JEOL

Solutions for Innovation

Poking fun at helpless Atoms –

Towards Consistency by a cut and paste solution with Delta Pulse Programming.

Early Lesson 1 – you get what you ask for…

- Phase = {0, 2, 1, 3} ; will get you zero, two, one and three degrees!

- Rather..
- Phase = {0, 180, 90, 270};

- Very handy for times when phases of 30, 45, 60, 135 might be desired. *Delta is a decidedly modern approach to such things.*

Delay and pulse Events – *consistency!*

time; … simple delay of duration in variable time..

time,(options); … an event of duration "time" with device defined in options..

x_pulse,(obs.gate, obs.phs.phase_x, obs.atn.x_atn); … simple obs channel pulse

grad_1,(fgz.gate,fgz.amp.grad_1_amp,fgz.shape.grad_shape); … Z-PFG pulse..

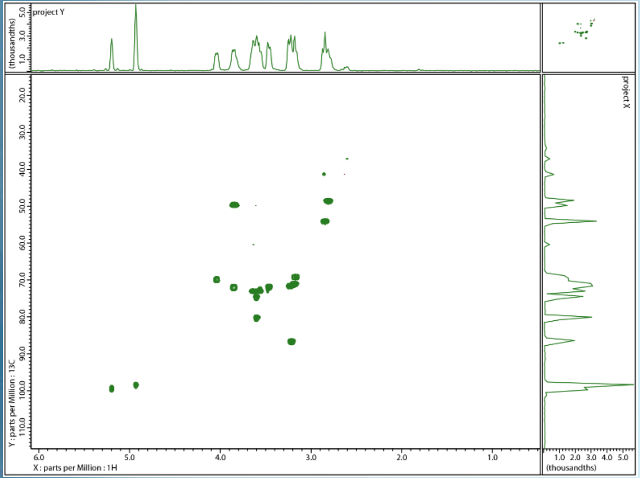
T/2 - (tau - epsilon) - y_pulse - tau - t1 ystep -1/(2*y_sweep),
(irr.gate.irr.rf_mod.irr_noise, irr.atn.irr_atn_dec);

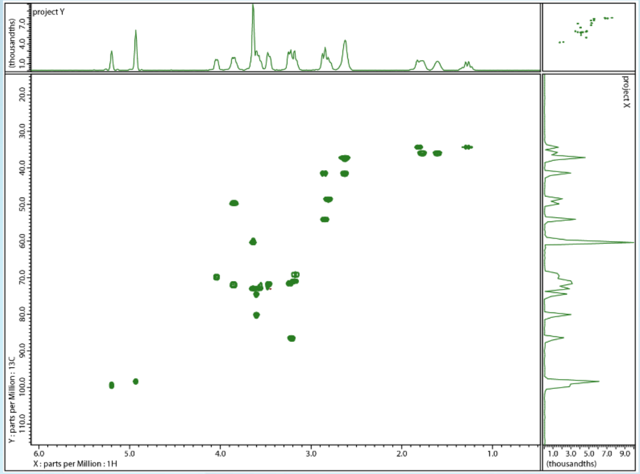
gHSQCAD & HSQCAD Edit Options -> Easy custom interface

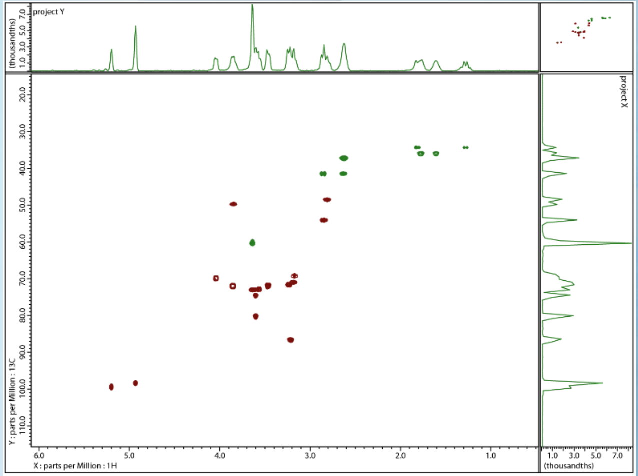
Pulse Delay and Options	
relaxation_delay	1.3[s]
multiplicity_edit_mode	CH_only
sspulse	☑
tango	☐

Pulse Delay and Options	
relaxation_delay	1.3[s]
multiplicity_edit_mode	off
sspulse	☑
tango	☐

Pulse Delay and Options	
relaxation_delay	1.3[s]
multiplicity_edit_mode	up_down
sspulse	☑
tango	☐



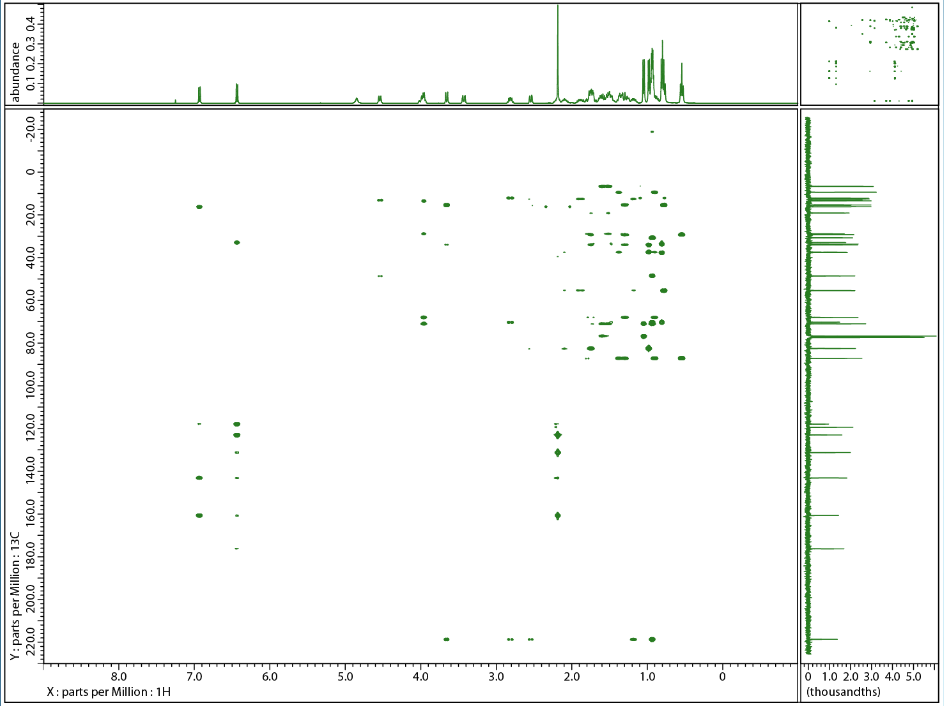




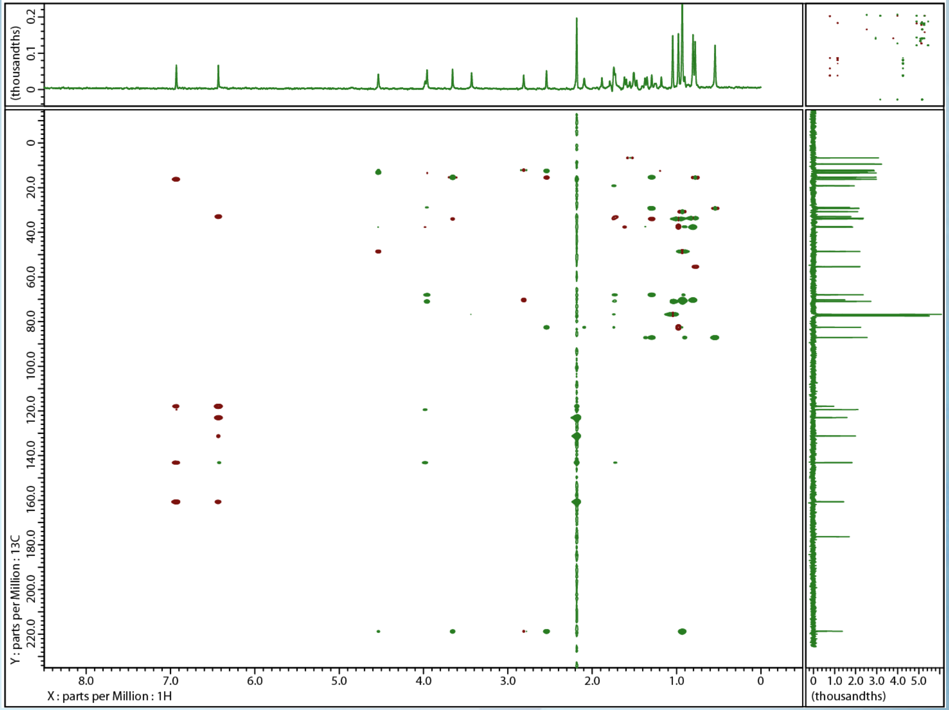

```
multiplicity_edit_mode => "up_down" ,("up_down", "off", "CH_only");

edit_multiplier    = if multiplicity_edit_mode = "up_down" then 2.0 else
                     if multiplicity_edit_mode = "CH_only" then 1.0 else
                     2.0;

multiplicity_edit = if multiplicity_edit_mode = "off" then FALSE else
                    TRUE;
```

Core experiment #2..

Vague "up/down" information but higher resolution



gHMBCAD av in F2 / phased F1

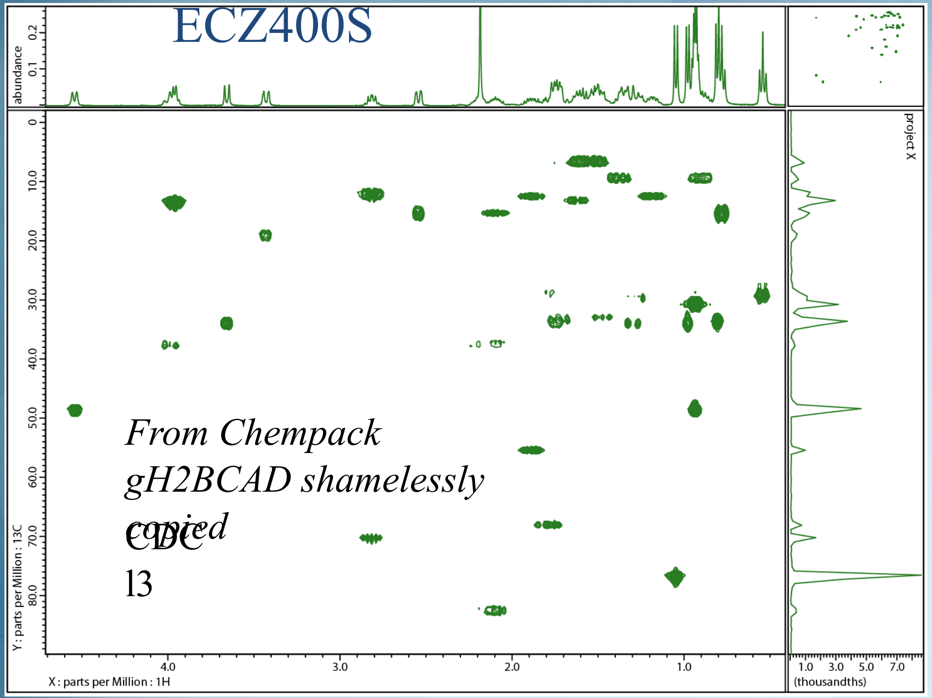


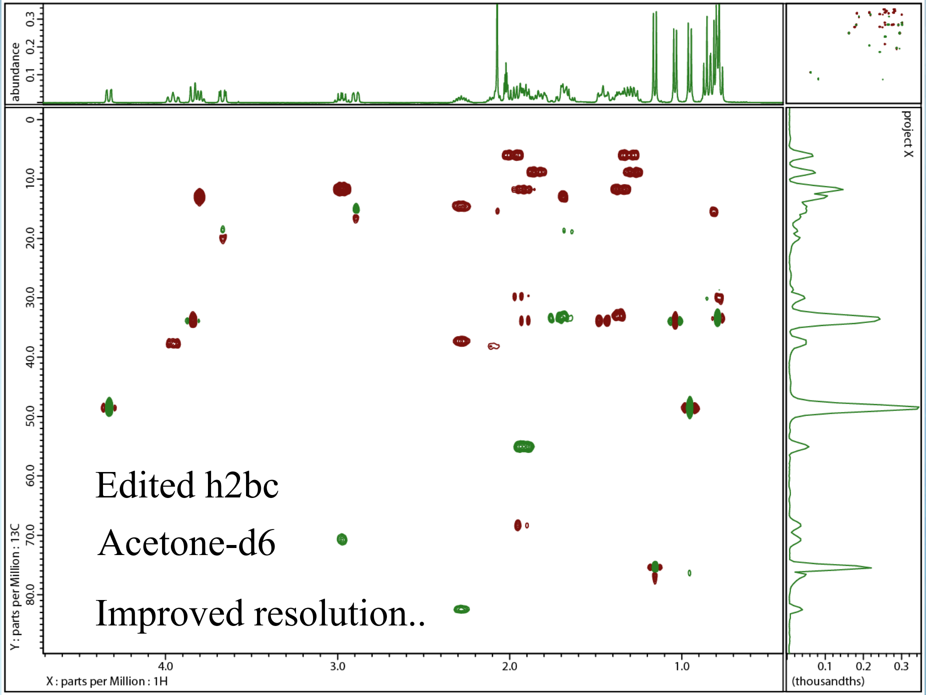
LR-HSQMBC with PSYCHE 1D as projection

Phased both F1/F2

{13C}

Asking around.. H2BC – under utilized! Why? Lasalocid-A example





Constant-time experiment
Compliments simple COSY..!

N. T. Nyberg, J. O. Duus, O. W. Sorensen, JACS, 127, 6154-6155, 2005.
N. T. Nyberg, J. O. Duus, O. W. Sorensen, Mag Res Chem 43, 971-974, 2005.

Experiment to Probe edited 1*J*cc and n*J*cc while observing 1H

Journal of Magnetic Resonance 236 (2013) 126–133

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

ELSEVIER

JMR

CrossMark

Broadband inversion of $^{1}J_{CC}$ responses in 1,*n*-ADEQUATE spectra ☆

Mikhail Reibarkh [a,*], R. Thomas Williamson [a], Gary E. Martin [b,*], Wolfgang Bermel [c]

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[b] Merck Research Laboratories, Discovery and Preclinical Sciences, Process and Analytical Chemistry, Structure Elucidation Group, Summit, NJ 07901, USA
[c] BrukerBioSpin GmbH, Silberstreifen, 76287 Rheinstetten, Germany

(1) *1,1-ADEQUATE:*
$f(J) = \sin^2(J * \pi * \Delta)$.
Δ is usually optimized to match J of ~40–60 Hz.

(2) *1,n-ADEQUATE:*
$f(J) = \sin^2(J * \pi * \Delta)$.
Δ is usually optimized to match J of ~5–7 Hz.

(3) *Single-optimization* $^{1}J_{cc}$*-edited 1,n-ADEQUATE:*
$f(J) = \sin(J * \pi * ((2 * m + 1)/(2 * {}^{1}J_{opt}) + \delta)) *$
$\sin(J * \pi * ((2 * m - 1)/(2 * {}^{1}J_{opt}) + \delta))$.

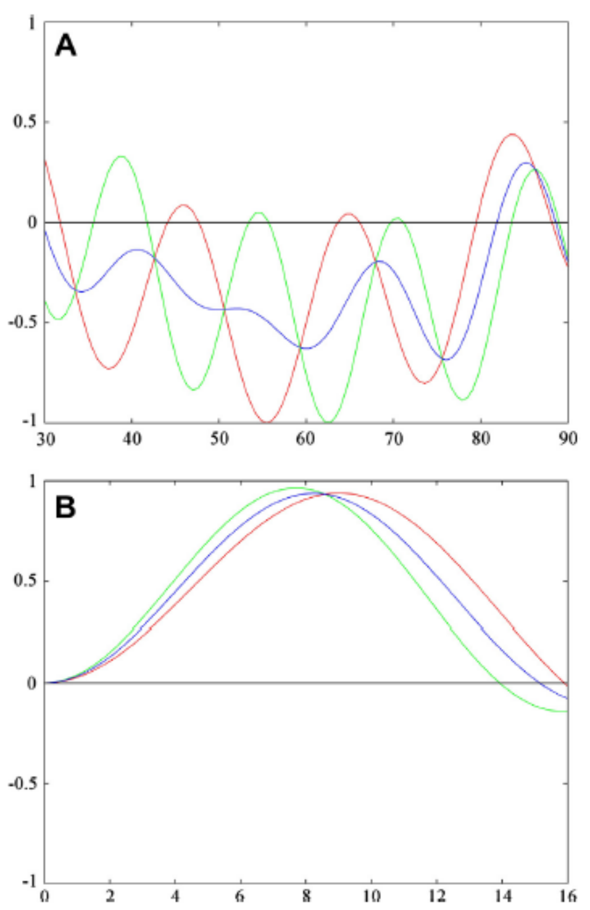
Here, m is the truncated integer of $^{1}J_{opt}/(2 * {}^{n}J_{opt})$. $^{1}J_{opt}$ and $^{n}J_{opt}$ are the optimization values for $^{1}J_{CC}$ and $^{n}J_{CC}$, respectively. δ Is the sum of all additional delays in the pulse sequence (primarily due to the length of shaped pulses).

(4) *Dual optimization* $^{1}J_{cc}$*-edited 1,n-ADEQUATE:*
$f(J) = 0.5 * [\sin(J * \pi * ((2 * m_A + 1)/(2 * {}^{1}J_A) + \delta)) *$
$\sin(J * \pi * ((2 * m_A - 1)/(2 * {}^{1}J_A) + \delta)) +$
$\sin(J * \pi * ((2 * m_B + 1)/(2 * {}^{1}J_B) + \delta)) *$
$\sin(J * \pi * ((2 * m_B - 1)/(2 * {}^{1}J_B) + \delta))]$.

nJ1J edited adequate has poor sensitivity *but*
With dual optimization the sensitivity is *greatly* improved!
1-1-Adequate clearly defines 1JCC connectivities
N-J-Adequate adds long-range connectivities

JEOL

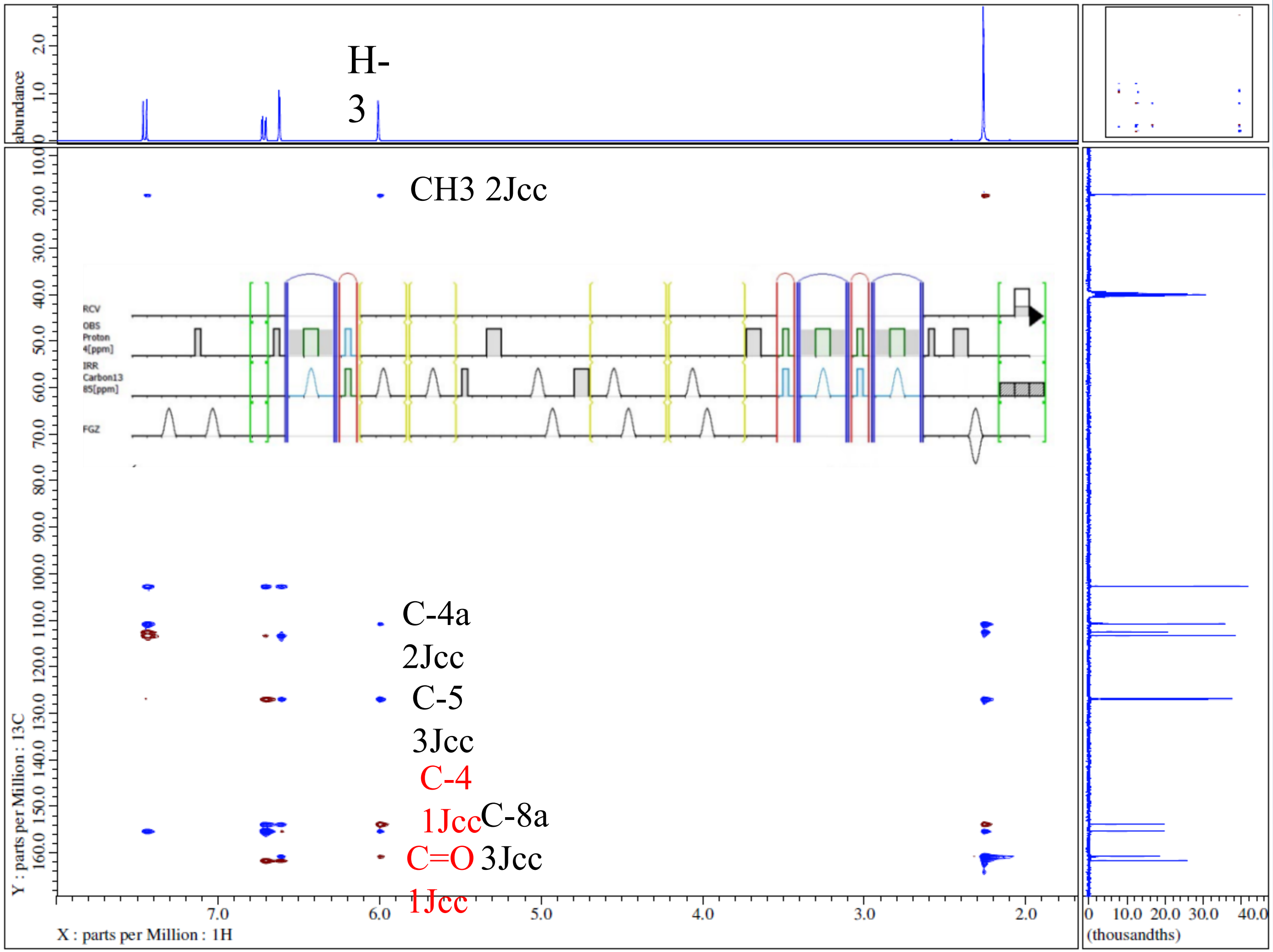
Fig. 2. Calculated amplitude transfer curves governing the response intensity of an inverted $^{1}J_{CC}$ 1,*n*-ADEQUATE experiment modified for dual optimization of the carbon–carbon delays (blue). The two optimizations were $^{1}J_{CC} = 57$ with $^{n}J_{CC} = 9.5$ Hz (red), and $^{1}J_{CC} = 64$ with $^{n}J_{CC} = 8$ Hz (green). (A) This panel shows the response intensity of the $^{1}J_{CC}$ correlations as a function of the optimization. The blue trace shows the calculated, summed, and normalized response intensity across the optimization range from 30 to 90 Hz. One-bond correlations are inverted across the range from 29 to 82 Hz, which encompasses most commonly encountered one-bond carbon–carbon [34,35]. (B) This panel shows the response intensity for the $^{n}J_{CC}$ correlations as a function of the optimization across the range from 0 to 16 Hz. The blue trace shows the summed and normalized long-range carbon–carbon response intensity. This dual optimization pair gives usable response intensity (>0.5) across the range from approximately 4 to 12 Hz, which encompasses most structurally useful $^{n}J_{CC}$ in the authors' experience [20].

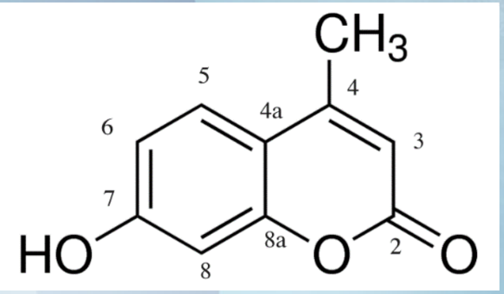


From J. Magn. Reson. 236 (2013) 126-133

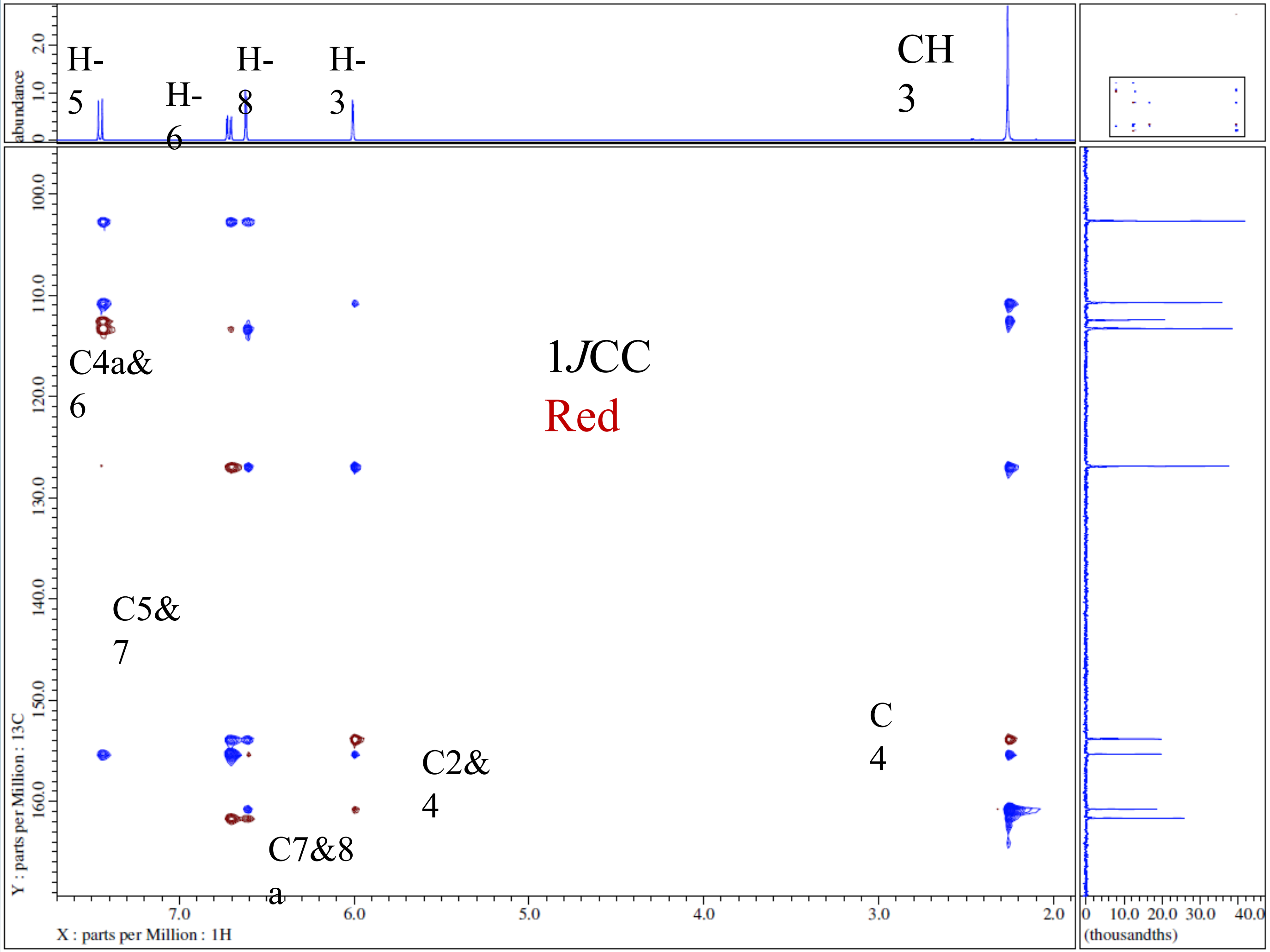
Dual-optimization greatly improves the 1-1 and the N-J receptivity.

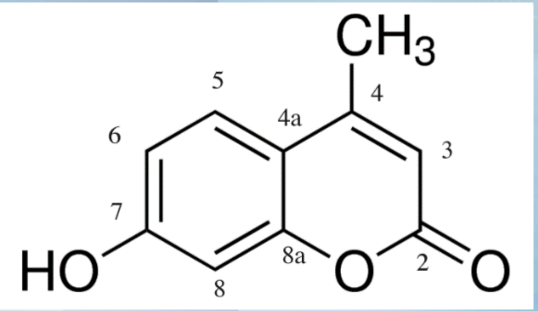
I simply cut/pasted the delay declarations from Bruker code and retained those variable names to avoid any possibility of confusion!





1JnJ-edited ADEQUATE with Dual 1/NJcc optimization


H-5
H-6
H-8
H-3
CH3
C4a&6
1JCC
Red
C5&7
C2&4
C4
C7&8a
abundance
Y : parts per Million : 13C
X : parts per Million : 1H
(thousandths)

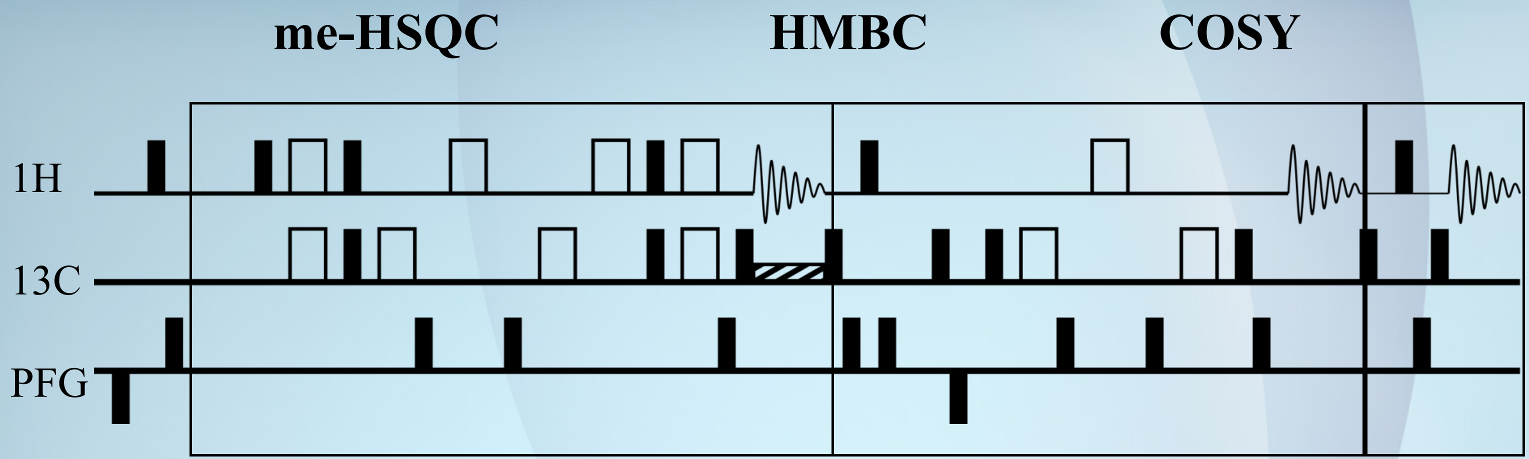

CH3
HO
O
O
5
4
4a
6
3
7
8a
2
8

(time to shamelessly steal some slides).

Dr. Adolfo Botana has done some interesting cut/paste activities..

Multi-2D data sets from single acquisition.. NOAH

NOAH *all 3 core experiments at once*

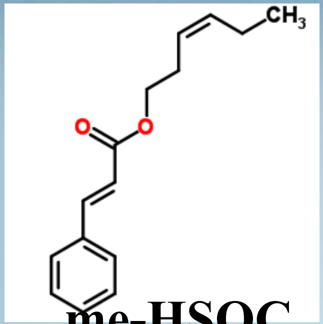


3 datasets or more from 1 experiment

Can easily be acquired by a Chemist with no NMR skills. In automation 3 3Ds are returned with no action required.

Ē. Kupče, T. D. W. Claridge, Angew. Chem. Int. Ed. 2017, 56, 11779.

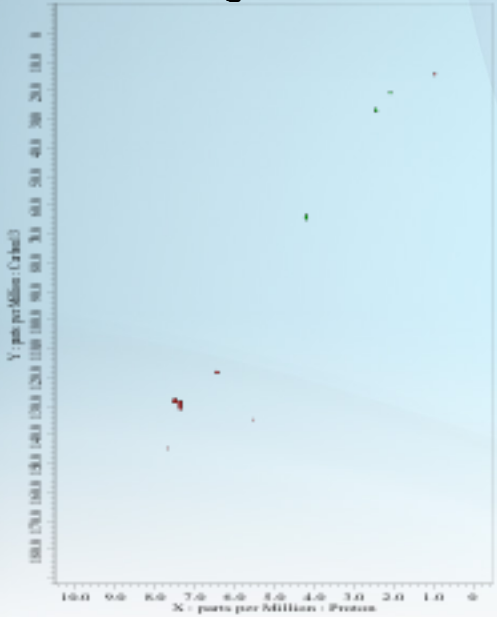
NOAH + NUS on hexenyl cinnamate



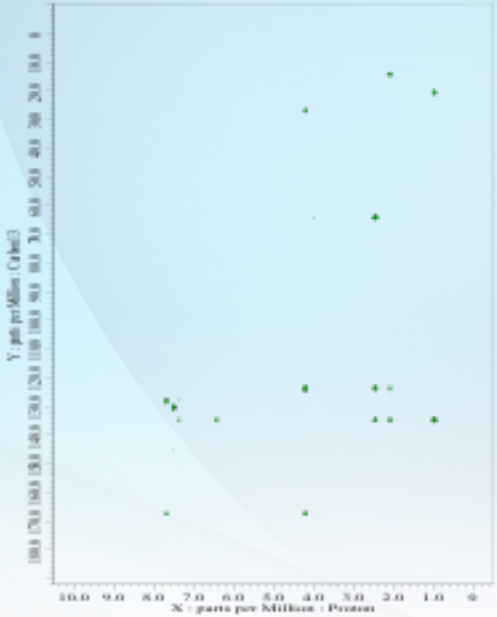
2 minutes total acquisition time!

32 increments inflated to 128
1 scan per increment
JEOL ECZ-400S

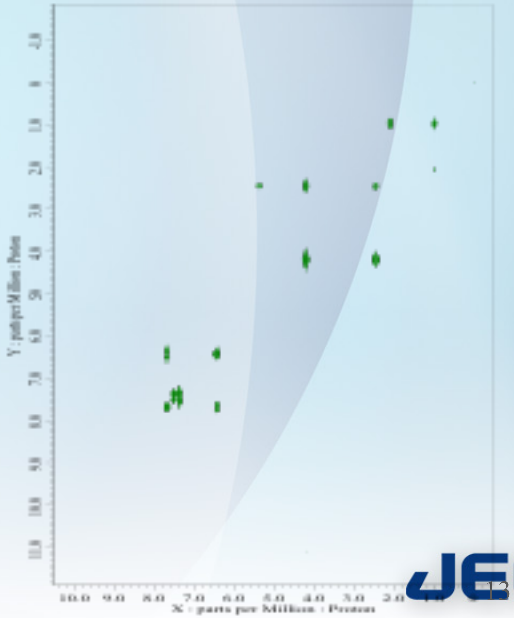
me-HSQC



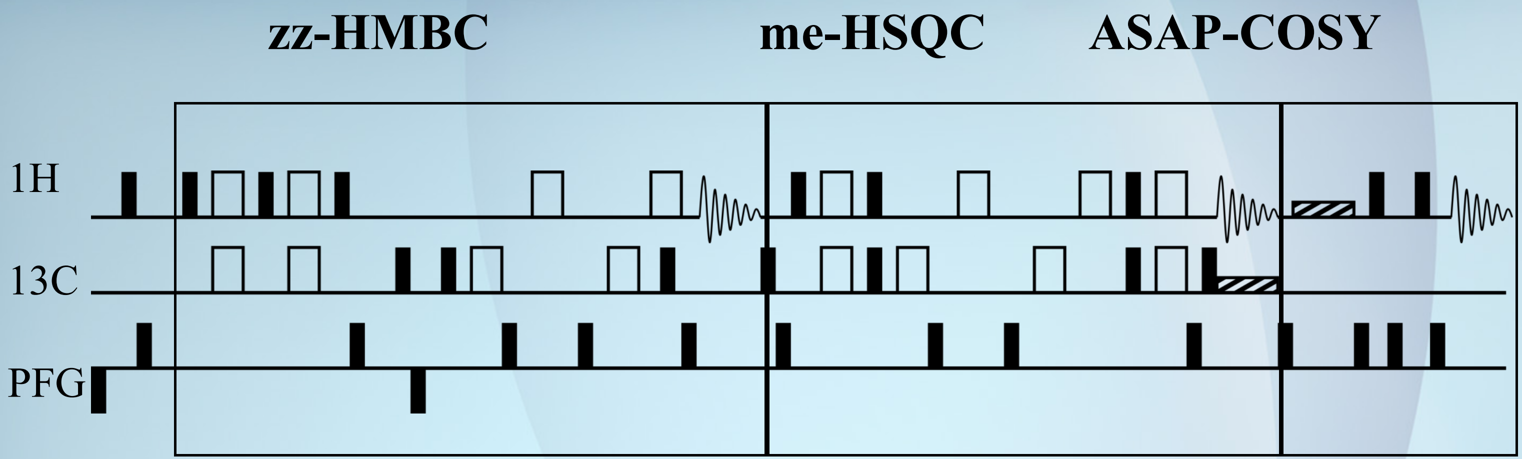
HMBC



COSY

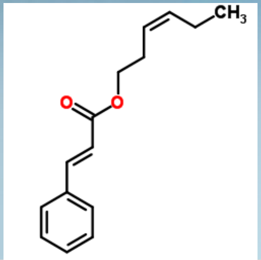


NOAH+ASAP



Ē. Kupče, T. D. W. Claridge, Chem. Commun., 2018, 54, 7139-7142

NOAH + ASAP + NUS

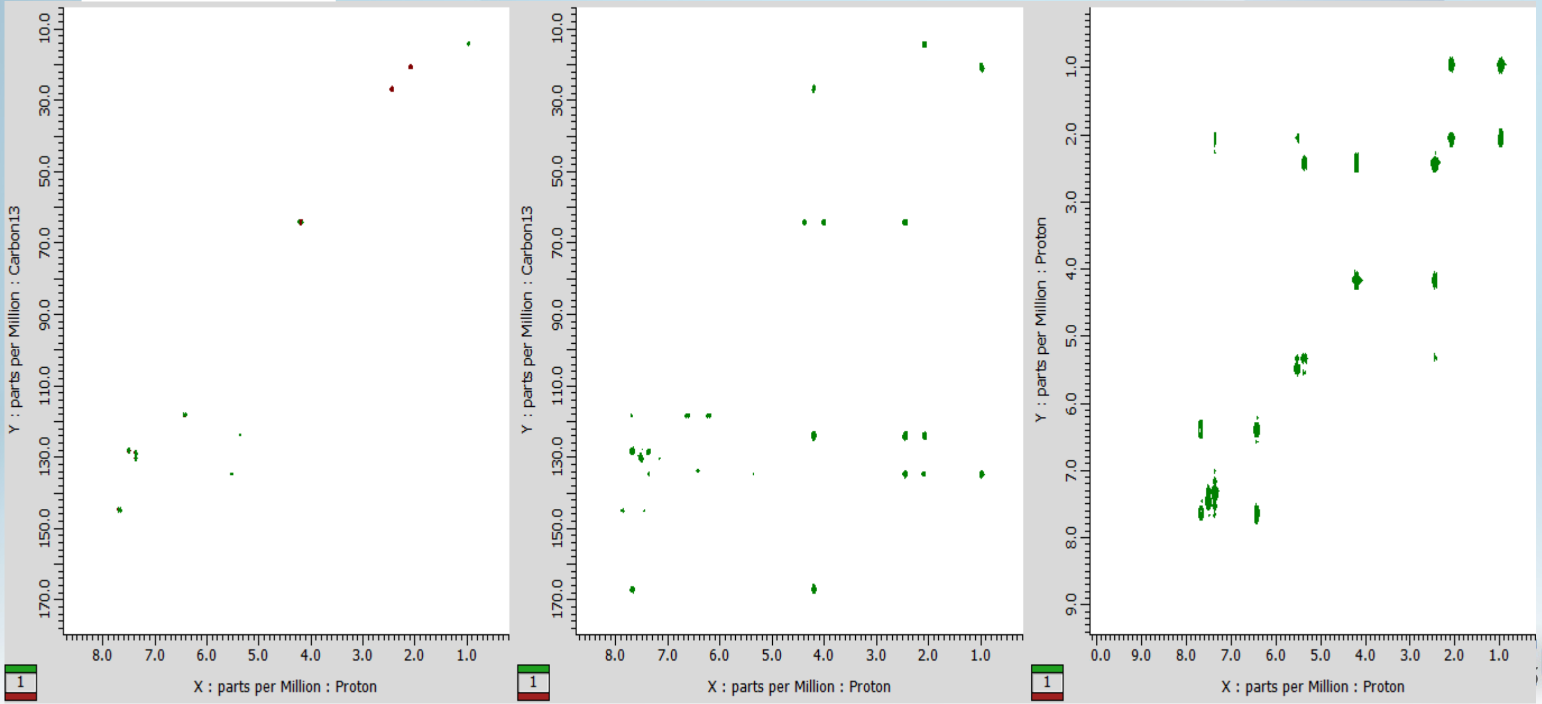


2 minutes total acquisition time!

32 increments inflated to 128

1 scan per increment

JEOL ECZ-400S



JEOL ROYAL Probe *(transition to HFX..)*

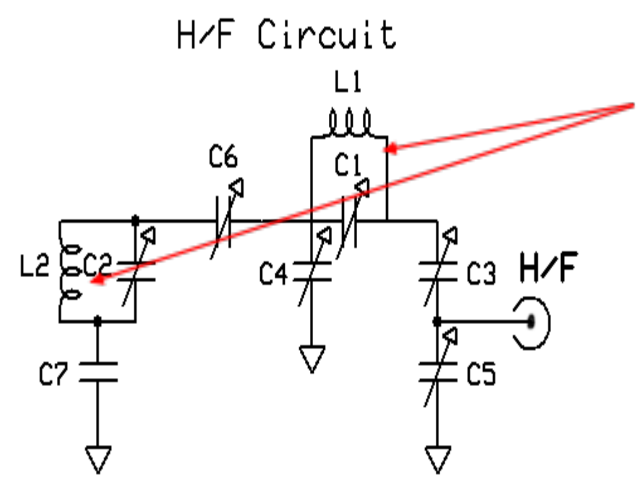
High Sensitivity both LF and HF channel

Multinuclear observation

1H,19F observation

How to tune H, F and X nuclei?

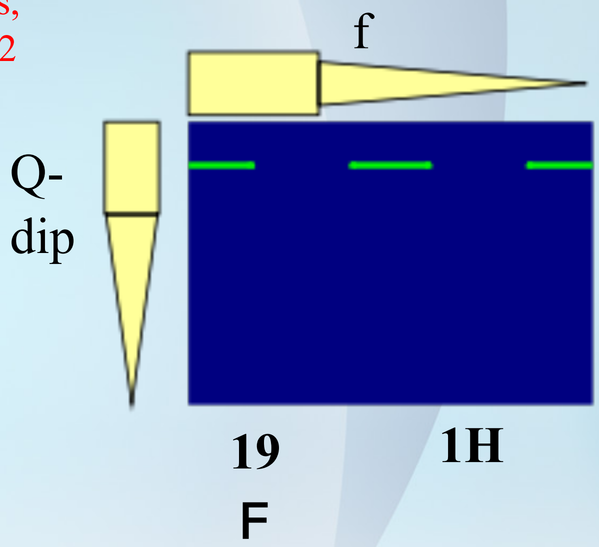
4N Nalorac Probe was capable of HFC experiments.



Simultaneously Observe 1H/19F

Two inductors, two modes, √2 loss!

You could not defeat this loss !

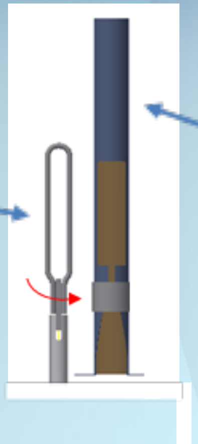


M. D. Schnall, V. H. Subramanian, J. S. Leigh, Jr., and B. Chance, J. Magn. Reson. 65, (1985) 122-129.

JEOL ROYAL HFX Probe

"magnetic switch"

Idler Coil



Alderman – Grant Coil

Smart Autotuning!
The software automatically tunes in the most sensitive way

Using an inductive coupling coil technique, it achieves a double tuning for 1H and 19F without loss of sensitivity – plus *bias towards 1H or 19F easily done.*

Royal mode - Uncoupled

f

1H or 19F

← High frequency coil tuning →

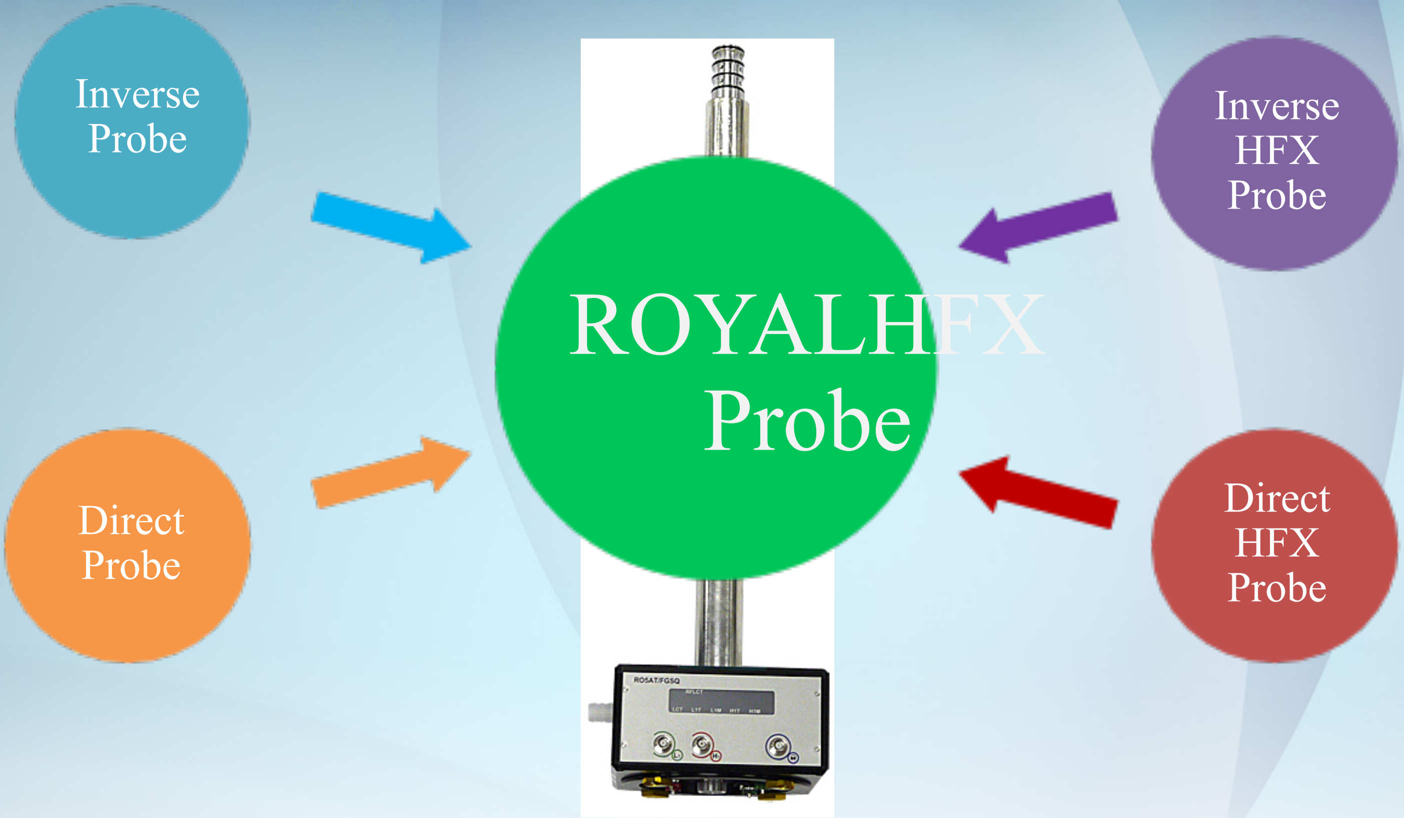
HFX mode - Coupled

f

19 F **1 H**

JEOL

JEOL ROYAL HFX Probe



No sensitivity loss
compared with double resonance probes

Performance improvement over
triple resonance probes

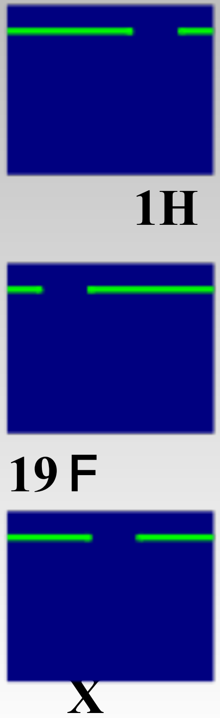
HFX experiments

On a 3-channel system

RF port 1
(1H)

RF port 2
(19F)

RF port 3 (X)

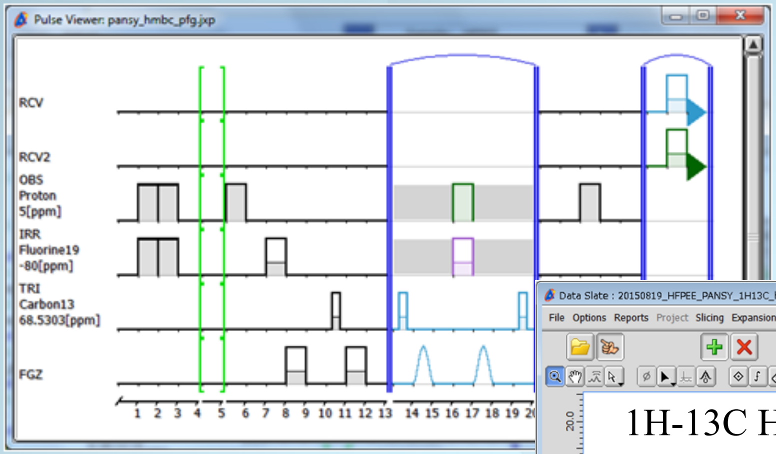


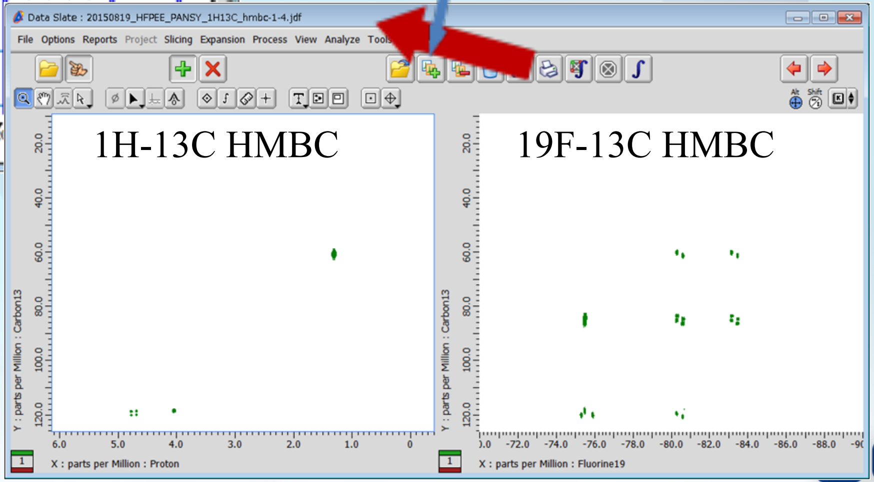
(probe has single "HF" port – cavity res combiner..
This configuration provides independent H/F receivers
for a total of 3 receivers in this example..

Multi receiver

ECZ spectrometer can have more than two receivers
and control each one independently – *extremely simple to program*.

Run 1H-13C HMBC and19F-13C HMBC
simultaneously





Couple of years ago we showed ET's H/C H/N F/C F/N HMBC simultaneously..

PANSY
HMBC

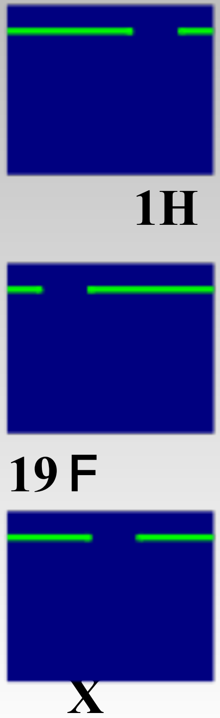
HFX experiments

On a 2-channel system

RF port1 (1H / 19F)

RF port2 (X)

(single high-band receiver but no cavity resonator required!)

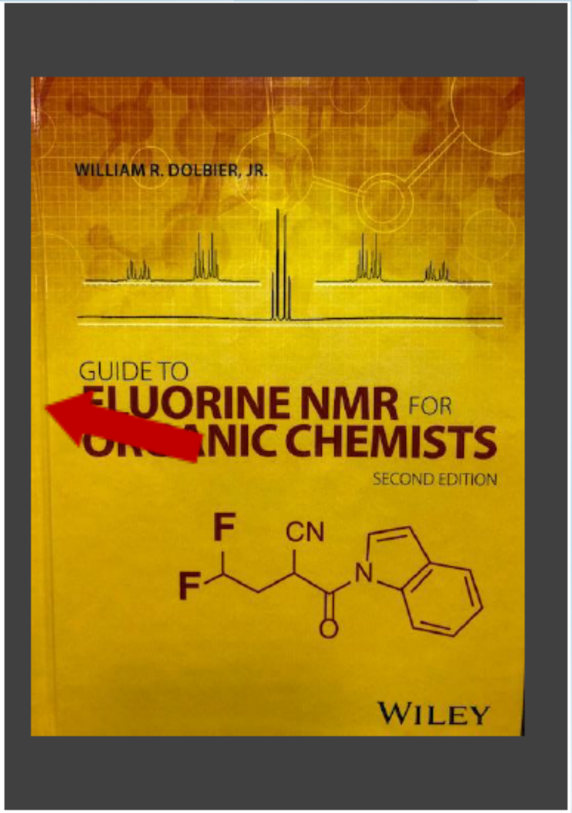


HFX in a 2 channel system?

Slide stolen from **Peter Grice, University of Cambridge**. UKMRM meeting 20/6/2018

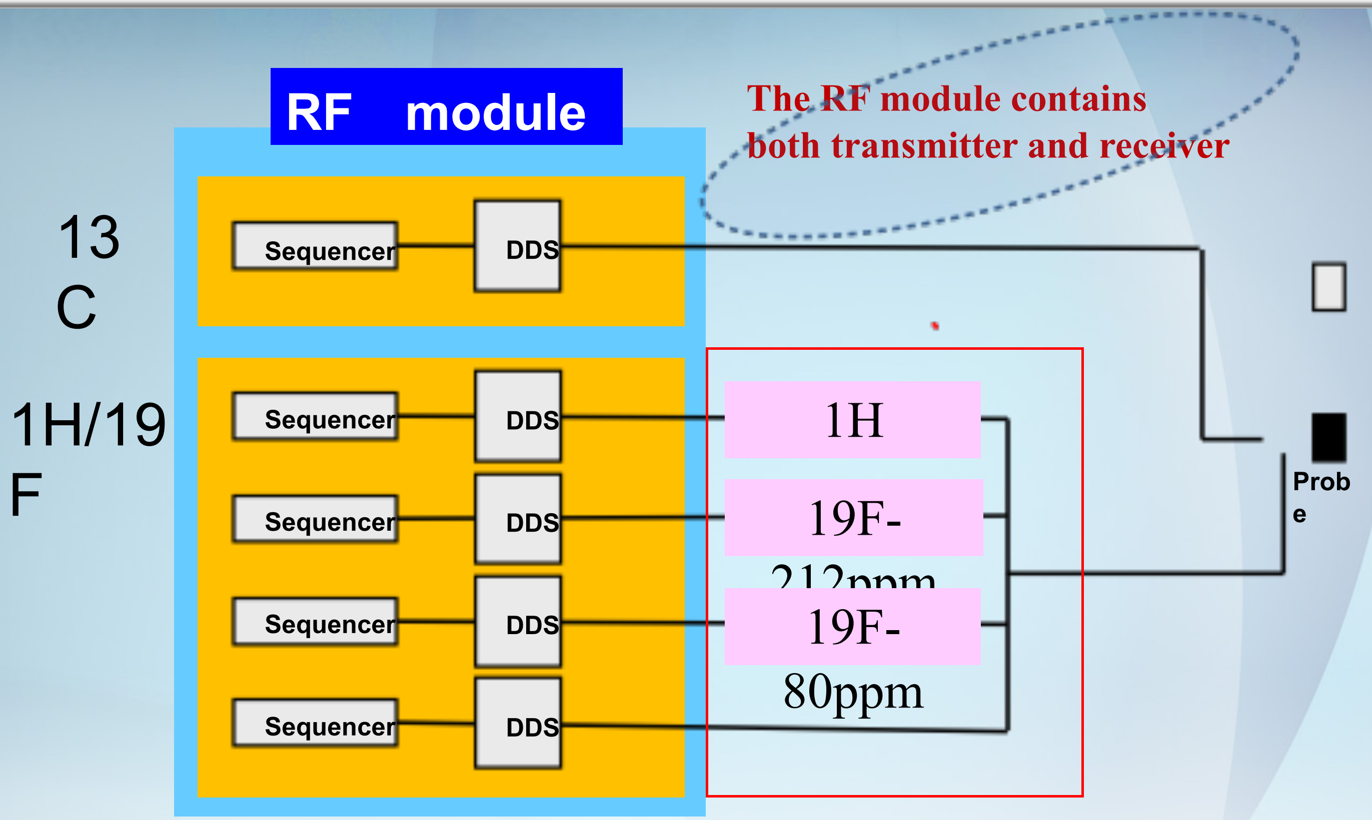
https://u-of-o-nmr-facility.blogspot.com/2018/06/the-limitations-of-19-f-garp-decoupling.html?m=1

The picture, I lifted off Twitter, got me a hmmm and beard scratch from Pete Gierth



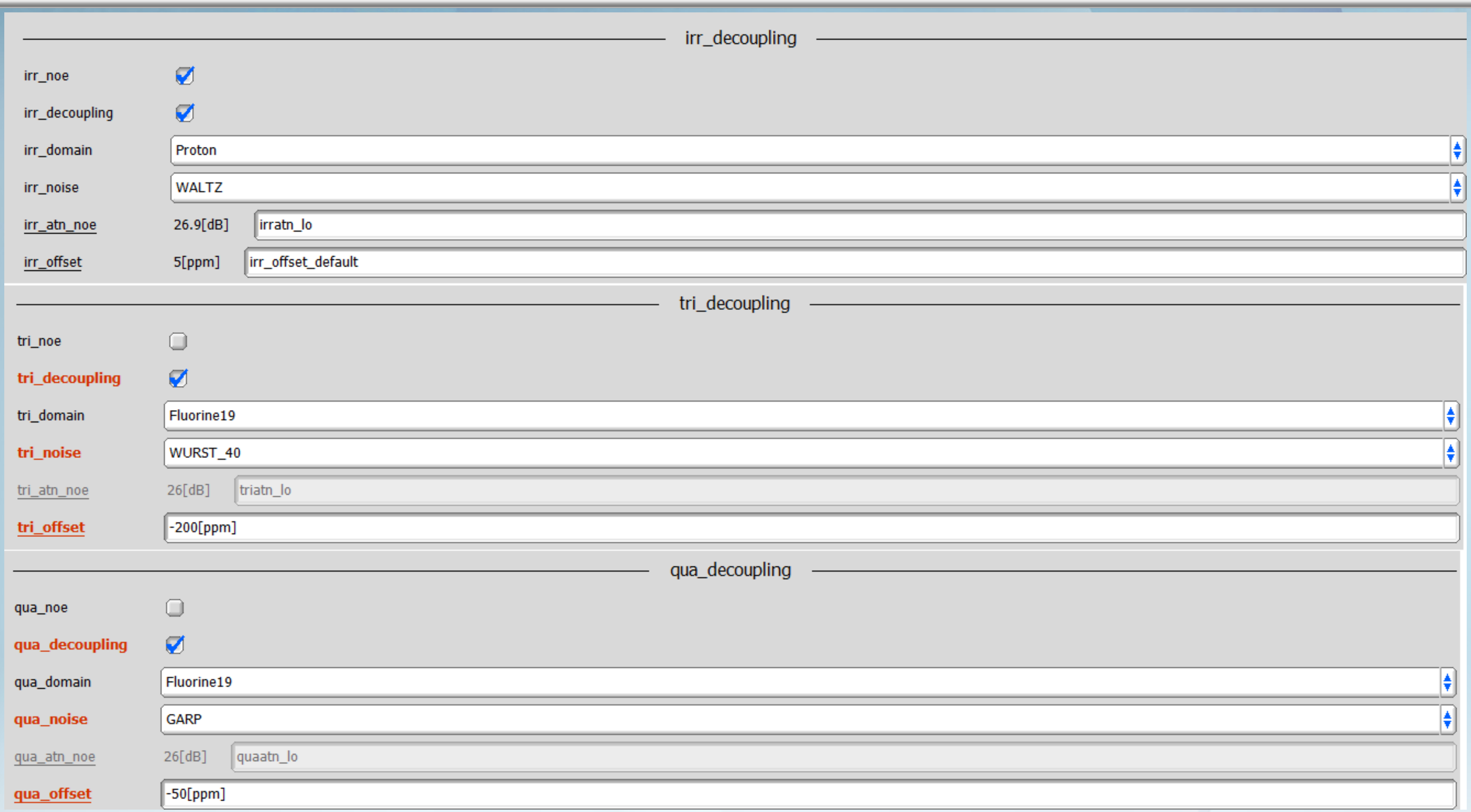
(slide now "twice stolen")

HFX with only 2 channels!



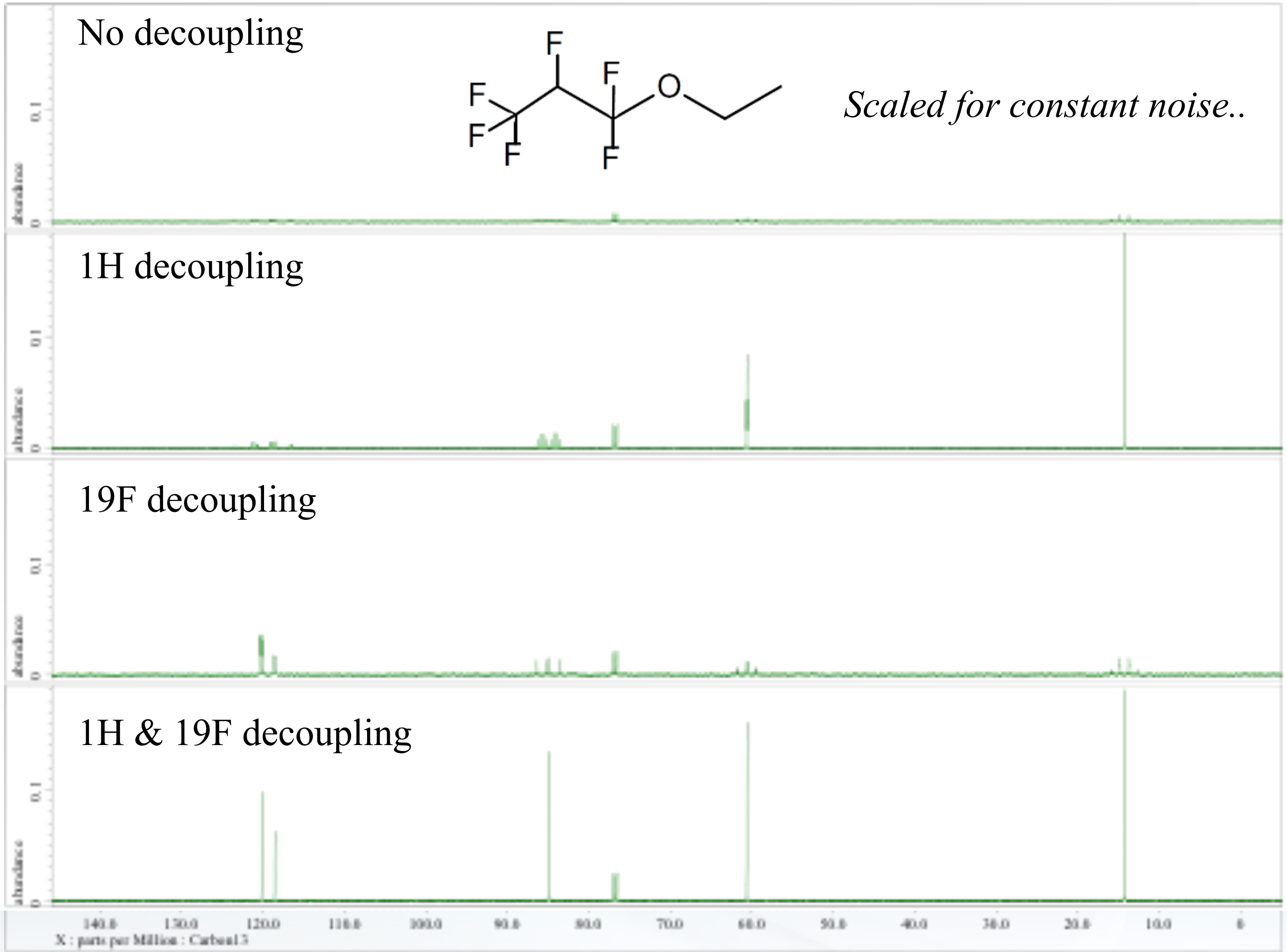
The maximum offset range of RF module is 50MHz, that covers both 1H and 19F.
A standard 2ch ECZ can run 13C-{1H}{19F} experiments with a HFX probe

Easy multiple decoupling setup



Just choose, nuclei, offsets and decoupling schemes.
No need to recalculate pulses – (*these few lines of experiment code easily copied*)

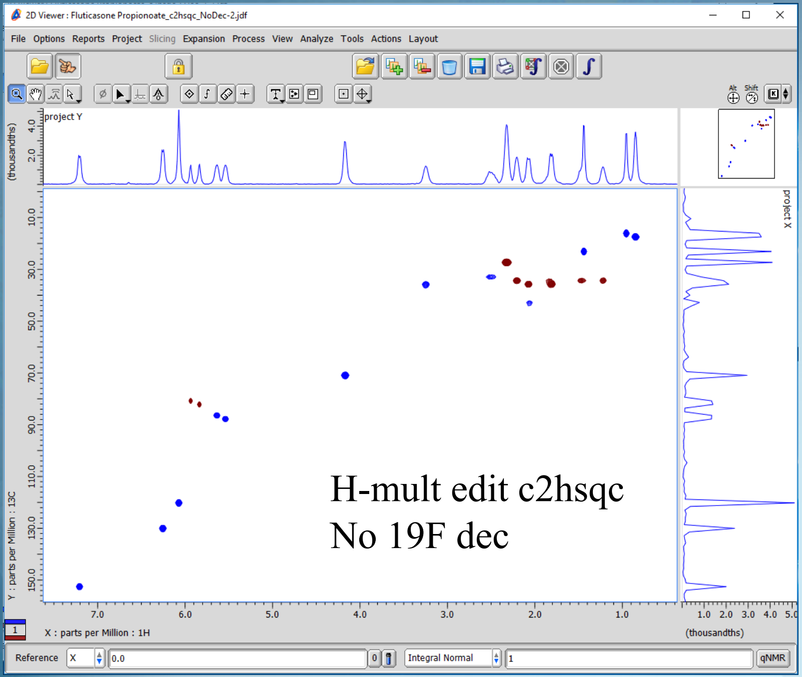
13C (Hexafluoro propyl ethyl ether)

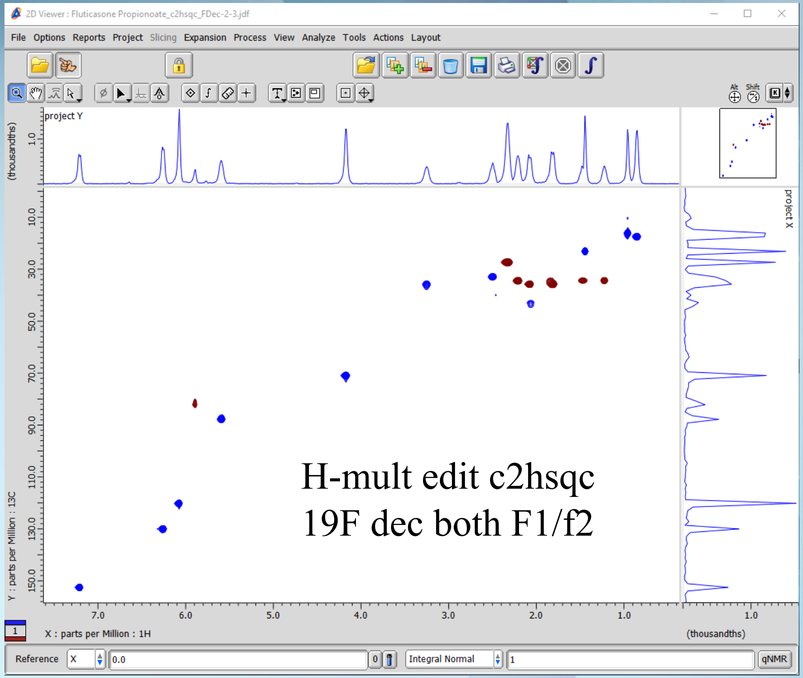


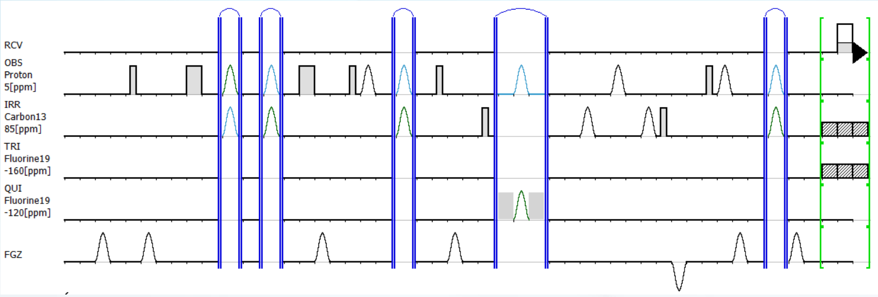
Decoupling 19F and/*or* 1H in HFX – 2D..

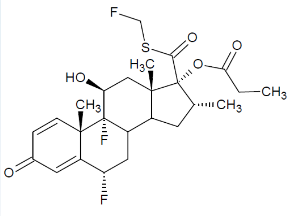
- **Must keep single quantum or multiple quantum in mind.**
- **BIP shape our easy choice for evolution decoupling 180**

- ***In 2D HFX, decoupling 19F or 1H much more important in F1 than F2 to resolve chemical questions.***



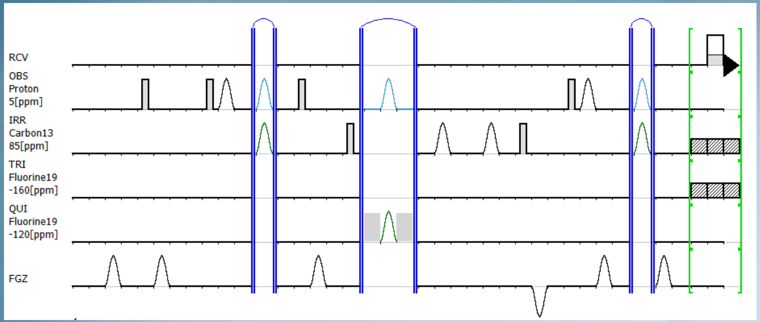




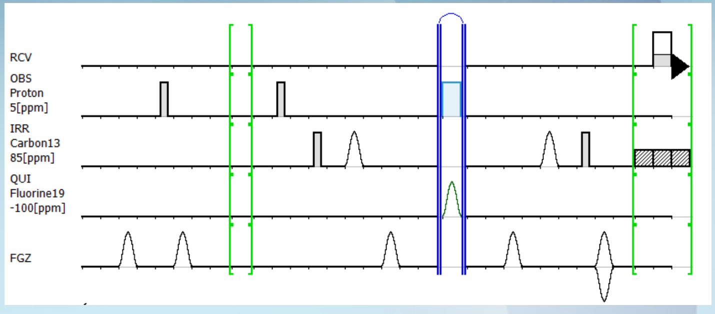


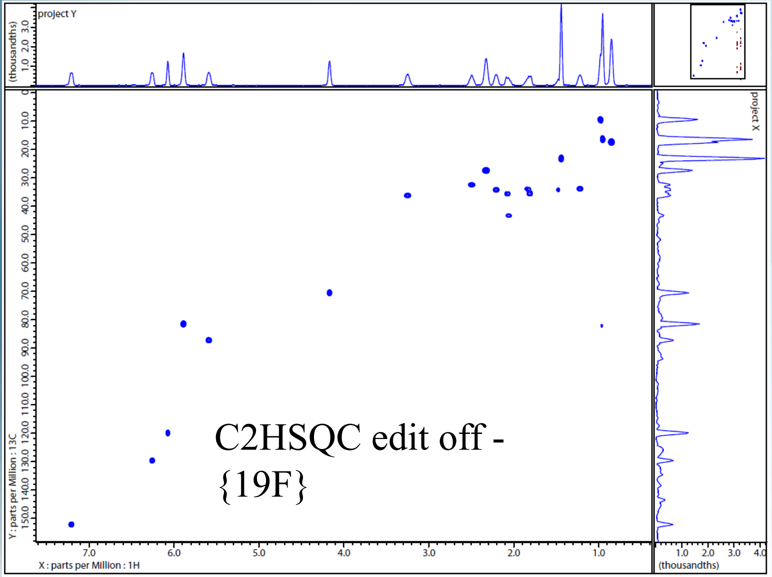
Fluticasone Propionate

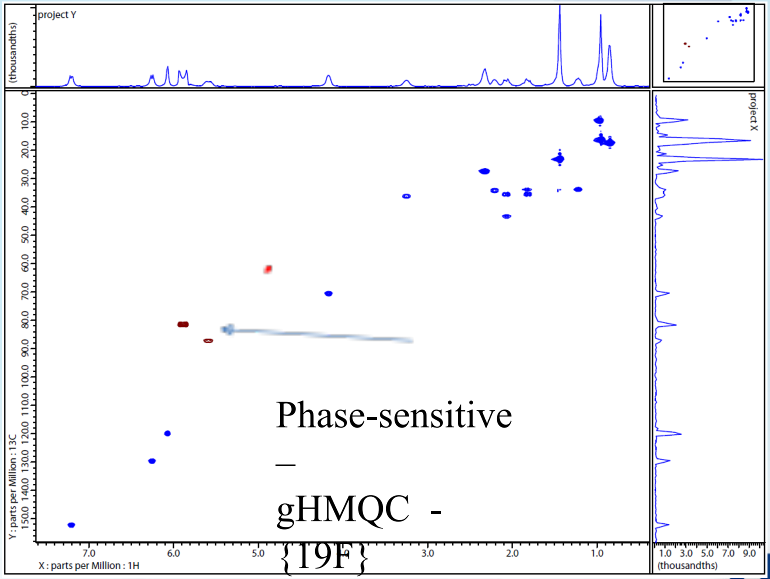
SQ – 19F J's refocused in INEPT period

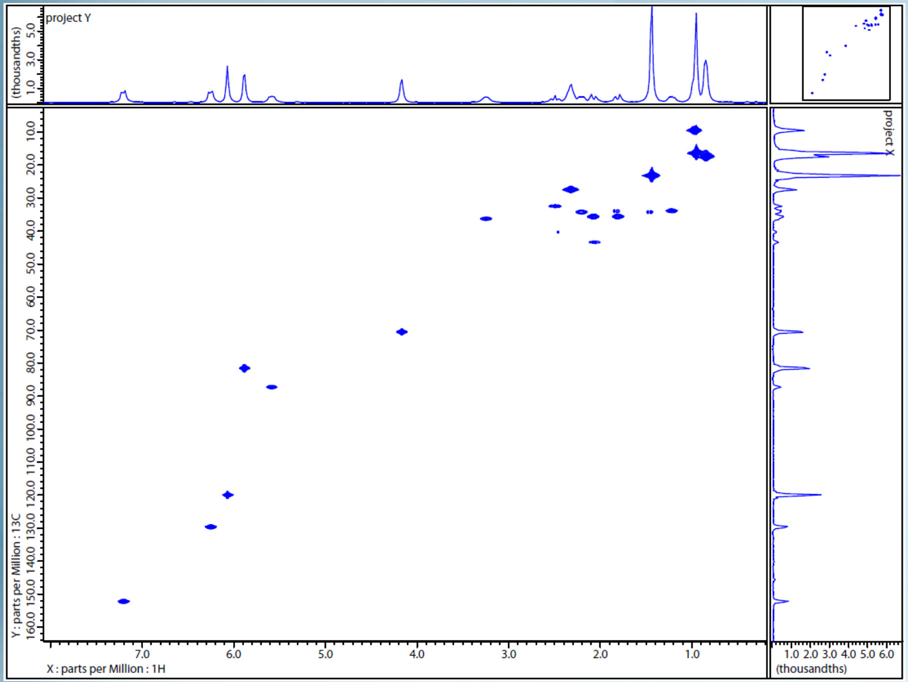


MQ – No INEPT refocusing so 19F 180 inverts 1H!

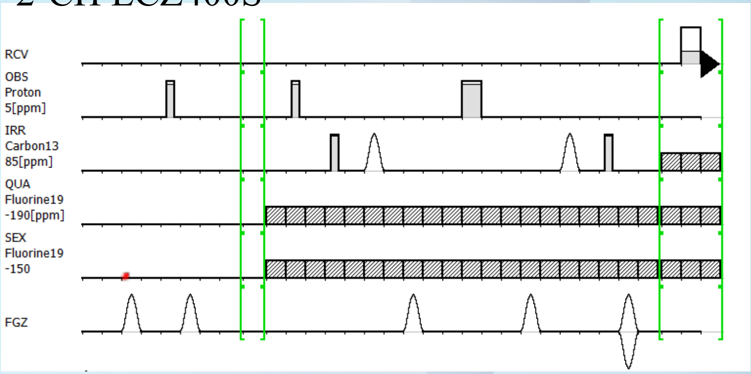


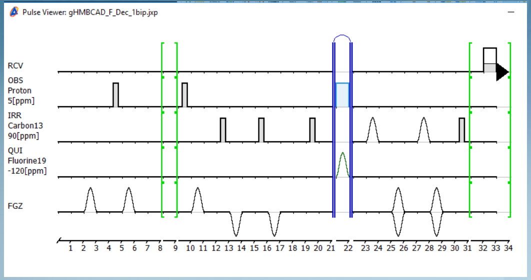


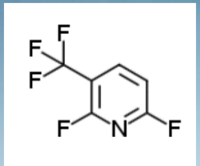




Phase-sensitive gHMQC with 19F decoupling
Using sequencers and multiple-bands.
2-CH ECZ400S

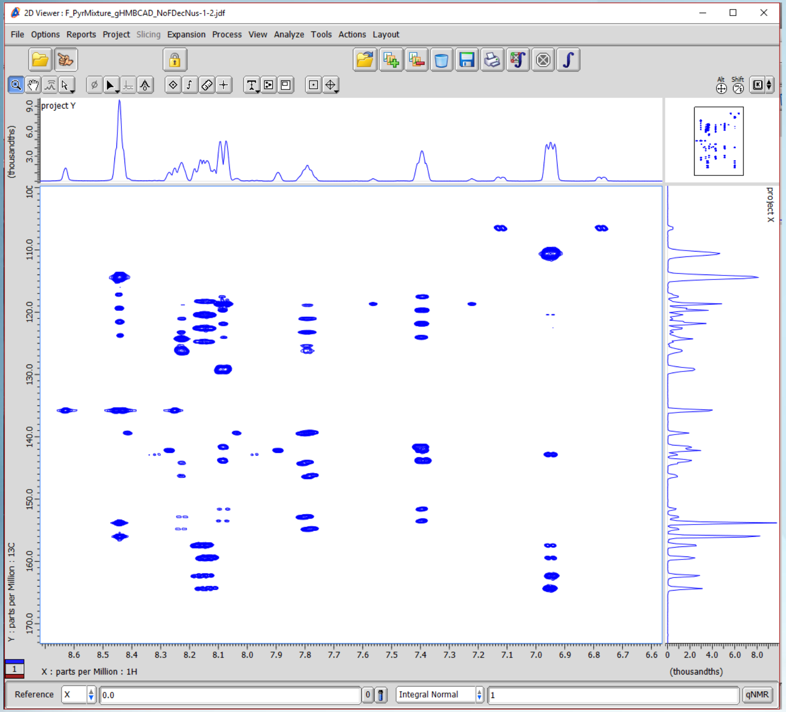


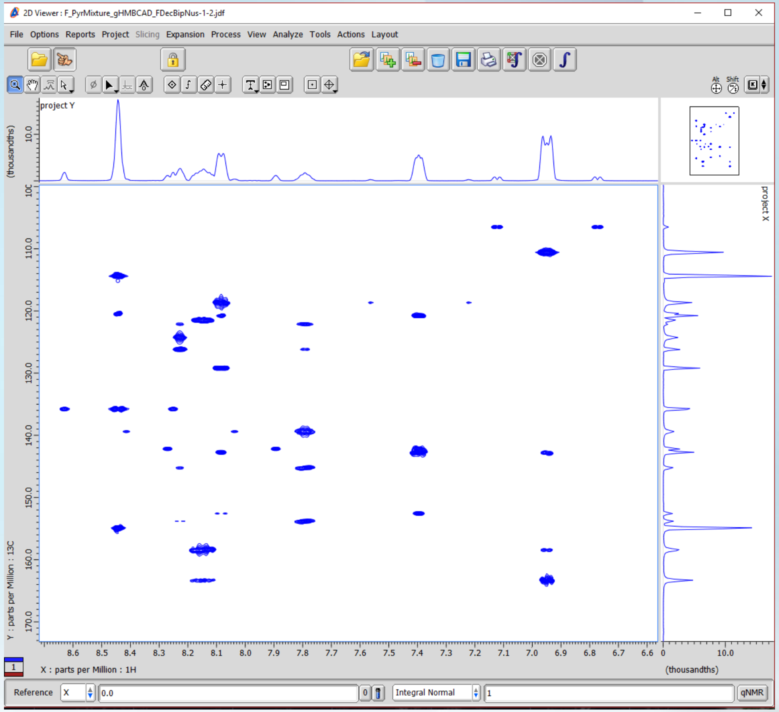


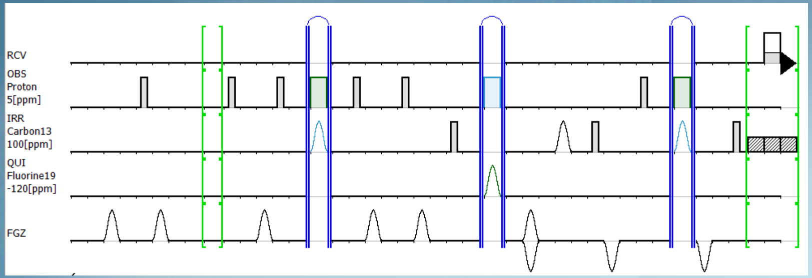


Decoupling 19F in F1-only answers ***Chemical*** question.. *2 CH console with HFX Royal*!

No setup required.





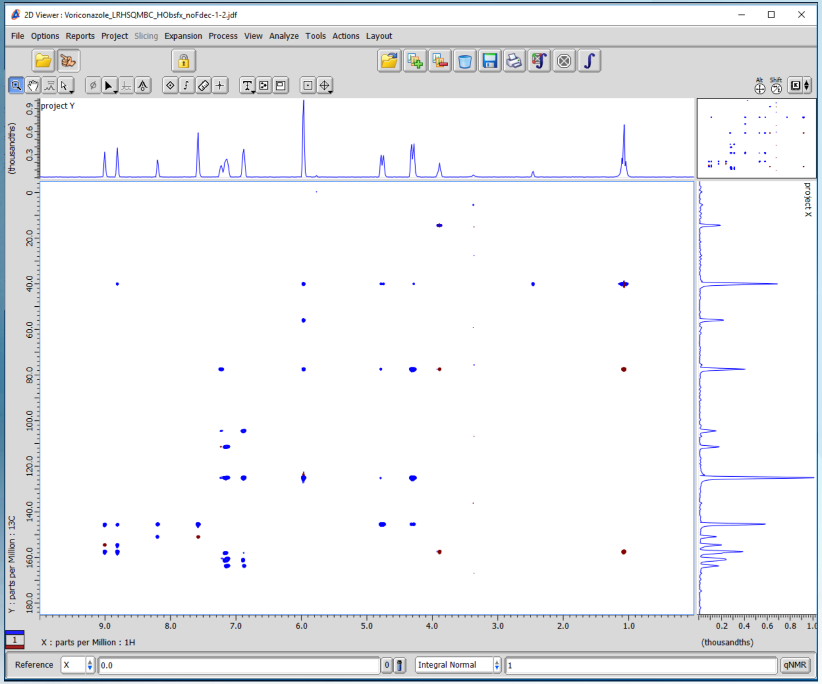


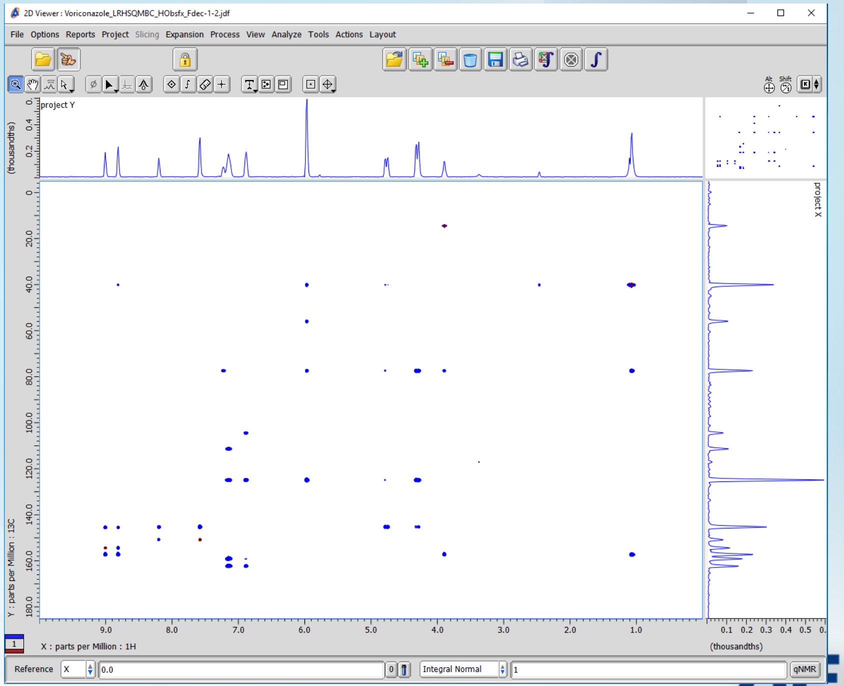
LRHSQMBC – With and without {19F} in evolution

Better linewidth and resolution than gHMBCAD.

{13C}

ECZ400S – Again.. 19F decoupling in <u>evolution only</u> answers the ***chemical*** question!





ELECTRON MICROSCOPES • SEM • ESR • E-BEAM •

TEM • SAMPLE PREP • MICROPROBES • NMR • MASS SPEC

JEOL

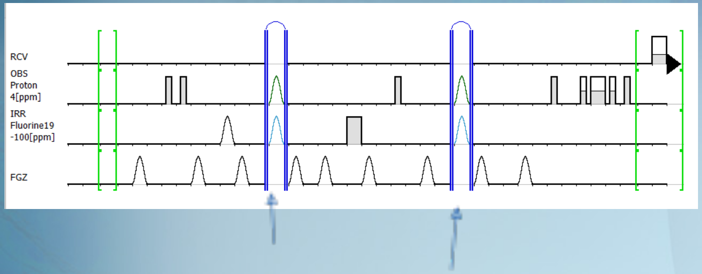
Solutions for Innovation

19F Filtered Mixtures

A modification of SRI-FESTA experiment for broadband use.

SRI-FESTA Experiment

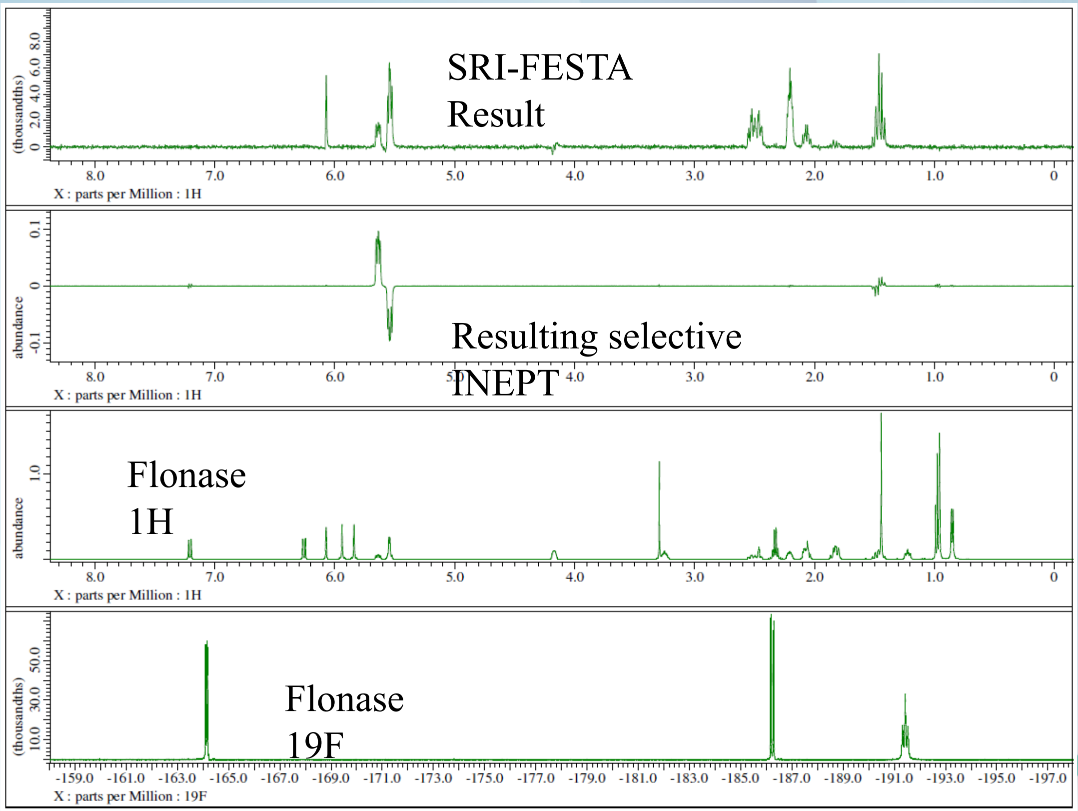
- http://dx.doi.org/10.1021/acs.analchem.8b00753
- {Laura Castanar et al.}
- SRI-FESTA uses a selective 19F -> 1H Polarization transfer followed by a selective labelling of a single 1H. F-H Inept only selected by menu.
- The experiment finishes with a TOCSY transfer to reveal all the protons which are coupled to the selected 1H revealed by the 19F transfer.
- Powerful and highly selective method but it required knowledge to choose the selective excitation points for both 19F and 1H.



Selective pulses required:
Both 19F and 1H

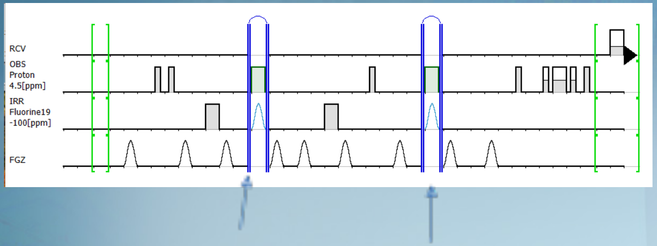
Because there are highly selective 180 degree pulses flanked by pulse-field gradients there is a "perfect echo" leading to a very clean result.

SRI-FESTA was more about structure fragments than screening per-se.

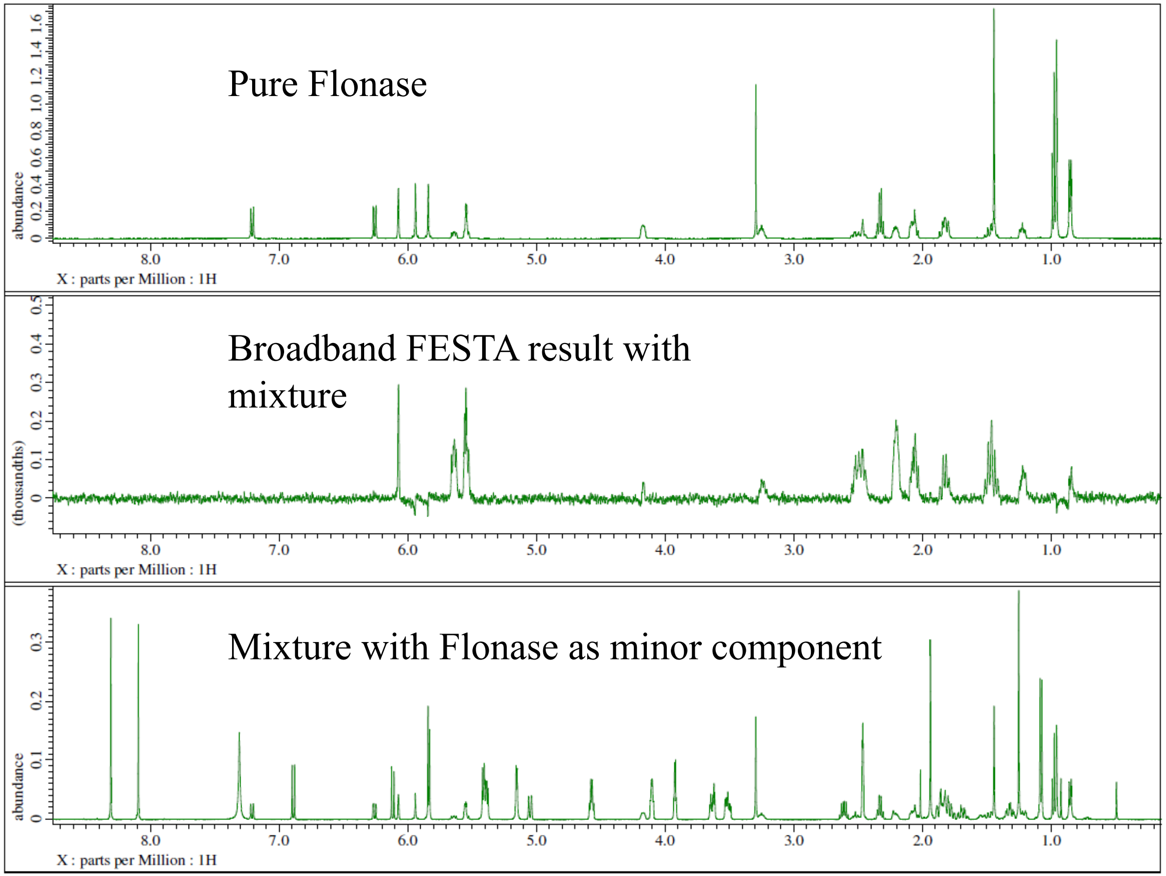


Broadband Modification

- Replace all selective pulses with the complete opposite idea – highly broadband pulses.
- This will give a very messy result because of the loss of the perfect echos.
- The phase cycle can be modified to select the desired pathway resulting in an experiment which effectively reveals the 19F filtered 1H TOCSY on complex mixture without requirement of the spectroscopist to first identify and then choose sites for band-selection.

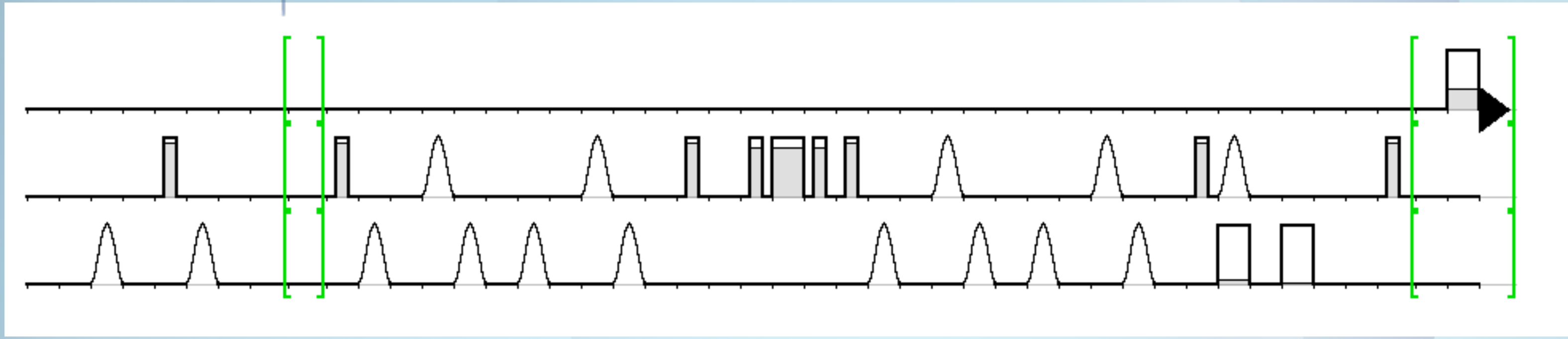


Broadband pulses both 19F & 1H



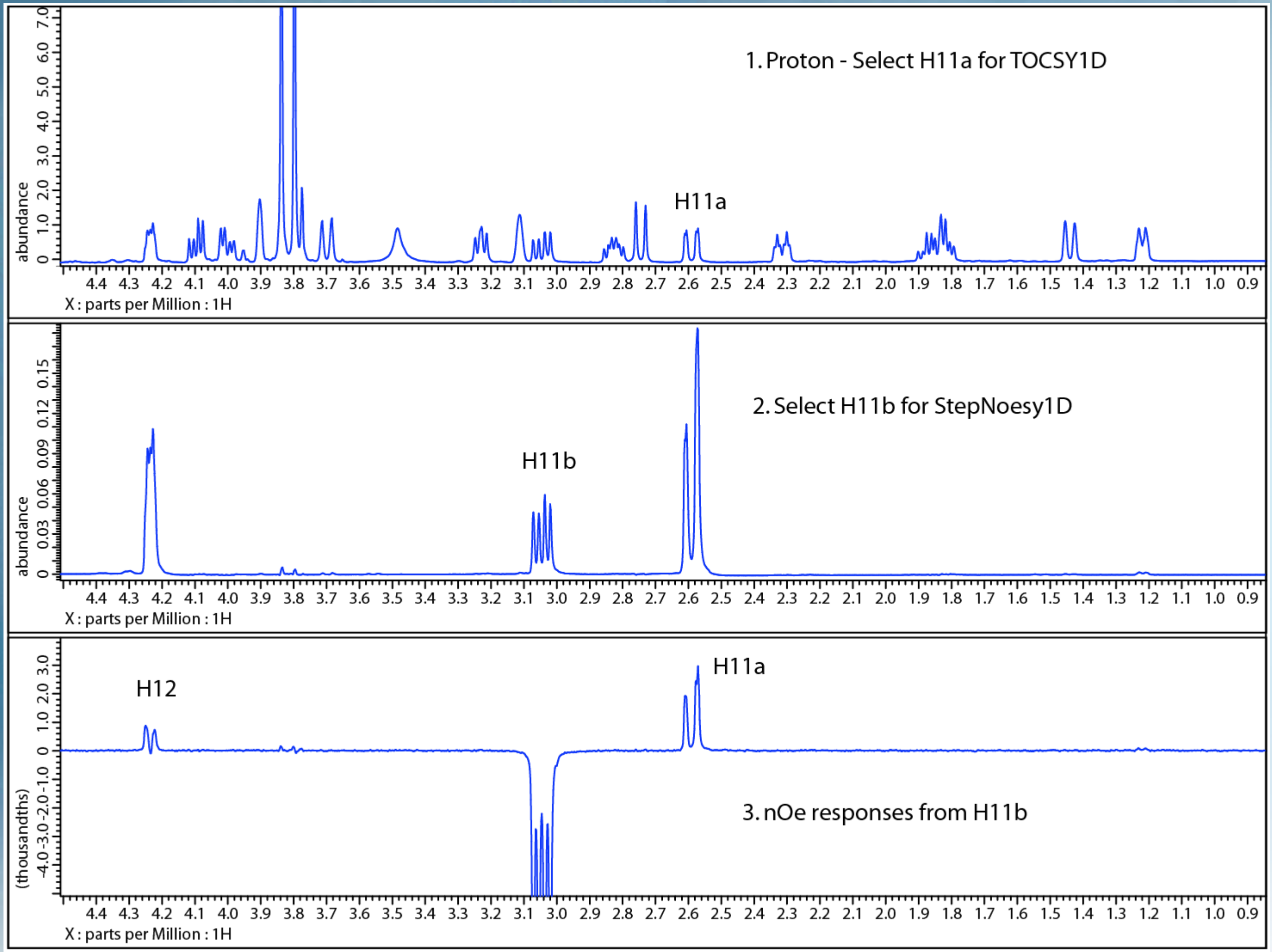
Dealing with Overlap in 1-Dimension

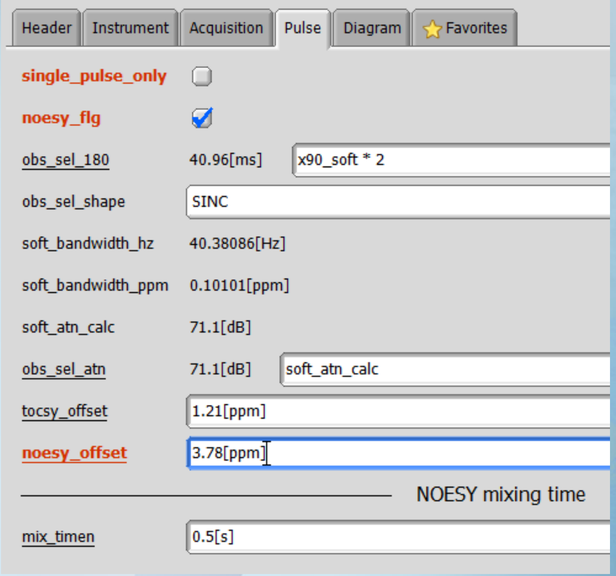
Step-Noesy



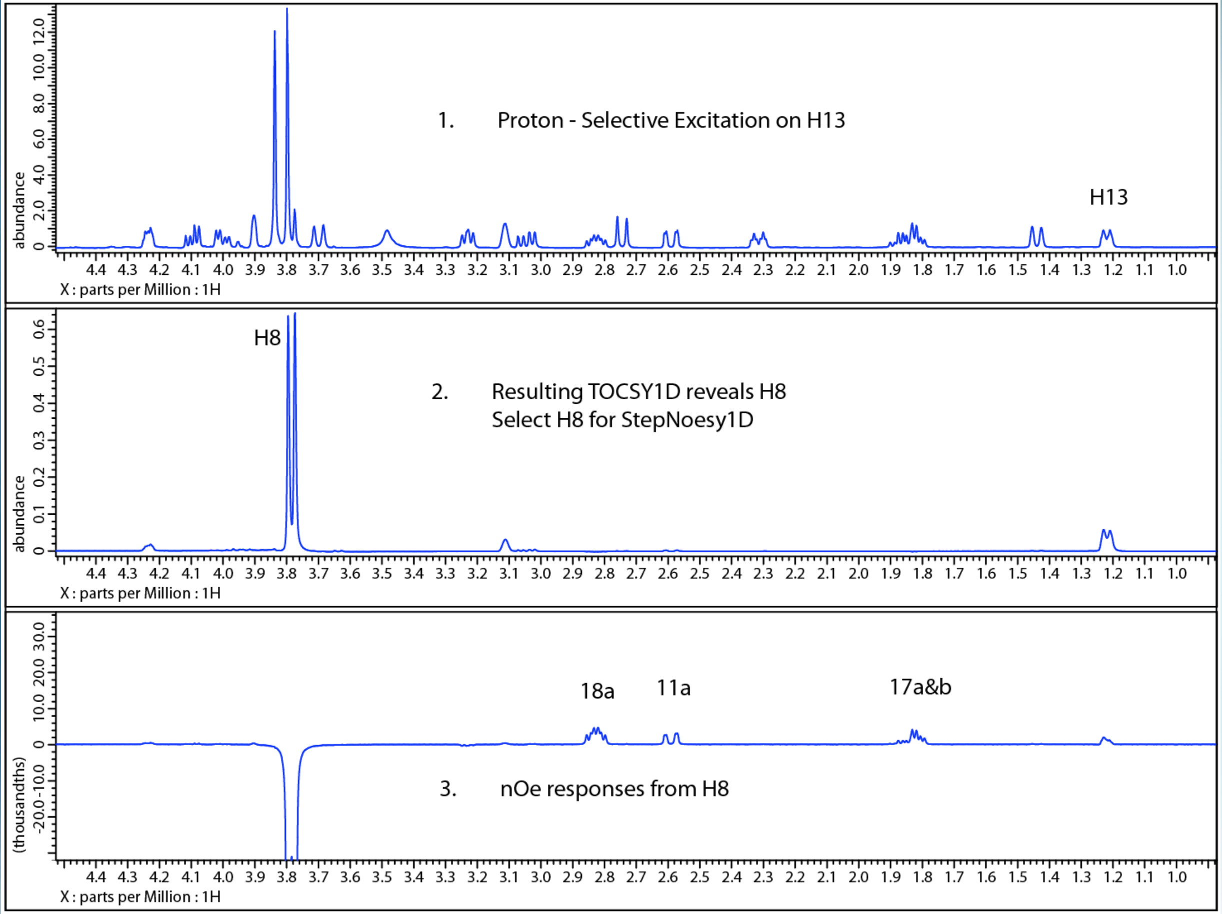
tocsy1 → Noesy1

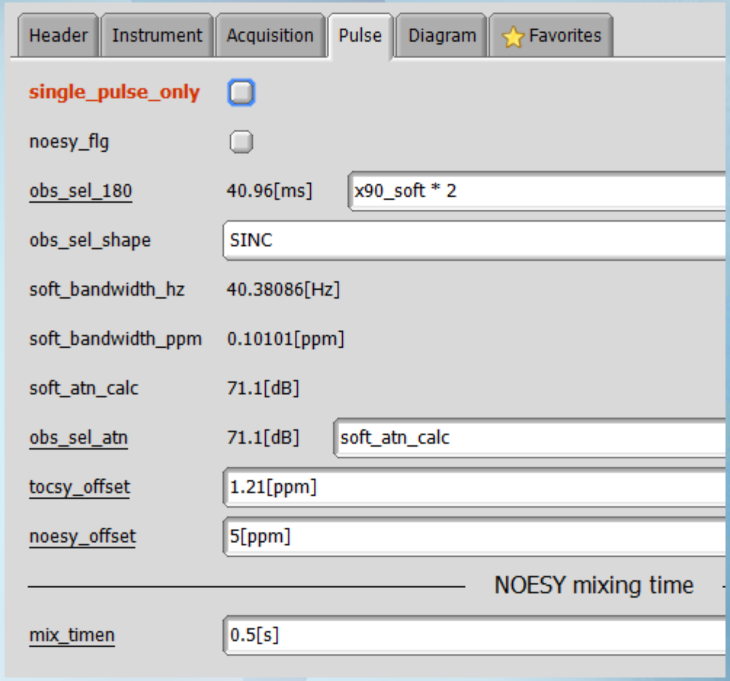
D D





Easy for Chemist to use.


1. Proton - Selective Excitation on H13
H13
abundance
X : parts per Million : 1H
H8
2. Resulting TOCSY1D reveals H8
Select H8 for StepNoesy1D
abundance
X : parts per Million : 1H
18a
11a
17a&b
3. nOe responses from H8
(thousandths)
X : parts per Million : 1H

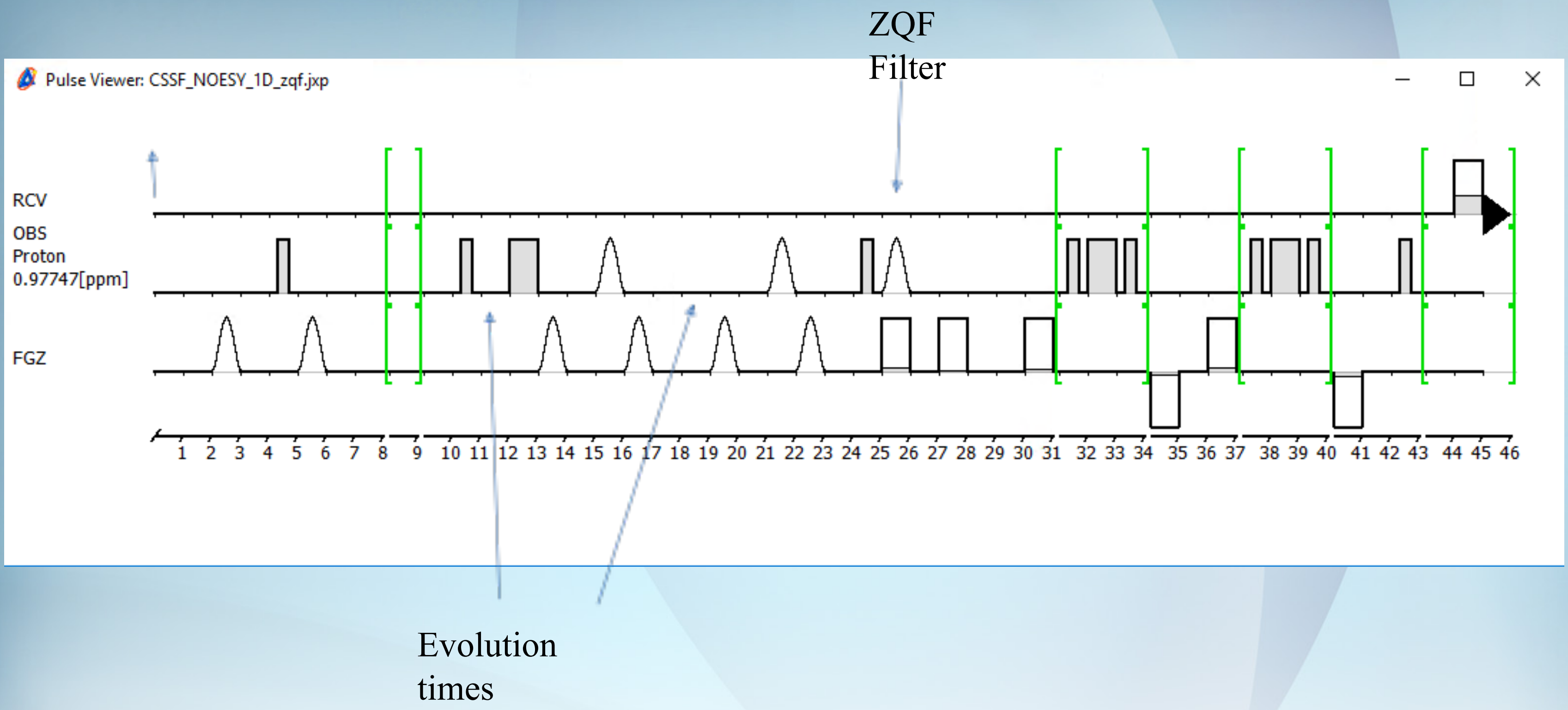

Header
Instrument
Acquisition
Pulse
Diagram
Favorites
single_pulse_only
noesy_flg
obs_sel_180 40.96[ms] x90_soft * 2
obs_sel_shape SINC
soft_bandwidth_hz 40.38086[Hz]
soft_bandwidth_ppm 0.10101[ppm]
soft_atn_calc 71.1[dB]
obs_sel_atn 71.1[dB] soft_atn_calc
tocsy_offset 1.21[ppm]
noesy_offset 5[ppm]
NOESY mixing time
mix_timen 0.5[s]

Chemical Shift Filter. Almost forgotten exp!

- **The concept of Chemical shift filtering can be used to greatly enhance selectivity in DPFGSE NOESY or TOCSY.**

- **CSSF Reference - P.T. Robinson, T. Nghai Pham and D. Uhrin, JMR, Vol. 179. p. 97-103 (2004).**

- **Application with DPFGSE 1D Spectroscopy – Sara J. Duncan, Richard Lewis, Michael A. Bernstein, Peter Sandor . Magn. Reson. Chem. Vol 45, Issue 4 p 283-288 (2007).**



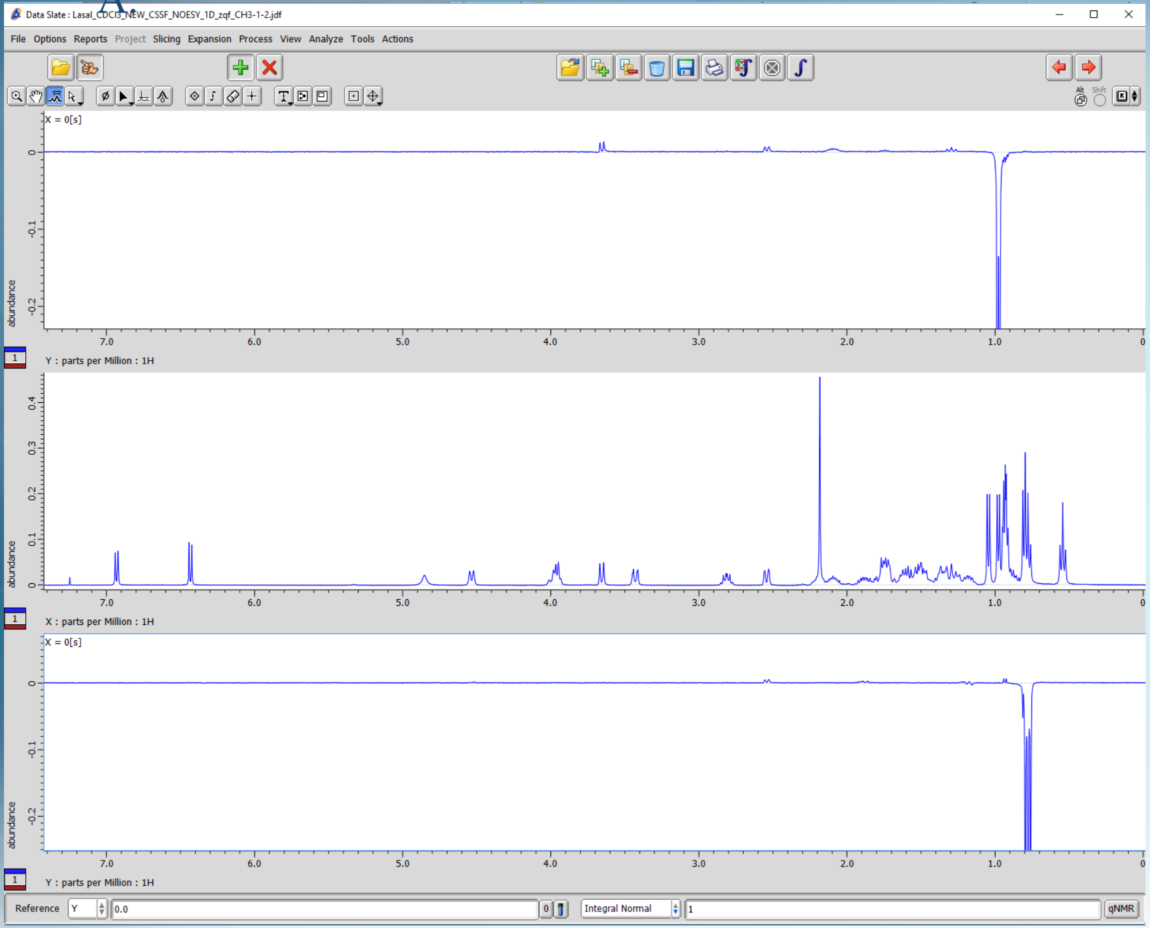
Constant time experiment set with parameter ct_flag = y.

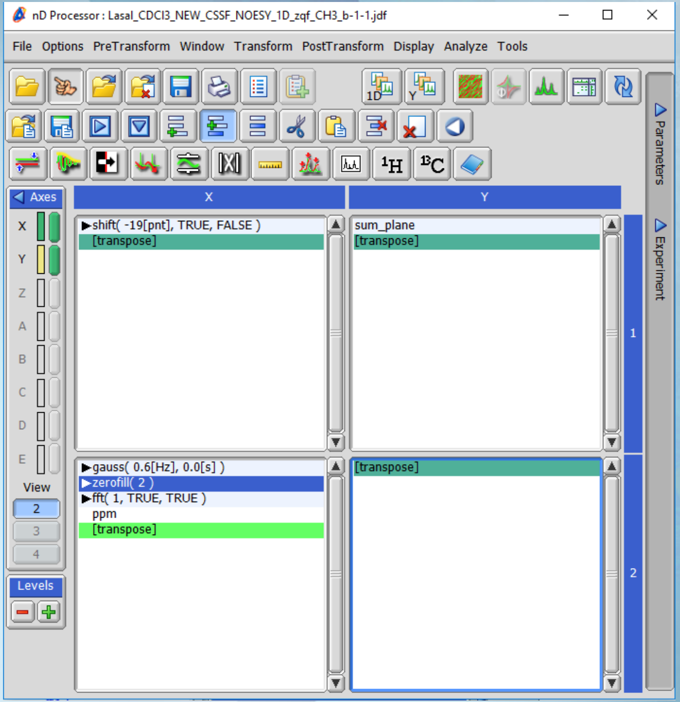
Max evolution is set automatically based on number of increments. (12 by default).

Principals

- Do a 1D NOESY or TOCSY with a chemical shift filter between the DPFGSE pair of selective pulses. A very low number of t1 increments is used .. ~12.
- Transmitter position must remain exactly at position of desired selection throughout experiment. (*PSYCHE!!*)
- Shifted-laminar selective pulses cannot be used.
- With constant time option best result is obtained.
- Result is processed simply by summing all of the fids as a 1D experiment.

Example result for 2 different CH3's in Lasalocid A

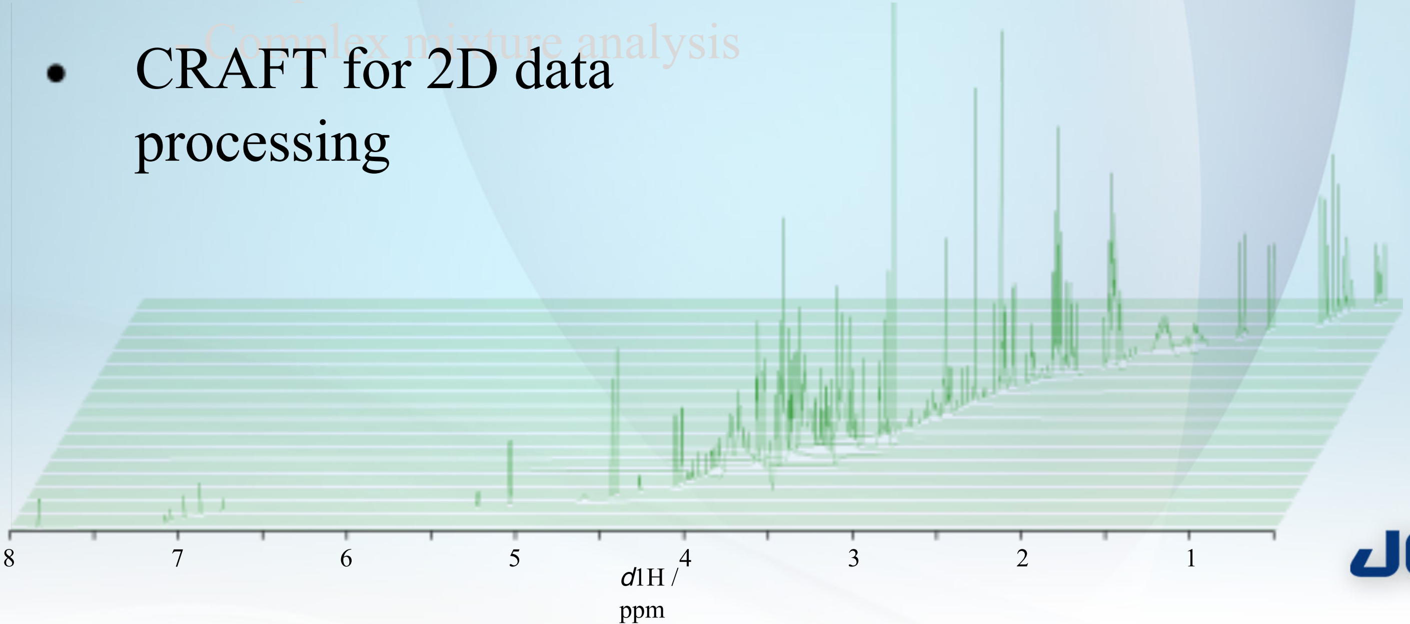




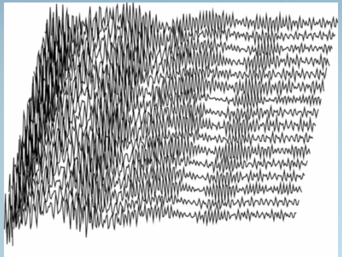
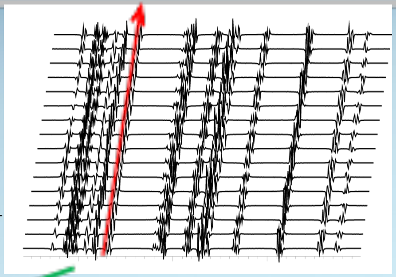
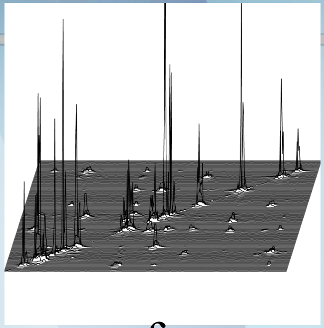
Processing list to simply sum all pseudo 2D fids. Provided as "CSSF_Proc.list".

Quest for Y (F1) resolution…

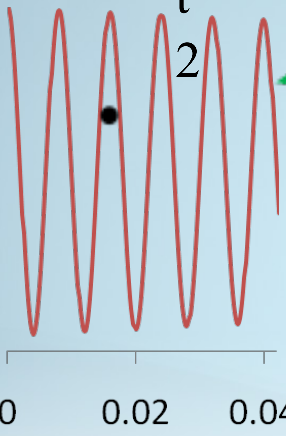
- What is CRAFT?
- CRAFT for Practical Applications
  - Simple mixture of APIs
  - Complex mixture analysis
- CRAFT for 2D data processing



CRAFT for 2D data processing

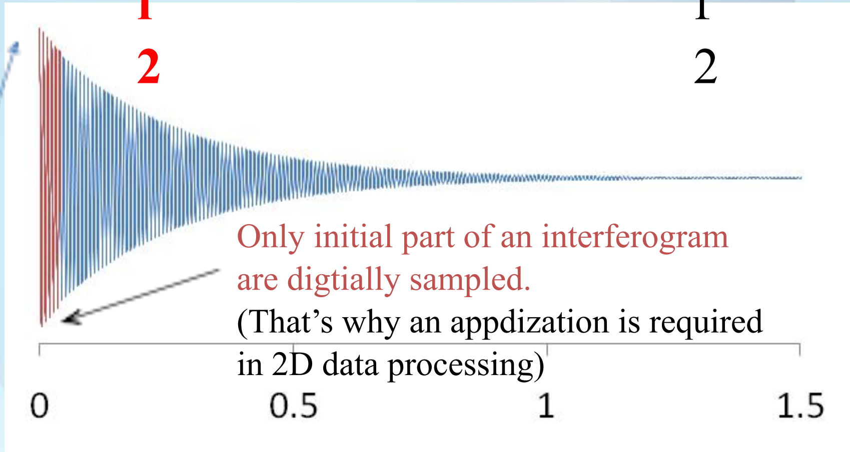
t1  FT ⇨ t1  FT ⇨ f1 

t2 f2 f2



typical interferogram

actually…
only initial part
is typically
used.

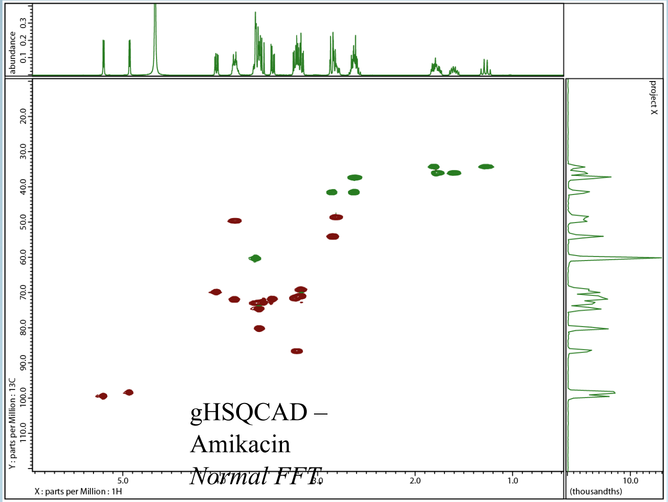


[1] K. Krishnamurthy, A.M. Sefler, D.J. Russel, *Magn. Reson. Chem.* 2017, **55** (3), 224–232.

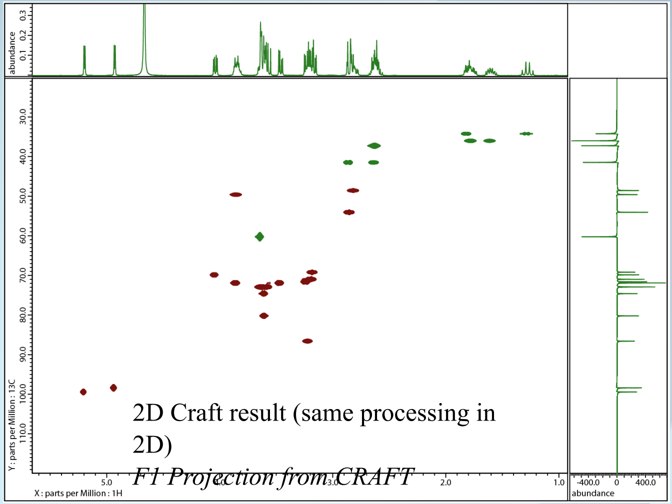
In the 2D CRAFT[1], frequency, amplitude, phase, decay rate of interferogram are estimated.

Y resolution is no longer limited by acquisition time of an interferogram.

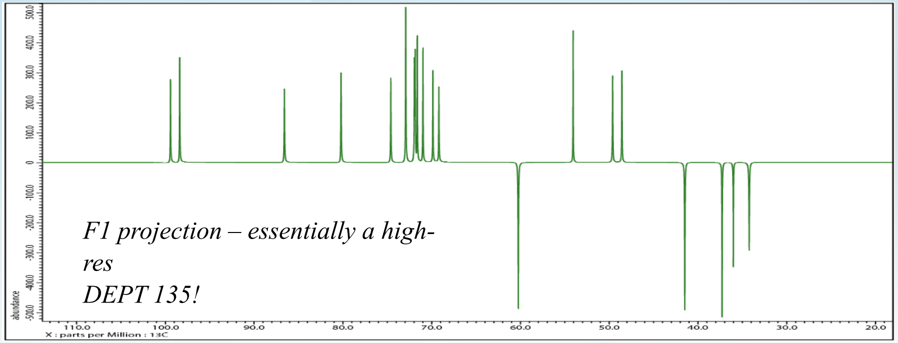
2D Craft – *use the available resolution*



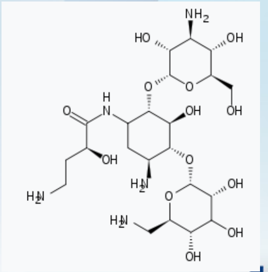
gHSQCAD – Amikacin
Normal FFT.



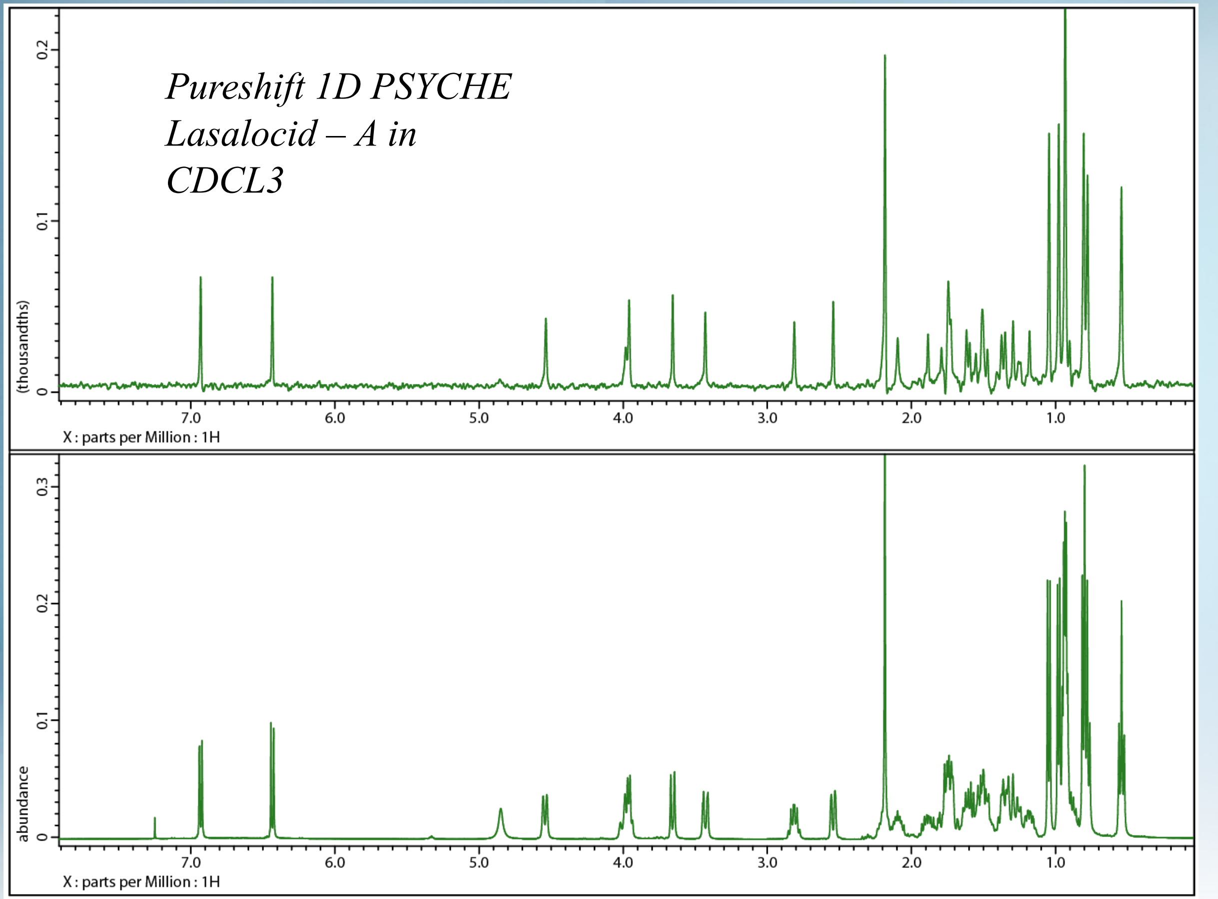
2D Craft result (same processing in 2D)
F1 Projection from CRAFT

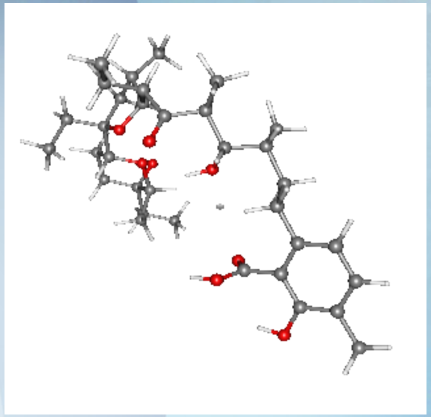


F1 projection – essentially a high-res DEPT 135!



So what about resolution in simple 1D?





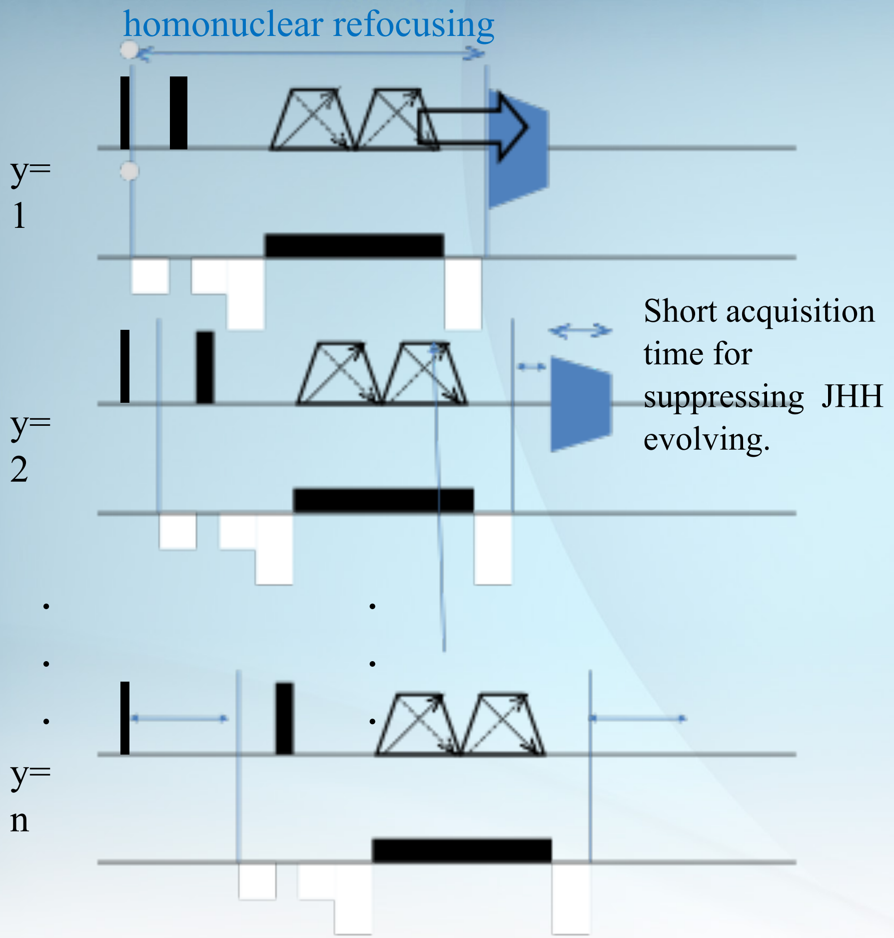

Galaxy of pureshift
Too large to
imagine…

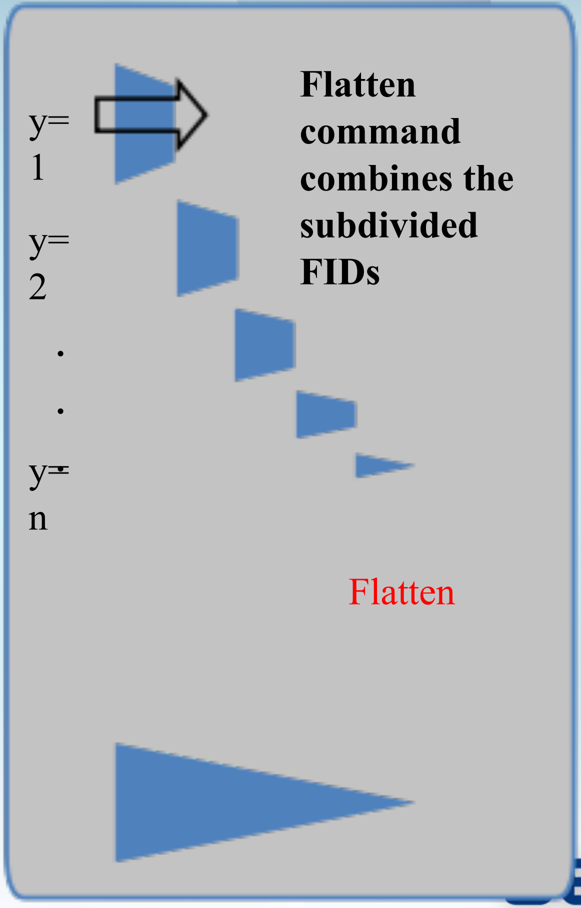
JEOL
Solutions for Innovation

Pureshift Additions

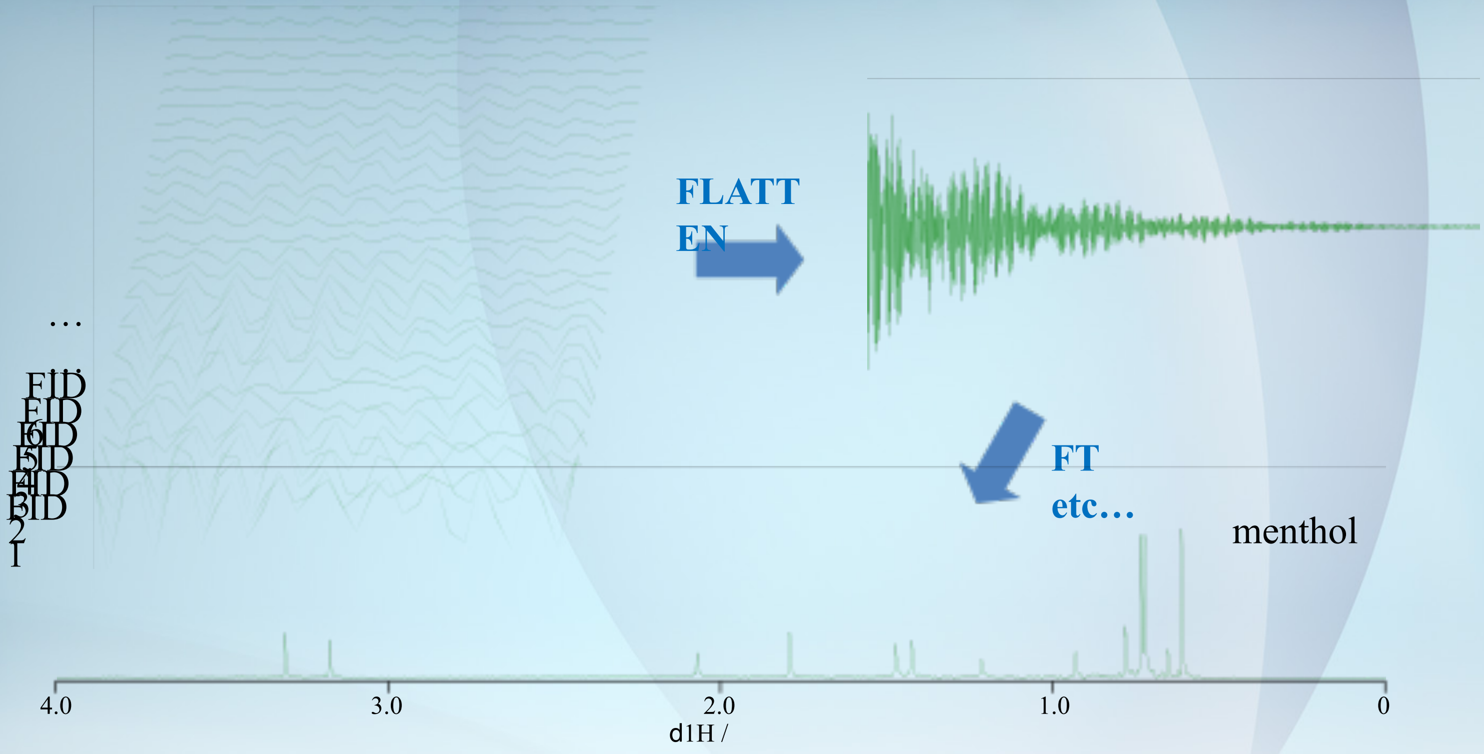
Hiroaki Utsumi has been very busy!

Measurement example requiring Flatten： Pure shift NMR





Pure shift NMR： menthol



As an example, PSYCHE spectrum of menthol is shown.
Unprocessed data is a two-dimensional format in which FIDs are aligned in the Y-axis direction, but is combined with one-dimensional FID by Flatten.
homo nuclear decoupled spectrum by Fourier transform of bound FID.

Pure shift sequence name (1) of DeltaV5.31

Sequence name	paper	1 D/2D	commnet
Pureshift_1d_PSYCHE	2014	1D	Good
Pureshift_1d_ZS	?	1D	original
Pureshift_1d_ZSsl	?	1D	original
Pureshift_1d_PEPSIE	2018	1D	Good /New ZS?
Pureshift_1d_TSE_PSYCHE	2015	1D	Good /
Pureshift_1dacq_hobs	2013	1D real time	Band selective
Pureshift_1dacq_ZS	2015	1D real time	Real time
pureshift_1d_TOCSY_PSYCHE	2016	1D	
pureshift_1d_NOESY_PSYCHE		1D	Bad SNR
pureshift_1d_ROESY_PSYCHE		1D	Bad SNR
pureshift_2d_bppste_dosy_psyche	2010	2D	PSYCHE xchane
Pureshift_2d_f1_tocsy_psyche	2014	2D	covariance

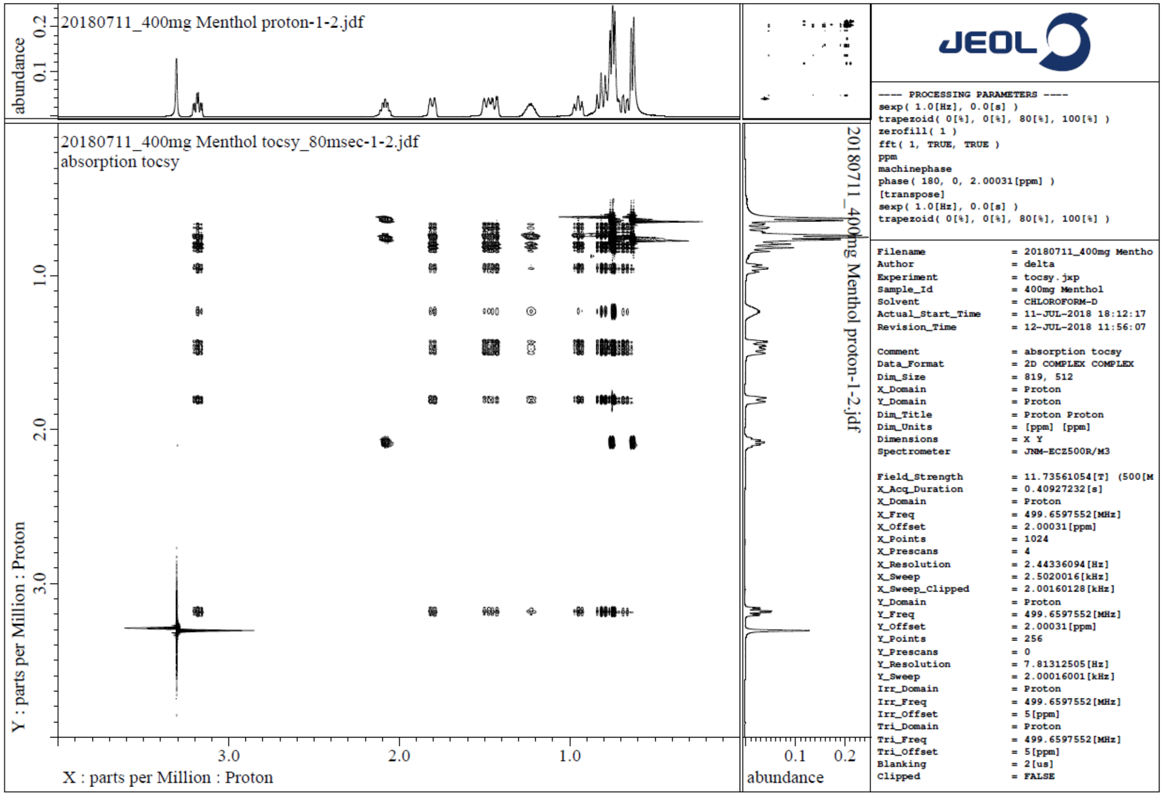
Process list	Commnet
Pureshift_1d	Pureshift 1d process list
Pureshidt_2d_homo_2d_phase	

Sequence name & process list (2) of DeltaV5.31

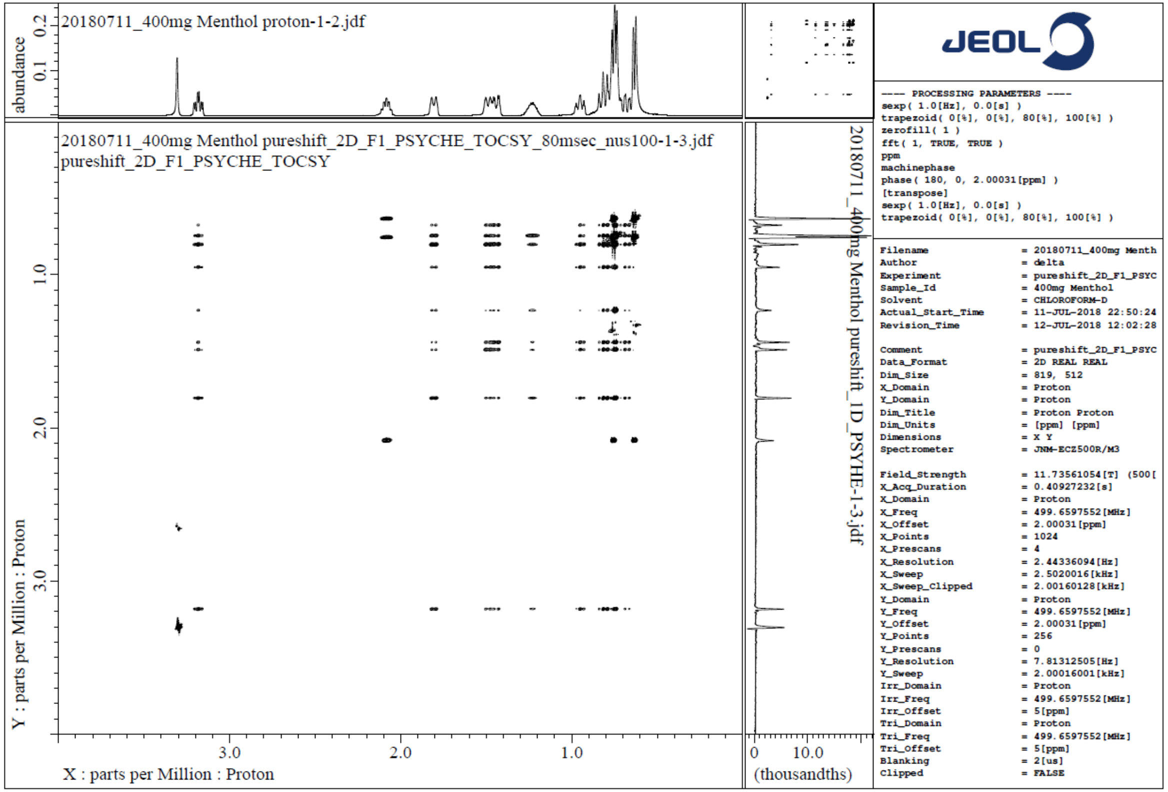
Sequence	paper	1D/2d	Pureshift
Pureshift_1d_edit_cpmg_hsqc	2015		2D type / Bird pulse
Pureshift_1dacq_edit_cpmg_hsqc	2013		Real time/ Bird pulse
Pureshift _2d_edit_cpmg_hsqc_pn	2015		3D type/ Bird pulse
Pureshift_2dacq_edit_cpmg_hsqc	2013		Real time / bird
Cpmg_hsqmbc_pn			Normal sequence

Process list	Comment
Pureshift_2d_inverse_pn_A.list	Real time hsqc pureshift
Pureshift_2d_inverse_pn_Z.list	3D type hsqc pureshift

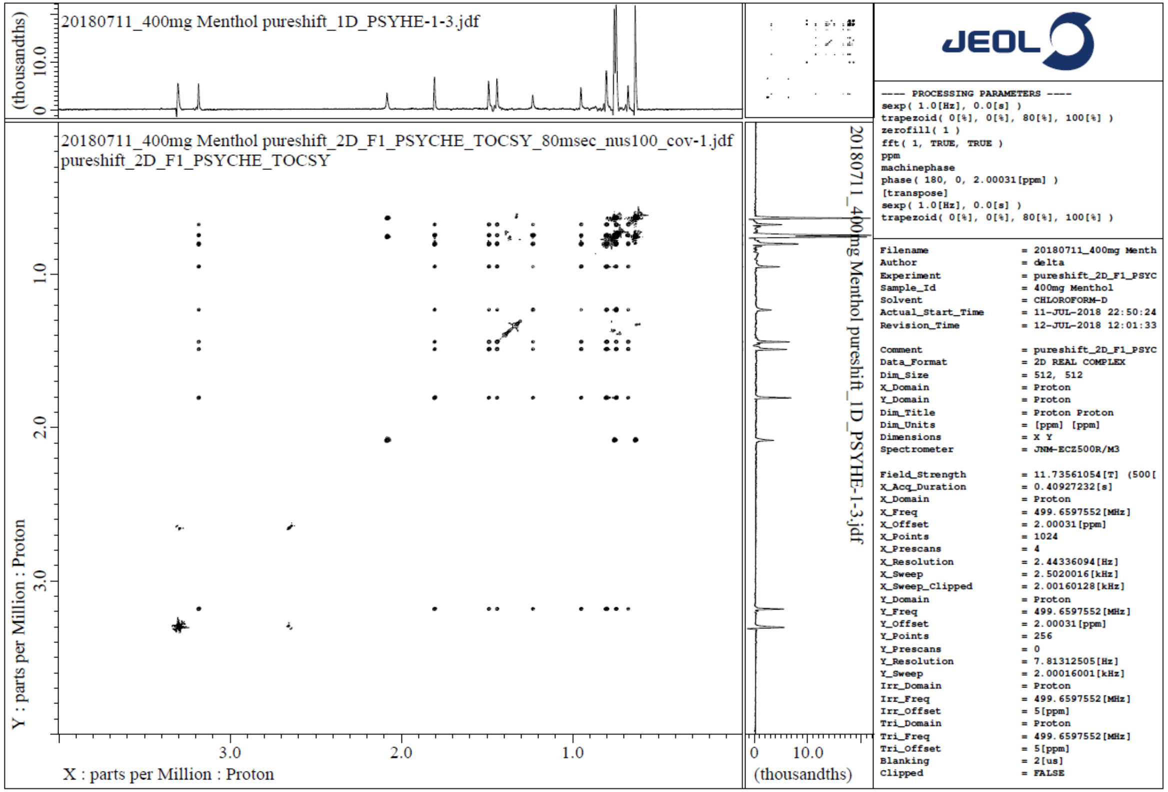
2D TOCSY reference example..



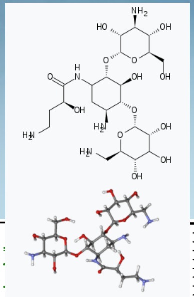
Pureshift_2d_f1_psyche_tocsy nus100%



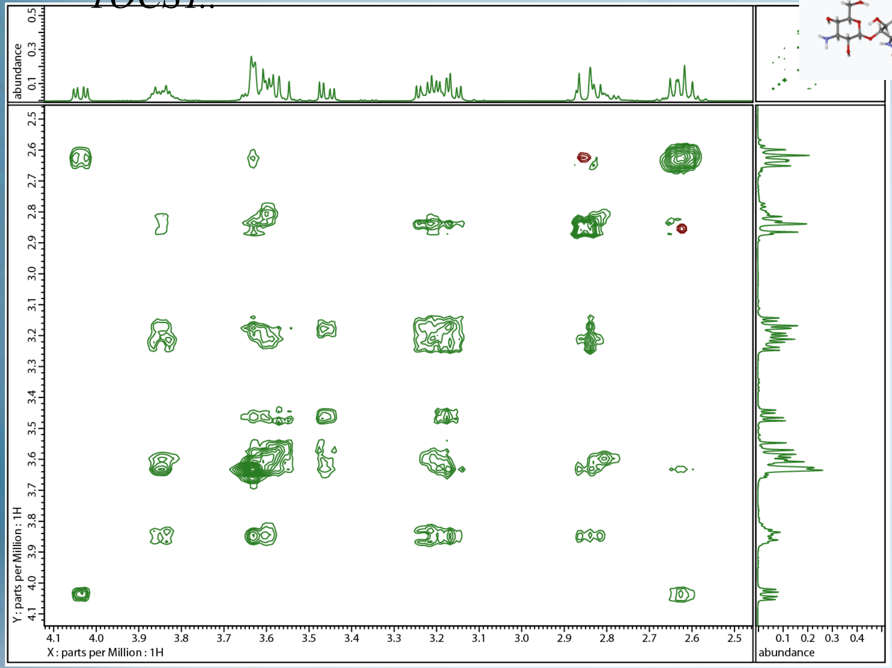
Pureshift_2d_f1_psyche_tocsy nus100%+covaricance



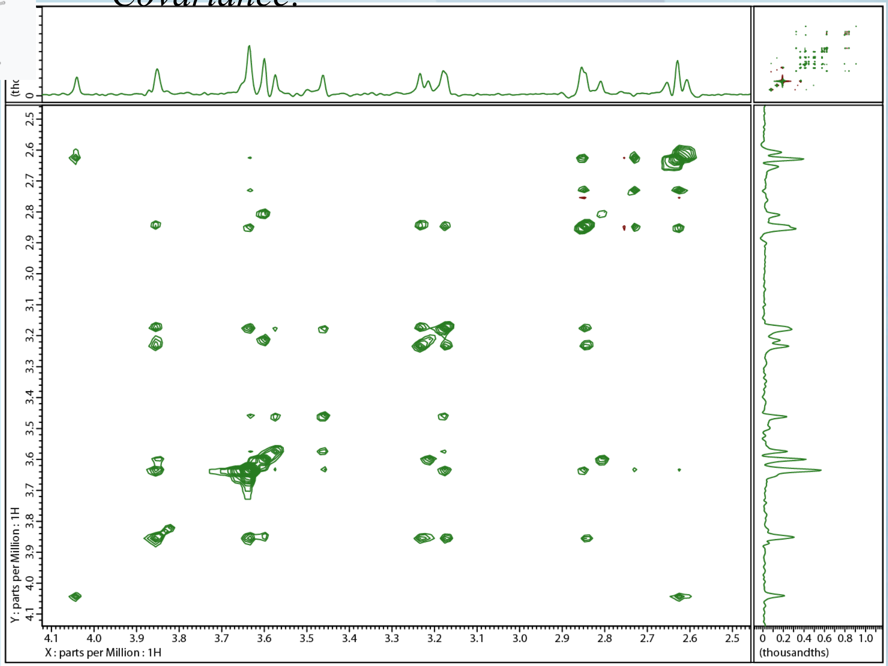
Amikasin – ECZ400S



Expansion from quick 2D TOCSY..



Same region 2D PSYCHE-TOCSY-Covariance!



Final example – an old favorite..before "ipap"

NMR Spectroscopy

PSYCHE CPMG–HSQMBC: An NMR Spectroscopic Method for Precise and Simple Measurement of Long-Range Heteronuclear Coupling Constants

István Timári,[a] László Szilágyi,[b] and Katalin E. Kövér*[a]

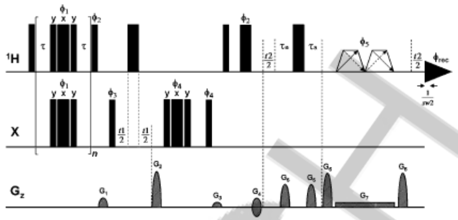


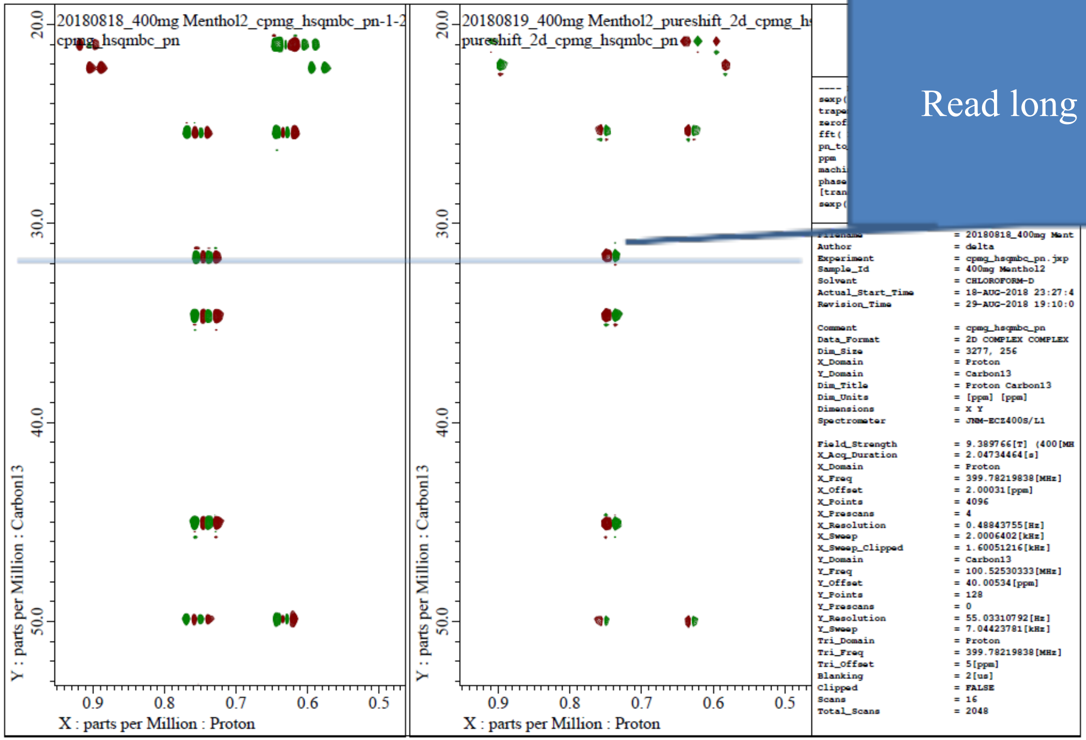
Figure 1. Pulse sequence of the PSYCHE CPMG–HSQMBC experiment proposed for the measurement of long-range heteronuclear coupling constants. Narrow and wide filled bars correspond to 90° and 180° pulses respectively, with phase *x* unless indicated otherwise. Low flip angle (β), frequency-swept Chirp pulses are shown as trapezoids with diagonal arrows. To improve the sensitivity of the experiment, Chirp pulses which sweep frequency in opposite directions can be used simultaneously, as indicated by the dotted arrows. ϕ_1 is incremented according to XY-16 cycles within the CPMG sequence, thus *n* should ideally be adjusted to a multiple of 16. Other phases are $\phi_2 = y$; $\phi_3 = x, -x$; $\phi_4 = x, x, -x, -x$; $\phi_5 = x, x, x, x, y, y, y, y$; and $\phi_{rec} = x, -x, -x, x, -x, x, x, -x$. Delays are set as follows: $\tau = 140$–150 µs, $\tau_a = 1/(4 \cdot sw2)$. Coherence order selection and echo–antiecho phase sensitive detection in the *x* dimension are achieved with gradient pulses (G_2 and G_4) in the ratio 80:20.1 for ^{13}C and 80:15.257 for ^{77}Se, respectively. Purging gradient pulses (G_1 and G_3) are set to 19%, 11% of maximum gradient strength (50 G cm^{-1}). Coherence selection gradient pulses (G_5 and G_6) used in the extra PSYCHE dimension have 49% and 77%. Sine-bell-shaped gradient pulses of 1 ms duration are utilized, followed by a recovery delay of 200 µs (G_1–G_4) and 1000 µs (G_5, G_6). Weak magnetic field gradient (G_7) used under the Chirp pulses is adjusted for 1.8% of maximum gradient strength.

Chem. Eur. J. **2015**, *21*, 1 – 5

Not real time
3D type

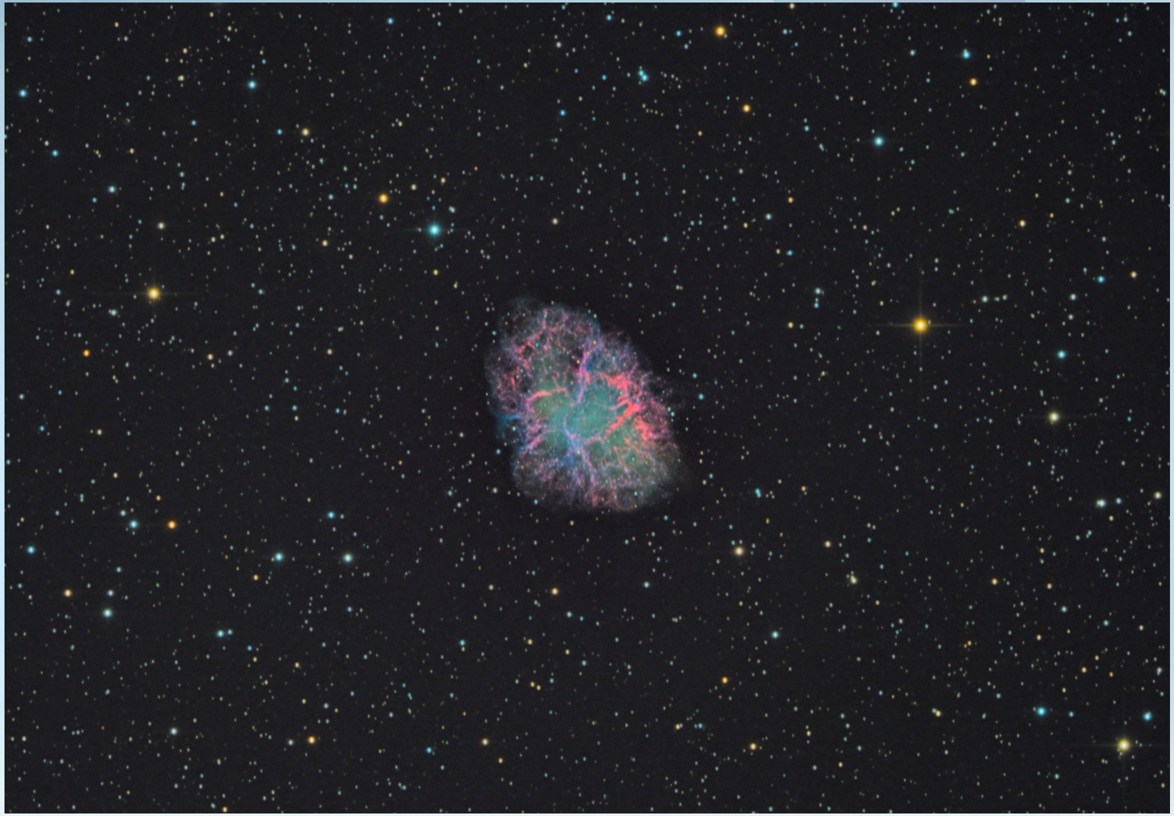
Hsqmbc Normal vs pureshift

Normal pureshift



Read long range JCH

Update from the observatory



Supernova remnant from 1054 event. *Still expanding!*

Many thanks!!

- Thanks to JEOL for helping me learn and play.
- Tim, ET, Ashok, Komatsu san, Mike, Toby. Many more..

- Special thanks to Dave Russell for coming up with ideas to try!

- *Thank you all for coming today!!*