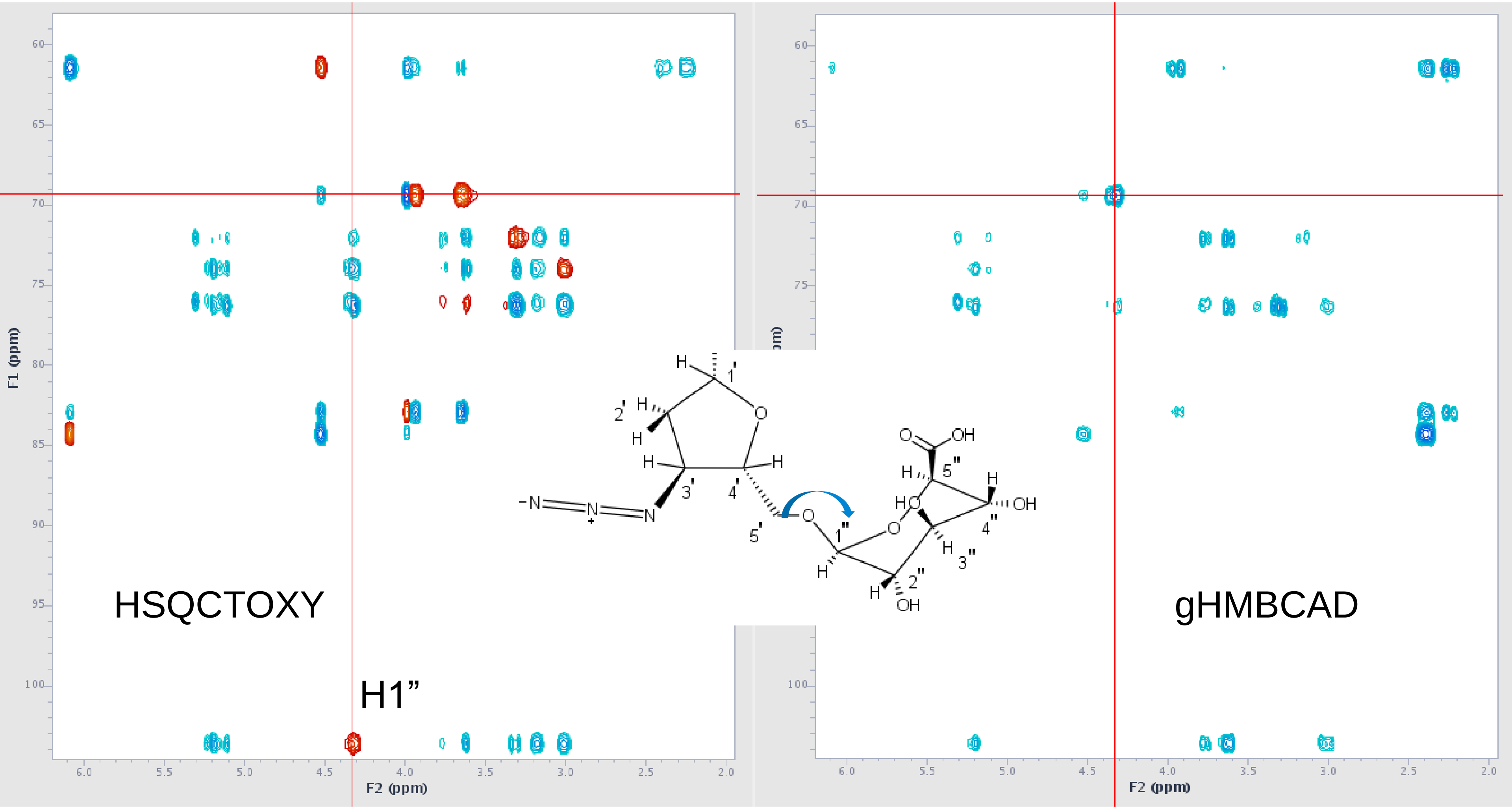


Key gHMBCAD Information…



“Highly over-determined/Redundant”

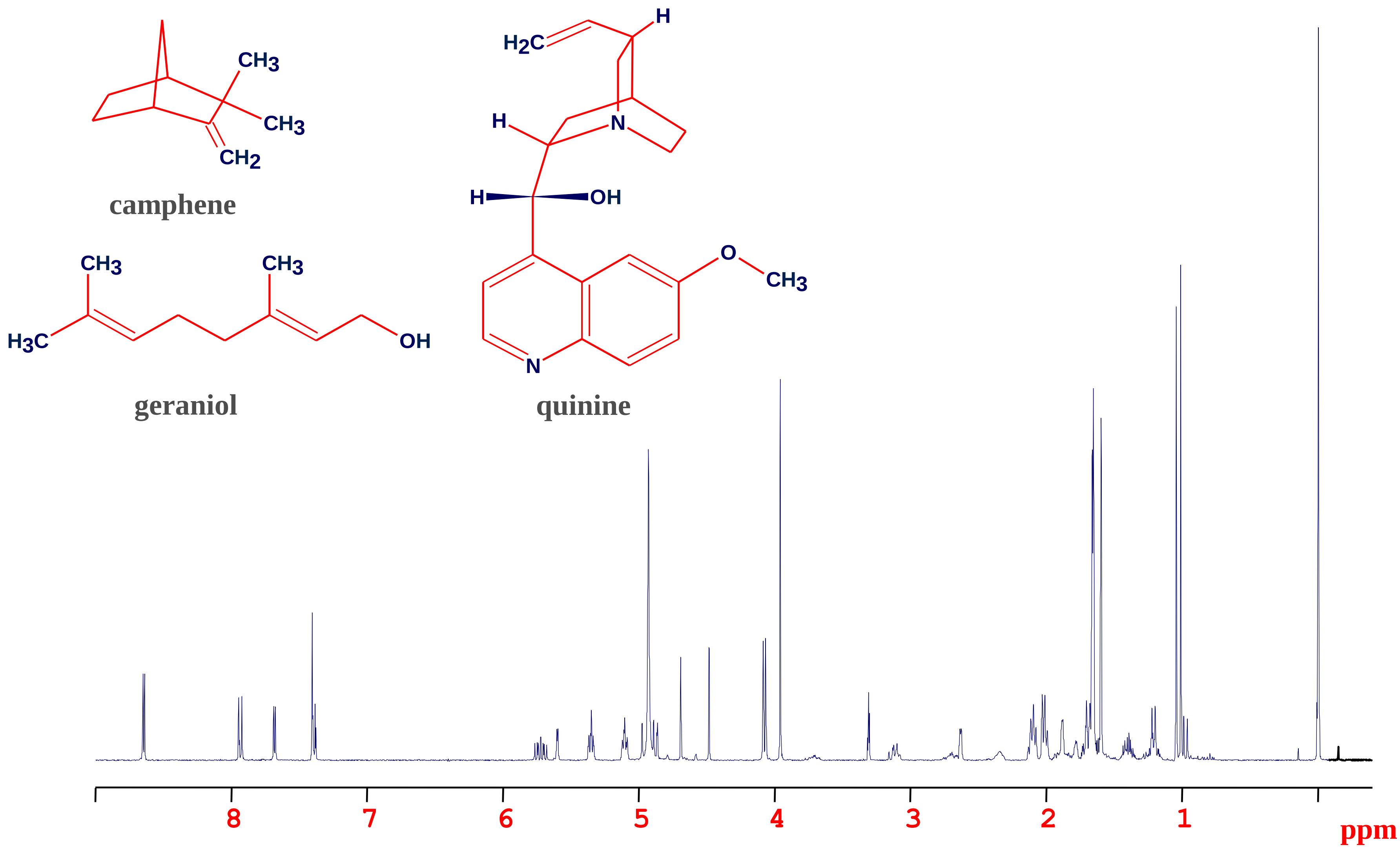
Shows H-1” is connected to C-5’. Connects Sugar-sugar attachment!!



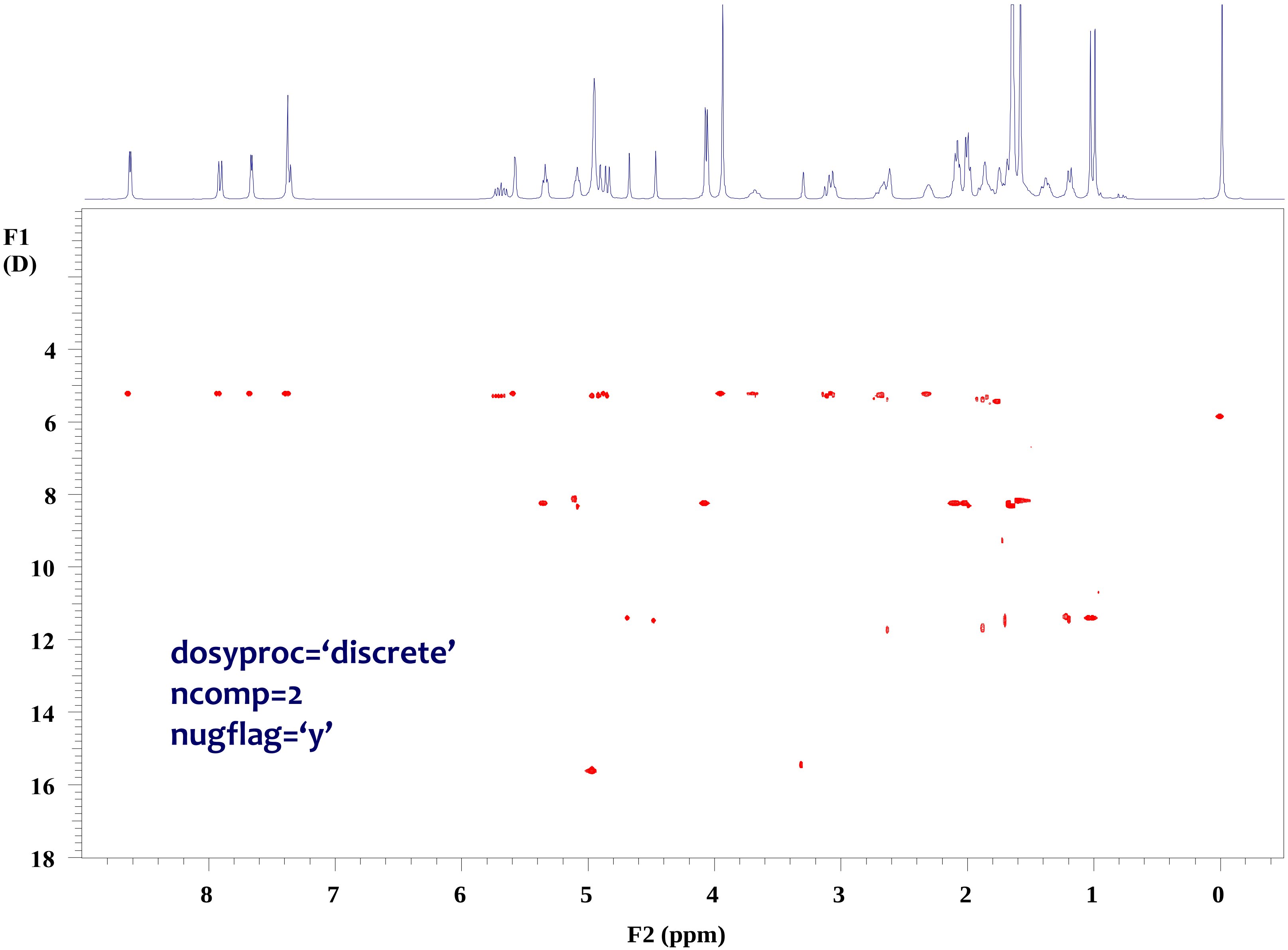
DOSY – Diffusion-Ordered Spectroscopy

In simplified terms.. Pulsed field gradient methods can allow the separation of molecules of differing sizes into distinct NMR spectra without requiring an actual chemical separation.

Structures and ^{1}H spectrum of the QGC mixture (in CD_3OD)

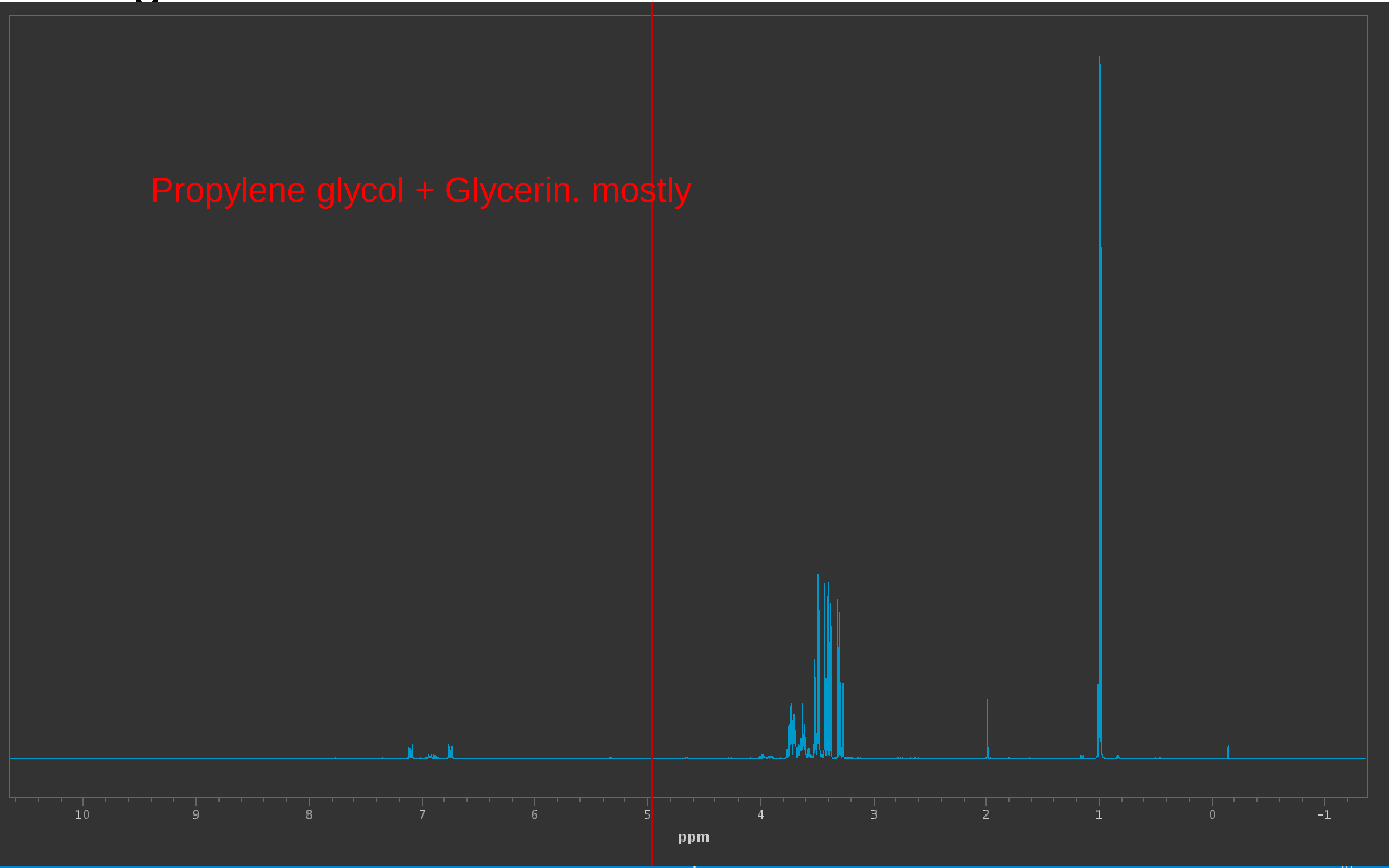


2D DOSY (Doneshot) of the QGC mixture

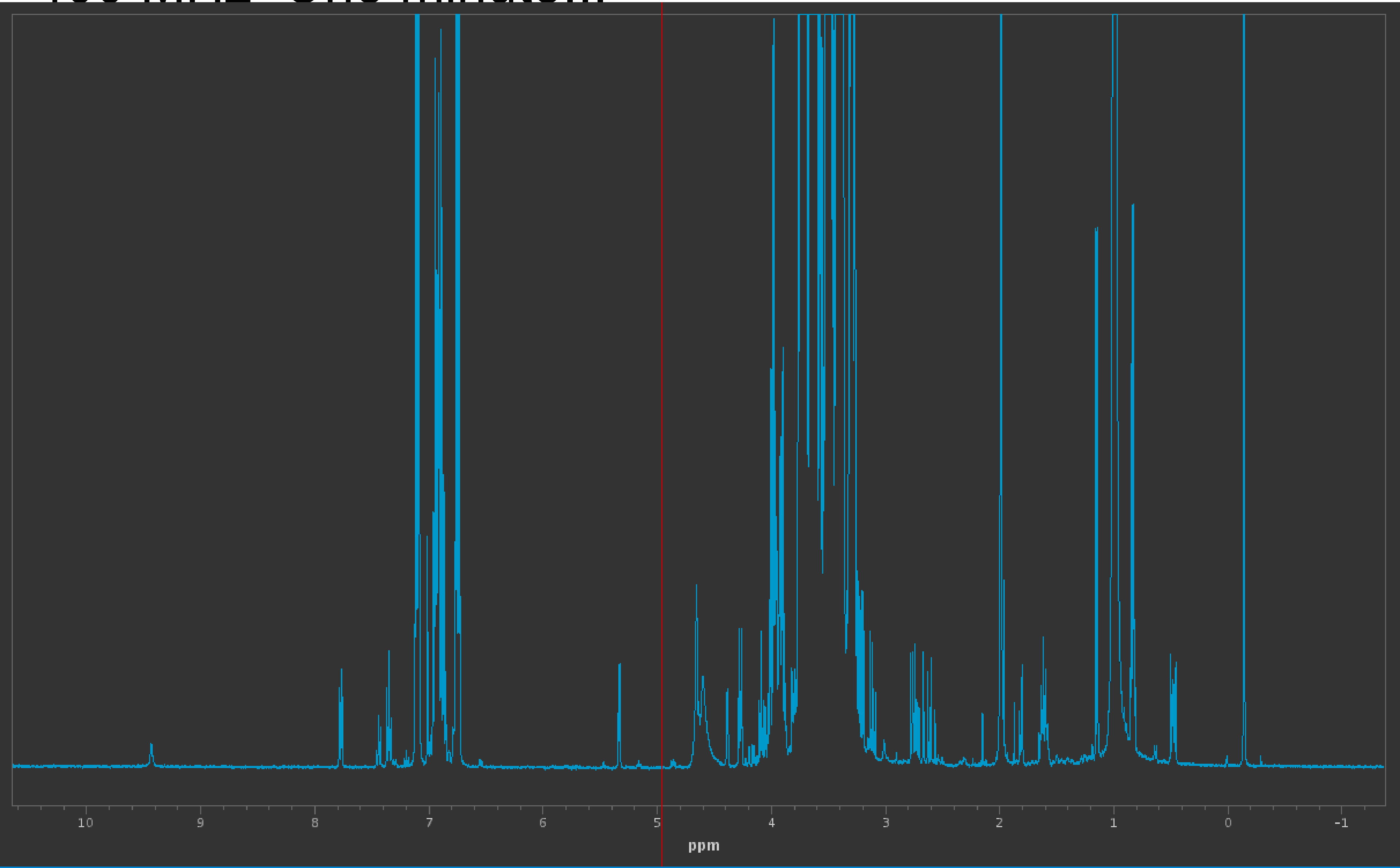


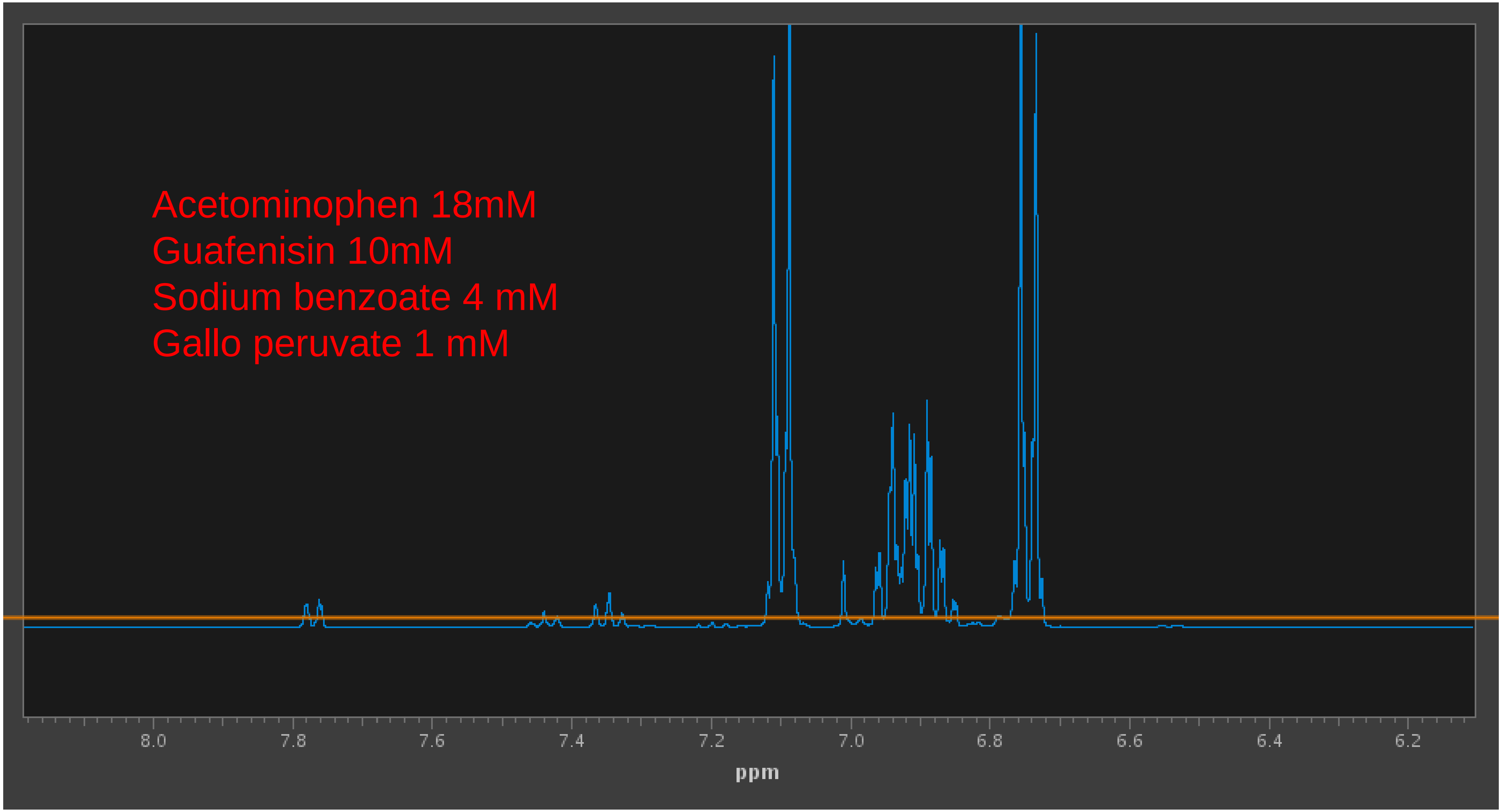
dosyproc='discrete'
ncomp=2
nugflag='y'

Cough Medicine – 150ul in d2o. Presat the water.



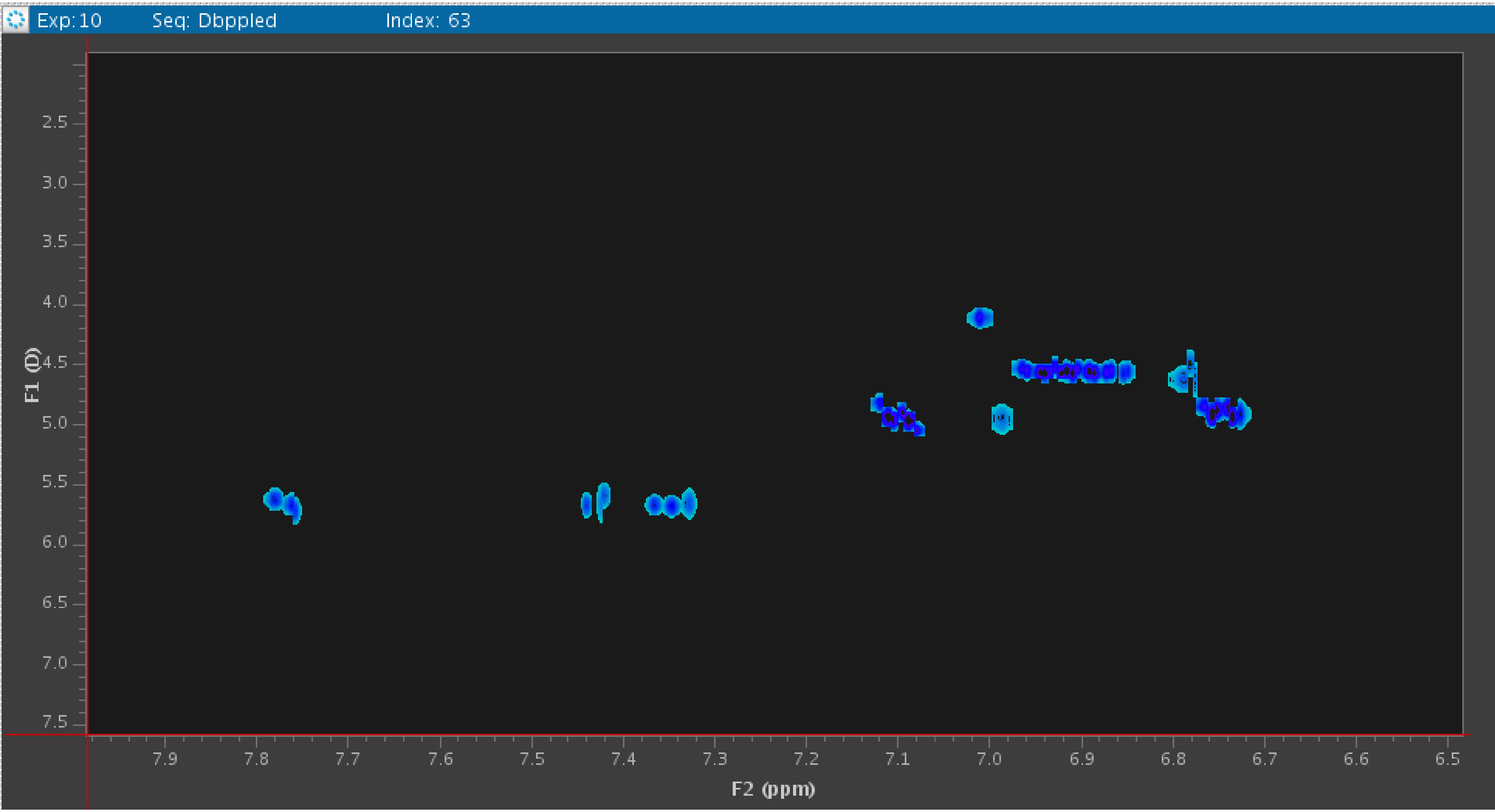
400 MHz One minute...





Acetominophen 18mM
Guafenisin 10mM
Sodium benzoate 4 mM
Gallo peruvate 1 mM

Big D 80ms little D 4ms Dbbpled – 16 G/cm max.



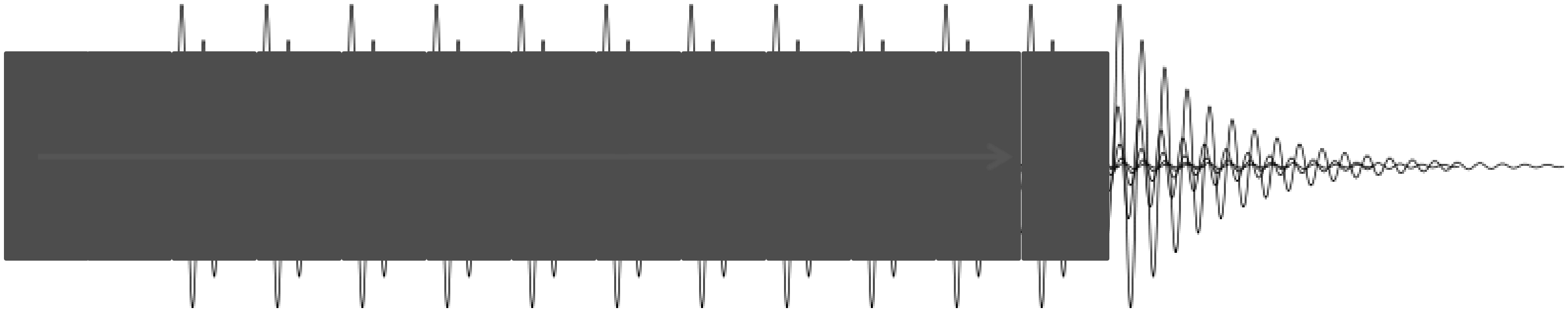
Conventional Sampling in 2D Spectroscopy

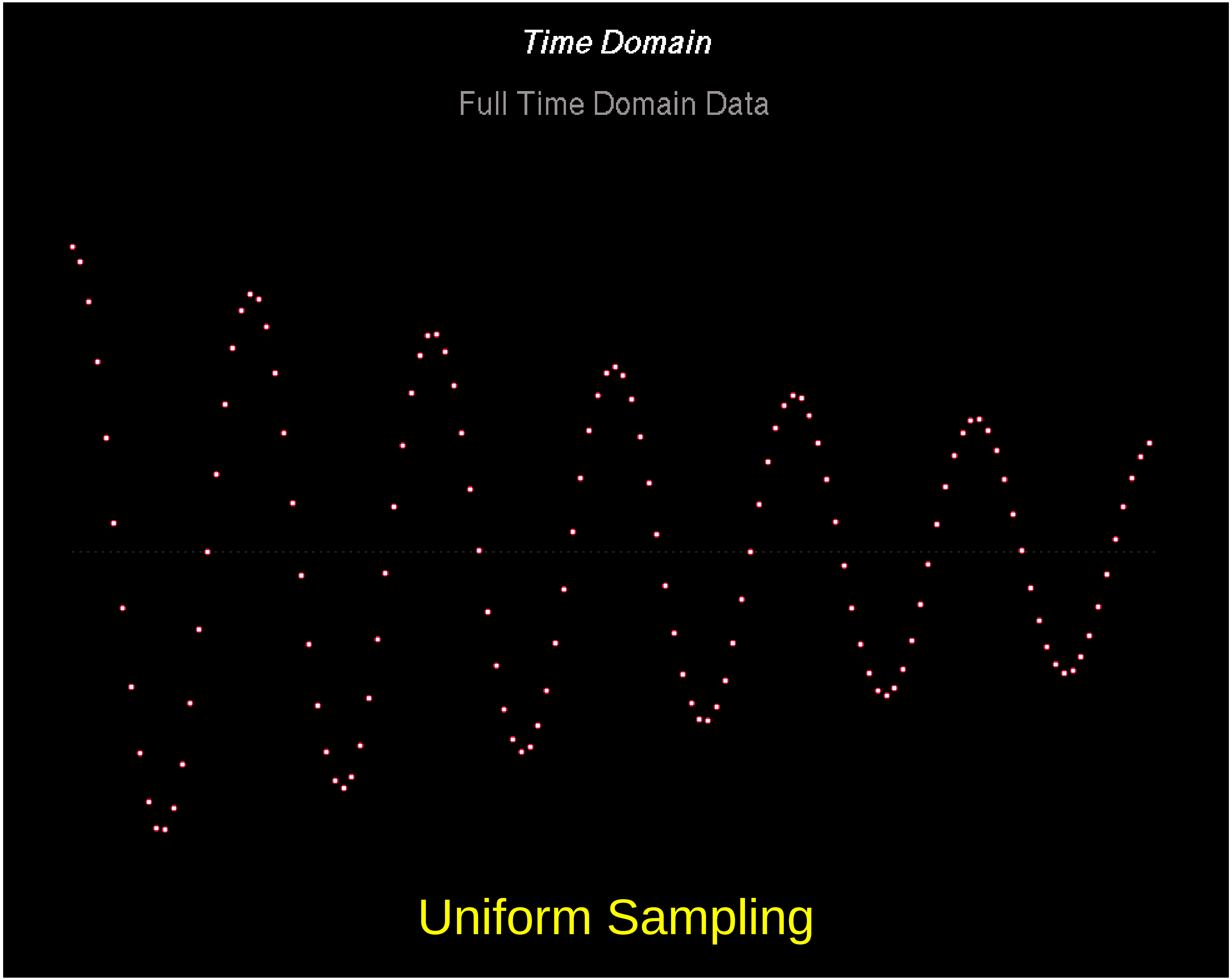
- t_1 period is incremented linearly (uniformly):

  0, 1/sw1, 2/sw2,…,(ni-1)/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 (ni-1)/sw1

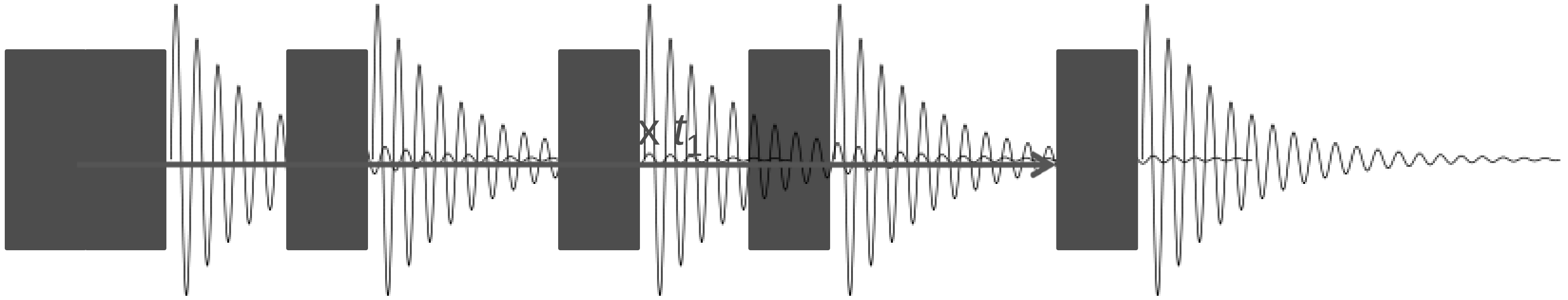




Uniform Sampling

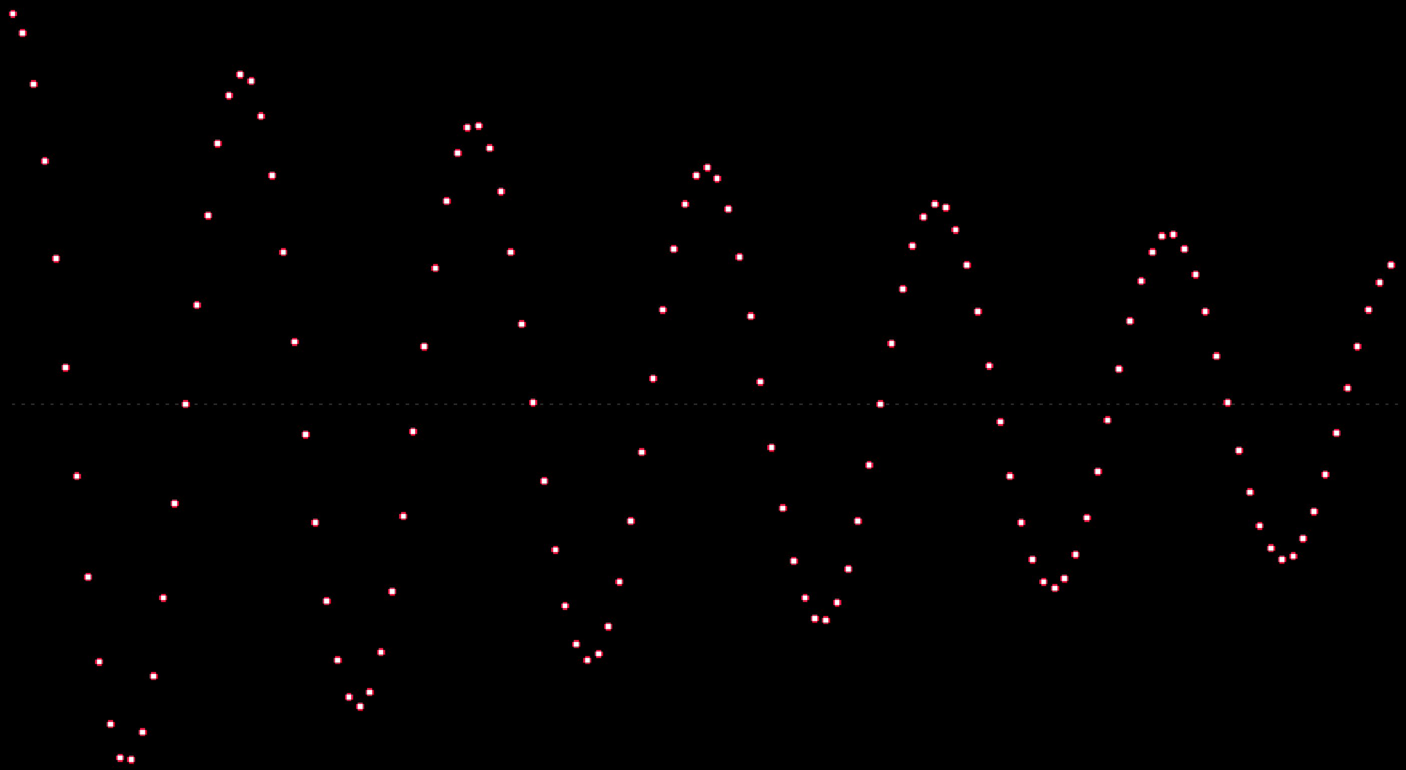
Do we need to sample every t_1 increment?

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

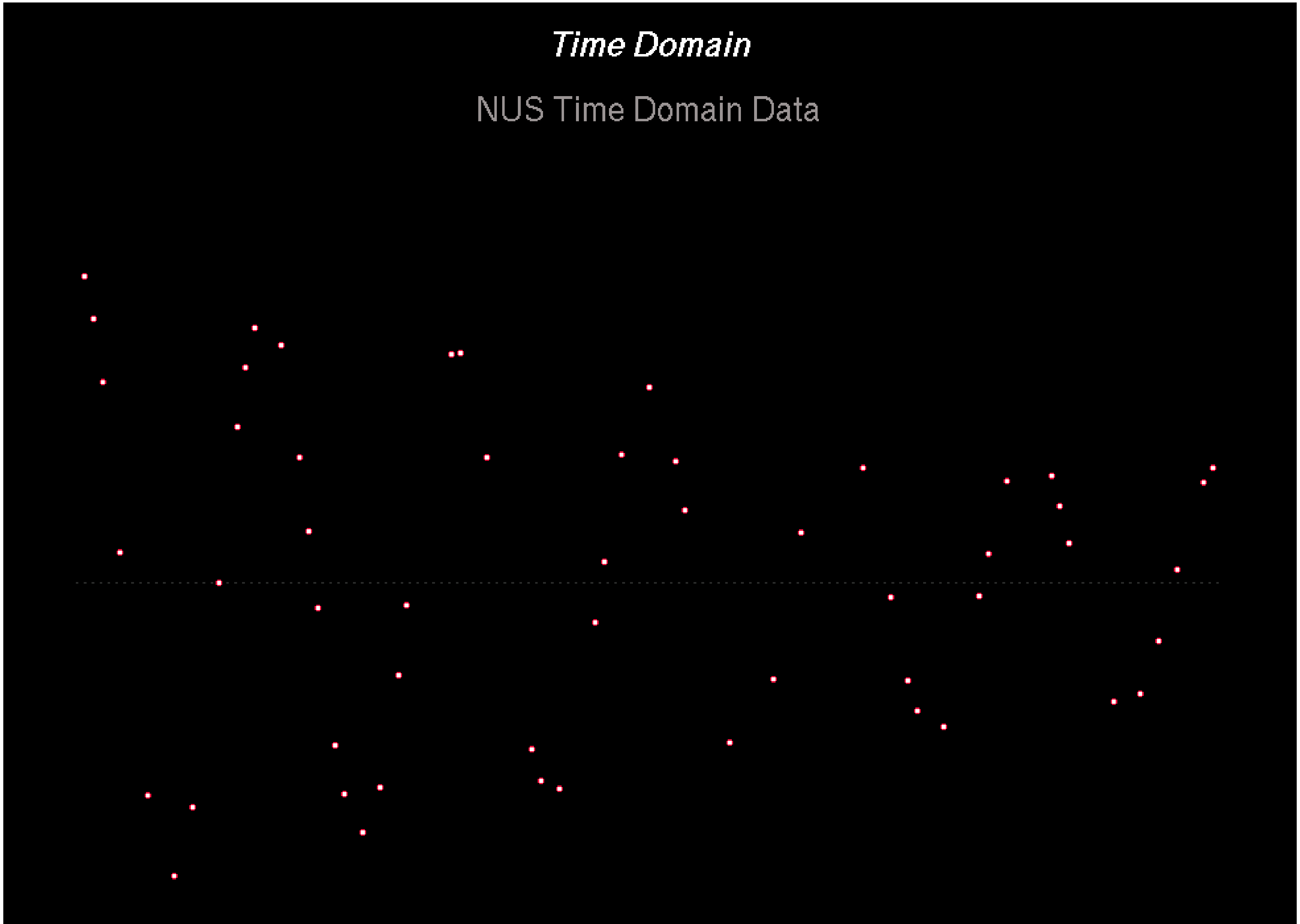


Time Domain

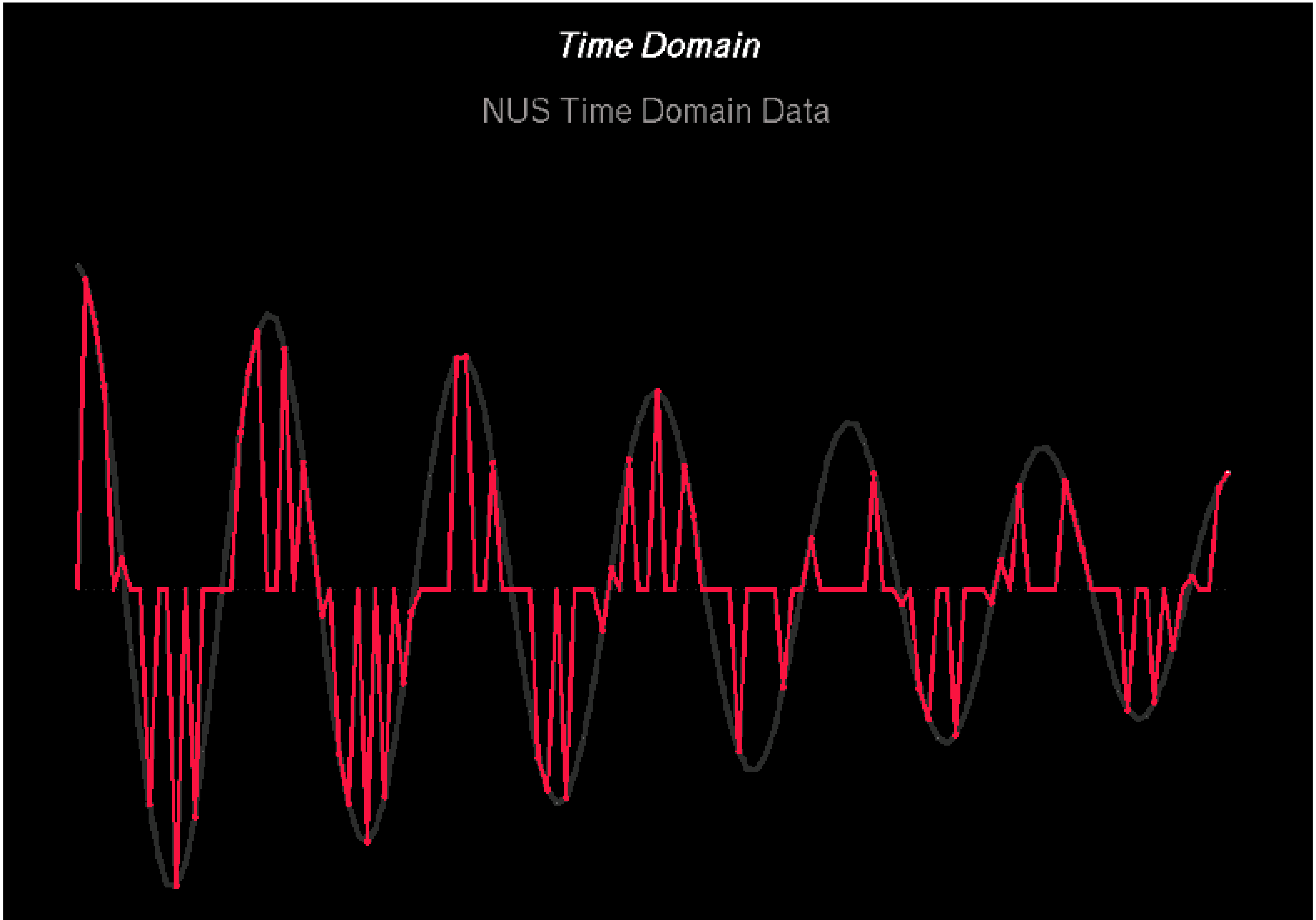
Full Time Domain Data



Uniform Sampling



Non-Uniform Sampling: Skip a Fraction of the Points

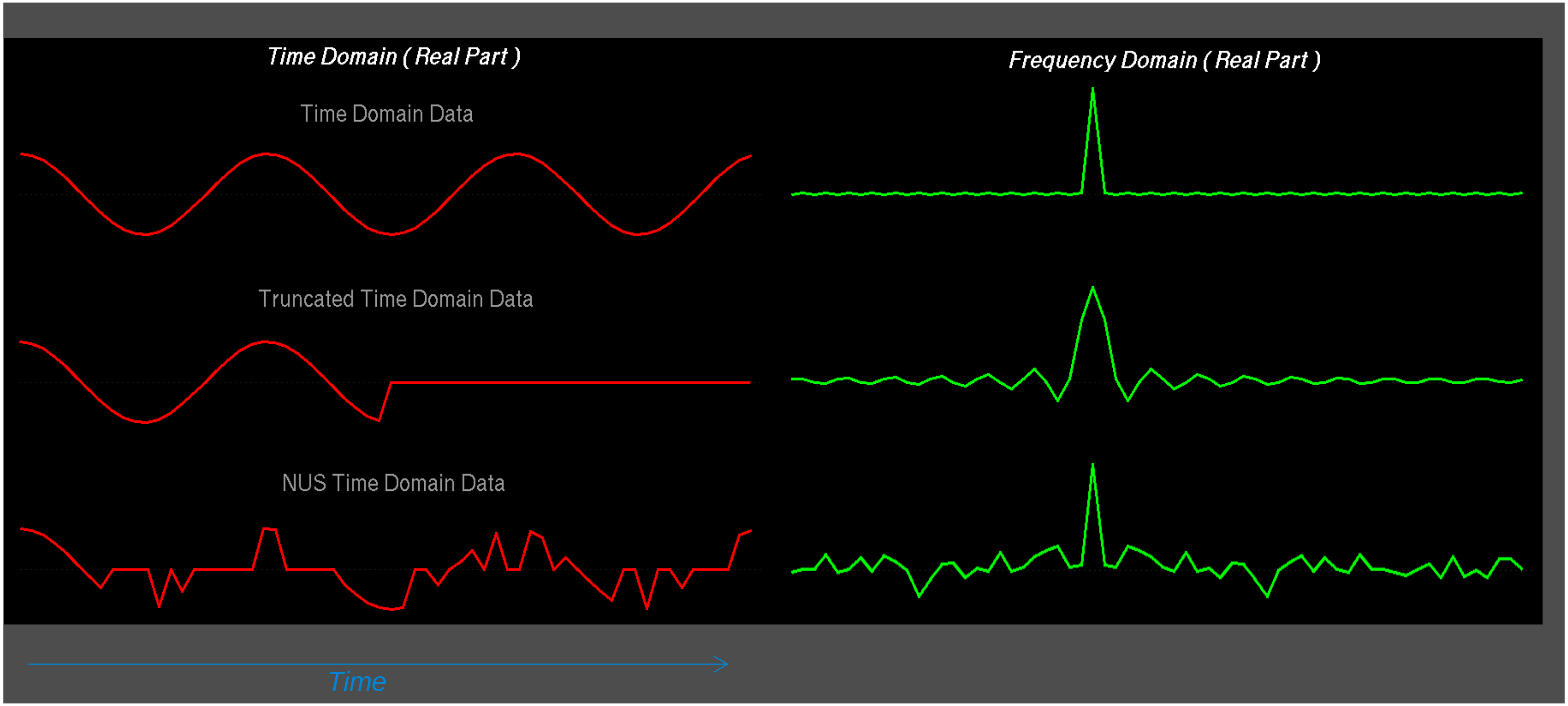


Non-Uniform Sampling:

for Fourier Transform, Replace Missing Points with Zeros

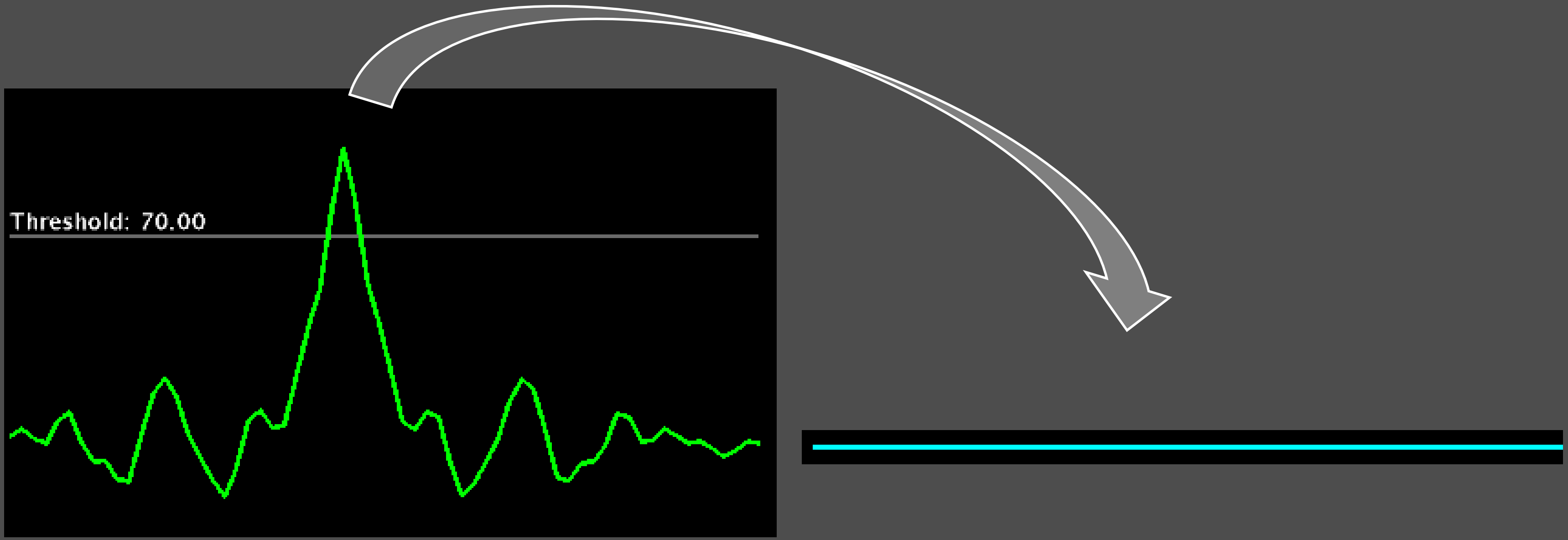
Linear versus Non-Uniform Sampling

Linear Sampling: Broad Line and Periodic Truncation Wiggles
Non-Uniform Sampling: Narrow Line and Random Noise-Like Artifacts



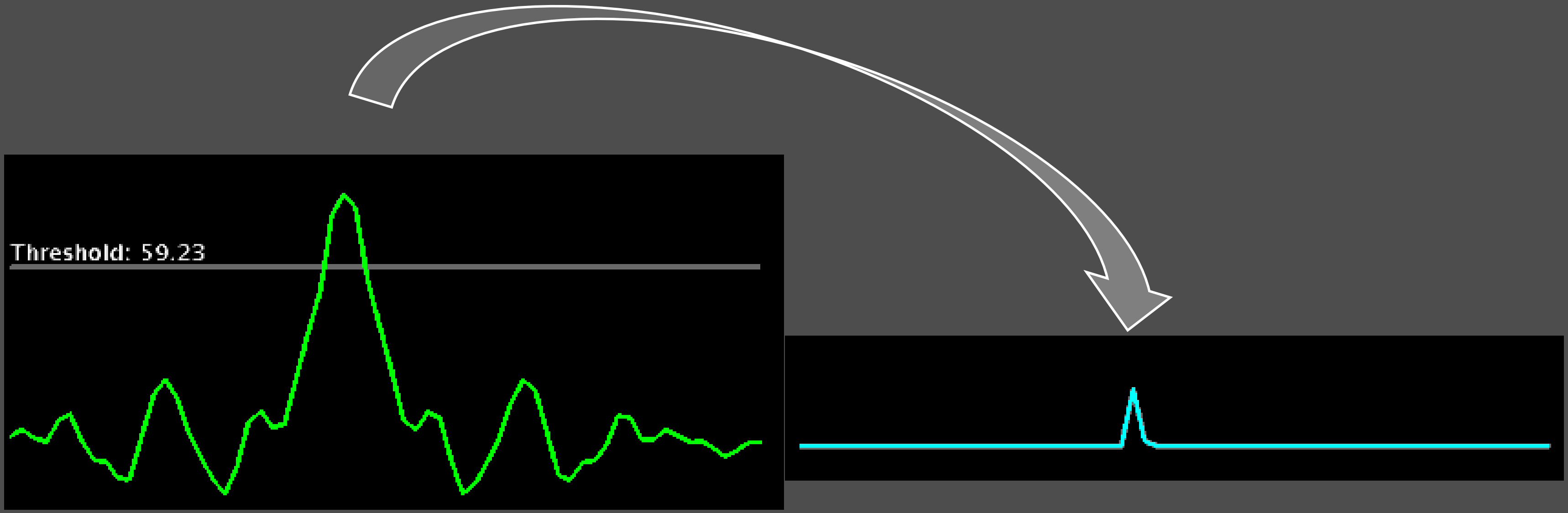
Non Uniform Sampling

IST – Iterative Soft Thresholding



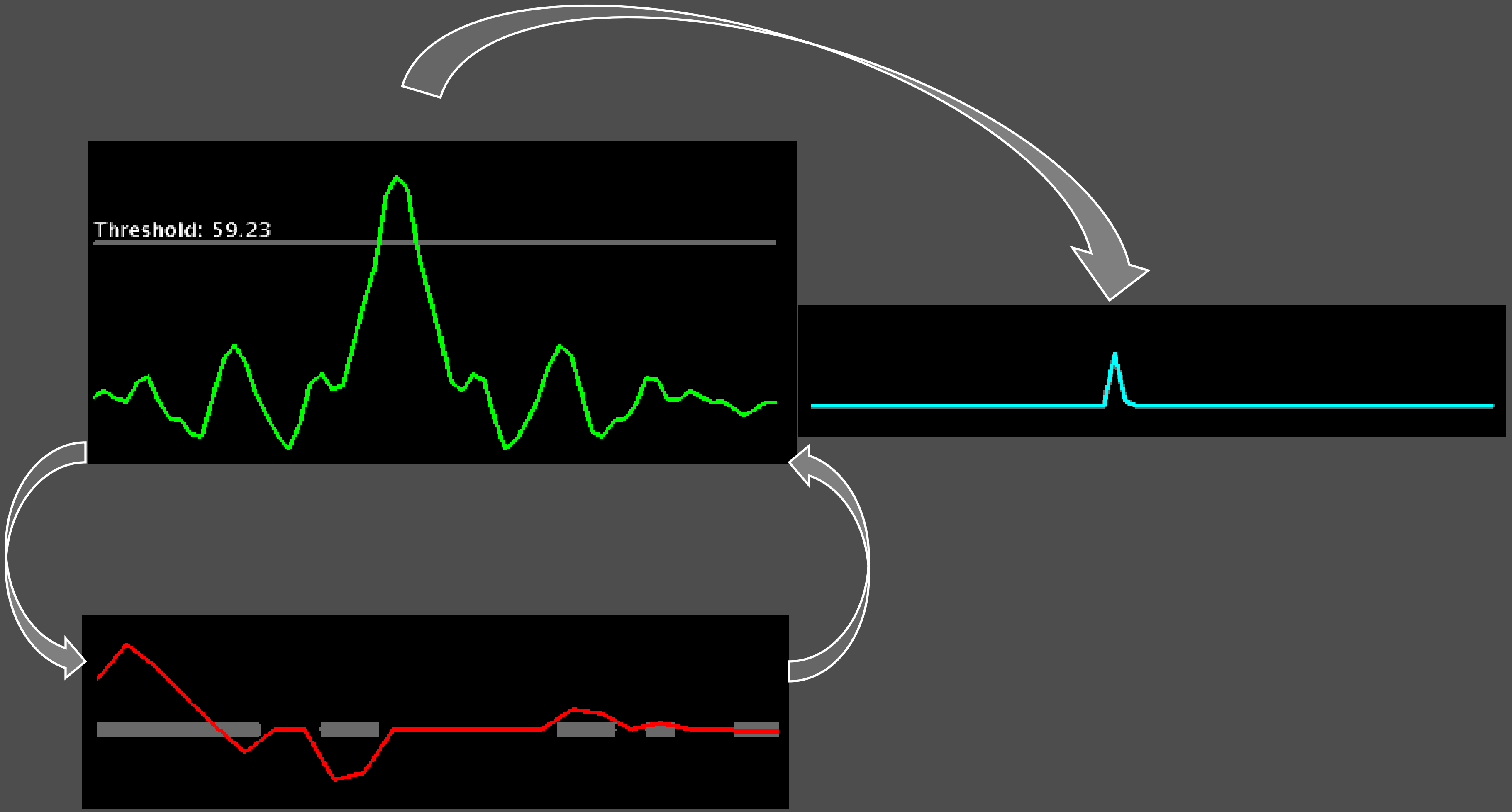
Non Uniform Sampling

IST – Iterative Soft Thresholding



Non Uniform Sampling

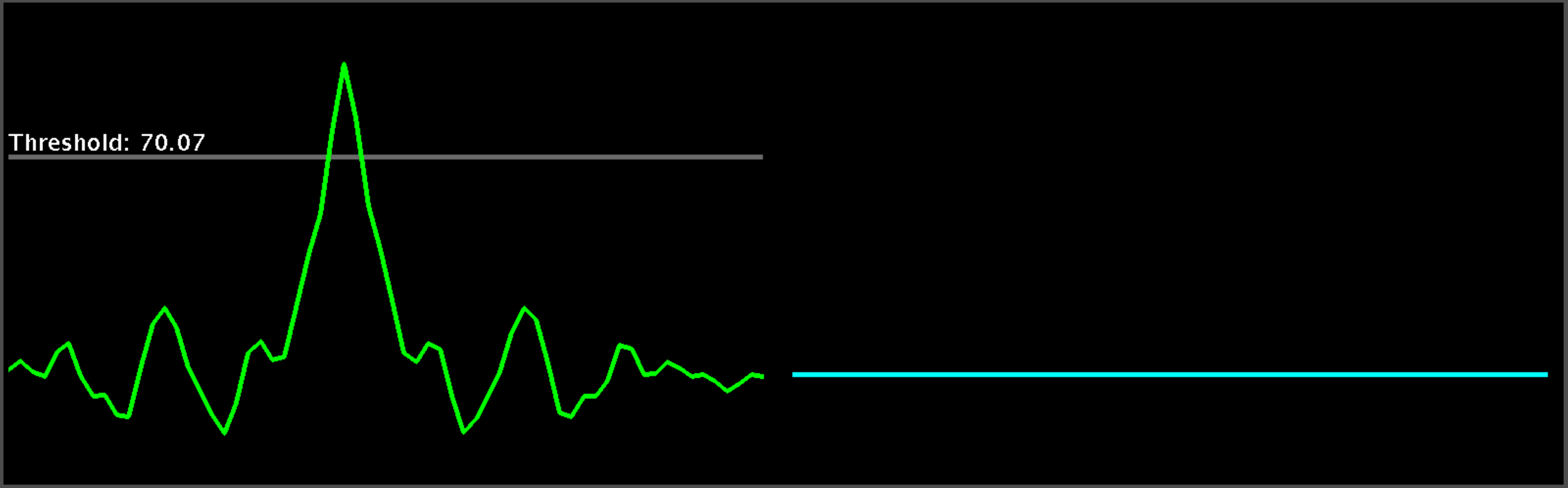
IST – Iterative Soft Thresholding



Non Uniform Sampling

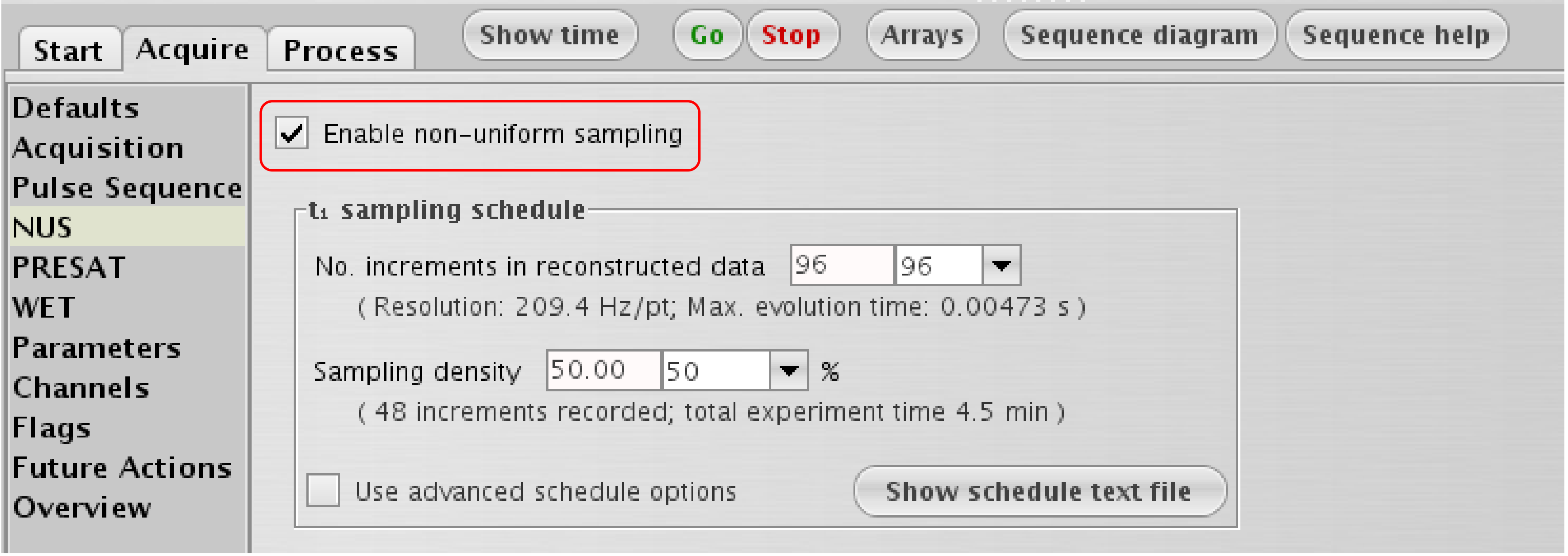
IST – Iterative Soft Thresholding

Fourier Spectrum **IST Spectrum**



Turning it on is just a mouse click

- NUS “Acquire” panel



☑ Enable non-uniform sampling

t_1 sampling schedule

No. increments in reconstructed data 96 96

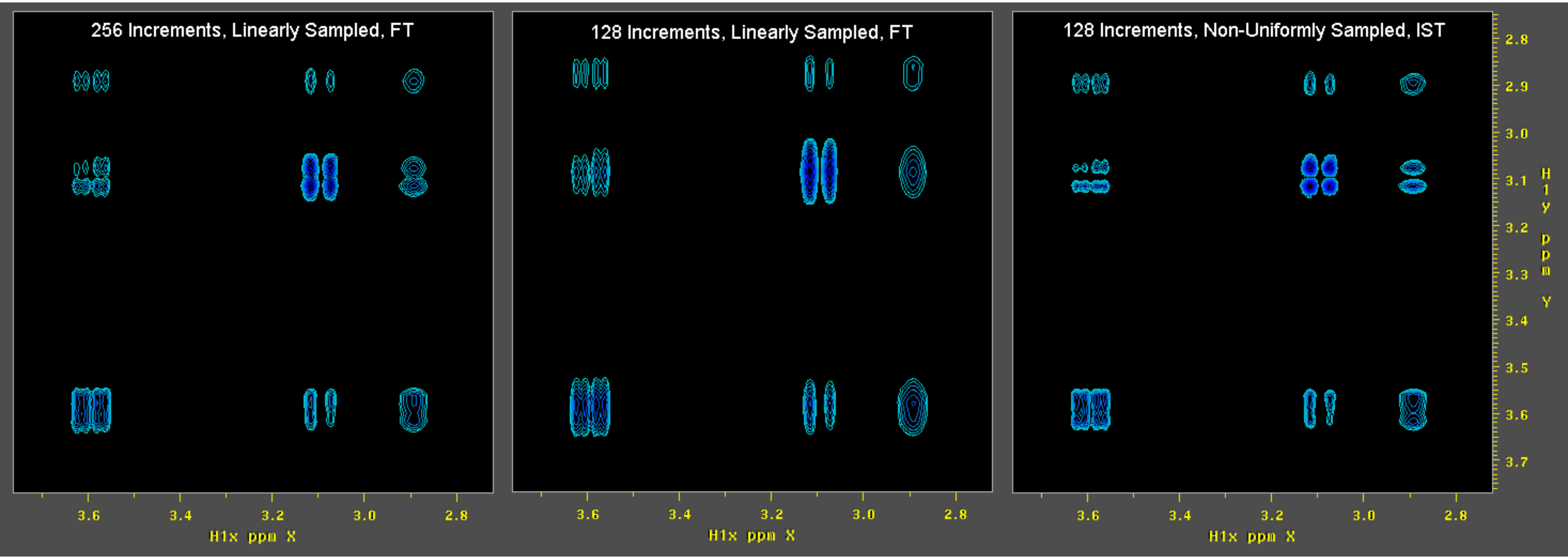
(Resolution: 209.4 Hz/pt; Max. evolution time: 0.00473 s)

Sampling density 50.00 50 %

(48 increments recorded; total experiment time 4.5 min)

☐ Use advanced schedule options

Example Results

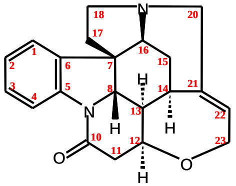


Conventional Linearly-Sampled

Conventional – Half of Original Measurement Time

NUS – Half of Original Measurement Time

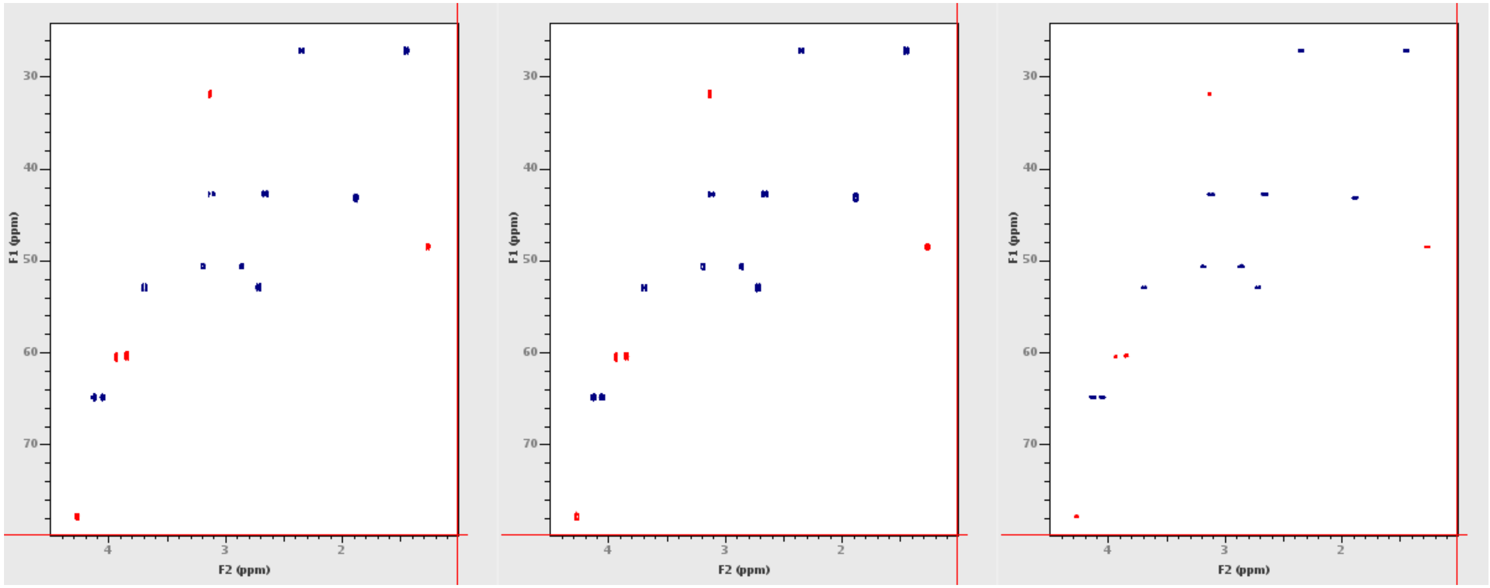
Non-Uniform Sampling (NUS) – gHSQCAD II.



Sample: Strychine (MW: 334, F:$C_{21}H_{22}N_2O_2$), concentration: 20 mmolar (6.84 mg/ml), solvent: $CDCl_3$

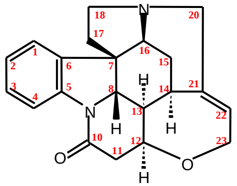
HSQCAD spectra of strychnine (aliphatic region)
sw1=11300 (90 ppm), phase=1,2 nt=2

NUS sampling | Conventional sampling | NUS sampling



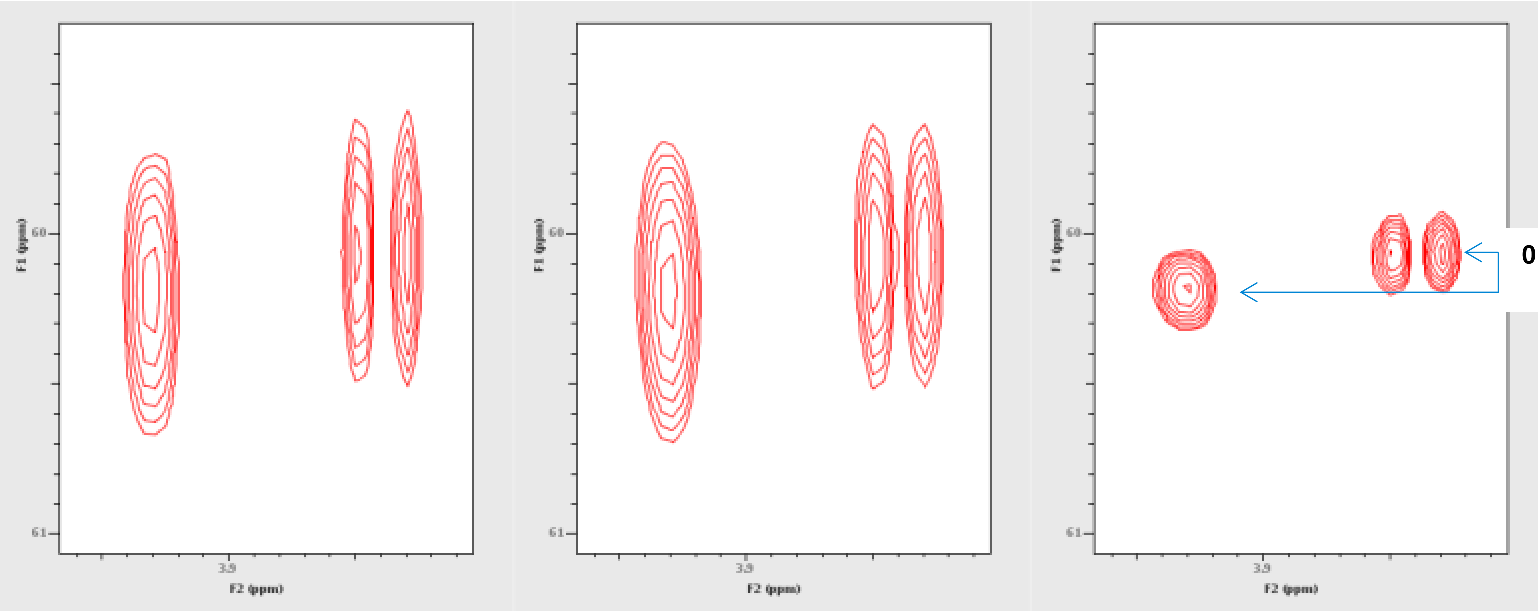
ni (max) =	128	← gain time	128	gain resolution →	512
SD =	25% (32)		100% (128)		25% (128)
Exp. Time =	4 min		16 min		16 min

Non-Uniform Sampling (NUS) – gHSQCAD IV.



Sample: Strychine (MW: 334, F:$C_{21}H_{22}N_2O_2$), concentration: 20 mmolar (6.84 mg/ml), solvent: $CDCl_3$

HSQCAD spectra of strychnine (aliphatic region – expansion #2)
sw1=11300 (90 ppm), phase=1,2 nt=2

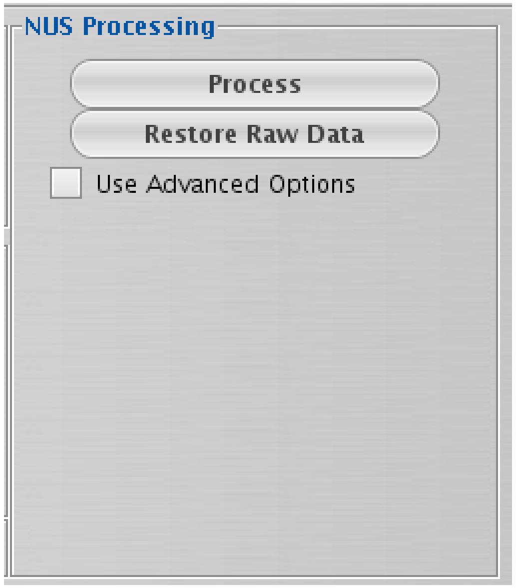


0.11 ppm
(14 Hz)

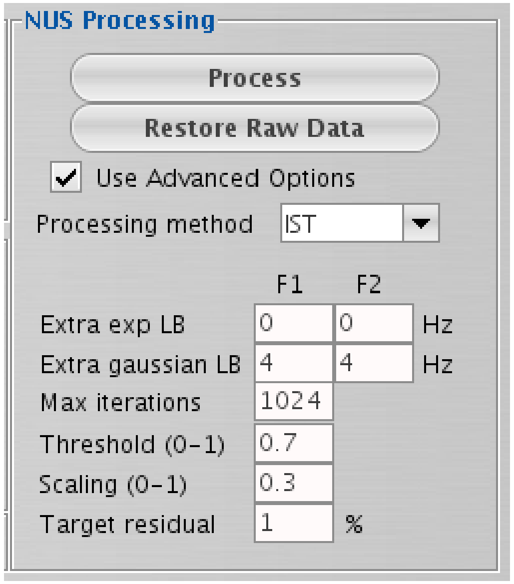
ni (max) =	128		128		512
SD =	25% (32)	← gain time	100% (128)	gain Resolution →	25% (128)
Exp. Time =	4 min		16 min		16 min

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



☐ Use Advanced Options



☑ Use Advanced Options

Processing method: IST

	F1	F2	
Extra exp LB	0	0	Hz
Extra gaussian LB	4	4	Hz
Max iterations	1024		
Threshold (0-1)	0.7		
Scaling (0-1)	0.3		
Target residual	1		%

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! ☺

Summary

NMR Applications are incredibly broad and diverse.

Currently very sophisticated and powerful NMR experiments exist to allow the determination of both primary and secondary structure for very complex molecules.

NMR is a powerful complementary addition to Mass Spectrometry and optical analytical methods.

Modern data acquisition and processing schemes such as linear prediction and non-uniform sampling can greatly reduce experiment time and are very easy to use and applicable to almost every 2D experiment.

Thank You!!!

Once again I apologize for the many omissions and I will try to answer any questions!